ORIGINAL ARTICLE

Nano-fats for bugs: the benefits of lipid nanoparticles for antimicrobial therapy

Chelsea R. Thorn[1,2,3] · Nicky Thomas[1,2,3] · Ben J. Boyd[3,4] · Clive A. Prestidge[1,3]

Abstract

Bacterial infections are an imminent global healthcare threat evolving from rapidly advancing bacterial defence mechanisms that antibiotics fail to overcome. Antibiotics have been designed for systemic administration to target planktonic bacteria, leading to difficulties in reaching the site of localized bacterial infection and an inability to overcome the biological, chemical and physical barriers of bacteria, including biofilms, intracellular infections and antimicrobial resistance. The amphiphilic, biomimetic and antimicrobial properties of lipids provide a promising toolbox to innovate and advance antimicrobial therapies, overcoming the barriers presented by bacteria in order to directly and effectively treat recalcitrant infections. Nanoparticulate lipid-based drug delivery systems can enhance antibiotic permeation through the chemical and physical barriers of bacterial infections, as well as fuse with bacterial cell membranes, release antibiotics in response to bacteria and act synergistically with loaded antibiotics to enhance the total antimicrobial efficacy. This review explores the barriers presented by bacterial infections that pose bio-pharmaceutical challenges to antibiotics and how different structural and functional mechanisms of lipids can enhance antimicrobial therapies. Different nanoparticulate lipid-based systems are presented as valuable drug delivery systems to advance the efficacy of antibiotics, including liposomes, liquid crystalline nanoparticles, solid lipid nanoparticles, nanostructured lipid carriers and lipid nanocarriers. In summary, liquid crystalline nanoparticles are emerging with the greatest potential for clinical applications and commercial success as an "all-rounder" advanced lipid-based antimicrobial therapy that overcomes the multiple biological, chemical and physical barriers of bacteria.

Keywords Antibiotics · Antimicrobial resistance · Biofilms · Intracellular infections · Lipid-based drug delivery · Liposomes · Lipid nanoparticles · Liquid crystalline nanoparticles

Introduction

Bacteria contribute to one of the greatest causes of death worldwide—infectious diseases [1]. As adaptive organisms, the virulence and fitness of bacteria are rapidly evolving, intensifying the critical healthcare issue of infectious diseases [2, 3]. Bacteria have three crucial mechanisms that control their virulence and survival, i.e. biofilm formation, intracellular survival and antimicrobial resistance. These mechanisms are associated with numerous biological, chemical and physical barriers preventing effective antimicrobial therapy. As a result, persistent, lifelong infections are highly prevalent in the clinic, for example, in cystic fibrosis, pneumonia, rhinosinusitis, non-healing chronic wounds and urinary tract infections [4, 5].

There is an urgent need to develop innovative antimicrobial therapies against these persistent bacterial infections. While efforts have primarily focused on developing new chemical entities, re-purposing and re-formulating compounds already available is considered a faster strategic approach in the current urgent situation. Consequently, the development of new antimicrobial therapies has recently shifted towards improving the delivery of commercially available antibiotics by using advances in nanotechnology

✉ Clive A. Prestidge
  clive.prestidge@unisa.edu.au

1 Clinical and Health Science, University of South Australia, City East Campus, Adelaide, SA 5000, Australia

2 The Basil Hetzel Institute for Translational Health Research, Woodville, SA 5011, Australia

3 ARC Centre of Excellence in Convergent Bio-Nano Science & Technology, University of South Australia, SA 5000 Adelaide, Australia

4 Drug Delivery Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Pde, Parkville, VIC 3052, Australia

to direct antibiotics to the site of infection. Drug delivery systems based on polymers, inorganic materials, metals, lipids and composites of synthetic and natural materials are all reported to improve the performance of existing antimicrobial compounds [6–8].

Drug delivery systems can address the various challenges faced by antibiotics, including; bypassing antimicrobial resistance mechanisms such as enzymatic inactivation and restricted permeation and overcoming multiple chemical and physical barriers that limit the access of antibiotics, such as biofilm and intracellular infections [9–11]. While drug delivery technologies cannot directly overcome the modifications induced by resistant bacteria associated with the molecular target of the antibiotics, they can overcome the physicochemical barriers from the bacteria and host, exposing bacteria to therapeutically appropriate antibiotic concentrations through directed and targeted therapy.
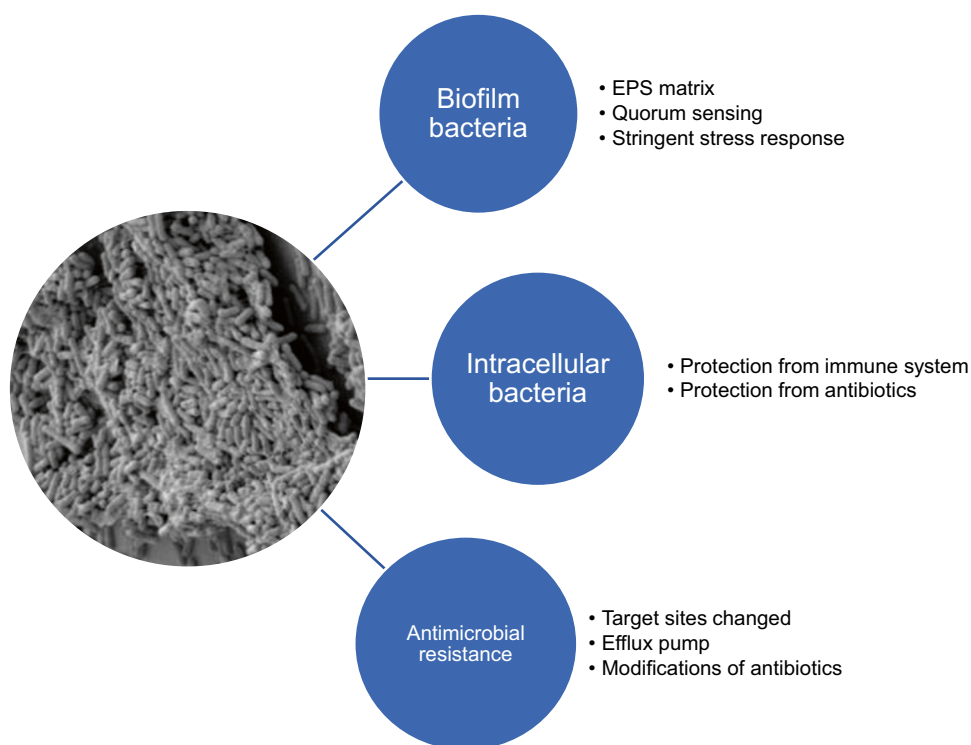
Lipids are particularly promising building blocks for the design of drug delivery systems. With inherent antimicrobial properties and the ability to self-assemble into structural vehicles for drug delivery [12, 13], amphiphilic lipids serve as a promising tool and solution to innovate and advance antimicrobial therapy against recalcitrant bacterial infections. Amphiphilic lipids are unique biologically active molecules containing both hydrophobic and hydrophilic portions with properties that drive specific structural and functional characteristics mimicking the chemistry of cellular vesicles. As biocompatible and biomimetic drug delivery systems, lipid-based drug delivery systems have been highly

