Development of Nanoparticles for Antimicrobial Drug Delivery

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Abstract: This review focuses on the development of nanoparticle systems for antimicrobial drug delivery. Numerous antimicrobial drugs have been prescribed to kill or inhibit the growth of microbes such as bacteria, fungi and viruses. Even though the therapeutic efficacy of these drugs has been well established, inefficient delivery could result in inadequate therapeutic index and local and systemic side effects including cutaneous irritation, peeling, scaling and gut flora reduction. Nanostructured biomaterials, nanoparticles in particular, have unique physicochemical properties such as ultra small and controllable size, large surface area to mass ratio, high reactivity, and functionalizable structure. These properties can be applied to facilitate the administration of antimicrobial drugs, thereby overcoming some of the limitations in traditional antimicrobial therapeutics. In recent years, encapsulation of antimicrobial drugs in nanoparticle systems has emerged as an innovative and promising alternative that enhances therapeutic effectiveness and minimizes undesirable side effects of the drugs. Here the current progress and challenges in synthesizing nanoparticle platforms for delivering various antimicrobial drugs are reviewed. We also call attention to the need to unite the shared interest between nanoengineers and microbiologists in developing nanotechnology for the treatment of microbial diseases.

Keywords: Antimicrobial delivery, microbes, liposomes, polymeric nanoparticles, solid lipid nanoparticles, dendrimers.

INTRODUCTION

Upon invasion of the epithelial surfaces, infectious microorganisms spread throughout the body *via* the circulatory system. They are then removed from the blood by macrophages which are present in all major organs such as liver, spleen and bone marrow [1]. After being phagocytosed by macrophages, the infectious microorganisms are trapped in phagosomes, which then fuse with lysosomal granules inside cell cytoplasm forming phagolysosomes. Subsequently, oxygen-dependent or oxygen-independent bacterial killing mechanisms induced by enzymes inside the phagolysosomes occur to digest the trapped microorganisms. However, many microorganisms are able to evade the macrophage digestion *via* escaping from the phagosomes, inhibiting the phagosome-lysosome fusion, withstanding the lysosomal enzymes, or resisting oxidative and non-oxidative killing mechanisms. These bacterial defense mechanisms make intracellular infections difficult to eradicate resulting in infectious diseases that range from staph infections to tuberculosis [1].

An antimicrobial refers to a substance that kills or inhibits the growth of microorganisms. Since the discovery of antimicrobial drugs in the 1960s [2], many infectious diseases have been overcome. Typically, antimicrobials kill bacteria by binding to some vital compounds of bacterial metabolism, thereby inhibiting the synthesis of functional biomolecules or impeding normal cellular activities. For instance, β-lactams such as penicillins and cephalosporins inhibit bacteria cell wall synthesis; tetracyclines, macrolides, and clindamycin inhibit protein synthesis; metronidazole and quinolones inhibit nucleic acid synthesis; and sulphonamides and trimethoprim have an inhibitory effect on enzyme synthesis. Some antimicrobials such as penicillin are only effective against a narrow range of bacteria, whereas others, like ampicillin, kill a broad spectrum of Gram-positive and Gram-negative bacteria [3]. Despite the great progress in antimicrobial development, many infectious diseases, especially intracellular infections, remain difficult to treat. One major reason is that many antimicrobials are difficult to transport through cell membranes and have low activity inside the cells, thereby imposing negligible inhibitory or bactericidal effects on the intracellular bacteria. In addition, antimicrobial toxicity to healthy tissues poses a significant limitation to their use. Aminoglycosides, for instance, cause ototoxicity and nephrotoxicity and have to be given in controlled dosages. Another major issue with antimicrobials stems from the acquired resistance of infectious microbes. In 2002, more than 70% of bacteria causing hospital-acquired infections were resistant to at least one common antimicrobial in the United States. To address these issues, alternative antimicrobial drug delivery strategies have been proposed.

Over the last few decades, the applications of nanotechnology in medicine have been extensively explored in many medical areas, especially in drug delivery. Nanotechnology concerns the understanding and control of matters in the 1-100 nm range, at which scale materials have unique physicochemical properties including ultra small size, large surface to mass ratio, high reactivity and unique interactions with biological systems [4]. By loading drugs into nanoparticles through physical encapsulation, adsorption, or chemical conjugation, the pharmacokinetics and therapeutic index of the drugs can be significantly improved in contrast to the free drug counterparts. Many advantages of nanoparticle-based drug delivery have been recognized, including improving serum solubility of the drugs, prolonging the systemic circulation lifetime, releasing drugs at a sustained and controlled manner, preferentially delivering drugs to the tissues and cells of interest, and concurrently delivering multiple therapeutic agents to the same cells for combination therapy [4-6]. Moreover, drug-loaded nanoparticles can enter host cells through endocytosis and then release drug payloads to treat microbes-induced intracellular infections. As a result, a

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number of nanoparticle-based drug delivery systems have been approved for clinical uses to treat a variety of diseases and many other therapeutic nanoparticle formulations are currently under various stages of clinical tests [4, 7]. Knowing the vast scope of nanoparticle drug delivery, here we will only focus on the development and application of nanoparticles for antimicrobial drug delivery through various mechanisms (Fig. (**1**)). As listed in Fig. (**2**), A few types of nanoparticles including liposomes, polymeric nanoparticles, solid lipid nanoparticles and dendrimers have been widely investigated as antimicrobial drug delivery platforms, of which several products have been introduced into pharmaceutical market. This review will summarize the current status, mechanisms of action, and structure-activity relationship of these nanoparticle-based antimicrobial delivery systems.

