Nanostructures as Promising Tools for Delivery of Antimicrobial Peptides

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Abstract: Antimicrobial peptides have been extensively investigated for their potential applications as therapeutics and food biopreservatives. The antimicrobial activity may be impaired by the susceptibility for proteolytic degradation and undesirable interactions of the antimicrobial peptide in the biological environment. Development of nanostructures for entrapment and delivery of antimicrobial peptides may represent an alternative to the direct application of these substances. Lipid nanovesicles have been developed for encapsulation of antimicrobial peptides. Phosphatidylcholine is often employed in liposome manufacture, which is mostly achieved by the thin-film hydration method. Nanofibers may allow different physical modes of drug loading, including direct adsorption on the nanofiber surface or the assembly of drug-loaded nanoparticles. Self-assembled peptides reveal attractive features as nanostructures for applications in drug delivery and promising as antimicrobial agent for treatment of brain infections. Magnetic nanoparticles and nanotubules are also potential structures for entrapment of antimicrobial peptides. Nanoparticles can be also chemically modified with specific cell surface ligands to enhance cell adhesion and site specific delivery. This article reviews the most important nanostructures as promising tools for peptide delivery systems.

Keywords: Antimicrobial, bacteriocin, bioactive peptides, drug delivery, encapsulation, liposome, nanostructure, nanoparticle.

INTRODUCTION

Nanotechnology involves the development of materials or structures sized between 1 to 100 nanometers. Nanotechnology has rapidly developing in many different fields and the remarkable potential uses of nanoscale materials and devices in medicine and biological sciences have been depicted [1-3].

Nanostructures hold enormous potential as effective drug delivery systems. Many nanosystems have been investigated for drug and gene delivery applications. Nanostructures consist of different biodegradable materials such as natural and synthetic polymers, lipids and metallic particles in the nanometric size range [4,5]. With the continued development of controlled release technology, the need for materials with more specific drug delivery properties has arisen and new polymeric composite materials have been constantly developed [6,7]. Nanoparticles are taken up by cells more effectively than larger micrometric particles and then could be used as effective transport and delivery systems. It has been observed that a great number of nanoparticles cross the epithelium than do microparticles. Also, targeting the drug to the desired site would improve therapeutic efficiency and also permit to reduce the drug amount used. Diverse nanoparticulate systems are being used for the nanoencapsulation of peptides to improve their accumulation inside target cells due to easy and efficient cellular internalization [8-10]. Other nanostructured systems have been also suggested as promising tools for drug delivery. Nanofibers produced by the electrospinning method are considered for diverse biomedical applications, and nanofibers incorporating antimicrobials could be an interesting option for direct delivery to sites of skin infection [11]. Nanotubes are also an emerging alternative for transporting therapeutic molecules with a great potential in the field of nanobiotechnology [12]. The most relevant nanostructures for biomedical applications and some examples of potential uses are summarized in Table **1**.

Bioactive peptides have their medical uses limited by low bioavailability, which is related to their poor stability to proteolysis and hydrolysis, low permeability across barriers, and short shelf-life in the circulatory system [9]. Nanoparticles appear to be a promising alternative for bioactive peptide administration and storage because of their versatility for formulations, subcellular size, biocompatibility and continued release properties. These systems have acquired increasing attention as a tool to target drugs to its site of action or to optimize drug circulation *in vivo*, allowing reduction of side effects as well as making treatments easier [16,17].

Antimicrobial peptides have unique characteristics that can overcome the limitations of existing antibiotics. Many antimicrobial peptides demonstrate a rapid bactericidal and/or fungicidal effect and have reduced immunogenicity. The antimicrobial peptide database (APD, available at http://aps.unmc.edu/AP/main.html) provides detailed information on more than 1,500 different antimicrobial peptides, illustrating the huge therapeutic potential of these compounds. Thus, the entrapment of antimicrobial peptides by nanostructures might represent an alternative to overcome some problems related to the direct application of these

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Table 1. Examples of Nanostructures as Potential Drug Carriers

Nanostructure	Example of Potential Utilization
(a) Nanoparticles	
Nanovesicles	Increased bioavailability of cyclosporine A encapsulated in poly(isohexylcyanoacrylate) nanovesicles [9]
Nanospheres	Prolonged therapeutic effect of octreotide, a long-acting somatostatin analogue [9]
Metallic nanoparticles	Combined effect of silver nanoparticles with antimicrobial peptides in coatings for medical instruments [13]
(b) Nanofibers	Inhibition of skin pathogens by poly(L-lactic acid) and poly(lactate-co-glycolide) nanofibers incorporating antimicrobial agents [11]
(c) Nanotubes	
Carbon nanotubes	Increased uptake of conjugated paclitaxel on single walled carbon nanotubes by breast tumor cells [14]
Polymer-based nanotubes	Controlled release of dexamethasone by nanotubes prepared with poly(lactide-co-glycolide) [15]

substances, such as proteolytic degradation or undesirable interaction in biological systems. In this context, the present article discusses the potential applications of nanoscale particles to transport and delivery of antimicrobial peptides.