prominent as potential delivery systems for antibiotics [6]. This review will focus on the biological, chemical and physical barriers associated with bacterial infections that pose significant challenges to antimicrobial therapies and details how lipid-based drug delivery systems can overcome these. Through the following sections, we provide an updated perspective on the use of lipids as antimicrobial compounds and delivery systems, specifically liposomes, liquid crystalline nanoparticles, solid lipid nanoparticles, lipid nano-carriers, and nanostructured lipid carriers. The discussion is divided across four principal advantages that lipid-based nanoparticles offer antibiotics, namely (1) innate and synergistic antimicrobial effects, (2) fusion with bacterial membranes, (3) attractive particulate surface characteristics and (4) bacterial triggered antibiotic release.

Bacterial barriers: biofilms, intracellular infections and antimicrobial resistance

Survival is the primary focus of bacteria. Through creating barriers that protect, nourish and sustain the microbe's lives, bacteria can adaptively withstand most environments within the human body. Biofilms, intracellular survival and antimicrobial resistance are key examples of advanced survival strategies (Fig. 1) [3, 14]. Biofilm fortresses or the intracellular space of crucial immune effector cells protect bacteria from noxious stimuli, while advanced communication between bacteria nourishes their survival and encourages the

Fig. 1 Bacterial mechanisms that create biological, chemical and physical barriers and limit effective antimicrobial therapy

- Biofilm bacteria
  - EPS matrix
  - Quorum sensing
  - Stringent stress response
- Intracellular bacteria
  - Protection from immune system
  - Protection from antibiotics
- Antimicrobial resistance
  - Target sites changed
  - Efflux pump
  - Modifications of antibiotics

development of new defensive strategies [14, 15]. The barriers created by bacteria lead to highly pathogenic, persistent and detrimental diseases in humans. Through understanding the underlying defensive strategies of bacteria, better antimicrobial therapies can be developed.

Biofilms

Biofilms are syntrophic clusters of microorganisms, adhered to biotic or abiotic surfaces and protected by a self-produced matrix. The notion of bacteria living as predominantly free-floating, planktonic cells was rebuffed by Costerton and colleagues [16], upon finding that bacteria predominately existed in these complex communities of biofilms (Fig. 2). The bacteria in the biofilm are phenotypically different from those in the planktonic state, harbouring significantly higher virulence, and can tolerate up to 1000-fold higher concentrations of conventional antibiotics [4, 16]. The protective, extracellular polymeric substance (EPS) matrix is composed of extracellular DNA, proteins, nucleic acids and polysaccharides, which maintain the intimate environment and restrict access to the bacteria [14]. The phenotypically different inner bacterial community has lower
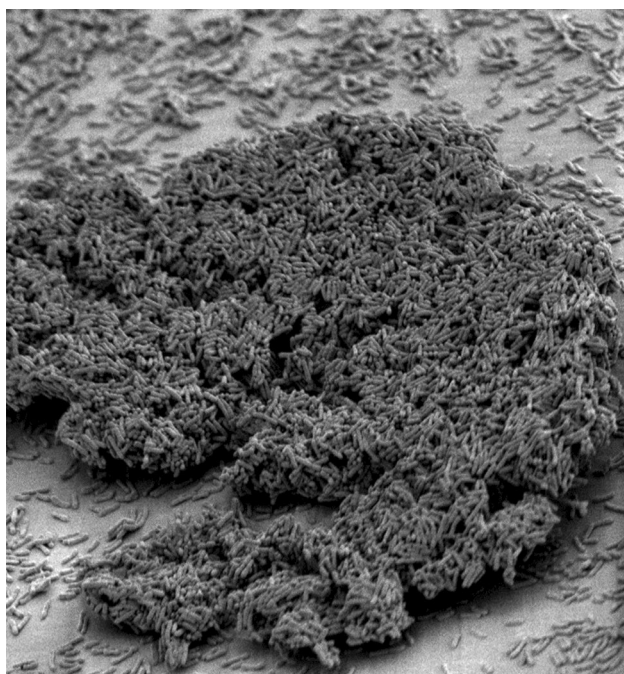

Fig. 2 Scanning electron micrograph of *Pseudomonas aeruginosa* biofilm surrounded by planktonic bacteria attached to polystyrene pegs

metabolic states that reduce their susceptibility to antibiotics, which typically kill or inhibit bacteria when in the metabolically active, planktonic state [17]. In humans, biofilms cause persistent and chronic tissue-related infections, for example, from *Pseudomonas aeruginosa* (*P. aeruginosa*) colonisation in the lungs, sinuses or wounds and implant-related infections and from *Staphylococcus aureus* (*S. aureus*) contaminating joint prosthetics and catheters [18–20]. The syntrophic consortium of bacteria in biofilms is a fortress with advanced strategies to withstand the effects of antibiotics.

Biofilms are underpinned by a host of impressive stress responses and quorum sensing abilities, enabling tightly coordinated responses and exerting dominance in any environment. The "stringent stress response" is a vital stress-coping mechanism from the accumulation of secondary-messenger nucleotides, and it diverts cellular energy away from growth and division so the biofilm can withstand environmental stressors [21–23]. The stringent stress response also activates quorum sensing, leading to the tightly controlled communication through genetic regulation in the dense population of bacteria in the biofilm [24]. Quorum sensing further results in a multitude of virulence factors, for example, the production of specific chemicals to cause host damage such as LasB elastase in *P. aeruginosa* [25–27]. The social cooperation within biofilms enables strategic accumulation of resources, with the primary goal of survival [14]. Overall, the unwavering dominance of biofilms leads to their highly pathogenic and persistent role in human infectious diseases.

