Advanced trends in protein and peptide drug delivery: a special emphasis on aquasomes and microneedles techniques

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Abstract
Proteins and peptides have a great potential as therapeutic agents; they have higher efficiency and lower toxicity, compared to chemical drugs. However, their oral bioavailability is very low; also, the transdermal peptide delivery faces absorption limitations. Accordingly, most of proteins and peptides are administered by parenteral route, but there are many problems associated with this route such as patient discomfort, especially for pediatric use. Thus, it is a great challenge to develop drug delivery systems for administration of proteins and peptides by routes other than parenteral one. This review provides an overview on recent advances adopted for protein and peptide drug delivery, focusing on oral and transdermal routes. This is followed by an emphasis on two recent approaches adopted as delivery systems for protein and peptide drugs, namely aquasomes and microneedles. Aquasomes are nanoparticles fabricated from ceramics developed to enhance proteins and peptides stability, providing an adequate residence time in circulation. It consists of ceramic core coated with poly hydroxyl oligomer, on which protein and peptide drug can be adsorbed. Aquasomes preparation, characterization, and application in protein and peptide drug delivery are discussed. Microneedles are promising transdermal approach; it involves creation of micron-sized pores in the skin for enhancing the drug delivery across the skin, as their length ranged between 150 and 1500 μm. Types of microneedles with different drug delivery mechanisms, characterization, and application in protein and peptide drug delivery are discussed.

Keywords Proteins · Peptides · Delivery · Aquasomes · Microneedles

Introduction
Protein and peptide drugs (PPDs) have a great potential as therapeutic agents because they have higher efficacy and lower toxicity, compared to chemical drugs [1]. Some common therapeutic PPDs are revealed in Table 1. PPDs have poor oral bioavailability due to their physicochemical properties represented by having high molecular weight, hydrophilicity, instability as a result of sensitivity to both enzymes and pH. Accordingly, most of PPDs are administered parenterally [13], but parenteral route is not favorable for most patients. In addition, PPDs face systemic instability due to proteases, opsonization, rapid metabolism, and agglutination [14]. Therefore, much attention has been focused on enhancement of PPDs oral bioavailability and stability [14]. Unfortunately, there are many barriers for oral delivery of PPDs such as gastrointestinal protease enzymes, causing their degradation [13, 15], epithelial barriers formed from a single layer of columnar epithelial cells that slows the absorption [16], and efflux pumps which can pump the PPDs back to gastrointestinal lumen [15]. The main challenge for enhancing oral bioavailability of PPDs is to overcome these barriers. Thus, there are many trends adopted for oral PPDs delivery such as their structural modification, co-administration of enzyme inhibitors, penetration enhancers, and carrier systems [14]. One of the recent carrier systems adopted for enhancement of PPDs stability is aquasomes technique. Aquasomes are recently emerged as nanoparticulate solid drug carrier systems that have three-layered structures which are core, coating, and drug. It consists of ceramic core coated with poly hydroxyl oligomer, on which drug can be adsorbed [17, 18]. Poly hydroxy oligomer film protects PPDs from changing shape and being damaged when they are surface bound [19].

Despite the oral route, numerous researches have been focused on PPDs administration by other routes alternative to parenteral one such as transdermal [20], intranasal [21], buccal
pulmonary [23], and rectal [24] routes, aiming at increasing the biological action of PPDs with enhanced stability. Regarding transdermal PPDs delivery, it has the advantages of avoiding the harsh environment of gastrointestinal tract (GIT) and it is a non-invasive route of administration that lead to better patient compliance. However, transdermal delivery faces the problem of absorption limitation due to skin barrier which tends to prevent the passage of drug molecules having a size greater than 500 Da [25], especially the molecules having a hydrophilic nature [26]. Thus, there are many trends adopted for transdermal PPDs delivery, namely active and passive delivery. Active delivery includes thermal ablation, electroporation, sonophoresis, iontophoresis, and microneedle technology. Regarding passive delivery, it includes chemical enhancers, nanocarriers (such as transfersomes, ethosomes, microemulsions, and nanoparticles) as well as miscellaneous approaches like prodrugs [27]. One of the promising approaches adopted for transdermal PPDs is microneedles (MNs) which create micron-sized pores in the skin for enhancing the transdermal drug delivery [28]. The main advantage of MNs, compared to hypodermic needles, is that they do not cause stimulation to nerves that are associated with pain; this results in enhanced patient compliance [29].

Some of recent trends adopted for PPDs delivery are approved by Food and Drug Administration (FDA) and came to the market, but others are still under clinical investigations. For example, FDA has recently approved Semaglutide, in 2019, branded as Rybelsus™; it is glucagon-like peptide-1 receptor agonist used for oral management of type 2 diabetes [30, 31]. Semaglutide is the structural modification of natural glucagon-like peptide-1, in order to be protected against GIT degradation enzymes such as dipeptidyl peptidase-4 [32]. This review discuss recent advances adopted for oral and transdermal PPDs delivery, shedding the light on recent promising carriers for PPDs delivery, namely, aquasomes and microneedles techniques.

### Oral delivery of PPDs

Oral delivery is the most favorable and convenient route for most patients; however, there are many barriers for oral delivery of PPDs such as gastrointestinal protease enzymes causing their degradation [13, 15], in addition to pH values of GIT resulting in deactivation due to pH-induced hydrolysis, deamination, or oxidation [33]. Also, epithelial barriers formed from a single layer of columnar epithelial cells can slows the absorption [16], in addition to the efflux pumps which can pump the PPDs back to gastrointestinal lumen [15]. Even after drug absorption, it undergoes first pass metabolism by liver resulting in a decrease of the drug fraction that reaches the systemic circulation [34]. Great efforts have been performed to overcome these barriers aiming at increasing the oral bioavailability of PPDs. Accordingly, several strategies for oral delivery of PPDs has been adopted [14].

### Trends adopted for oral delivery of PPDs

#### 1- Structural modification

For efficient oral delivery of PPDs, their physicochemical properties such as (molecular weight, hydrophobicity, and pH stability) as well as biological barriers such as (proteolytic enzymes and poor membrane permeation) should be

| Generic name         | Size       | Indications                                | Route delivered | Ref.       |
|----------------------|------------|--------------------------------------------|-----------------|-----------|
| Etanercept           | 934 AA, 150 kDa | Rheumatoid arthritis, plaque psoriasis, psoriatic arthritis, ankylosing spondylitis | Intradermal, Parenteral, IV, SC, Topical | [2]       |
| Insulin glargine     | 53 AA, 6.1 kDa    | Type 1 and 2 diabetes mellitus              | SC              | [3]       |
| Pegfilgrastim        | 175 AA, 39 kDa  | Neutropenia                                | SC              | [4]       |
| Salmon calcitonin    | 32 AA, 3.4 kDa  | Osteoporosis                               | Oral            | [5]       |
| Cyclosporine         | Cyclic, 11 AA, 1.2 kDa | Prophylaxis, solid organ rejection          | IV, oral        | [6]       |
| Otebrootide (Somatostatin analogue) | 8 AA, 1.02 kDa | Gigantism, acromegaly, symptomatic relief of carcinoid syndrome | IV, IM, SC (depot) | [7]       |
| Liraglutide          | 31 AA, 3.8 kDa  | GLP-1 agonist for treatment of type II diabetes mellitus | SC              | [8]       |
| Bivalirudin          | 20 AA, 2.2 kDa  | Anticoagulant                              | IV              | [9]       |
| Desmopressin         | 9 AA, 1.2 kDa   | Nocturnal enuresis                         | Intra nasal     | [10]      |
| Serratiopeptidase    | 470 AA, 52 kDa  | Anti-inflammatory and antibacterial enzyme  | Oral            | [11]      |
| Influenza vaccine    | Influenza virus particle ~ 80–120 nm | Influenza vaccination | IM, Intradermal, SC | [12]      |

AA amino acid, GLP glucagon-like peptide, IM intramuscular, IV intravenous, SC subcutaneous
considered. Structural modification of PPDs improves their enzyme stability and membrane permeation.