LIPOSOMES FOR ANTIMICROBIAL DRUG DELIVERY

Liposomes are spherical lipid vesicles with a bilayered membrane structure consisting of amphiphilic lipid molecules [8]. Liposome structure was first described in 1965 [9], and they were proposed as a drug delivery nanoparticle platform in 1970s [10]. After extensive studies on their fundamental properties including lipid polymorphisms, lipid-protein and lipid-drug interactions, and mechanisms of liposome disposition in 1980s, the application potential of liposomes as a drug delivery vehicle was thoroughly recognized and started being transferred to practice. Liposomes were initially introduced to the cosmetic market by Dior in 1986. In 1995, Doxil (doxorubicin liposomes) became the first liposomal delivery system approved by the Food and Drug Administration (FDA) to treat AIDS associated Kaposi 's sarcoma [11, 12]. Liposomal drug delivery system can be made of either natural or synthetic lipids. One of the most commonly used lipids in liposome preparation is phosphotidylcholine, which is an electrically neutral phospholipid that contains fatty acyl chains of varying degrees of saturation and length. Cholesterol is normally incorporated into the formulation to adjust membrane rigidity and stability. Structurally, liposomes can be classified into multilamellar vesicles (MLVs), which consist of multiple phospholipid bilayer membranes, and unilamellar vesicles (ULVs), which have a single lipid bilayer. ULVs can be further classified into small unilamellar vesicles (SUVs) and large unilamellar vesicles (LUVs) depending on their size range [13]. Methods for preparing liposomes can take into consideration parameters such as the physicochemical characteristics of the liposomal ingredients, materials to be contained within the liposomes,

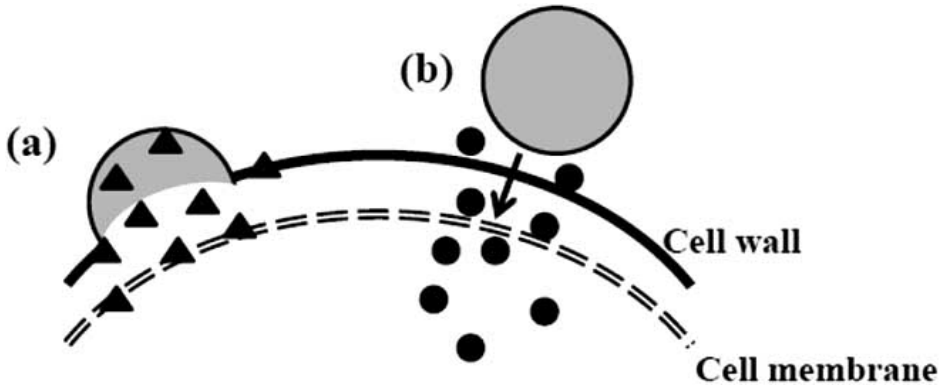

Fig. (1). Mechanisms of nanoparticle-based antimicrobial drug delivery to microorganisms: (**a**) nanoparticles fuse with microbial cell wall or membrane and release the carried drugs within the cell wall or membrane; (**b**) nanoparticles bind to cell wall and serve as a drug depot to continuously release drug molecules, which will diffuse into the interior of the microorganisms.