Intracellular infections

In tissue-related infections, bacteria typically survive in the extracellular space, growing on epithelial surfaces to form biofilms and stimulate an aggravated host immune response [28]. The host up-regulates an inflammatory response to defend itself against the bacteria, with mononuclear phagocytes (i.e. monocytes and macrophages) acting as the key effector cells. To withstand the effects of the inflammatory response, bacteria can also conceal and protect themselves in these critical effector cells of the immune system. Bacteria survive in the intracellular space of mononuclear phagocytes by diverting the endosomal pathway, entering directly into the host cells' cytoplasm [29]. *S. aureus* and *Mycobacterium tuberculosis* (*M. tuberculosis*) are organisms renowned for their ability to cause intracellular infections. Inside professional and non-professional phagocytes, bacteria are concealed from the innate immune system, evading attack from antibodies, antibiotics and obtain a direct supply of nutrients that would have otherwise been restricted [5, 30]. Intracellular infections are persistent and difficult to detect and treat due to the bacteria utilising the host's barriers to conceal

themselves, including the hydrophobic epithelial barrier that may have a heterogenous coating of mucus.

Antimicrobial resistance

Beyond these innate growth and survival mechanisms of bacteria, antimicrobial resistance is a highly adaptive response by bacteria to protect themselves from antibiotics. Antimicrobial resistance is a global healthcare crisis that has evolved from inappropriate and excessive use in humans and animals, enabling bacteria to develop mechanisms to overcome the effect of many antibiotics. The leading cause of nosocomial infections are resistant to multiple antimicrobial therapies, collectively known by the acronym ESKAPE pathogens (e.g. *Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter* spp., *P. aeruginosa* and *Enterobacter* spp.) [31]. These notorious pathogens create fatal disease due to their sophisticated resistance mechanisms [32] and are causing a catastrophic healthcare crisis.

The increased selective pressures of improper and excessive antibiotic use promote the biological responses that improves the survival of bacteria in the presence of otherwise harmful substances. Antimicrobial resistance mechanisms include the ability of bacteria to modify antibiotics, express efflux pumps to prevent the entry of antibiotics or to actively remove antibiotics from the intracellular space and alter the target sites of antibiotics [33]. These mechanisms are highly specific concerning the bacterial species and antibiotics used, but inhibit the effectiveness of most of the first-line antibiotics available today [34]. Antimicrobial resistance is a leading contributor to deaths worldwide, and within the next 30 years, the predicted global annual death toll from antimicrobial drug-resistant infections is 10 million deaths per year [35]. The global catastrophe demands alternations in the way antibiotics are used.

Challenges in the development of antimicrobial compounds

While biofilm formation and intracellular survival are innately inscribed in bacteria, antibiotics were designed to kill single (planktonic) colonies of bacteria, without an understanding of biofilms and intracellular survival. Altogether, biofilms, intracellular survival and antimicrobial drug resistance creates several biological, chemical, and physical barriers to effective antimicrobial therapy. The biological barriers include; the advanced mechanisms in biofilm formation (i.e. quorum sensing and the stringent stress response) and antimicrobial resistance (i.e. efflux pumps and target modifications). The chemical barriers include the protective matrix of the biofilm, and antibiotic modifications from

antimicrobial resistance and the physical barriers include the host cell membrane and up-regulated host responses (i.e. mucus production and inflammation). Antibiotics cannot target these mechanisms or lack physicochemical properties to bypass these barriers of bacteria, leading to significant treatment challenges.

Limitations of the physicochemical properties

The physicochemical properties of drugs affect their trajectory after administration. While drugs need to be soluble in bodily fluids to cause an effect at the target tissue, they need reasonably high partition coefficients to pass through hydrophobic and biological barriers. While many antibiotics are water-soluble, their permeability across biological barriers including the bacterial cell envelopes varies with dose and pH [36]. For example, clinically relevant antibiotic classes (e.g. aminoglycosides and glycopeptides) are characterized by high solubility but demonstrate low permeability across hydrophobic biological barriers, while amoxicillin can have high predicted permeability but the solubility depends on the dose [36, 37]. Conversely, some poorly water-soluble antibiotics show good permeation across biological barriers (e.g. rifamycins and macrolides) [38]. At the site of action, antibiotics need to permeate the bacterial cell envelope, which is particularly challenging for Gram-negative bacteria due to the presence of the outer lipopolysaccharide- and phospholipid-rich membrane and inner plasma membrane [39–41]. Resistance mechanisms that restrict the permeation of antibiotics through altered expression of porins and up-regulation of multidrug efflux pumps are furthermore a considerable challenge for all antibiotics [42, 43]. The restricted permeability into the bacterial cell envelope decreases the bioavailability of the antibiotic at the site of action and constitutes one of the largest physicochemical challenges for the effectiveness of antimicrobials.

Depending on the site of administration, drugs require different physicochemical properties to reach the intended site of action. Typically, the preferred route of administration for antibiotics is the oral route, due to its non-invasiveness, ease of administration and patient compliance. Oral drug delivery relies on the drug being soluble and permeable to cross the intestines and to be absorbed to cause an effect. Poorly permeable and poorly soluble drugs are challenging to develop as their properties typically lead to low absorption and decreased bioavailability following oral administration. Low bioavailability of antibiotics limits the attainment of therapeutically relevant drug concentrations at the site of infection, compromising antimicrobial efficacy. While lower antibiotic plasma concentrations are required to clear acute infections with planktonic bacteria, biofilm-associated and chronic infections prevail in up to 80% of infectious diseases [44]. In this scenario, high and prolonged concentrations of

antibiotics are required at the site of infection and are usually required to be dosed via intravenous administration.

Inadvertently, the systemic exposure to antibiotics from intravenous administration decreases the effective concentration at the site of infection compared with direct, local treatment. Moreover, systemic administration increases the development of resistance by exposing bacteria to sub-therapeutic levels of antibiotics [45]. Achieving supra-therapeutic concentrations of antibiotics via systemic administration is difficult due to broad, non-specific distribution and consequent increased risk of off-target side effects (e.g. cardiotoxicity, nephrotoxicity, ototoxicity and peripheral neurotoxicity) [4]. The shortcoming of low bio-availability from oral administration is not eliminated by intravenous administration and highlights the importance of local administration to the infection site.