AN OVERVIEW ON ANTIMICROBIAL PEPTIDES

Antimicrobial peptides are widespread produced among living organisms. Production of antimicrobial peptides is found as a prevalent strategy used by plants, animals and microorganisms to combat pathogenic microorganism. Besides the variable structural characteristics, these peptides are mostly cationic, showing an amphipathic nature, containing less than 50 amino acid residues in a linear or cyclic arrangement [18,19]. These peptides are divided in several groups according their molecular masses, secondary and tertiary structures, presence or absence of disulfide bridges. Many of them, such as cecropin A, β-defensins, magainins and iturin A show superior microbiocidal activity compared to synthetic and semi-synthetic antibiotics, and present a remarkably broad antimicrobial spectra in killing bacteria, fungi, parasites and even viruses [20-22]. These peptides have been also associated with inhibition of multiresistant bacteria like methicillin-resistant *Staphylococcus aureus* and antitumoral activities [23].

The emergence of multidrug-resistant pathogens that caused serious problems in hospitals worldwide has intensified the search for novel drugs, in order to replace or to be used in complement with the existing antibiotics. In this concern much interest has been addressed to antimicrobial peptides, which are less likely to cause pathogen resistance [21]. This fact is very appealing for their use as coating materials in medical devices. Antimicrobial peptides, such as nisin, epidermin and Pep5 have been used as effective barriers against bacterial adhesion to catheters [24,25].

Most antimicrobial peptides share some common physico-chemical properties, such as small molecular mass, cationic character, and being often membrane active [26]. Although different peptides may act in different ways and the exact mechanisms are not completely elucidated, most antimicrobial peptides kill target cells by membrane permeabilization through peptide-lipid interactions. Various mechanisms have been proposed, including the formation of the discrete channels that dissipate ion gradients across the membrane, disturbance of the lipid bilayer as a result of carpet-like peptide binding, phase separation due to specific peptide-lipid interaction, and detergent-like solubilization of the membrane [20,27].

Bacteria may represent the most viable alternative to obtain antimicrobial peptides at a commercial level. Indeed, these peptides are produced by different classes of bacteria, including enterobacteriaceae, lactic acid bacteria, coryneforms and other [28,29]. Several antimicrobial peptides representing diverse chemical structures are produced by *Bacillus*, being cyclic peptides like gramicidin S and bacitracin, and lipopeptides like iturins, bacilomycins and fengicins typical secondary metabolites produced by this genus [30]. Several species of *Bacillus* also produce bacteriocins or bacteriocin-like substances [31,32]. *B. subtilis* produce diverse antimicrobial peptides, some of them synthesized during the exponential phase like subtilosin, subtilin, sublancin, TasA, and other during the stationary phase, like surfactin, bacilysin and iturins [30,33]. Bacteriocins produced by lactic acid bacteria, such as nisin, pediocins and lactacins, attain enormous potential for use in the perspective of food protection against pathogenic and spoilage bacteria [34,35]. Most bacteriocins present strong antilisterial activity and inhibition of bacterial strains resistant to conventional antibiotics has been related to some bacteriocins, indicating potential medical applications for these peptides. Despite the enormous potential of such bacterial antimicrobial peptides, relatively few studies have been developed on their encapsulation in nanoparticles [36].

NANOENCAPSULATION

Encapsulation in nanoparticles may offer a potential solution to protect antimicrobial peptides, enhance their

efficacy and stability in practical applications. Nanoparticles can be defined as solid colloidal particles that contain the active substance, and are a combined name for nanospheres and nanovesicles (or nanocapsules). Nanovesicles are vesicular systems where the active substance is confined to a cavity or inner liquid core enclosed by a polymeric membrane (Fig. **1**). The active substances may be dissolved in the inner core or may be also adsorbed at the surface of nanovesicles. Nanospheres have a matrix-type structure [9,37].

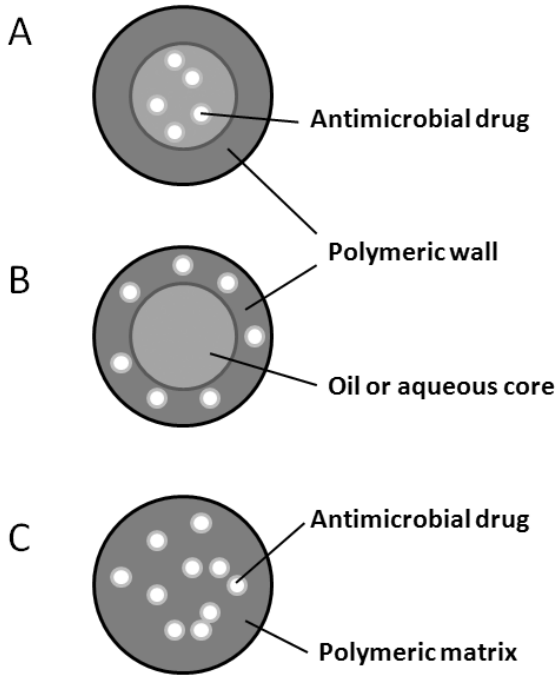

Fig. (1). Schematic representation of nanoparticles. In nanovesicles, the antimicrobial drug can be dispersed in the oil/aqueour core (**A**) or embedded in the wall (**B**). (**C**) Nanospheres have a matrix-type structure where the drug may be absorbed at their surface or entrapped.