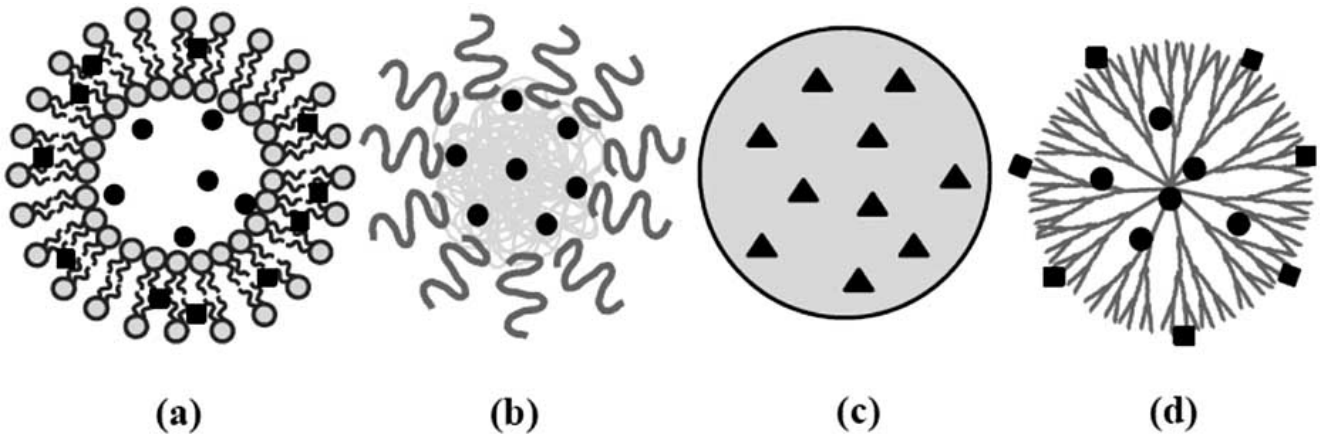

Fig. (2). Schematic illustration of four nanoparticle platforms for antimicrobial drug delivery: (**a**) liposome, (**b**) polymeric nanoparticle, (**c**) solid lipid nanoparticle, and (**d**) dendrimer. Black circles represent hydrophobic drugs; black squares represent hydrophilic drugs; and black triangles represent either hydrophobic or hydrophilic drugs.

particle size, polydispersity, surface zeta potential, shelf-time, batch-to-batch reproducibility, and the possibility for large-scale production of safe and efficient products. Liposomes, ULVs in particular, do not form spontaneously. Rather, liposomes form when a sufficient amount of energy (e.g., *via* sonication, homogenization, shaking, or heating) is supplied to phospholipids placed in water. Typical methods for generating liposomes include sonication method [14], (e.g., low shear rates can result in MLVs and high shear rates can generate ULVs), extrusion method, and heading method [15].

Currently, liposomes are the most widely used antimicrobial drug delivery system. One of the distinguishing features of liposomes is its lipid bilayer structure, which mimics cell membranes and can readily fuse with infectious microbes. By directly fusing with bacterial membranes, the drug payloads of liposomes can be released to the cell membranes or the interior of the bacteria. The unique structure of liposomes, a lipid membrane surrounding an aqueous cavity, enables them to carry both hydrophobic and hydrophilic compounds without chemical modification. In addition, the liposome surface can be easily functionalized with "stealth" material to enhance their *in vivo* stability or targeting ligands to enable preferential delivery of liposomes. For example, polyethylene glycol (PEG) has been frequently conjugated to liposome surface to create a stealth layer that prolongs the circulation lifetime of liposomes in the blood stream. Specifically, the PEG coating forms a hydration layer that retards the reticuloendothelial system (RES) recognitions of liposomes through sterically inhibiting hydrophobic and electrostatic interactions with plasma proteins. On the other hand, by attaching targeting ligands such as antibody, antibody segments, aptamer, peptides and small molecule ligands to the surface of the liposomes, they can selectively bind to microorganisms or infected cells and then release the drug payloads to kill or inhibit the growth of the microorganisms.

AmBisome(NeXstar Pharmaceuticals, San Dimas, USA) is an FDA approved liposomal formulation of amphotericin B (AMB), which has been widely used in the clinic to treat *Candida* spp, *Aspergillus* spp, *Fusarium* spp, and other fungi infections in neutropenic, visceral leishmaniasis, and methylmalonic acidaemia patients [16-18]. In the AmBisome formulation, AMB are intercalated into the phospholipid bilayer of liposomes consisting of hydrogenated soy phosphaticylcholine, cholesterol, and distearoyl phosphatidylglycerol (DSPG) [19]. Freeze-fracture electron microscopy results have shown that AmBisome delivered its drug content through an absorption mechanism. For example, AmBisome attached to the outer cell wall of *Candida glabrata* [20] and released AMB to disrupt fungal membranes [21, 22]. Adler-Moore J. *et al*. have observed great specificity of AmBisome to the site of fungal infections. Following the injection of fluorescently-labeled AmBisome into *Candida*-infected mice, the localization of fluorescent liposomes has been observed at the sites of fungal infections [23]. Because of its liposomal structure, AmBisome has shown greater pharmacokinetics than free AMB drug, including prolonged systemic circulation half-life, reduced plasma clearance rate, decreased renal toxicity, and most importantly, enhanced therapeutic efficacy [24].

Polymyxin B-loaded loposome represents another successful example of liposomal antimicrobial drug delivery. Polymyxin B has been recognized for treating *P. aeruginosa* related infections (e.g., pneumonias and chronic bronchopneumonia's of cystic fibrosis). However, its systemic use has been limited due to toxic side effects such as nephrotoxicity, ototoxicity and neuromuscular blockade. It has been reported that liposomal encapsulation of polymyxin B dramatically diminished the drug's side effects and improved its antimicrobial activity against resistant strains of *P. aeruginosa* [25]. The action mechanism of liposomal polymyxin B against *P. aeruginosa*is has been recognized as membrane fusion. Transmission electron microscopy (TEM), flow cytometry and fluorescent resonance energy transfer studies have revealed lipid reorganizations in *P. aeruginosa* membranes upon incubation with polymyxin B-loaded liposomes [26]. Membrane fusion between liposomes and bacteria is a rapid and spontaneous process driven by non-covalent forces such as van der Waals force and hydrophobic interactions that minimize the system's free energy. Antibiotic efflux is a widely accepted mechanism of microbial drug resistance, in which proteinaceous transports located in bacterial membranes preferentially pump antimicrobial drugs out of the cells [27]. When liposomes fuse with cell membranes, a high dosage of drug contents is immediately delivered to the bacteria, which can potentially suppress the antimicrobial resistance of the bacteria by overwhelming the efflux pumps, thereby improving drug's antimicrobial activity.