Local delivery of antibiotics to the site of infection is an essential therapeutic consideration and could be the preferred option to achieve a spatially confined high concentration of antibiotic, minimising unnecessary systemic exposure, reducing toxicity and the development of resistance [4]. As infections primarily begin at a localized site, directed therapy to the site of infection will provide the greatest therapeutic benefit. However, multiple other factors need to be considered for the local delivery of antibiotics. For example, for adequate absorption via the pulmonary route of administration, the lung physiology, particle deposition, residence times, clearance mechanisms and drug permeability all need to be taken into account [46]. For topical delivery, the therapeutic response is driven by the release of the drug from the dosage form and penetration/diffusion across the upper and deeper skin layers [47]. This is co-dependent on the drug's physicochemical properties and also the dosage form or vehicle used. Importantly, further pharmaceutical development is required to produce effective local delivery systems for antibiotics, where currently, there are a limited number of antibiotics formulated and used for local administration.

Inability to overcome biological, chemical, and physical barriers

The trend of high hydrophilicity or low partition coefficients generally results in low permeability of antibiotics through biological barriers, including across cell membranes needed to treat intracellular infections or epithelial tissue surfaces (i.e. skin, sinus and pulmonary infections). The up-regulated host barriers, including inflammatory responses and mucus production, add further challenges to these bio-pharmaceutically challenged antimicrobial drugs. The dehydrated, thick mucus becomes a significant barrier for drug permeation across the epithelium, particularly in cystic fibrosis and other respiratory and gastrointestinal tract infections [48]. The EPS matrix is the first-line barrier in biofilms, where due to the overall negative charge of the EPS, cationic antibiotics (i.e. aminoglycosides and polymyxins) are bound to the outer matrix, rendering them highly ineffective against the bacterial community residing inside the biofilm (Fig. 3) [49, 50]. At slightly less than neutral pH (pH 6–7), lipophilic and uncharged antibiotics (e.g. rifamycins and fluoroquinolones) can penetrate the EPS matrix, although changes in bacterial phenotype may alter the target sites of antimicrobial drugs [51]. The inhibition of bacterial cell division by fluoroquinolones has a limited effect on biofilm-based bacteria due to their switching to a slow growth rate [14, 52]. The unique interplay of genetic dominance and chemical barriers manifests as tolerance towards antimicrobial drugs that differ functionally and mechanistically from antimicrobial resistance [4, 17].
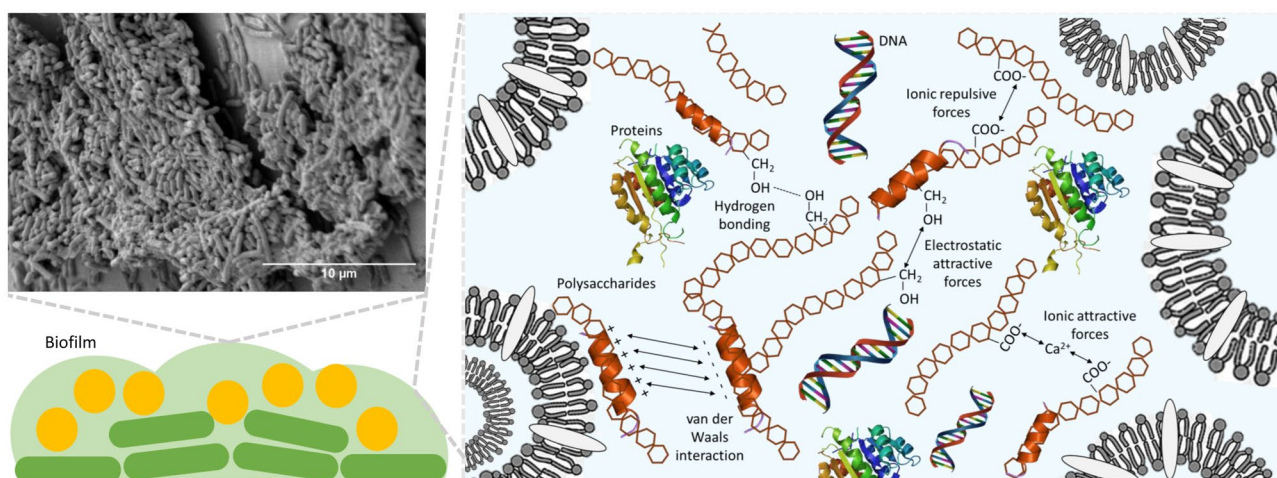

Fig. 3 The chemical barriers of the EPS matrix of biofilms, as adapted from Flemming and Wingender [53], Copyright 2010, with permission from Springer Nature

As schematically demonstrated in Fig. 4, delivery of the antibiotic to the bacteria requires penetration across several biological, chemical and physical barriers, including the biofilm's EPS matrix, hydrophobic cell membranes in intracellular infections and host factors such as mucus and bodily secretions in the skin, eyes, noses, ears or lung. Due to their physicochemical properties, achieving sufficient penetration may not be possible for antibiotics with high permeability coefficients without adequate formulation.

The structure and function of lipids as drug delivery systems

The physical chemistry of lipids

The structure and function of lipids are important in all biological systems and are primarily driven by the interaction between lipids within aqueous systems to form bilayers and other self-assembly structures. The assembly of amphiphilic lipids is driven by the hydrophobic effect, whereby hydrophilic solvents dissolve in similar solutes but repel hydrophobic solutes [54]. The unique structures formed are dictated to a less extent by the interplay of intermolecular forces between the amphiphiles [55]. This self-assembly phenomenon of lipids is vital to their biological functions, for example, forming cellular membranes, storing energy, providing insulation and transferring messaging between cells [56]. These properties also drive the function of lipids as soaps, detergents, emulsifiers and surfactants. At the interplay between chemistry and biology, lipids also serve as a critical ingredient in sanitation, food, cosmetic and pharmaceutical products.

In 1968, Small [57] classified biological lipids based on their interaction with water as either non-polar or polar. Non-polar lipids are long-chained alkanes or unsubstituted aromatic compounds either present as crystals or oils in water and do not spread as a monolayer on a surface. Polar lipids having at least one hydrophilic head group along the hydrocarbon chain can be split into three classes. Class I polar lipids are water-insoluble and non-swelling amphiphiles that remain as crystals or oils in a bulk aqueous environment and can spread at an aqueous surface to form a monolayer. Class II polar lipids are insoluble but can swell to form liquid crystals in water, due to the aliphatic chain becoming partly liquid at a specific temperature. Class III polar lipids are soluble amphiphiles that form unstable films at aqueous interfaces. Type IIIA soluble amphiphiles form liquid crystals in small volumes of water, before being solubilized. In contrast, type IIIB soluble amphiphiles do not form liquid crystals (Table 1) [57].