Structural modification of PPDs for oral delivery could be attained by several means, as illustrated in Fig. 1.

Cyclization involves formation of disulfide bonds, lanthionine, dicarba, hydrazine, or lactam bridges between side chains or ends of PPDs. The mechanism of enhanced PPDs oral bioavailability, by cyclization, could be explained by resistance to proteolytic enzymes, decreased flexibility, and reduction in intermolecular hydrogen bonds. The later reduces hydrophilicity of PPDs, allowing their permeation through the gut wall [35]. Cyclosporine, somatostatin, and encephaline are examples of cyclic proteins revealing improved oral absorption [14]. However, not all proteins are amenable to cyclization; in this case, direct PEGylation can be employed which involves covalent conjugation of PPDs with polyethylene glycol (PEG), a safe and non-immunogenic polymer. PEGylation of protein confers benefits regarding the protection of PPDs against protease as well as enhancement of their systemic stability [36]. For example, PEGylated insulin was formulated into mucoadhesive tablets revealed enhanced and prolonged biological effect. Blood glucose level was dropped by 50% 3 h after oral administration; some activity was prolonged till 30 h [37].

PEGylation of salmon calcitonin, at Lys(18)-amine, make it resistant to both proteolytic and systemic clearance [38]. Another structural modification of PPDs is protein lipidization, in which PPDs is conjugated with fatty acids which was reported to improve passage across biological membranes, higher stability, in addition to longer plasma half-live [39, 40]. Salmon calcitonin was lipidized by this technique which enhanced its stability against the intestinal enzymes, increased intestinal absorption, and slowed systemic clearance compared with the free form of salmon calcitonin [40]. Medium chain fatty acids such as caprates was reported to enhance paracellular diffusion of Class III drugs comprising high-soluble and low-permeable molecules such as peptides, and can be used to avoid first pass metabolism [41].

Substitution of natural L-amino acids with D-amino acids, in the peptide backbone, is another promising approach of PPDs structural modification. This substitution makes peptides stable against cleavage by chymotrypsin, elastase, pepsin, trypsin, and carboxypeptidases [42]. It has been previously reported that replacement of natural L-amino acids with D-amino acids resulted in protection of MUC2, a mucin glycoprotein, from proteolytic enzymes [43].

2- Enzyme inhibitors

Enzymatic degradation of PPDs is one of the major obstacles that face their oral delivery. Several types of enzymes such as trypsin, chymotrypsin, pepsin, elastase, and carboxypeptidases are responsible for the cleavage of amino acid chains; each type of enzyme is specific for the cleavage of certain links of amino acids [44]. Co-administration of PPDs with enzyme inhibitors can increase their oral bioavailability. A common enzyme inhibitor is soybean trypsin inhibitor, FT-448, a potent inhibitor of chymotrypsin. When orally co-administered with insulin to rats and dogs, increasing of insulin absorption was attained [45]. Another example of enzyme inhibitors is aprotinin; it is used to reduce bleeding during surgeries, branded as Trasylol™. Co-administration of aprotinin with insulin resulted in decreased blood glucose level by 30%, compared with administration of insulin alone [46]. A novel class of enzyme inhibitor is chicken and duck ovomucoids. The developed formulation of insulin and duck ovomucoids revealed 100% protection against the action of trypsin and α-chymotrypsin [47]. Also, conjugation of mucoadhesive polymer such as sodium carboxymethyl cellulose (Na CMC) with enzyme inhibitor revealed a promising result concerning protein stability. A previous study demonstrated that incorporation of insulin in a mixture of two polymer-inhibitor conjugates, namely Na CMC-Elastatinal and NaCMC-Bowman-Birk inhibitor revealed in vitro protection against proteolytic enzymes. After 4 h of incubation, about 33% of the therapeutic protein remained stable against enzymatic degradation [48]. Unfortunately, enzyme inhibitors may be toxic and damage GIT after prolonged administration [44], as they can prevent the normal absorption of dietary

![Fig. 1 Structural modifications of PPDs for oral delivery](https://example.com/fig1.png)
peptides [49]. Moreover, it is thought that enzyme inhibitors could stimulate the body to produce more proteases, resulting in hyperplasia and hypertrophy of the pancreas [44].

3- Absorption enhancers

The ideal absorption enhancer should be safe at the effective concentration that provides permeation enhancing effect on the intestinal wall. One example of such class is chitosan which is a polymer derivative of chitin, FDA approved, nontoxic, and biocompatible that improves the absorption of hydrophilic macromolecule drugs [50]. In addition, it has a limited absorption from GIT, as it has high molecular weight, thus systemic side effects are limited [51]. Chitosan has been reported to improve the absorption of some drugs, namely insulin, atenolol, and 8-R-vasopressin [51]. The proposed mechanism of absorption enhancement is that the protonated chitosan (pH < 6.5) enhances paracellular permeability through tightly binding to the epithelium by positive charges of chitosan which in turn results in redistribution of tight junction and cytoskeletal F-actin [1, 52]. A previous study revealed that absorption of octreotide into the duodenum was increased by a threefold when co-administered with chitosan [51]. Chitosan derivatives, such as trimethyl chitosan chloride (TMC), have found to overcome the limited solubility and effectiveness of chitosan as absorption enhancer at neutral pH values such in the intestine. TMC enhances the intestinal permeability by the same mechanism of protonated chitosan. In vivo studies in both rats and juvenile pigs revealed that co-administration of TMC with peptide drugs resulted in enhancement of their oral bioavailability [50].

The medium chain fatty acids are another class of ideal absorption enhancers [53]. Caprylate, caprate, and laurate; C8, C10, and C12 fatty acids, respectively can enhance paracellular permeability of hydrophilic drugs through inducing dilation of tight junction [54].

Toxins could also be used as absorption enhancer, so long as they are safe. For example, Zonula occludens toxin, a toxin produced by *Vibrio cholera*, revealed an increase in insulin permeability into Caco-2 cells by 6.3-fold [55].