The encapsulation of peptides and proteins in nanostructures have been attained by diverse methods including emulsification-polymerization, interfacial polymerization, solvent evaporation, salting out, coacervation, combination of sonication and layer-by-layer technology, surface-functionalized particles and other. Methodologies for nanoencapsulation of peptides and proteins have been recently reviewed [10]. Natural and synthetic polymers and metallic nanoparticles have been investigated for protein and peptide encapsulation. Among natural polymers, chitosan has attracted particular attention as a biodegradable material for mucosal delivery systems. Chitosan nanoparticles have low toxicity and appear to increase systemic absorption of hydrophobic peptides such as cyclosporine A [38]. Gelatin nanoparticles are also an interesting material to exploit controlled release in food and medicinal applications, since it is nontoxic and biodegradable nature. Gelatin molecules consist of both cationic and anionic groups, and this functionality warrants usefulness for encapsulation of both acidic and basic peptides [39]. Comparison of protein, lipid and chitosan nanoparticles for delivery of ciprofloxacin indicate that chitosan and solid lipid nanoparticles can act as promising carriers for sustained ciprofloxacin release in ocular and skin infective conditions [40]. The biodegradable and biocompatible nature of poly(ε-caprolactone) and poly(L-lactic acid) deserve these synthetic polymers the most successfully used for the development of delivery systems in nanomedicine. Also, amine functionalized polymeric nanoparticles are being used to enhance charge directed targeting of lysozyme nanoparticle conjugates to bacteria [41].

Nanovesicle encapsulation may provide protection against degradation or interaction with undesirable compounds, resulting in enhanced therapeutic activity of the antimicrobial peptide by sustained activity and improved stability. The antimicrobial peptide P34 encapsulated in phosphatidylcholine (PC) nanovesicles showed higher residual activity after exposition to Maillard reaction products in comparison to the free peptide [42]. Phytoglycogen subjected to β-amylolysis and subsequent succinate and octenyl succinate substitutions was used to develop nanoparticles as carriers for nisin. The inhibitory activity against *Listeria moncytogenes* was monitored during 21 days, and the nanoparticles led to prolonged retention of nisin activity [43].

Lipid Nanovesicles

Lipid nanovesicles can be defined as small liposomes with size ranging the nanometric level. Liposomes are colloidal structures having an internal aqueous pool formed by self-assembly of amphiphilic lipid molecules in solution. Due to the presence of both lipid and aqueous phases in the structure of lipid vesicles, they can be utilized in the entrapment, delivery, and release of water-soluble, lipid-soluble, and amphiphilic materials [44,45]. The challenge in making nanovesicles is to achieve the formation of vesicles with the right size, satisfactory polydispersity, elasticity, structure, and encapsulation efficiency [45-47].

Encapsulation of antimicrobial peptides into lipid nanovesicles is mainly reported to be achieved by the thin-film hydration method (Fig. **2**). By this technique, a pre-formed lipid film is hydrated with an aqueous buffer containing the antimicrobial peptide, at a temperature above the phase transition temperature of lipids. The resulting heterogeneous population of multilamellar vesicles can be further processed (membrane extrusion, sonication), resulting in small unilammelar vesicles of uniform size [47,48]. Alternatively, in the reversed-phase method (Fig. **2**), an aqueous solution of the antimicrobial peptide is dropped into the lipid solution to form water in oil emulsion, which is sonicated yielding a homogeneous opalescent dispersion of reverse micelles. The organic solvent is evaporated, resulting a highly viscous organogel, which is reverted to nanovesicles after addition ultrapure water [49]. These two methodologies were compared to encapsulate the antimicrobial peptide nisin in PC nanovesicles, also testing both probe-type and bath-type ultrasound. Film hydration using bath-type ultrasound resulted in liposomes of smaller size and with full