Many other liposome-based antimicrobial drug delivery systems have also been developed for various applications [28]. Ampicillin-loaded liposomes have shown elevated drug stability and higher antimicrobial activity than free drug against *Salmonella typhimurium* [29, 30]. Benzyl penicillin-loaded liposomes have shown complete growth inhibition of penicillin-sensitive strain of *Staphylococcus aureus* at lower drug concentration and shorter exposure time than free benzyl penicillin [31]. Liposomal ciprofloxacin, a fluoroquinolone antibiotic, has effectively inhibited the number of *Salmonella dublin* in mouse spleen [32]. Liposomal gentamicin and streptomycin, which belong to aminogylcoside antibiotics, have successfully treated mice and guinea pigs infected with *Brucella* spp. [33]. It has also been reported that liposomal vancomycin and teicoplaninhas have significantly enhanced intracellular killing of methicillin-resistant *Staphylococcus aureus* (MRSA) [34]. Table **1** summarizes other antimicrobial liposomes, many of which have been in clinical use for years.

POLYMERIC NANOPARTICLES FOR ANTIMICROBIAL DRUG DELIVERY

Biocompatible and biodegradable polymers have been used extensively in the clinic for controlled drug release. The annual worldwide market of polymer-based controlled release systems is about $60 billion and they are given to over 100 million patients each year [35]. The first polymer-based drug delivery system was developed by Langer and Folkman in 1976 for macromolecule delivery[36]. However, the initial polymeric nanoparticles possessed poor therapeutic efficacy because of their rapid clearance by the reticuloendothelial system (RES) after intravenous administration. This limita-

Table 1. Liposomes for Antimicrobial Drug Delivery

Formulation	Drug	Targeted Microorganism	Activity	References
hydrogenated soy phosphatidylcholine, cholesterol, and distearoylphosphatidylglycerol (DSPG)	amphotericin B	*Aspergillus fumigatus*	targeted drug delivery at infection site	[73]
1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and cholesterol	polymyxin B	*Pseudomonas aeruginosa*	1) decreased bacteria count in lung 2) increased bioavailability 3) decreased lung injury caused by bacteria	[74]
soybean phosphatidylcholine (PC) and cholesterol	ampillicin	*Micrococcus Luteus* and *Salmonella typhimurium*	1) increased stability 2) full biological activity of Ampicillin was observed	[29]
dipalmitoyl-phosphatidylcholine, dipalmitoyl-phosphatidylglycerol, and cholesterol	ciprofloxacin	*Salmonella dublin*	1) decreased motality of animals 2) distribution of liposomes to all areas of infection	[32]
dipalmitoylphosphatidylcholine (DPPC), cholesterol, and dimethylammonium ethane carbamoyl cholesterol (DC-chol)	benzyl penicillin	*Staphylococcus aureus*	lower drug concentrations and shorter time of exposure were required	[31]
phosphatidylcholine, cholesterol, and phosphatidylinositol	netilmicin	*Bacillus subtilis* and *Escherichia coli*	1) reduction in toxicity 2) increased circulation half-life 3) increased survival rate of animal model	[75]
partially hydrogenated egg phosphatidylcholine (PHEPC), cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-(polyethylene glycol-2000) (PEGDSPE)	gentamicin	*Klebsiella pneumoniae*	1) increased survival rate of animal model 2) increased therapeutic efficacy	[76]
phosphatidyl glycerol, phosphatidyl choline, and cholesterol	streptomycin	*Mycobacterium avium*	increased antimicrobial activity	[77]
hydrogenated soy phosphatidylcholine, cholesterol, and distearoylphosphatidylglycerol (DSPG)	amikacin	gram-negative bacteria	prolonged drug and exposure	[78]
stearylamine (SA) and dicetyl phosphate	zidovudine	Human immunodeficiency virus	enhanced targeting of ZDV to lymphatics	[79]
egg phosphatidylcholine, diacetylphosphate, and cholesterol	vancomycin or teicoplanin	methicillin-resistant *Staphylococcus aureus* (MRSA)	1) enhanced each drug uptake by macrophages 2) enhanced intracellular antimicrobial effect of each drug	[34]

tion was overcome after the discovery of long-circulating stealth polymeric nanoparticles in 1994 [37].