4- Carrier systems

Several drug carrier systems have been developed to entrap PPDs aiming at increasing their oral bio-availability. Generally, these carrier systems are based on either lipids, polysaccharides, polymeric, cell-penetrating peptides, or inorganic materials [1]. Figure 2 illustrates the main types of carriers used for oral PPDs delivery.

i- Lipid-based carriers

Lipid-based carriers have advantage of excellent biocompatibility regarding crossing the intestinal barrier [56]. Bilosomes (bile salts stabilized vesicles) are examples of recent investigated lipid based carriers for oral delivery of PPDs [57]. They are vesicles composed of phospholipid bilayer membrane incorporated with bile salts like deoxycholate. Bilosomes have the ability to resist disruption by physiological bile salts in GIT, thus protects the entrapped PPDs from GIT enzymes, and absorbed in intact form with subsequent release of entrapped peptides [57, 58].

Self-emulsifying drug delivery system (SEDDS) is a general terminology for both self-micro-emulsifying and self-nano-emulsifying drug delivery systems (SMEDDS/SNEDDS) [59]. It is oil in water nanoemulsion that is formed spontaneously by mixing the mixture of oil, surfactant, and co-surfactant with water [60]. SEEDS are recently discovered for oral administration of hydrophilic macromolecules such as PPDs. Incorporation of hydrophilic macromolecular drugs into SEDDS protects them from the enzymatic and sulfhydryl barrier of GIT. Furthermore, SEDDS have the ability to permeate the mucus gel barrier to facilitate the passage of incorporated hydrophilic macromolecular drugs to the underlying epithelium [61]. Hydrophilic macromolecular drugs should be first dissolved in the oily phase of SEDDS, thus hydrophobic ion pairing (HIP) has been considered to be the most suitable technique. It involves ion pairing of PPDs with hydrophobic counter ion as an attempt to increase the lipophilicity of PPDs. Combination of HIP with SEDDS represents a promising approach for oral PPD delivery [62–64].

ii- Polysaccharide based carriers

Polysaccharides are natural biomaterials that have advantages of being highly safe, biocompatible, and biodegradable. The majority of polysaccharides have hydrophilic groups such as carboxyl, amino, and hydroxyl groups, which bind to intestinal mucus through the formation of non-covalent bonds; this in turn facilitates the absorption of PPDs [65]. Chitosan and its derivatives are the common example of natural polysaccharides. Chitosan is a polycation copolymer, obtained from deacetylation of chitin, embracing muco-adhesion through interaction with sialic acid residues present on mucosal surfaces in addition to having permeation enhancing effect through tight junction [52]. Accordingly, chitosan-based nanoparticles have attracted increased attentions concerning their abilities to oral delivery of PPDs [66, 67]. In fact, chitosan nanoparticles with small size revealed enhanced absorption and passage through GIT. Unfortunately, the ability of chitosan for opening tight junctions in neutral pH condition is inadequate, limiting its potential as penetration enhancer to be only in the duodenum. Furthermore, the chitosan is only dissolved in acidic media and has limited ability for mucoadhesion at neutral and basic pHs. Accordingly, several chitosan derivatives, namely trimethyl chitosan, O- and N-carboxymethyl chitosan, N-methylene phosphonic chitosan, carbohydrate branched
chitosan, and alkylated chitosan, are synthesized to overcome such problems [1, 68, 69]. Other examples of polysaccharides that has been previously reported to improve oral relative bioavailability of PPDs are dextran [70], alginate [71], and cellulose derivatives [72].

### iii- pH responsive polymeric carriers

pH-responsive carriers have been reported to improve the stability of PPD in the stomach and reveal controlled release in intestine [73]. Generally, pH-responsive carriers should protect PPD from low pH and enzymes in the stomach. One of pH-responsive carriers approaches is crosslinked hydrogel; it has a network structure that enables PPD protection from low pH and enzymes in the stomach [74]. Hydrogels may be based on synthetic polymers, like poly (acrylic acid) and poly (methacrylic acid), or natural polymers such as alginate, hyaluronic acid, and guar gum [73]. Another approach of pH-responsive carriers is nanoparticles that exhibiting pH-responsive swelling. pH-sensitive polymeric nanoparticles are formulated mainly with polyanions, polycations, or their mixtures [73]. Eudragits are the common example of pH-sensitive polyanion polymers that are widely used for pH-responsive nanoparticle formulations [75, 76]. Chitosan is the main cationic polymer used for preparation of pH-sensitive nanoparticles; it is soluble at low pH of stomach but insoluble at higher pH of intestine [77]. Accordingly, the solubility of chitosan encounters some limitations for intestinal drug delivery. To overcome these limitations, different chitosan derivatives are developed revealing the desired solubility characteristics such as carboxylated chitosan [78] and N-trimethylated chitosan [79] for oral delivery of insulin and octeriotide, respectively. pH-sensitive polymeric nanoparticles formulated from both polyanions and polycations does not require cross-linker and homogenizer in the formulation procedure due to the presence of oppositely charged polymers; this helps to prevent protein denaturation [80, 81].

### iv- Cell-penetrating peptides based carriers

Cell-penetrating peptides (CPPs) are short peptides, comprising positively charged amino acid fragments (< 30 amino acids). They have excellent ability for membrane penetration, carrying macromolecules or nanoparticles into cells [82]. The mechanism of CPPs for enhanced membrane penetration could be attributed to the presence of abundant basic residues viz., arginine and lysine, resulting in electrostatic interactions with negatively charged molecules viz., glycosaminoglycans and sialic acids present at the cell surface. Furthermore, the presence of hydrophobic amino acid residues viz., tryptophan enables membrane translocation of CPPs due to interaction with the lipid bilayer of the cell membrane; this in turn facilitates cellular uptake of CPPs by endocytosis [83]. In fact, enhancement of PPDs intestinal permeation is attained by either covalent conjugation or simple physical mixing of CPPs with PPDs [82, 83]. For instance, transepithelial permeation of insulin, across the Caco-2 cell line, was reported to be enhanced via covalent conjugation with transactivator of transcription (Tat) peptide which is one of CPPs [84]. Another study revealed that penetratin, which is another example of CPPs, act as efficient carrier for improving intestinal permeation of co-administered insulin [85].
v- Inorganic particles

In contrary to organic matrices, inorganic particles reveal obvious stability in acidic and enzymatic environment [14]. Accordingly, some of inorganic nanocarriers have been successfully employed for oral delivery of PPDs such as silica [86], titanium dioxide [87], zirconium phosphate [88], and hydroxyapatite nanoparticles [89]. Aquasomes are example of inorganic particles that will be discussed later in details.

Transdermal delivery of PPDs

Delivery of PPDs by transdermal route has many advantages such as avoiding of both GIT degradation and first pass hepatic metabolism of drugs, better patient compliance due to the easiness of administration with low frequent dosing, as a result of the prolonged and continuous drug release unique to these systems [90, 91].