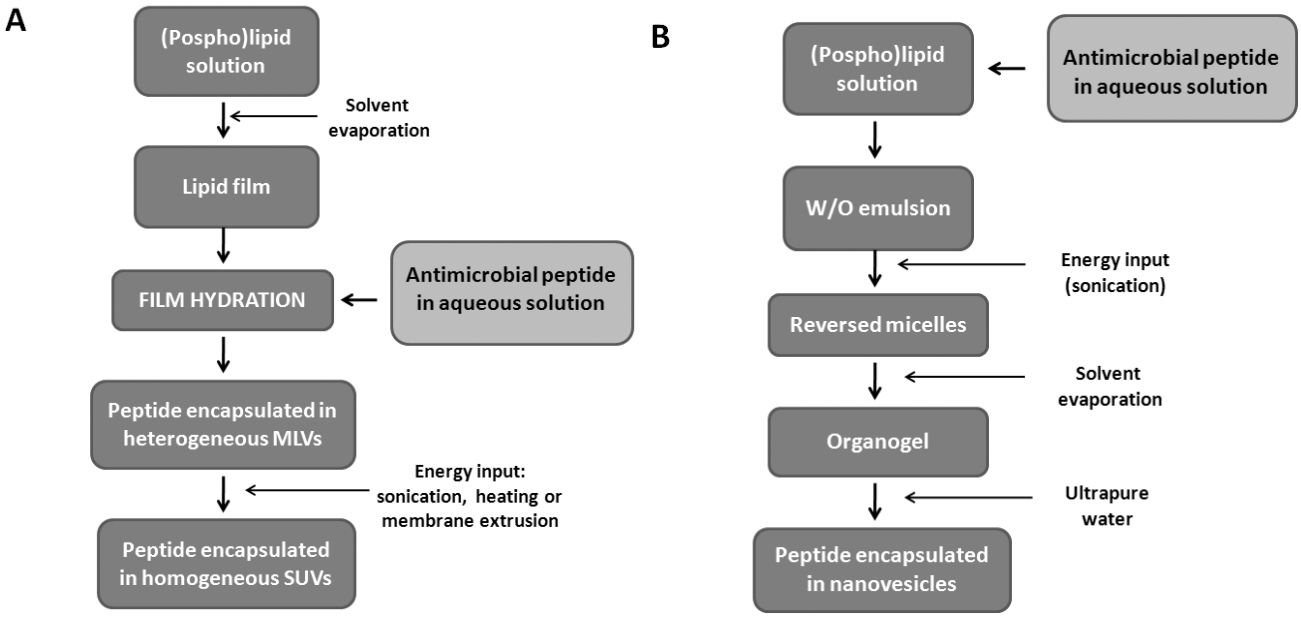

Fig. (2). Methods for nanoencapsulation of peptides. (**A**) Thin-film hydration method and (**B**) reversed-phase method for encapsulation of antimicrobial peptides into nanoliposomes.

maintenance of antimicrobial activity [50]. Nisin-loaded nanovesicles, manufactured by the thin-film hydration method followed by bath-type ultrasound, presented high encapsulation efficiency, displaying enhanced antimicrobial activity. These nanovesicles have been applied in milk as food model, inhibiting *L. monocytogenes* growth [51].

The concentration of compounds that can be entrapped is a function of lipid composition and may be attributed to electrostatic and hydrophobic interactions between antimicrobials and phospholipids [52]. Most antimicrobial peptides are cationic amphiphilic molecules and therefore, it may be encapsulated in the inner aqueous phase of liposome and also be immobilized into liposome membranes. The effect of lipid composition of the nanovesicle on the entrapment efficiency of nisin has been recently reviewed [36], and is summarized in Table **2**.

Nisin-loaded PC nanovesicles caused no significant inhibition of target pathogens in comparison to free nisin, whereas PC:PG (8:2 and 6:4) liposomes produced a significant inhibition, suggesting PG-containing nanovesicles to release their contents more efficiently [53]. Liposomes prepared from proliposomes with lower contents of negatively charged phospholipids were less susceptible to the nisin-membrane destabilizing action in comparison with other liposomes tested [54]. As the bacterial cell has a negative charge, electrostatic repulsion might occur between PG-containing nanovesicles and the cell surface, preventing direct contact between nanovesicles and pathogens, and the subsequent release of antimicrobials. This hypothesis gains further importance since the mechanism of interaction

Table 2. Some Characteristics of Nisin-Loaded Nanovesicles.[a]

Nanovesicle Composition[b]	Size (nm)	Entrapment Efficiency (%)
PC, PC:CH (7:3), PC:PG:CH (5:2:3)	131-182	63, 54, and 59% for PC, PC:CH, and PC:PG:CH, respectively
PC, PC:CH (7:3), PC:PG:CH (5:2:3)	NR[c]	> 54% (not specified)
PC, PC:PG (8:2), PC-PG (6:4)	123-310	NR[c]
PC; PG; PC:PG (8:2, 6:4)	SUV	NR[c]
Proliposomes (Pro-lipo® C, Duo, S and H) with or without CH (0-20%)	140-2400	9.5-47
DPPC; phospholipon 90H; phospholipon 100H; with or without CH, DCP, SA	190-284	11.7-54.2
Soy lecithin	148-190	94.1

[a]Compiled from [36].
[b]Abbreviations: PC, phosphatidylcholine; PG, phosphatidylglycerol; CH, cholesterol; DPPC, dipalmitoyl-PC; SA, stearylamine; DCP, dicetylphosphate; SUV: small unilamellar vesicles.
[c]Not reported.

between nanovesicles and bacteria seems to involve membrane fusion [55,56]. In this regard, bovine lactoferricin (LfcinB) is a cationic antimicrobial peptide with potent cytotoxic activity against cancer cells. The antimicrobial activity of LfcinB resides in its RRWQWR amino acid sequence (LfcinB6). However, free LfcinB6 did not kill T-leukemia or breast cancer cells but LfcinB6 was strongly cytotoxic when delivered to the cytosolic compartment by fusogenic liposomes [57]. Electron microscopy studies on the mode of action of PC nanovesicles containing the peptide P34 showed that the liposomes adhered but not fuse with the cell wall of *L. monocytogenes*, suggesting that the antimicrobial is released from nanovesicles to act against the microorganism [58].