Polymeric nanoparticles possess several unique characteristics for antimicrobial drug delivery. Firstly, polymeric nanoparticles are structurally stable and can be synthesized with a sharper size distribution. Secondly, particle properties such as size, zeta potentials, and drug release profiles can be precisely tuned by selecting different polymer lengths, surfactants, and organic solvents during the synthesis. Thirdly, the surface of polymeric nanoparticles typically contains functional groups that can be chemically modified with either drug moieties or targeting ligands. For targeted antimicrobial delivery, polymeric nanoparticles have been frequently decorated with lectin, which is a protein that binds to simple or complex carbohydrates present on most bacterial cell walls. For example, lectin-conjugated gliadin nanoparticles were studied for treating *Helicobacter pylori* associated infection diseases. It has been found that lectin-conjugated nanoparticles bind specifically to carbohydrate receptors on cell walls of *H. pylori* and release antimicrobial agents into the bacteria [38].

There are currently two major types of polymeric nanoparticles for antimicrobial drug delivery. One is formed *via* spontaneous self-assembly of diblock copolymers consisting of hydrophilic and hydrophobic segments. The hydrophobic segment forms a polymeric core containing the drugs while the hydrophilic segment shields the core from osponization and degradation. The rate of drug release can be tuned by varying the length of the hydrophobic chain. A variety of biodegradable polymers have been used to form the hydrophobic polymeric core, including poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(lactide-co-glycolide) (PLGA), poly (ε-carprolactone) (PCL), and poly(cyanoacrylate) (PCA), whereas polyethylene glycol (PEG) has been commonly used as a hydrophilic segment. Diblock copolymer nanoparticles are typically prepared through solvent displacement. In this process, polymers and drugs are first dissolved in a water-miscible organic solvent such as acetonitrile. The polymer-drug mixture is then added to an aqueous solution. As the organic solvent evaporates, the block copolymers and drugs undergo nanoprecipitation to form nanoparticles consisting of a hydrophobic core and a hydrophilic shell. Polymeric nanoparticles are primarily used to carry and deliver poorly water soluble drugs because of the hydrophobic nature of the nanoparticle core [39, 40].

The other type of polymeric nanoparticles consists of linear polymers such as polyalkyl acrylates and polymethyl methacrylate that form nanocapsules through an emulsion polymerization method. In this process, monomers are first

dissolved in polymerization media in the presence of surfactants. Polymerization initiators are then added to the solution to trigger polymerization resulting in the formation of nanocapsules. Antimicrobial drugs can be either absorbed to the nanocapsules during the polymerization process or covalently conjugated to the surface of the nanoparticles after they are formed. The absorption process favors hydrophobic drugs as it requires dissolving the drugs to an oil phase. Hydrophilic drugs are usually attached to the particle through covalent conjugations. It is worth noting that in the case of covalent linkage, antimicrobials can be inactivated and need to be verified of their activity before use. For instance, in a study on treating *staphylococcal* infections, Abeylath *et al*. have observed that β-lactam and ciprofloxacin retained their potency whereas penicillin was inactivated upon covalent attachment to nanoparticles [41].

Polymeric nanoparticles have been explored to deliver a variety of antimicrobial agents to treat various infectious diseases and have shown great therapeutic efficacy. For example, the antimicrobial activity of amphotericin B-loaded poly(ε-caprolactone) nanospheres coated with non ionic surfactant poloxamer 188 have shown greater therapeutic efficacy against *Leishmania donovani*has compared to the free drug counterparts [42]. Rifampicin-loaded polybutylcyanoacrylate nanoparticles have shown enhanced antibacterial activity both *in vitro* and *in vivo* against *Staphylococcus aureus* and *Mycobacterium avium* due to an effective delivery of drugs to macrophages [43]. In another example, ampicillin-encapsulated polyisohexylcyanoacrylate nanoparticles have been studied against *Listeria monocytogenes*in mouse peritoneal macrophages [44]. Other examples of polymeric nanoparticles for antimicrobial drug delivery are summarized in Table **2**.

SOLID LIPID NANOPARTICLES FOR ANTIMICROBIAL DRUG DELIVERY

Solid lipid nanoparticles (SLNs) are another antimicrobial drug delivery platform that has attracted much attention since 1990s. SLNs are typically particulate systems with mean diameters ranging from 50 nm up to 1000 nm for various drug delivery applications [45]. SLNs are mainly comprised of lipids that are in solid phase at the room temperature and surfactants for emulsification. Solid lipids utilized in SLN formulations include fatty acids (e.g. palmitic acid, decanoic acid, and behenic acid), triglycerides (e.g. trilaurin, trimyristin, and tripalmitin), steroids (e.g. cholesterol), partial glycerides (e.g. glyceryl monostearate and gylceryl behenate) and waxes (e.g. cetyl palmitate). Several types of surfactants are commonly used as emulsifiers to stabilize lipid dispersion, including soybean lecithin, phosphatidylcholine, poloxamer 188, sodium cholate, and sodium glycocholate. The typical methods of preparing SLNs include spray drying [46], high shear mixing, ultra-sonication [47], and high pressure homogenization (HPH) [48].