In fact, skin is the main important barrier in transdermal delivery [90]; it tends to prevent the passage of drug molecules having a size greater than 500 Da [25], especially the molecules having a hydrophilic nature [26]. Generally, the biological function of the skin is mainly to prevent foreign substances entry. Therefore, transport across the skin to enable drug entry is very important for efficient transdermal delivery [92]. The skin consists of three essential layers; the outermost layer is stratum corneum that is composed mainly of dead cells (keratinocytes); the second layer is known as viable epidermis below which the third layer, known as dermis, is present [93]. The dermis is a fibrous layer, has a thickness of about 1–2 mm; it comprises blood capillaries by which the drug can enter to the circulation [14]. Accordingly, successful transdermal delivery of large hydrophilic molecules such as PPDs requires physical and/or chemical enhancement strategies. Conventional enhancements in transdermal delivery should penetrate the stratum corneum which is the main physical barrier [91, 94, 95]. Methods for enhancing the transdermal PPDs delivery are either via active or passive delivery (Fig. 3). Active delivery includes thermal ablation, electroporation, sonophoresis, and microneedle technology; the latter will be discussed in details. Regarding passive delivery, it includes chemical enhancers, nanocarriers (such as transfersomes, ethosomes, microemulsions, and nanoparticles) as well as miscellaneous approaches like prodrugs [27].

A) Active delivery of PPDs

1- Thermal ablation

It involves short pulses of high heat, about 100 °C, creating small reversible channels, through the stratum corneum, having a size in micron range [96]. Subsequently, drug can be administered to the treated area to penetrate into the circulation.

2- Electroporation

It involves very short pulses of high voltages (10–100 V) in order to perforate the skin [97]. Application of an electric current destroys the structure of the lipid layer, surrounding the dead cells of stratum corneum; this allows molecules to bypass the skin.

3- Sonophoresis

It is also known as cavitational ultrasound which depends on the skin exposure to sound waves, ranged between 20 and 100 kHz, aiming at increasing its permeability [96].

4- Iontophoresis

It utilizes the principles of electrorepulsion and electroosmosis for charged and uncharged peptides, respectively, acting on the drug molecules themselves not on the skin [98]. Iontophoresis involves placing of a device on the skin, allowing generation of an electric current, similar to that of a battery. Upon delivering negatively charged peptides, the battery generates a negative strong charge at the anode, which would be present on the skin together with the drug molecules. Charge-charge repulsion allows the negatively charged peptide to be driven into the skin [90, 99].

However, thermal ablation, electroporation, sonophoresis, and iontophoresis are unlikely to be used as a result of their complex working mechanisms as well as certain irreversible skin damage [27].

B) Passive delivery of PPDs

Passive delivery of PPDs is simple to be used; it does not involve injuries to the skin, i.e., non-invasive. It includes the following approaches:

1- Penetration enhancers

i- Chemical penetration enhancers

They are one of the classes of auxiliary chemical moieties incorporated with drug molecules and protein formulations for enhancing their penetration through the skin. The mechanism of penetration enhancing effect is thought to be association of chemical penetration enhancers with lipids of stratum corneum, resulting in formation of a microenvironment which facilitates the passage of drug through the skin [27]. Examples of commonly used chemical penetration enhancers are solvents such as ethanol and propylene glycol [100], fatty acids...
such as oleic acid and linoleic acid [101], terpenes such as menthol [102], and surfactants such as sodium lauryl sulphate [102].

**ii- Peptide chain mediated delivery**

Some of the peptides have the ability of good skin penetration enhancing effect; also, they can act as drug carriers for transdermal drug delivery. They include cell-penetrating peptides and antimicrobial peptides. Cell-penetrating peptides are amphipathic peptides made from up to 30 amino acids; all known cell-penetrating peptides have a net positive charge at physiological pH. Its skin-penetrating effect depends on its electrostatic interaction with the negatively charged glycoproteins of cell surface [103]. Examples of commonly used cell-penetrating peptides are penetratin [104], trans-activator of transcription (Tat) of human immunodeficiency virus [105] and multiple antigenic peptide (MAP) [105].

Regarding antimicrobial peptides (Magainin), it is a microbial peptide that consists of 23-amino acids that is isolated from the African frog skin (Xenopus laevis) having a net +4 charge. This charge enables it to bind with negatively charged phospholipid membranes due to electrostatic interactions. Magainin has the ability to form pores in the bacterial cell membranes with consequent increase of the permeability of lipid bilayers. Taking into account the interaction between magainin and the lipid membranes, the potential use of magainin as a skin penetration enhancer was assessed by Kim et al. [106] However, magainin alone is unable to enhance transport across the skin. They require co-administration of surfactants for optimal transport.

**2- Nanocarriers**

Novel nanocarriers have been developed to help penetration of molecules through the deep skin layers. The capacity of penetration-enhancing effect utilizing some nanocarriers has been proved to be much potent than that of chemical penetration enhancers [107]. Nanocarriers commonly employed for PPDs delivery are illustrated in fig. 3 [27].

**3- Prodrug**

Prodrug is a reversible chemical modification to alter physicochemical properties of drugs to enhance the solubility, bioavailability, and stability compared to the original compound with preserving its pharmacological actions [27]. For instance, Thyrotropin-releasing
hormone (TRH) has successfully transported through human skin. This is achieved by using the lipophilic prodrug technique for TRH, namely N-octyloxycarbonyl-TRH [108].

After the previous overview regarding recent advances adopted for oral and transdermal PPDs delivery, it is worthy to shed the light on recent promising carriers for PPDs delivery, namely aquasomes and microneedles techniques.

A) Aquasomes as promising carriers for improving PPDs stability

Aquasomes are recently emerged as nanoparticulate solid drug carrier systems that have three-layered structures which are core, coating, and drug. It consists of ceramic core coated with poly hydroxyl oligomer, on which drug can be adsorbed [17, 18]. Generally, the layers that forms aquasomes are assembled through non-covalent bonds, ionic bonds, and van der Wals forces [109].

The structure of aquasome is illustrated in Fig. 4.

Composition of aquasomes

1- Solid core materials

Ceramics are mainly used as core material; they provide structural regularity and high degree of order as they are crystalline in nature. This in turn provides high surface energy, leading to efficient carbohydrate binding onto its surface resulting in stable structure of aquasomes. Common materials used as ceramic core in aquasomes are nanocrystalline tin oxide, brushite (calcium phosphate dehydrate), carbon ceramic (diamond particles), and hydroxyapatite. Calcium phosphate and hydroxyapatite have an advantage of revealing ideal biodegradability, biocompatibility, safety and stability [18].

2- Coating by carbohydrates materials

Carbohydrates coating provides glassy molecular layer capable to adsorb small molecule or therapeutic protein without modifications [18]. Carbohydrates deliver environment that resembles water to the bioactive drug, but keeping it in dry solid state, that protects the three-dimensional conformations of drug molecule [110, 111]. Carbohydrates that are mainly adopted for coating are pyridoxal-5-phosphate, trehalose, cellobiose, lactose, and sucrose; carbohydrate coating is mainly attained by adsorption onto core.

3- Bioactive drug

They have the ability to interact with coating film by non-covalent and ionic interactions.