Comparisons of nisin-containing PC and PC:cholesterol (7:3) nanovesicles showed that the addition of cholesterol reduced leakage [52], and although concentrations of nisin encapsulated into PC vesicles were higher than that in PC:cholesterol (7:3), antimicrobial activities against *L. monocytogenes* were similar [59]. Cholesterol could be beneficial as a stabilizing component in liposome compositions since it promotes increased ordination of lipid chains, decreasing the ability of nisin to perturb the permeability and the structure of liposomes [60,61]. Similar results were reported with different antimicrobial peptides, such as temporin L [62], the antimicrobial decapeptide KSL [63], plantaricin A [64] and the amphibian peptide DDK [65]. However, liposomes manufactured with hydrogenated PC:cholesterol (8:2) presented higher nisin encapsulation efficiency when compared to liposomes of hydrogenated PC only [53].

Other factors, such as the pH might present significant effects on the entrapment of antimicrobial peptides into nanovesicles. Modification of pH may alter the ionization of certain groups in the peptide and certain lipid constituents, which influence both solubility and the interactions between the peptide and lipids and, therefore the entrapment. Nisin is about 228 times more soluble at pH 2.0 than at pH 8.5 [66], and its antimicrobial activity is high at acid pH, but lost at pH values above 7 [35]. For instance, a pH reduction (pH range: 3.6-6.6) showed a positive effect in the amount of nisin entrapped into nanovesicles prepared with some proliposome preparations [54]. Liposomes can be expected to retain the encapsulated material if the phospholipids used to formulate the liposomes maintain their charge regardless of the pH of systems in which they are applied. Thus, the knowledge on the pK_a values of the phospholipids utilized for liposome manufacture is critical [67].

Nanofibers

Nanofibers are produced from polymers treated in a specific manner to form filaments of nanometers in diameter. Although these ultra fine fibers can be produced by self-assembly of polymers, template synthesis or phase separation, electrospinning is the most cost-effective and easiest method to produce large amounts of nanofibers [68,69]. Electrospun nanofibers have been investigated for their potential uses in biomedical applications, and most studies have pointed the promising relevance for development of tissue engineering scaffolds [70]. Diverse natural and synthetic polymers have been used to develop nanofibers. These include chitin, silk fibroin, chitosan acetate, polyurethane, poly(ε-caprolactone), poly(L-lactic acid), poly-vinyl alcohol, among other. Nanofibers may be also surface-modified to enhance the immobilization of hydrophilic and charged macromolecular drugs like proteins and nucleic acids [69].

Nanofibers also hold great potential for drug delivery, since the large surface to volume ratio and manipulation of surface properties makes nanofibers ideal to drug loading. The modes of drug loading on the surface of electrospun nanofibers are illustrated in Fig. (**3**). Several therapeutic agents such as antibiotics, anticancer drugs, proteins and growth factors have been formulated within the bulk phase or on the surface of electrospun nanofibers and their topical release within a period of time was investigated [71,72]. The highest release of the antimicrobial lysozyme encapsulated in poly(ε-caprolactone) and polyethylene oxide nanofibers (90:10) was 87% over 12 days [73]. Layer-by-layer assembly was used to obtain a polypeptide multilayer antimicrobial nanofilm constituted by negatively charged layers of poly(L-glutamic acid) and positively charged layers of egg white lysozyme, which is widely employed as a food preservative [74]. These nanofilms were effective to inhibit growth of *Micrococcus luteus*. The viability of incorporating bacteriocins into electrospun nanofibers has been reported. The bacteriocin plantaricin 423 maintained its antimicrobial activity after electrospinning and inhibited the growth of *Enterococcus faecium* and *Lactobacillus sakei* [75]. Also, nisin maintained its antimicrobial activity for up to 45 days when loaded in poly(L-lactic acid) nanostructure by semicontinuous compressed CO_2 antisolvent precipitation method [76].

Antimicrobial peptides incorporated in nanofibers have been proposed as delivery systems for use as wound dressings [11]. A nanofiber scaffold consisting of ionic, self-complementary peptides with 16 amino acids that undergo self-assembly into hydrogels containing 99.5% w/v water when exposed to physiological media or salt solution, was investigated as a bioactive wound dressing. The nanoscale peptide scaffolds combined with epidermal growth factor (EGF) modulate the wound healing rate in tissues that closely mimic the human wound response *in vivo* [77]. Since wound infection is frequently associated to chronicity of wound repair, it may be possible to utilize multi-functionalized nanofibers incorporating antimicrobial peptides that could both provide anti-bacterial activity and wound healing stimulants such as EGF, to provide wound protection while stimulating wound closure.