Several unique properties of SLNs make them a promising antimicrobial drug delivery platform, leading to a few cosmetic and pharmaceutical products for skin care applications. Firstly, SLNs contain occlusive excipients that, upon application on skin, readily form a thin film to reduce water

Table 2. Polymeric Nanoparticles for Antimicrobial Drug Delivery

Formulation	Drug	Targeted Microorganism	Activity	References
Poly (D,L-lactide) (PLA) Nanospheres	arjunglucoside	*Leishmania donovani*	reduced toxicity	[80]
Poly lactic-co-glycolic acid (PLGA) nanoparticles	Phosphorothioate antisense oligonucleotide	HIV	Protection of oligonucleotides from degradation	[81]
Poly(ethylene oxide) (PEO)-modified poly(epsilon-carprolactone) (PCL) nanoparticle	saquinavir	HIV	1) Protect the drug from cytochrome C metaboish 2) bypass P-gp efflux pump.	[82]
Alginate nanoparticle	Rifampicin, isoniazid, pyrazinamide, and ethambutol.	*Mycobacterium Turberculosis*	1) High drug payload 2) Improved pharmacokinetic 3) High therapeutic efficacy	[83]
Poly-lactide-co-glycolide (PLG) nanoparticle	Rifampicin, isoniazid, pyrazinamide, and ethambutol.	*Mycobacterium Turberculosis*	1) Enhanced bioavailability 2) Improvied pharmacodynamic	[84]
Poloxamer 188 coated poly(epsilon-caprolactone) (PCL) nanosphere	Amphotericin B.	*Candida albicans*	Lower *in vivo* toxicity due to reduced accumulation in kidney and liver	[85]
Polyethylene glycol (PEG)-PLA nanocapsule	halofantrine	*Plasmodiumberghe*	Prolonged circulation half-life	[86]
Poly (isohexylcyanoacrylate) (PIHCA) nanospheres	primaquine and ampicillin	*Leishmania donovani, Salmonella typhymurium and Listeria monocytogenes*	Particle itself exhibits antimicrobial activity	[87, 88]
Glycosylated polyacrylate nanoparticle	Beta-lactam/ciprofloxacin	Staphylococcus aureus and Bacillus anthracis	1) Improved bioavailability 2) Higher therapeutic efficacy	[41, 89]

evaporation and retain skin moisture. This occlusive property promotes molecule penetrations into the skin. For instance, SLNs encapsulated antimicrobial agents such as retinol and retinyl palmitate have shown better drug penetration rate and slower drug expulsion than the free drug counterparts [49]. Secondly, SLNs are stable in water and dermal cream and therefore can be readily incorporated into cosmetic and skin care products [50]. Lastly, simple manufacturing techniques such as high pressure homogenization make it possible to produce SLNs in a large-scale and reproducible manner. Moreover, the preparation of SLNs does not require any organic solvents, which could be difficult to remove after nanoparticle synthesis.

SLNs are ideal for topical application as their occlusive property can induce film formation and prolong residence time on the stratum corneum [51, 52]. One application of SLNs is delivering azole antifungal drugs to superficial fungal infection patients. Commonly used azole antifungal family drugs such as clotrimazole, miconazole, econazole, oxiconazole and tioconazoleare are extremely water-insoluble [53]. It is therefore difficult to administer and deliver these drugs to infected sites. However, these lipophilic compounds can be efficiently encapsulated into SLNs. The occlusive effect of SLNs, together with its small particle size, can extend drug residence time on the epidermis and enhance drug penetration through the skin [54]. The advantages of using SLNs to deliver antifungal agents to superficial infections have been well documented. It has been reported that econazole nitrate-loaded SLNs with a diameter of about 150 nm have increased drug diffusion rate into deeper skin layer [55]. Another study has demonstrated that clotrimazole- and ketoconazoleare-loaded SLNs have high drug loading yield, long and sustained drug release profile, and significant physicochemical stability [56].

In addition to topical applications, SLNs in the forms of tablets, capsules and pellets can also be used for oral administration[57]. Tobramycin is an orally administered antimicrobial drug against *Pseudomonas aeruginosa* infections commonly parasitizing in the gastrointestinal tracts of cystic fibrosis (CF) patients [58]. The absorption rate of tobramycin by the intestinal cells is poor because P-glycoproteins (P-gp), an ATP-dependent drug efflux pump, on the brush border of small intestine actively export the drugs from the cells. In contrast, tobramycin-loaded SLNs can significantly suppress the P-gp efflux pump as they penetrate the intestinal linings through endocytosis rather than passive diffusion [59]. After being internalized through endocytosis, SLNs are carried away from the transmembrane drug-efflux pumps and release tobramycin payloads inside the cells. The drugs are thus more likely to take effects on the bacteria rather than being excreted out of the cells.