Properties of aquasomes [112]

1- Aquasomes are nanoparticles, so they have large surface area that can be loaded with considerable amount of bioactive drug. They act like reservoirs for drug release either in a continuous or a pulsatile pattern.

2- They are biodegradable as the core material comprises mainly calcium phosphate that is endogenous material present in the body.

3- They provide adequate environment for PPDs, hence protect them from denaturation. This property is due to the coating of inorganic cores with polyhydroxyl compounds that impart the hydrophilic characteristics.

4- They enhance the therapeutic efficacy of the drug and protect it from phagocytosis by reticuloendothelial system and degradation by other environmental conditions.

5- Aquasomes can be used for several imaging tests as they can be combined with biological labels such as antibodies, nucleic acid, and peptides.
Aquasomes reveal many benefits as a vaccine delivery system. Antigens adsorbed onto the aquasomes surface result in triggering of both cellular and humoral immune responses.

**Mechanism of protein stabilization by aquasomes**

Disaccharide, namely trehalose, was previously reported to induce stress tolerance in bacteria, yeasts, fungi, insects, and some plants. Trehalose protects protein within the plant cells during dehydration process, thus preserves cell structures, colors, flavors, and textures [17, 113]. Kaushik and Bhat explained the mechanism by which trehalose can stabilize protein [114]; they observed that trehalose increased the transition temperature of protein resulting in its increased stability. Furthermore, the hydroxyl groups of carbohydrates interact with polar and charged groups of the proteins, in a similar way to water molecules alone preserving the aqueous structure of proteins upon dehydration. Upon drying, the large quantity of hydroxyl group provided by carbohydrates helps to replace the water around polar groups in proteins, thus maintaining their integrity [115]. In addition, polyhydroxy oligomer film protects PPDs from changing shape and being damaged when they are surface bound. These surface-modified nanoparticles resulted in conformational stabilization to proteins [19].

**Preparation of aquasomes**

The method of aquasomes preparation requires three steps, namely formation of an inorganic ceramic core, then coating of the core with carbohydrates (polyhydroxy oligomer), and finally the drug is loaded to this assembly [17]. Figure 5 represents a diagrammatic illustration of aquasomes preparation.

1- **Formation of an inorganic ceramic core**

Calcium phosphate, hydroxyapatite, and diamond are generally used as ceramic cores; they can be fabricated by colloidal precipitation and sonication. Ceramic materials are characterized by having regular structures, offering a high surface energy that enables them to be bound with polyhydroxy oligomer material. The precipitated cores are separated by centrifugation, and then washed with a sufficient amount of distilled water to get rid of sodium chloride that is formed during the reaction. The precipitates are re-suspended in distilled water, and then passed through a fine membrane filter to obtain the particles of specific size [17]. The reaction equation is as follows:

\[
4\text{Na}_2\text{HPO}_4 + 3\text{CaCl}_2 \rightarrow \text{Ca}_3(\text{PO}_4)_2 + 2\text{NaH}_2\text{PO}_4 + 6\text{NaCl}
\]

2- **Coating of the core with carbohydrates (polyhydroxy oligomer)**

The coating is carried out by a simple mixing of carbohydrate and the aqueous dispersion of the cores under sonication. This is followed by lyophilization to aid an irreversible adsorption of carbohydrate onto the core surface. The amount of un-adsorbed carbohydrate is removed by centrifugation. The effect of core to coat ratio, sonication time, and sonicator power on particle size and shape has been investigated. Core:coat ratio of 1:4 or 1:5 resulted in assembly of spherical-coated particles. Increasing of the power of sonicator (till 15 W/20 W) led to assembly of small spherical discrete particles of less than 200 nm. Upon increasing of sonication time (till 60 min), assembly of small, spherical particles of less than 200 nm were attained, but sonication at 90 min resulted in appearance of small aggregates [19].

![Fig. 5 A diagrammatic illustration of aquasomes preparation](image)
3- Loading of the drug

The drug is loaded to the coated core by adsorption method [19, 111, 116]. This can be achieved by incubation of the drug in the coated core solution; adsorption involves non-covalent and ionic interactions [116]. Briefly, coated particles are dispersed into a solution of known concentration of drug having a suitable pH. The dispersion is kept at low temperature overnight, lyophilized after certain time in order to obtain the drug-loaded aquasomes [17]. It was reported that the factors affecting drug loading are drug concentration and incubation temperature. It was documented that drug loading is proportional to the drug concentration. But unusual sudden increase in drug loading was observed at a certain concentration, as a result of crystallization of drug. Thus, it is necessary to confirm that the drug is loaded by adsorption technique [117].

Disadvantage of aquasomes

According to the method of preparation of aquasomes, it could be deduced that the preparation is time consuming. In addition, the concentration of the drug solution should be carefully adjusted to not exceed a certain point at which the drug is crystallized, resulting in a false increase in the drug loading.

Characterization of aquasomes

1- Morphological analysis and size distribution

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) techniques are used for morphological and size analysis. Mean particle size and zeta potential of the particles can also be assessed by using photo correlation spectroscopy [19, 111]. Damera, DP et al. [118] have demonstrated the image of bovine serum albumin loaded aquasomes, using SEM (Fig. 6) that reveals a spherical shape.

The prepared hydroxyapatite core revealed a size ranged between 30 and 50 nm. This size was increased to be around 200 nm after coating with carbohydrate, namely cellobiose that forms plain aquasomes. The size of aquasomes was further increased to be around 480 nm upon loading of bovine serum albumin [118]. In another study [119], the size of hydroxyapatite core was found to be 90.1 ± 2.3 nm, which was increased to be ranged from 98.5 ± 4.3 to 125.3 ± 3.2 nm according to the type of carbohydrate (oligomer) used in the preparation of aquasomes.

2- Structural analysis

Structural analysis is assessed by Fourier-transform infrared spectroscopy (FT-IR) in the wave number range of 400–4000 cm⁻¹. The characteristic observed peaks, in aquasomes formulations, are then matched with the reference ones. FT-IR structural analysis revealed the characteristic peaks of ceramic core, sugar, and drug in aquasomes formulations which indicates loading of sugar and drug over the ceramic core. Moreover, FT-IR structural analysis revealed the formation of hydrogen bonds between drug and sugar [111, 116].

3- Crystallinity

Generally, X-ray diffraction study is employed to estimate the amorphous or crystalline nature of a compound. Hence, diffraction study of individual components of aquasomes are done and compared to the entire aquasomes. In a previous study [120], it was observed that the individual components of aquasomes gave typical sharp peaks for crystallinity, but X-ray diffraction of carbohydrate coated cores revealed peaks that represent an amorphous structure. This may be resulted from the coating technique that involves dissolving of carbohydrate in solvent followed by lyophilization.

4- Carbohydrate coating

Coating of ceramic core with the sugar is confirmed by concanavalin A-induced aggregation method that estimates the amount of sugar coated over core or by anthrone method that estimates the residual sugar remained after coating. In addition, zeta potential measurement can be utilized to confirm the adsorption of sugar over the core [111, 121].