Silk fibroin provides an important option for biomaterials because of its impressive mechanical properties, environmental stability, biocompatibility and biodegradability. Many studies emerged on its application as nanofiber in biological and biomedical fields. Silk fibroin films modified by a cecropin B antimicrobial peptide show improved antimicrobial activity. The bacteria reductions of surface-modified silk fibroin for 2 h at ambient temperature were 93.5% and 91.6% against *Escherichia coli* and *S. aureus*, respectively [78]. Surface-modified polyurethane nanofibers

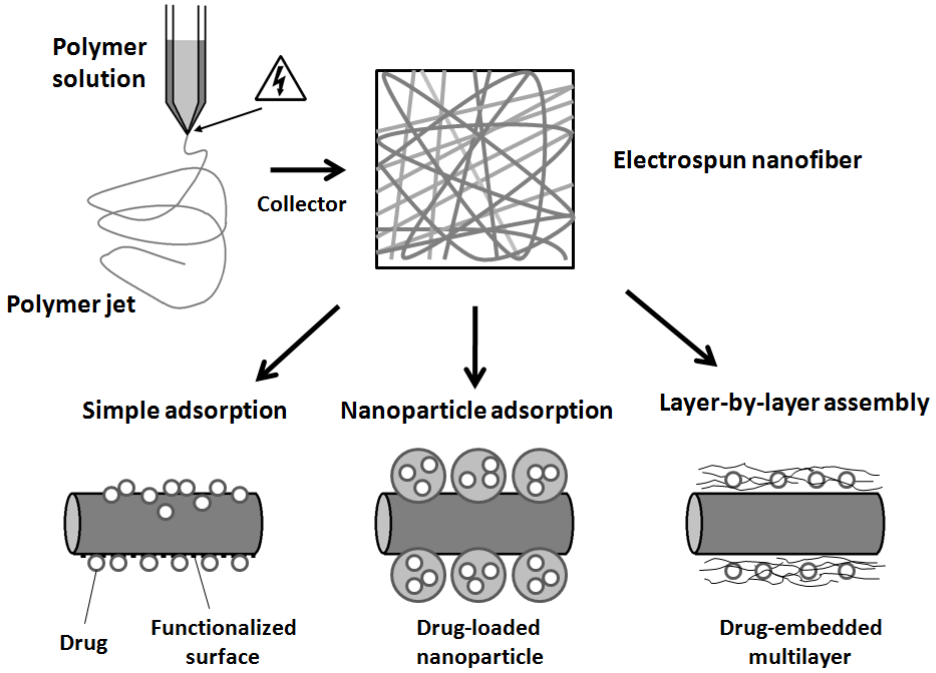

Fig. (3). Schematic representation of electoppining and methods of drug loading on the surface electrospun nanofibers. Antimicrobial peptides can be loaded on the surface of nanofibers by simple physical adsorption or adsorption to surface functionalized nanofibers. Functional nanoparticle containing antimicrobial peptides can be embedded within or adsorbed onto nanofiber mesh. Drug loading can be also realized by layer-by-layer polyelectolyte multilayer assembly [69].

are endowed with antibacterial activity. The efficacy of polyurethane-modified fibers for *S. aureus* and *E. coli* were 99.999% and 99.9%, respectively after 4 h contact, indicating a highly effective antibacterial activity [79]. Functionalized nanofibers were also developed by conjugation of an antimicrobial peptide, which consists of three repeating units of amino acids serine, glutamic acid, glutamic acid $(SEE)_3$ to polyethylene oxide [80].

Metallic Nanoparticles

Metallic nanoparticles may serve as potential nanocarriers for antimicrobial peptides. Silver is known for its antimicrobial properties and has been used for years in the medical field for antimicrobial applications and even has shown to prevent HIV binding to host cells. Furthermore, silver has been used in water and air filtration to eliminate microorganisms [81]. The determined effective concentration of Ag nanoparticles is at nanomolar levels while Ag^+ ions are effective at micromolar levels, suggesting that Ag nanoparticles seem to be more efficient than Ag^+ ions in performing antimicrobial activities. The bacteriolytic activity of lysozyme and the biocidal properties of silver nanoparticles were combined in colloidal suspensions and tested as coatings for medical instruments. Coatings exhibited antimicrobial activity against a range of bacterial species, reducing cell viability by at least 3 log within 1.5 h for *Klebsiella pneumoniae*, *Bacillus anthracis* Sterne, and *Bacillus subtilis* and within 3 h for *S. aureus* and *Acinetobacter baylyi* [13].

Magnetic nickel nanoparticles uniformly coated with a nanolayer biofilm of polyacrylic acid were used to immobilize the antimicrobial peptide LL-37. The modified nickel nanoparticles immobilizing a certain concentration of LL-37 could kill the bacteria *E. coli* effectively [82]. Iron oxide magnetic nanoparticles can be coated with various polymers, especially biopolymers such as polysaccharides. Multifunctional particles can be created through entrapment of specific molecules like proteins, peptides, drugs, and other ligands [83]. This development opens a range of applications for iron oxide nanoparticles as nanocarriers for biomedical and biotechnological applications.