The geometric self-assembly of polar lipids in an aqueous system is driven by the critical packing parameter (CPP), which is a dimensionless ratio of the hydrophobic chain volume (V_s) to the product of the area of the hydrophilic head group (a_0) and the hydrophobic chain length (l) [58, 59].

$$CPP = \frac{V_s}{a_0 l}$$

When the CPP is less than $\frac{1}{3}$, spherical micelles form with a positive curvature of the lipid, and the hydrophilic headgroups face the outside (class IIIb). If CPP is higher than $\frac{1}{3}$, but less than $\frac{1}{2}$, non-spherical, rod-like structures form, while at a CPP $> \frac{1}{2}$, planar aggregates form in a bilayer (class IIIa). As the CPP increases (> 1), corresponding to the hydrophobic tail being more significant than the hydrophilic head group, inverted structures (e.g. inverse micelles) begin to form, including higher orders of liquid crystals (class II) [60]. The CPP is an arbitrary classification and is also affected by environmental factors, including ionic strength, pH, water concentration and temperature [61–64].

Lamellar and non-lamellar nanostructured liquid crystalline particles

The polar class II and class IIIa lipids that self-assemble into liquid crystals (LC) upon the addition of an aqueous solvent form various unique nanostructures, dependant on the CPP of the lipid [55]. As described by Kaasgaard and Drummond [65], the "ideal" amphiphile self-assembles with
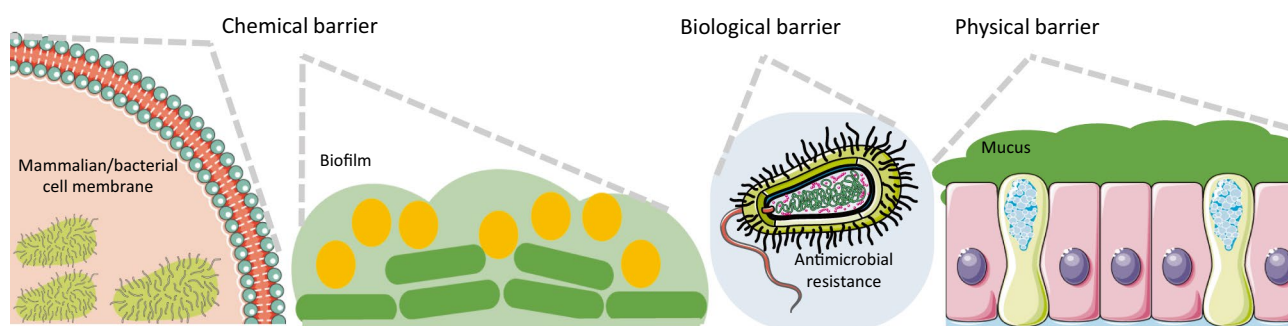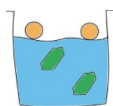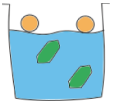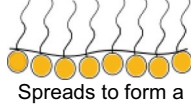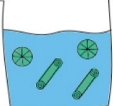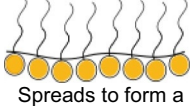

Fig. 4 The hydrophobic cell membrane and EPS matrix of the biofilm are chemical barriers to antibiotics, while antimicrobial resistance mechanisms are biological barriers and the mucosal epithelia are physical barriers

Table 1 Classification of biological lipids in aqueous systems adapted from Small [57], Copyright 1968, with permission from John Wiley and Sons

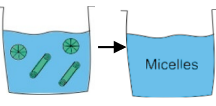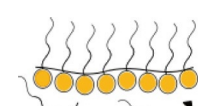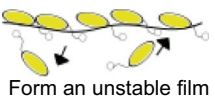| Class	Interaction with water		Examples
	Bulk	**Surface**	
Non-polar lipid	Crystal or oil in water	Will not spread to form a monolayer	Paraffins, large aromatic hydrocarbons
Polar lipids			
I. Insoluble non-swelling amphiphiles	Crystal or oil in water	Spreads to form a stable monolayer	Di-, triglycerides, long-chain protonated fatty acids, waxes, sterols
II. Insoluble swelling amphiphiles	Liquid crystals (LC) in water	Spreads to form a stable monolayer	Cell membrane lipids (i.e. lecithins), monoglycerides, acid soaps
III. Soluble amphiphiles			
A. Lyotropic mesomorphism	Solid → LC → micelles	Forms an unstable film	Anionic, cationic and non-ionic detergents
B. No lyotropic mesomorphism	Solid → micelles	Form an unstable film (higher surface tension)	Sulphated bile salts in fish and higher animals, steroids

increasing concentration spans through micelles, micellar cubic, hexagonal, bicontinuous cubic, lamellar, inversed bicontinuous cubic, inversed hexagonal, inversed micellar cubic and inversed micelle mesophases. Fig. 5 a depicts the "normal" mesophases and the varied shape of the amphiphile (including single carbon chained fatty acids and dual carbon chained diglycerides). The "normal", lamellar and "inverse" assembly are represented in Fig. 5b. These unique nanostructures are excellent drug delivery systems, where drugs can be loaded in the aqueous or lipophilic compartments according to the drug's physicochemical properties.

Positive curvature in the self-assembled lipid (i.e. CPP < 1) results in simpler nanostructures, including micelles up to lamellar phase structures. Dispersion of the lamellar phase creates one of the most extensively used drug delivery systems, liposomes [66]. The lipid bilayer shell of liposomes comprises phospholipid combinations that encase an inner aqueous cavity to load water-soluble drugs. Targeting ligands (e.g. proteins, antibodies or peptides) or stabilising compounds (e.g. cholesterol, or polyethylene glycol) can be integrated into the lipid bilayer to improve the liposome function, in addition to loading hydrophobic or amphiphilic drugs. Drug loading is typically limited by rapid drug leakage from amphiphilic drugs partitioning in and out of the lipid membrane, as well as lower hydrophobic and hydrophilic interfacial regions, and limited solubilities [67].