5- Glass transition temperature

The effect of carbohydrate on the drug loaded to aquasomes can be analyzed by differential scanning calorimetry (DSC)
which has been employed to study glass transition temperature of carbohydrates and proteins. The transition from glass to rubber state can be estimated as a change in temperature upon melting of glass [111].

6- Zeta potential measurement

Zeta potential measures the electrostatic attraction or repulsion between particles. It is the best indicator for the stability of dispersions such as suspension and emulsion. The value of zeta potential depends on the type of carbohydrate (oligomer) used in the preparation of aquasomes. A previous study [119] revealed that zeta potential values of aquasomes prepared from trehalose, cellobiose, and pyridoxal-5-phosphate were found to be $-15.6 \pm 1.15$, $-20.4 \pm 0.9$, and $-23.2 \pm 1.26$ mV, respectively. This could be explained by the existence of a lot of electronegative atoms in the chemical structure of pyridoxal-5-phosphate, compared to trehalose and cellobiose [119]. It can also be utilized to confirm the adsorption of sugar over the core [111, 118]. It was revealed that a decrease in zeta potential value is resulted from the increase of the saturation process of carbohydrate on to the hydroxyapatite core. Coating of hydroxyapatite core with cellobiose resulted in a decrease of zeta potential value from $+15.6$ to $-18.2$ mV due to the presence of numerous OH$^-$ groups of cellobiose. ZP value was further decreased to be $-25.3$ mV upon loading of bovine serum albumin resulting from the presence of COO$^-$ groups of bovine serum albumin [118].

7- Drug loading efficiency

After incubation of coated cores in a known concentration of the drug solution for 24 h at 4 °C, the aquasomes suspension was subjected to high-speed centrifugation for 1 h at low temperature. The supernatant is then separated for estimation of the amount of remained unloaded drug by a suitable method of assay [19]. Kaur, K et al. [119] have studied aquasomes bearing recombinant human interferon-α-2b; loading capacity of the polypeptide drug was found to be ranged between 20.4 ± 3.1 and 48.3 ± 2.3 μg/10 mg of aquasomes. Another study [109] revealed that the adsorption efficiency of ovalbumin was found to be about 60.2 μg mg$^{-1}$ of hydroxyapatite core.

8- In vitro drug release study

In-vitro release study is performed at 37 °C in buffer media of suitable pH with constant stirring. Sample is withdrawn at time intervals, replaced with the same volume of buffer, and analyzed for the amount of drug released [111]. There is a variation in the results of in vitro release; a previous study revealed that about 90% of the adsorbed ovalbumin was released after 50 min from aquasomes prepared using trehalose as a carbohydrate coat [109]. Another study [119] revealed that more than 95% of recombinant human interferon-α-2b was released from aquasomes prepared using trehalose, cellobiose and pyridoxal-5-phosphate as a carbohydrate coat, after 4, 6, and 8 h, respectively. This could be attributed to the nature of the drug as well as the materials used in aquasomes preparation.

Application of aquasomes in PPDs delivery

1- Insulin delivery

Cherian et al. [19] prepared aquasomes for the parenteral delivery of insulin, employing a calcium phosphate ceramic core. The core was coated with several disaccharides such as trehalose, cellobiose, and pyridoxal-5-phosphate. This is followed by the drug loading to the coated cores via adsorption. The biological effect of aquasome formulations of insulin was assessed using albino rats. Pyridoxal-5-phosphate-coated particles were superior to particles coated with trehalose or cellobiose regarding effectiveness in reducing blood glucose levels. This could be explained by the high degree of structural stabilization by pyridoxal-5-phosphate. In addition, there was prolonged activity as a result of slow release of drug from the carrier as well as structural stability of the peptide.

2- Oral delivery of enzyme

Rawat et al. [122] have developed a nanosized ceramic core-based system for oral administration of serratiopeptidase; the acid-labile enzyme. The core was prepared at room temperature by colloidal precipitation with the aid of sonication. Subsequently, the core was coated with chitosan under stirring at a constant rate, and then the enzyme was adsorbed over this coat. The enzyme was further protected via encapsulation of the enzyme-loaded core into alginate gel. The particles revealed a size of 925 nm. The enzyme-loading efficiency on to the particles was about 46%. Both stability and integrity of enzyme during formulation steps was evaluated by in vitro proteolytic activity. The results revealed the good potential of aquasomes to protect the structural integrity of enzymes, resulting in a more potent therapeutic effect.

3- As oxygen carrier

Khopade et al. [121] prepared hydroxyapatite core, coated with trehalose followed by hemoglobin adsorption. In vivo studies in rats revealed that aquasomes indicate good potential to be used as an oxygen carrier with maintaining its activity for 30 days.
In another study, Patil et al. [123] have prepared hydroxyapatite ceramic cores which were subsequently coated with many sugars such as cellobiose, maltose, trehalose, and sucrose. Hemoglobin was subsequently adsorbed onto the coated ceramic core, followed by determination of the drug loading. The capacity of aquasome formulation as oxygen carriers was observed to be as that of fresh blood. The aquasome formulations did not induce hemolysis of the red blood cells; furthermore, the time of blood coagulation was not altered.

4- Antigen delivery

Vyas et al. [111] have prepared aquasomes by self-assembling of hydroxyapatite employing the co-precipitation method. Trehalose and cellobiose have been used as coating materials; subsequently, bovine serum albumin, a model antigen, was adsorbed onto the coated core. The antigen-loading efficiency was about 20–30%. The prepared formulation of bovine serum albumin revealed more potent immunological activity compared to that of plain bovine serum albumin, after SC injection.

In the light of these results, aquasomes were stated to have potential for preserving surface immutability, as they protect the conformation of protein structure to be presented to immune cells that triggers a better immunological response.

B) Microneedles as a smart approach for PPDs transdermal delivery

In fact, most vaccines and biotherapeutics are injected by a hypodermic needle. Injection provides many advantages such as a low-cost, rapid, and direct way for delivery of almost all types of molecule into the body. However, there are disadvantages concerning use of hypodermic needles, as it is difficult to be used by patients themselves [124] as well as the limited patient compliance due to pain and needle phobia [125]. Accordingly, other routes of administration have also been explored, but they did not provide the same efficacy of direct injection by a needle. So, it is necessary to shrink the needle to be in micron dimensions so as to maintain its powerful delivery potential in addition to improve patient compliance and safety [126]. From this point of view, MNs have become an interesting research subject since the mid-1990 when their manufacture could be employed by the use of microfabrication technology [126]. MNs create micron-sized pores in the skin for enhancing the transdermal drug delivery [28]. The main advantage of MNs, compared to hypodermic needles, is that they do not cause stimulation to nerves that are associated with pain. Accordingly, MNs enhance patient compliance and the patients can administer the drug by themselves [29].

Figure 7 indicates the difference between the classical hypodermic needles (Intradermal, ID; Subcutaneous, SC; and Intramuscular, IM) compared to transdermal MNs regarding the anatomy of the skin. It is revealed that the hypodermic needle penetrates deeply into the dermis at which pain receptors are present. Thus it is very painful, resulting in poor patient compliance. On the contrary, MNs patch penetrates the barrier of stratum corneum, resulting in the direct drug delivery into the epidermis or upper dermis layer without causing pain [127].