Another prominent example of SLNs-based drug delivery is pulmonary delivery of antimicrobials to treat tuberculosis, a serious lung infection caused by *Mycobacterium tuberculosis*. In some severe cases, tuberculosis infection spreads from the lungs and affects the lymphatic systems. SLNs can facilitate the delivery of anti-tuberculosis drugs such as rifampin, isoniazidand pyrazinamide to the lungs as well as to the lymphatic systems [60]. Once these SLNs enter the lungs, they are phagocytosed by alveolar macrophages and subsequently transported to the lymphoid tissues [61]. SLN translocation mechanism and biodistribution through pulmonary delivery have been investigated using radio-labeled aerosol SLNs in rats. Effective uptake of radio-labeled SLNs by the lungs after inhalation and considerable particle accumulation in the periaortic, axillary, and inguinal lymph nodes have been observed. The SLNs can provide a sustained release of the carried antimicrobial payloads, which then can effec-

Table 3. Solid Lipid Nanoparticles (SLNs) for Antimicrobial Drug Delivery

Formulation	Drug	Targeted Microorganism	Activity	References
stearic acid	rifampicin, isoniazid, pyrazinamide	*Mycobacterium tuberculosis*	1) increased residence time 2) increased drug bioavailability 3) decreased administration frequency	[60]
stearic acid , soya phosphatidylcholine, and sodium taurocholate	ciprofloxacin hydrochloride	gram-negative bacteria, gram-positive bacteria, and mycoplasma	prolonged drug release	[90]
stearic acid , soya phosphatidylcholine, and sodium taurocholate	tobramycin	*Pseudomonas aeruginosa*	increased drug bioavailability	[91]
glyceryl tripalmitate and tyloxapol	clotrimazole	fungi (e.g. yeast, aspergilli, dermatophytes)	1) prolonged drug release 2) high physical stability 3) high encapsulation efficiency	[92]
glyceryl behenate and sodium deoxycholate	ketoconazol	fungi	1) high physical stability 2) chemical instability when exposed to light	[93]
glyceryl behenate, propylene glycol, tween 80, and glyceryl monostearate	miconazole nitrate	fungi	1) high physical stability 2) high encapsulation efficiency 3) enhanced skin targeting effect	[94]
glycerol palmitostearate	econazole nitrate	fungi	1) high encapsulation efficiency 2) controlled drug release profile 3) enhanced drug penetration through stratum corneum	[55]

tively eliminate the infectious microbes harbored at these lymphatic sites [62].

Even though the development history of SLN-based antimicrobial drug delivery systems is relatively shorter than other nanoparticle systems such as liposomes and polymeric nanoparticles, SLNs have shown great therapeutic potentials. More examples of SLN-based antimicrobial drug delivery are summarized in Table **3**.

DENDRIMERS FOR ANTIMICROBIAL DRUG DELIVERY

Dendrimers are defined as highly ordered and regularly branched globular macromolecules produced by stepwise iterative approaches. The structure of dendrimers consists of three distinct architectural regions: a focal moiety or a core, layers of branched repeat units emerging from the core, and functional end groups on the outer layer of repeat units [63]. In 1978, the first iterative cascade synthetic procedure for branched amines was discovered by Vögtle *et al*. [64]. A few years later, highly branched l-lysine-based dendrimers were patented [65]. In 1984, Tomalia *et al*. reported the synthesis and characterization of the first family of polyamidoamine (PAMAM) dendrimers, which has become one of the most popular dendrimers since then [66].

Two synthetic approaches, divergent and convergent approaches, have been developed to synthesize dendritic systems for delivering various types of drugs. The divergent approach initiates the synthesis from a core and emanates outward through a repetition of coupling and activation steps. During the first coupling reaction, the peripheral functional groups of the core react with the complementary reactive groups to form new latent branch points at the coupling sites and increase the number of peripheral functional groups. These latent functional groups are then activated to couple with additional monomers. The activation of the latent functional groups can be achieved by removal of protecting groups, coupling with secondary molecules, or reactive functionalization. Large excess of reagents is required to drive the activation step to completion. The final resulted dendrimer products can be separated from the excess reagents by distillation, precipitation or ultrafiltration. Although the divergent approach is ideal for large-scale production, incomplete functionalization or side reactions can occur when the number of generation increases. These flawed dendrimers are usually difficult to be separated from the final products because of structural similarity [67]. In contrast, the convergent approach initiates the synthesis from the periphery and progresses inward. This approach starts with coupling end groups to each branch of the monomer, followed by the activation of a single functional group located at the focal point of the first wedge-shape dendritic fragment or dendron. Higher generation dendron is synthesized by the coupling of the activated dendron to an additional monomer. After repetition of coupling and activation step, a globular dendrimer is formed by attaching a number of dendrons to a polyfunctional core. Dendrimers thus synthesized can be effectively purified. However, synthesis of large dendrimers above the sixth generation is difficult [63].