Higher orders of the lyotropic lipid mesophases are non-lamellar and have a higher degree of negative curvature (i.e. CPP > 1). The higher CPP results in the formation of inverse nanostructures, beginning from the inversed bicontinuous cubic phase and beyond. For drug delivery, the (inverse bicontinuous) cubic and inverse hexagonal LC phases have received significant attention. These complex three-dimensional LC structures of the lipid have a higher interfacial surface area

Fig. 5 a Different phase structures of amphiphilic lipids and **b** the specific structures of the amphiphile, adapted from Kaasgaard and Drummond [65], Copyright 2006, with permission from the Royal Society of Chemistry
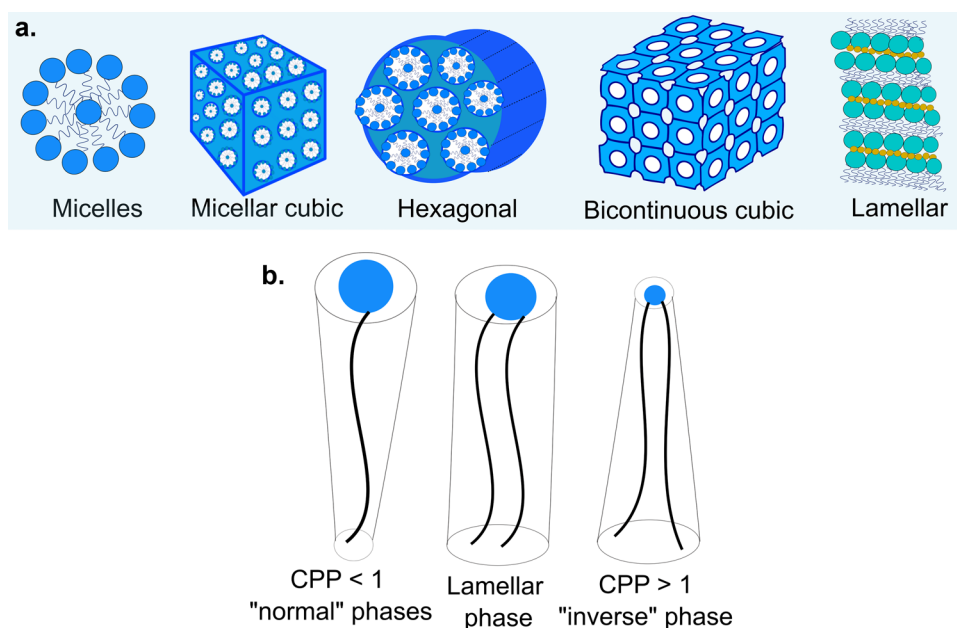
between aqueous domains and the ability to load considerable amounts of hydrophilic, hydrophobic and amphiphilic drugs [55, 61, 68]. Non-lamellar LC nanoparticles (hereafter denoted as LCNPs) are the LC mesophases dispersed as sub-micron particles and are often stabilized by synthetic block copolymers such as polyethylene glycol-polypropylene glycol-polyethylene glycol [68]. These LCNPs are also referred to as cubosomes and hexasomes, depending on their LC structure. Monoolein and phytantriol are two lipids primarily used to form the cubic or hexagonal phase LC structures. Obtaining the correct ratio and concentration of these lipid and aqueous components is essential for specific LC formation, in addition to temperature, pH and ionic strength.

The basic structural unit of liposomes and LCNPs are the LC lipid bilayers in different conformations and provide biomimetic features to both lipid nanoparticles. The complex structural geometry of the LCNPs offers advantages over liposomes, such as enhanced drug loads, increased stability, immediate- and sustained-drug release, although, by far, liposomes have been more prevalent in innovating formulations for antimicrobials. For an extensive literature review on the use of liposomes in antimicrobial applications, the interested reader is referred to Forier et al. [6].
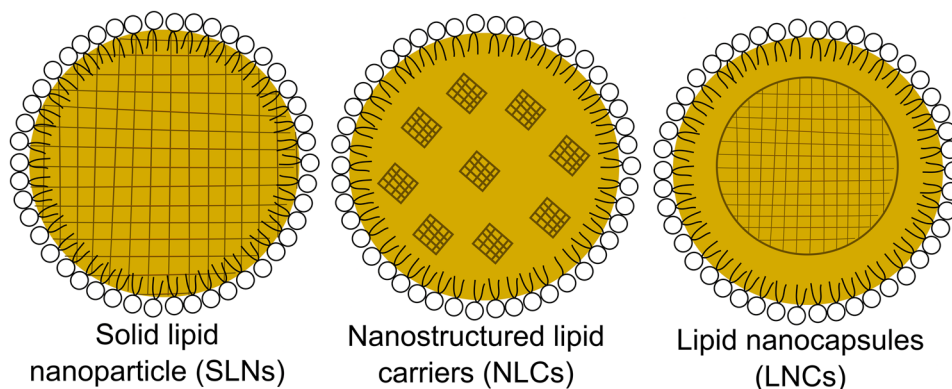
Solid lipid nanoparticles, nanostructured lipid carriers and lipid nanocapsules

The non-swellable polar class I lipids (i.e. mono-, di-, triglycerides or fatty acids) that remain as crystals or oils in water are also useful drug delivery systems. Additional surfactants can stabilize non-soluble glycerolipids as solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs) and lipid nanocapsules (LNCs) [69]. These lipid-based nanoparticles are preferred systems for loading lipophilic drugs and provide extended stability during storage by dissolving the drug in an amorphous or molecularly dispersed state in the lipid core, reducing its exposure to the aqueous environment. Fig. 6 schematically represents the physical differences between SLNs, NLCs and LNCs. SLNs contain only solid lipids (i.e. triglycerides, partial glycerides or saturated fatty acids) with rigid crystalline structures. In contrast, NLCs are a mixture of solid and liquid lipids (i.e. unsaturated fatty acids) with decreased crystallinity, which increases the drug loading capacity and release profiles [70]. LNCs are composed of a solid lipid core shielded by an oil or a lipophilic surfactant shell formed via a phase inversion temperature method.

When comparing the three systems, the crystalline structure of SLNs is less favourable than the disordered NLCs and LNCs with formless matrices allowing higher drug loading capacities [71]. The denser arrangement of lipids in these systems affords a greater lipophilic surface area to load bio-pharmaceutically challenging (low solubility and/or permeability) drugs, primarily through increasing the drug solubility [72–76]. Conversely, they are unable to load high amounts of water-soluble drugs and require alternative methods, such as actively loading hydrophilic compounds onto surfactant capsules or utilising lipophilic pro-drugs and hydrophobic ion-pairing [77, 78].