Dimensions of MNs

Since the thickness of epidermis is up to 1500 μm, accordingly the needle length till 1500 μm is suitable to release the drug into the epidermis. Larger needles can go deeply into the dermis with consequent damaging the nerves causing pain [101]. Generally, MNs length ranged between 150 and 1500 μm, while the width ranged between 50 and 250 μm with having 1–25 μm tip thickness [128].

Types of MNs and different drug delivery mechanisms

Generally, MNs can be classified into solid MNs, drug-coated MNs, dissolving MNs, hollow and hydrogel-forming MNs as revealed in Fig. 8; each type of MNs can deliver the drug by a certain mechanism [126].

1- Solid MNs for skin pretreatment

Microneedles can be used prior treatment, by formation of channels of micron size in the skin (Fig. 8). Sharp MNs penetrate into the skin for making holes through which drugs can transport, either for localized action in the skin or for
transdermal delivery to systemic circulation. Then, the drug can be administered to the skin surface over the formed holes by using a transdermal patch loaded with drug, or using a semisoloid topical formulation, such as cream, ointment, gel, or lotion [126]. Solid MNs deliver the drug by passive diffusion through the layers of the skin [129].

The fabrication of solid MNs should provide adequate mechanical strength through choice of MNs material, geometry, and reducing the force required to insert MNs into tissue via increasing the sharpness of the tip. Solid MNs have been fabricated from many materials including silicon [130], nondegradable polymers such as a copolymer of methylvinylether and maleic anhydride [131], polycarbonate [132], and polymethylmethacrylate [133], in addition to biodegradable polymers such as polyglycolic acid (PGA), poly-lactic-co-glycolic acid (PLGA), and polylactic acid (PLA) [134]. Metals including stainless steel [135] and ceramics [136] are also used for fabrication of solid MNs.

2- Coated MNs

Solid MNs can be used not only prior treatment, but also it can carry and deposit drug within the skin. This can be achieved by coating MNs with a drug solution that is suitable for coating and subsequent dissolution. Upon insertion of MNs, the desired dose of the drug is delivered by dissolution from the coating layer [137]. It should be to consider that the drug dose which can be administered by coated MNs is limited to the amount of the drug that can be coated onto MNs, which is typically < 1 mg for small MNs arrays [138]. Several processes were adopted for coating of MNs; most of them involve dipping or spraying MNs using an aqueous bioactive drug solution of high viscosity to be more retained on MNs during drying. Coating has been employed by dipping MNs into a large bath of coating solution once or repeatedly, or into microwells of coating solution for each individual microneedle [126]. Other techniques such as layer-by-layer coating technique have been employed for MNs coating [139, 140]. DNA or protein molecules have been coated onto polymer and metal MNs by alternately dipping into two solutions containing oppositely charged solutes, such as positively charged polymer and negatively charged DNA to form a polyelectrolyte multilayer.

MNs coating solution should have the following considerations [138, 141]:

(i) Higher viscosity and smaller contact angle of the coating solution with the substrate, by addition of surfactant, providing uniform coating as well as improve coating thickness.

(ii) The coating solution should be hydrophilic for fast and complete coating dissolution into the skin that has an aqueous environment.

(iii) The dried coating should has a high mechanical strength that is necessary to maintain the coating adherent to MNs during insertion into skin.

(iv) Safety of the coating solution additives and solvent for human use and should not damage the coated bioactive drug.
Several surfactants as well as thickening agents have been used to facilitate MNs coating. For example, Lutrol F-68 NF [141], Tween 20 [142], and poloxamer 188 [143] are surfactants that have been used to improve spreading on MNs surfaces. Carboxymethylcellulose (CMC) sodium salt [141], methylcellulose [143], sucrose, hyaluronic acid, sodium alginate polyvinylpyrrolidone (PVP), and glycerol [138] have been used as thickening agents for increasing coating thickness. Also Stabilizers such as trehalose, glucose, sucrose, dextran, and inulin can be added to coating solution to protect the bioactive drugs from damage during the coating/drying process [144].

3- Dissolving MNs

Dissolving MNs are fabricated from biodegradable polymers in which the drug is encapsulated; dissolution of the drug takes place after inserting MNs in the skin. MNs are not removed out after insertion; the polymer is degraded within the skin and controls the drug release. Polymer dissolution within the skin let it to be favored for long-term therapy as well as improve the patient compliance [137]. Homogenous distribution of the drug into MNs should be attained. Therefore, polymer-drug mixing is an important step in such fabrication [129]. Chen et al. [145] developed dissolving microneedles which revealed efficient and rapid drug delivery avoiding causing skin irritation.

Generally, dissolving MNs have been fabricated using micromolds filled by solvent casting, using water as the common solvent. Various materials including CMC [146], chondroitin sulfate [147], dextran [147], PVP, polyvinyl alcohol (PVA) [148], PLGA [149], and sugars [150] have been dissolved in water, and then filled into the mold cavities and let to be dried; additional use of vacuum can be sometimes employed.

4- Hollow MNs

Hollow MNs have hollow space filled with the dispersion or solution of the drug. They have holes at the tips; upon insertion into the skin, the drug is directly delivered into the epidermis or the upper dermis layer. It is used commonly for drugs having high molecular weight such as vaccines, proteins, and oligonucleotides [151]. These MNs have the ability of delivering a large dose of the drug because the hollow space inside MNs enables incorporation of more amount of drug. It is essential to keep a constant flow rate of the drug [152]; the main factor that affects the flow rate is the resistance of the dermal tissue during insertion of MNs tips [153]. Also, increase in the cavity of MNs results in an increase in the drug flow rate but lead to reduced strength and sharpness. It is necessary that hollow MNs reveals suitable mechanical strength and their cavities are not clogged during transdermal drug delivery [151]. A metal coat is sometimes applied on the MNs in order to increase their strength but this can cause sharpness of the needles [151]. Recently, Suzuki et al. [154] have developed hollow MNs that mimic the mosquitoes’ action, which showed enhanced penetration into the skin.

Hollow MNs have been directly fabricated from a material substrate, using microelectromechanical systems (MEMS) techniques such as laser micromachining [155], deep reactive ion etching of silicon [156], deep X-ray photolithography [157], an integrated lithographic molding technique [158], wet chemical etching, and microfabrication [159].

5- Hydrogel-forming MNs

They are recently developed; they are fabricated utilizing super-swelling polymers. The polymers provide the hydrophilic structure which allows it to be able of absorbing a large amount of water into their polymeric network structure. These polymers swell upon insertion into the skin by the aid of the interstitial fluid, resulting in the formation of microchannels between the drug patch and the capillary circulation. These MNs behave as a rate-controlling membrane upon swelling. They are characterized by easy sterilization, having flexibility in size and shape, and complete removal from the skin [160]. Hydrogel-forming MNs revealed effective and smart transdermal delivery of insulin [161] and Bevacizumab [162]. It is worthy to mention that hydrogel-forming MNs have been demonstrated to improve the ocular delivery of a model macromolecule, FITC-dextran [163].