Dendrimers possess several unique properties that make them a good nanoparticle platform for antimicrobial drug delivery. The highly-branched nature of dendrimers provides enormous surface area to size ratio and allows great reactivity with microorganisms *in vivo*. In addition, both hydrophobic and hydrophilic agents can be loaded into dendrimers. Hydrophobic drugs can be loaded inside the cavity in the hydrophobic core, and hydrophilic drugs can be attached to the multivalent surfaces of dendrimers through covalent conjugation or electrostatic interaction [68, 69]. Moreover, by using antimicrobial drugs as a building block, the synthesized dendrimers themselves can become a potent antimicrobial. Dendrimer biocides are such example that contains quaternary ammonium salts as functional end groups. Quaternary ammonium compounds (QACs) are antimicrobial agents that disrupt bacterial membranes. Dendrimer biocides have displayed greater antimicrobial activity against target bacteria than small drug molecules because of a high density of active antimicrobials present on the dendrimer surfaces. The polycationic structure of dendrimer biocides facilitates the initial electrostatic adsorption to negatively charged bacteria. The absorption then increases membrane permeability and allows more dendrimers for entering the bacteria, leading to leakage of potassium ions and eventually complete disintegration of the bacterial membrane [70].

Table 4. Dendrimers for Antimicrobial Drug Delivery

Formulations	Drug	Targeted Microorganisms	Activity	References
Polyamidoamine (PAMAM) dendrimers	Nadifloxacin and prulifloxacin	Various bacteria	Improved water solubility	[95]
	Niclosmide	Tapeworm	1)Improved water solubility 2) Controllable drug release	[96]
	Silver salts	Gram-positive bacteria*(Staphylococcus aureus, Pseudomonas aeruginosa, andEscherichia coli)*	1)High payload 2)Prolonged circulation half-life	[71]
	Sulfamethoxazole	Strep throat (streptococcus), staph infection (staphylococcus aureus), and flu (Haemophilus influenza).	1)Sustained drug release 2)Increased antibacterial activity	[41]
Pegylated lysine based copolymeric dendrimer	Artemether	Plasmodium falciparum	1) Increased drug stability 2) enhanced solubility 3) Prolonged drug circulation half-life	[97]

PAMAM is one of the most studied dendrimers for antimicrobial delivery because of its higher density of functional groups, which make the dendrimer more hydrophilic and more readily reactive to antimicrobial conjugation. Silver salts loaded PAMAM dendrimers have demonstrated significant antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* [71]. Incorporation of antibacterial agents such assulfamethoxazole (SMZ) into the ethylenediamine (EDA) core of PAMAM dendrimers has significantly improved the drug's aqueous solubility and antibacterial activity against *E. coli* [72]. Many other antimicrobial drugs have been successfully loaded into dendrimer nanoparticles and have shown improved solubility and therapeutic efficacy. Table **4** summarizes more dendrimer-based antibacterial drug delivery systems.

CONCLUSIONS

In summary, many antimicrobial drugs are difficult to administer because of their low water-solubility, cytotoxicity to healthy tissues, and rapid degradation and clearance in the blood stream. Their antimicrobial activities against intracellular microbes are also severely limited by poor membrane transport ability. Extensive studies have demonstrated that nanoparticles such as liposomes, polymeric nanoparticles, solid lipid nanoparticles and dendrimers are able to overcome these issues and facilitate antimicrobial delivery to microbial infection sites. While most of these nanoparticle-based antimicrobial drug delivery systems are currently in preclinical development, several have been approved for clinical use. With the ongoing efforts in this field, there is no doubt that nanoparticle-based drug delivery systems will continue to improve treatment to bacterial infections, especially in life-threatening diseases such as staph infections and tuberculosis.

OUTLOOK

In spite of the great progresses on nanoparticle-based antimicrobial drug delivery, here we call attention to the need to unite the shared interest between nanoengineers and microbiologists in developing novel nanotechnology targeting a few major unmet challenges of antimicrobial drug delivery. First, acquired microbial drug resistance remains a major challenge for infection treatment. One possible approach is to incorporate more than one antimicrobial drug to a single nanoparticle and then concurrently deliver the drugs to the same microbes. Combinatorial drug therapy is expected to have higher potency as multiple drugs can achieve synergistic effects and overwhelm microbial defense mechanisms. Secondly, premature drug release from the antimicrobial-loaded nanoparticles remains another major challenge, especially for treating systemic and intracellular infections. To minimize drug loss before the nanoparticles arrive at the infectious sites, an infection microenvironment-sensitive drug release nanoparticles can be developed. That is, negligible amount of antimicrobial drugs will be released when the nanoparticles circulate in the blood stream or encounter healthy tissues whereas triggered rapid drug release will occur after the nanoparticles get to the infectious cells or tissues. Potential drug-release triggers include pH value, enzyme and other unique characters of the infection microenvironment. Lastly, few of the current nanoparticle-based antimicrobial drug delivery systems can distinguish microbes or infectious cells from healthy cells due to the lack of the specific targeting ability, although targeted drug delivery has been extensively studied for other disease treatment such as cancers and cardiovascular diseases. It would be beneficial for infection treatment if antimicrobial nanoparticles could be modified with microbe antigen- or infectious cell antigen-specific ligands including antibodies, antibody fragments, aptamers and peptides.

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