Solid lipid nanoparticle (SLNs)

Nanostructured lipid carriers (NLCs)

Lipid nanocapsules (LNCs)

The mechanisms behind lipids and lipid-based drug delivery systems modulating the effects of antibiotics

The unique structural properties of lipids in aqueous environments drive their function in a biological environment and as drug delivery systems. The biological nature and amphiphilic properties of lipids render them excellent drug delivery systems to overcome the biological, chemical and physical barriers of bacteria and reduced permeability of antibiotics [79]. Lipid-based drug delivery systems (LBDDS) can increase antibiotics' permeability and availability at the site of infection, to obtain effective therapeutic antibiotic concentrations that lead to advance antimicrobial effects. This can reduce intravenous dosing requirements while maximising the antimicrobial effect and minimising off-target toxicity, as reviewed elsewhere [6, 7, 80–83]. These benefits of LBDDS for antimicrobial therapy arise due to the unique properties and mechanisms of the lipids, including (1) innate and synergistic antimicrobial activity, (2) fusion with the bacterial cell membrane, (3) nanoparticle characteristics that target bacteria and overcome barriers and (4) triggered antibiotic release in the presence of bacteria.

Innate and synergistic antimicrobial effects

A seemingly forgotten function of lipids is their inherent antimicrobial properties. The antimicrobial properties of lipids were first recognized in the 1800s, advancing the scientific understanding of their use as soaps and disinfectants [84]. Their function as a soap relies on selective adsorption of the lipid to the surface or soil (e.g. grime, bacteria, fungi and viruses) to displace the soil from the surface under agitation. Later, lipids were discovered to play a crucial role in the function of the human innate immune system, showing potential as the first therapy for bacterial infections [85–87]. However, with the rise of antibiotics in the early 1900s, lipids were soon superseded as antimicrobial compounds.

The lipids that primarily demonstrate antimicrobial activity are fatty acids and monoglycerides. The bactericidal mechanism of action is non-specific and involves the insertion of the fatty acid carbon chain into the bacterial cell membrane. This disrupts and interferes with transmembrane signal transduction or affects the electron transport chain and oxidative phosphorylation as summarized in Fig. 7 [13]. Compared with a mammalian cell membrane, bacteria have a cell envelope composed of the cell membrane and cell wall. The cell wall contains peptidoglycan and differs between Gram-positive and Gram-negative bacteria, with a thicker peptidoglycan layer in Gram-positive bacteria. In contrast, Gram-negative bacteria have a second outer cell membrane containing more lipopolysaccharides. Thus, Gram-negative bacteria are less susceptible to the action of lipids than Gram-positive bacteria [13]. While overexpression of the peptidoglycan layer produces a thicker cell wall that could inhibit the insertion of the fatty acid, phenotypes resistant to fatty acids have not been observed, which is an attractive feature for clinical use [88].

The structure and shape of the fatty acid are pivotal to the function. Medium-chain fatty acids (6–12 carbons in chain length) demonstrate the most potent antimicrobial activity, where the minimum inhibitory concentration (MIC) decreases as the chain length of the fatty acid increases [88]. Specifically, the free carboxyl group is deemed essential for antimicrobial activity; hence, the availability of the functional group is crucial for the antibacterial effect. The type, position and degree of unsaturation also affect the antimicrobial action of the fatty acid, where one or two *cis* double bonds increase the antibacterial effect [88]. There are other differences in the dynamics of the antimicrobial effect, where monoglycerides containing medium-chain fatty acids have time-dependent antimicrobial properties compared with the respective fatty acids, which are concentration-dependent [89]. Sequentially, the variation in antimicrobial function is linked to the structure of individual lipid.

Monolaurin, the monoglyceride of the medium-chain fatty acid, lauric acid, is one of the most well-known
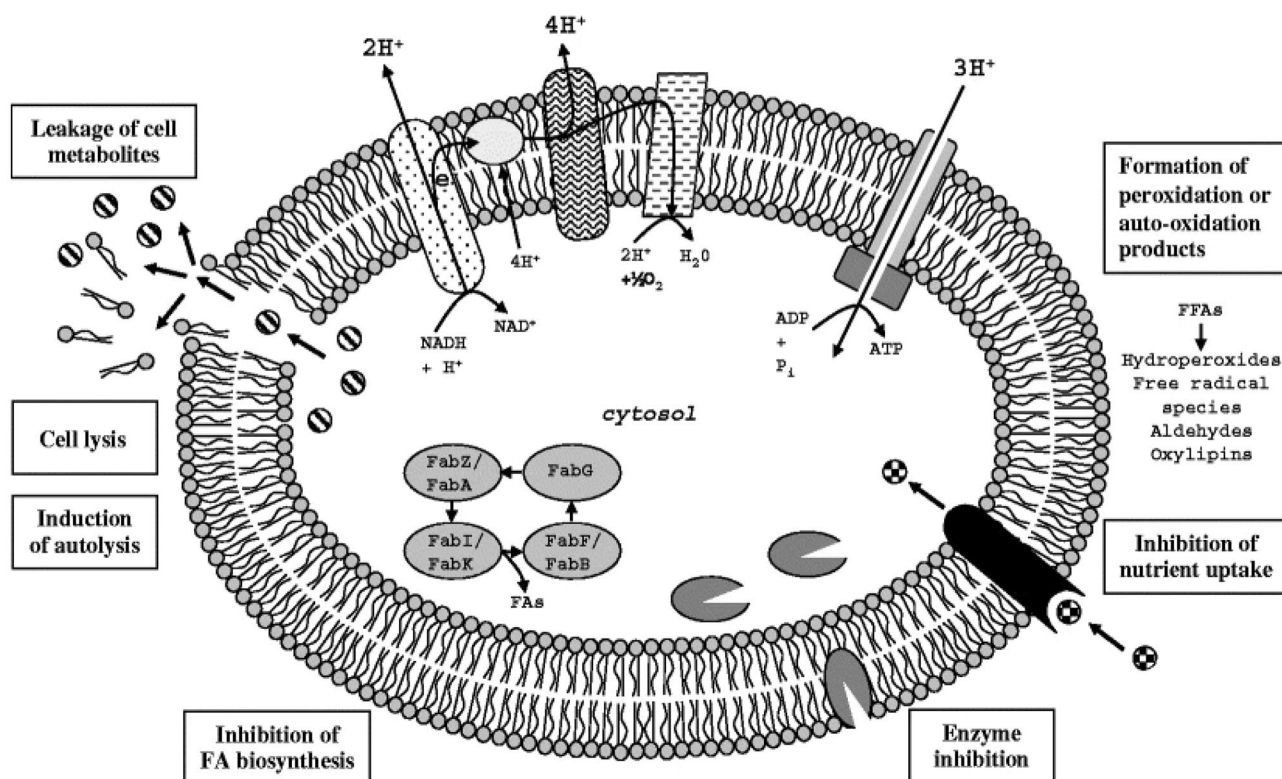
Fig. 7 Proposed mechanism and possible cell targets of antimicrobial lipids as described and reprinted from Desbois and Smith [13], Copyright 2009, Springer Nature