Characterization of MNs

1- Morphological analysis

The real shape of MNs is observed visually and photographed by digital camera; MNs could also be visualized by SEM for measuring the needles dimensions. Furthermore, confocal laser scanning microscope (CLSM) is utilized to determine the distribution of fluorescein coupled drug within MNs arrays [164, 165]. Figure 9 illustrates the morphological characterization of MNs developed by Pan et al. [164] for intradermal delivery of polyethyleneimine/STAT3 siRNA complexes aiming to treat skin melanoma.

Pan and co-workers have reported that the prepared MNs had a height of 650 μm, while the base and tip radii were 300 and 20 μm, respectively. CLSM revealed that polyethyleneimine/STAT3 siRNA complex was located preferentially in the upper tips of MNs [164].

2- Mechanical strength and the depth of MNs insertion into the skin

Mechanical property of MNs was assessed by texture analyzer, while the depth of MNs insertion into the skin was
determined by optical coherence tomography of skin samples after MNs insertion. The force required for MNs, prepared by Pan and co-workers, to puncture the rat skin, without MNs deformation, was 20 N. Thus the prepared MNs revealed a mechanical strength that is sufficient to puncture the rat skin for targeting of polyethyleneimine/STAT3 siRNA complex into the layers of basal epidermis and upper dermis, where melanoma cells were present [164].

3- In vitro drug diffusion study

In vitro diffusion study is conducted to assess the ability of MNs to deliver the drug via transdermal route. The prepared MNs arrays are inserted into a shaved animal skin, and then placed on the orifice of franz diffusion cell with the stratum corneum (MNs side) facing up. The receiver medium, namely phosphate buffer saline pH 7.4, is constantly stirred at 37 °C. At predetermined time intervals, samples from the receiver medium are withdrawn and replaced with fresh medium to assess the amount of permeated drug [162].

Applications of MNs in PPDs delivery

1- Peptide delivery

Peptide delivery through MNs has the main advantage concerning overcoming poor skin permeation of the peptides. For example, desmopressin, which is a synthetic form of vasopressin, is used for patients of low vasopressin level. It is used to treat bedwetting in young children, diabetes insipidus, and hemophilia A. Delivery of desmopressin using coated MNs technique was investigated; the results revealed more safety and efficacy of MNs delivery compared to other routes [142]. Another example is cyclosporin A, which is a water-insoluble and high molecular weight cyclic peptide, used to treat numerous skin diseases. Dissolving MNs for delivery of cyclosporine A was fabricated, by molding process, with the dimension of 250 μm width and 600 μm in length. Fabricated MNs with 10% cyclosporine A was inserted into the porcine skin for 60 min, showing dissolution of about 65% of MNs with 34 ± 6.5 μg drug delivery [167]. Liu et al. [168] have investigated the loading of GAP-26, a gap junction blocker, into polyethylene glycol diacrylate-based MNs for delivery through swelling effect. The fabricated MNs revealed improvement in the permeation of loaded peptide.

2- Hormone delivery

Delivering insulin employing MNs technique was proved to be more effective for lowering blood glucose levels [135]. Dissolving MNs encapsulating insulin have been deeply investigated in mice, diabetic rats, and dogs [169–171]; this approach enabled stable insulin encapsulation as well as effective insulin delivery for reduction of blood glucose levels. Hollow MNs, including silicon MNs made using MEMS-based etching techniques, have been fabricated for effective delivery of insulin [172]. Li et al. [173] have studied the delivery of insulin through solid MNs and by evaluating the effect on blood glucose levels in diabetic mice. The results revealed that blood glucose level was reduced to 29% of the initial level at 5 h, confirming the improved penetration of insulin to the skin using MNs.

Clinical study has been conducted for parathyroid hormone (I-34) coated MNs; the results revealed that $T_{\text{max}}$ and apparent $T_{1/2}$ were shortened by three and two times, respectively compared to conventional injection therapy [174].

3- Vaccine delivery

MN have been studied as a promising approach for needle-free immunization. MNs revealed successful clinical response for influenza vaccine [175]. Recently, dissolving microneedles are reported to improve the thermal stability of inactivated polio vaccine, compared with conventional liquid vaccine [176].
Clinical trials and safety of MNs

Several pre-clinical trials were performed to investigate the effectiveness of MNs in human subjects. Pre-clinical study developed by Vicente-Perez et al. [177] revealed that repeated application of microneedles does not change appearance and barrier function of the skin and does not cause any remarkable disturbance of infection, inflammation, or immunity serum biomarkers. Kaushik et al. [178] conducted the first clinical trial for MNs in human subjects in 2001. MNs were applied to the forearm of the 12 male and female healthy volunteers; the results indicated that the pain induced by MNs was less than that induced by the hypodermic needles.

Arya et al. [179] have conducted clinical trial, on 15 human subjects, to find whether MNs result in topical skin reactions and acceptable by patients or not. The study revealed that MNs did not induce any pain, erythema or swelling at the site of the patch application. The patients could apply the patches by themselves without any need of the applicator. MNs are more comfort to the human subjects in comparison with the conventional needles.

Approved MNs products

There are various approved medical and cosmetic products, using MNs, which are sold all over the world. Some of them are employed for cosmetically use [126]; however, there are medical microneedels products such as BD Soluvia® microinjection system [180]. It is a single hollow microneedle having a length of 1.5 mm, attached to a syringe, prefilled with influenza vaccine for intradermal vaccination.

Conclusion

PPDs have a great therapeutic potential, but it has some problems such as poor oral bioavailability and systemic instability. The oral route is simple and very acceptable by the most patients, but PPDs are degraded in GIT in addition to poor oral absorption due to their hydrophilic nature. Advanced strategies adopted in the oral delivery of PPDs involve the use of enzyme inhibitors, absorption enhancers, and direct structural modification of PPDs. Muco-adhesive polymers and nanocarriers have also been utilized to increase the stability of peptides in addition to increase their absorption.

Delivering PPDs by transdermal route has the advantage of avoiding instability in GIT, but a problem concerning PPDs through the skin is arisen. Recent strategies for enhancing the passage of PPDs through the skin have been studied including microneedle technology, thermal ablation, electroporation, sonophoresis, and iontophoresis. Passive delivery of PPDs transdermally has been deeply investigated. The transdermal technique that gets its way to the light is microneedles, where there are many products of microneedles present in the market for either cosmetic or clinical use. Another recent strategy for increasing the stability of PPDs is aquasomes which has been investigated regardless the route of administration. Unlike microneedles, aquasomes did not find its way to the market; this may be due to the long time consumed during the preparation of aquasomes. Generally, it can be concluded that challenges of finding alternatives to parenteral route for PPDs administration have started from several years ago. Therefore, a lot of researches have focused on this aim; this has resulted in the presence of commercial products for PPDs delivery by routes other than parenteral ones. The research is still in progress to increase the number of PPDs delivery systems that can find their way to the market.

Compliance with ethical standards

Conflict of interest

The author confirms that this article content has no conflict of interest.

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