SELF-ASSEMBLY PEPTIDES AS NANOSTRUCTURES

Current attention is devoted to the self-assembly structures that can be assumed by natural peptides or protein hydrolysates [84,85]. Self-assembled peptides exhibit several attractive features for applications in tissue regeneration, drug delivery, biological surface engineering as well as in food science, cosmetic industry and antibiotics [85].

Short peptides, from single dipeptides to small linear or cyclic peptides can self-assume nanotubular structures [86]. Cationic dipeptides NH_2–Phe–Phe–NH_3^+ self-assemble into nanotubes at neutral pH and rearrange into spherical structures of about 100 nm in diameter upon dilution [87]. The nanotubes can be absorbed by cells through endocytosis upon spontaneous conversion into vesicles. This property has been used to deliver oligonucleotides into the interior of the cells as a proof of concept of the potential applications of the system in drug delivery. The proposed self-assembly mechanism for diphenylglycine and diphenylalanine is illustrated in Fig. (**4**). Examples of self-assembled cyclic

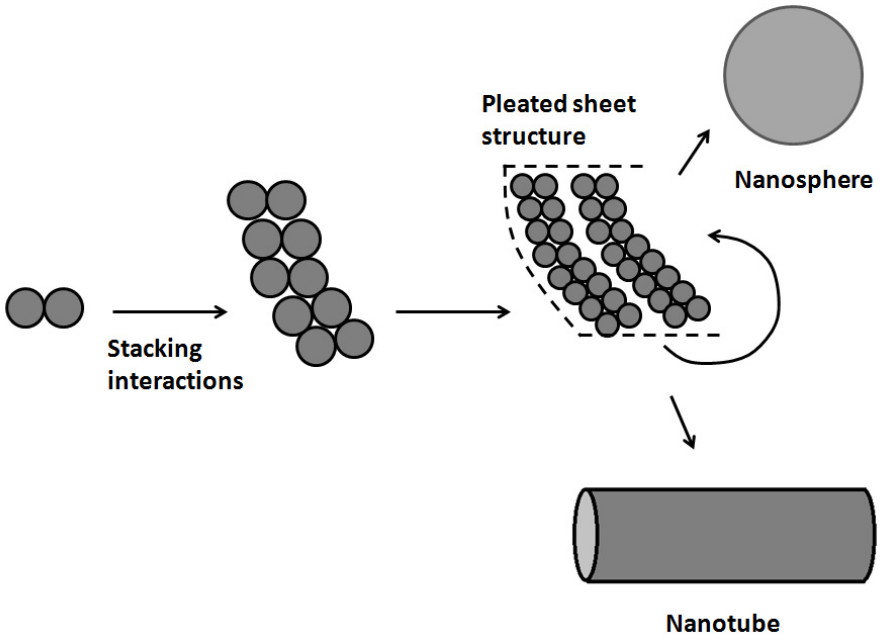

Fig. (4). Self-assembly mechanism of diphenylglycine and diphenylalanine peptides into nanostructures. Peptides initially associate through stacking interactions between aromatic groups to form an extended pleated sheet structure. The formation of nanotubes may result from a closure of the extended sheet along one axis of the two dimensional layer. The formation of nanospheres may occur by a closure of the sheet along two axes [88].

peptides have a number of alternating D and L amino acids, and stack through extensive intermolecular hydrogen bonding to form extended cylindrical structures with an anti-parallel β-sheet structure. The ability to adjust the outer surface properties enables nanotube arrangement in a variety of different environments, such as in bulk solution, in the solid state, and as transmembrane pores inside the cellular lipid bilayer, which can act as efficient ion channels [89]. The pores can be preferentially assembled inside bacterial rather than eukaryotic cell membranes and cause bacterial death. Multiple crown α-helical peptide nanostrutures designed to form artificial ion channels have been also described [90]. These works led to the development of new antimicrobial and cytotoxic agents, controlled-release drug delivery carriers, and new artificial ion channels whose transport properties are determined by peptide design [86,91]. Some examples of self-assembly peptides are listed in Table **3**.

The cationic decapeptide KSL was used as a model template molecule to catalyze self-immobilization into bionanocomposities that retain the antimicrobial properties of the peptide [92]. The resultant antimicrobial peptide nanoparticles retain biocidal activity, protect the peptide from proteolytic degradation, and facilitate a continuous release of the antibiotic over time. There was no statistical difference between the minimal inhibitory concentration of free KSL and Si-KSL determined for *Staphylococcus epidermidis*. The effect toward *S. aureus* suggests the bioinorganic composites of KSL exert a stronger biocidal effect than the free peptide.

A novel class of core-shell nanoparticles formed by self-assembly of an amphiphilic peptide has strong antimicrobial properties against a range of bacteria, yeasts and fungi [93]. The nanoparticles show a high therapeutic index against *S. aureus* infection in mice and are more potent than their unassembled peptide counterparts. Despite these nanoparticles have a broad spectrum of antimicrobial activity, they induce relatively low hemolysis and do not cause significant toxicity to the major organs. Using *S. aureus*-infected meningitis rabbits, the nanoparticles can cross the blood-brain barrier and suppress bacterial growth in infected brains [93]. These nanoparticles may provide an efficient antimicrobial agent in treating brain infections and other infectious diseases.

Cholesterol-conjugated G_3R_6TAT peptide form cationic

Table 3. Examples of Some Peptide Sequences Forming Nanostructures[a]

Nanostructure	Sequence[b]
Nanospheres	NH_2-Phg-Phg-COOH
	NH_2-Cys-Phe-Phe-COOH
Nanotubes	NH_2-D-Phe-D-Phe-COOH
	NH_2-L-Phe-L-Phe-COOH
	Ac-KLVFFAE-NH_2 ($A\beta_{16-22}$)
Nanofibril	NFGAI, NFLVH, NFGSV
	KLVSFFAE
	DFNK

[a]Compiled from [91].
[b]Phg, phenylglycine; Ac, acetyl.

nanoparticles *via* self-assembly, which demonstrated strong antimicrobial activities against various types of microorganisms *in vitro*. Biodistribution studies in rabbits revealed that the nanoparticles were able to cross the blood-brain barrier [94]. The efficacy of nanoparticles was evaluated in a *Cryptococcus neoformans* meningitis rabbit model. The nanoparticles suppressed the yeast growth in the brain tissues with similar efficiency as amphotericin B did. In addition, unlike amphotericin B, they neither caused significant damage to the liver and kidney functions nor interfered with the balance of electrolytes in the blood [94], indicating their promising as antimicrobial agent for treatment of brain infections caused by *C. neoformans*.

Despite this current interest on self-assembly property of some peptides, earlier description of nanostructures formed by antimicrobial peptides has been described. The bacteriocin linocin M18, produced by the surface-ripened cheese bacterium *Brevibacterium linens*, has been purified and characterized. The antimicrobial peptide has an actual molecular mass of 31 kDa but it eluted in the void volume of the Superose 6 column (>2,000 kDa). When the gel filtration fractions containing activity were observed by electron microscopy, particles of a size between 20-30 nm were observed [95]. Similarly, the antimicrobial lipopeptide iturin A produced by *Bacillus amyloliquefaciens* and *B. subtilis* showed great propensity to self-associate forming vesicles of an average size of 150 nm [96]. Other antimicrobial peptides from *Bacillus* spp. have been also described to elute at the void volume of gel filtration as large aggregates, such as the antimicrobial peptides P34 [97], P40 [98] and P45 [99]. Examination of concentrated samples of the bacteriocin lactacin F by transmission electron microscopy demonstrated the presence of globular structures resembling micelles with an average diameter of 25 to 50 nm [100]. This information suggests that some native antimicrobial peptides form aggregates of high molecular masses that range nanometer size.

CONCLUSION AND PERSPECTIVES

Antimicrobial peptides incorporated to nanocarriers may be suitable for controlling pathogenic bacteria, surviving the exposure to different environmental stresses typically encountered in biological systems, thus improving stability, efficacy, and also its distribution in tissues. The encapsulation of peptides in nanostructures has been achieved by diverse methods and several natural and synthetic materials have been successfully investigated for protein and peptide encapsulation. Alternative nanostructures such as metallic nanoparticles and carbon nanotubes may also have potential as peptide carriers in targeted delivery. A large surface-to-volume ratio and unique electronic properties made carbon nanotubes a welcome component for fabricating new antimicrobial drugs to the treatment of infectious diseases in the future [12,101]. Pristine single wall carbon nanotubes exhibited an antimicrobial effect in a size-dependent manner, indicating that they might be useful for antimicrobial therapeutics [102]. Organic modification on the surface of carbon nanotubes can generate sites for the attachment of bioactive molecules, whose secondary structure can be preserved. Composite films with embedded antimicrobial lysostaphin-carbon nanotube conjugates are effective against methicillin-resistant *Staphylococcus aureus* [103]. This material may be valuable to prevent the risk of staphylococci infection and biofouling of surfaces.

The potential of antimicrobial peptide nanofilms for development of electrochemical biosensors has been described. The antimicrobial peptide dermaseptin 01, from the skin secretion of *Phyllomedusa hypochondrialis* frogs was immobilized in nanostructured layered films in conjunction with nickel tetrasulfonated phthalocyanines. The immobilized molecules of this anti-Leishmanial peptide as a nanostructured film were valuable for detection of *Leishmania* cells [104].

Investigations on nanoparticle-encapsulated antimicrobial peptides generally show advantages in comparison to free peptides. The recent descriptions that self-assembly peptide nanoparticles may provide an efficient antimicrobial agent in treating brain infections indicates that nanoparticles can be successfully used to combat persistent microbial infections. These promising results should encourage intensive efforts focusing on novel materials and methodologies for development protein and peptide-loading nanostructures, aiming the development of valuable tools to combat infectious diseases.

CONFLICT OF INTEREST

None declared.

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