antimicrobial lipids to date. It is marketed as a nutraceutical (Lauricidin) by Med-Chem Laboratories Inc. and as a pharmaceutical product by Hennepin Life Science [88, 90]. As monoglycerides are poorly water-soluble, drug delivery systems are required to improve their solubility, activity and utility during pharmaceutical development. Currently, a non-aqueous gel composed of propylene glycol, polyethylene glycol and hydroxypropyl cellulose is under development [91]. In New Zealand white rabbits with a surgical wound infected with *S. aureus* (1×10^9 colony forming units (CFU)/mL), the 5% monolaurin gel completely eradicated the infection within 24 h. After 1 h, the 5% monolaurin gel also eliminated 3-log and 7-log of *P. aeruginosa* and *Acinetobacter baumannii*, respectively from the surgical wounds [91]. Currently, phase II clinical trials for monolaurin have begun for bacterial vaginosis and candidiasis. At the same time, preclinical testing has been completed for the urinary tract, skin and soft tissue infections, demonstrating advantages in the clinical environment [92].

Innate antimicrobial systems

Glycerolipids are a key ingredient in most LBDDS. In SLNs, NLCs and LNCs, glycerolipids are the backbone of the lipid nanoparticle's structure. Using antimicrobial-active glycerolipids can form innate antimicrobial SLNs, NLCs and LNCs in the absence of a loaded antibiotic. Umerska et al. [89] investigated a range of LNCs containing various fatty acids and monoglycerides. By substituting lecithin, a commonly used co-surfactant to form LNCs, with active antimicrobial monoglycerides and fatty acids, the LNCs had built-in antimicrobial properties. LNCs (without antibiotics) composed of caproic (C_6), caprylic (C_8), capric (C_{10}) and lauric acid (C_{12}) had a minimum inhibitory concentration (MIC) for *S. aureus* of 1.88, 0.938, 0.470 and 0.234 mg/mL, respectively. The antimicrobial performance of the LNCs corresponded to the properties of the included fatty acid, which was more potent against Gram-positive bacteria and increased with the carbon-chain length of the fatty acid

[13]. Correspondingly, when formed with monoglycerides, the antimicrobial effect of LNCs further increased against *S. aureus* [89]. As depicted in Table 2, solubilisation of the fatty acid or monoglyceride in LBDDSs further increased the antimicrobial activity compared with application in an organic solvent (i.e. dimethyl sulfoxide [DMSO]). The elimination of the organic solvent further extends the potential clinical application of the fatty acids and monoglycerides as antimicrobial therapies.

While fatty acids and monoglycerides imparted antimicrobial properties on the LNCs, in some cases, this also increased the toxicity of the lipid nanoparticles against healthy cells. Along with the ability to interfere and destabilize the bacterial cell membrane, lipids can destabilize eukaryotic cell membranes, causing cell lysis. While lipids are generally recognized as safe (GRAS) materials, monoglycerides and fatty acids have varying toxicity profiles, depending on concentration, cell line and conditions employed. The potential of lipids to destabilize membranes is more pronounced when in direct contact with a single-cell monolayer compared with topical application to tissue in an animal model [97]. LNCs containing capric, caprylic and caproic acids were haemolytic in horse erythrocytes at the same concentration that inhibited bacterial growth (Table 2) [89]. In this scenario, the advantage of the antimicrobial effect is outweighed by the toxicity of the LNCs. In comparison, monolaurin LNCs were non-toxic at their antimicrobial concentrations enabling use as both an antimicrobial agent and as an excipient for the design of antimicrobial

drug delivery system. All of which further demonstrates the strong clinical potential of monolaurin LBDDS to advance antimicrobial therapies.

Other LBDDS that are formed from glycerolipids, including LCNPs, have yet to demonstrate innate antimicrobial activities. Unlike other monoglycerides (e.g. monolaurin), the most commonly used lipid to form LCNPs, monoolein has not demonstrated antimicrobial activity at non-toxic concentrations, which is likely due to its long, saturated hydrocarbon chain. However, the aliphatic alcohol, phytantriol that is also commonly used to form LCNPs, is patented as an antimicrobial preservative in cosmetic preparations [98]. Yet, the concentration of phytantriol is imperative to the antimicrobial effect concentration in imperative to effect. Other fatty acid and monoglyceride combinations can form LCNPs which may be strategically designed to be intrinsically active, including a monolaurin LC system [99]. Similarly, innate antimicrobial activity has been previously achieved with liposomes doped with oleic and linolenic acid [94–96]. Harnessing the innate antimicrobial activity of specific lipids can be very advantageous to build smart, innately active LBDDS.

Synergistic antimicrobial systems

"Two is better than one" is an analogy that has not always been accepted in basic antimicrobial therapy guidelines. However, with the rise in antimicrobial resistance, targeting two or more functions of the bacteria has proven benefits in reducing further development of resistance [100].

Table 2 Minimum inhibitory concentration (MIC) of various fatty acids and monoglycerides against *S. aureus* from American Type Culture Collection (ATCC), NI = not inhibitory and NA = not assessed

Glycerolipid	Chain length	Formulation	MIC *S. aureus* (mg/mL)	Haemolytic concentration (mg/mL)	Ref
Caproic acid	C6, saturated	LNCs	1.9	0.9	[89]
		DMSO soln	NI	-	
Caprylic acid	C8, saturated	LNCs	0.94	0.45	[89]
		DMSO soln	NI	-	
Capric acid	C10, saturated	LNCs	0.45	0.45	[89]
		DMSO soln	0.50		[88]
Lauric acid	C12, saturated	LNCs	0.23	0.45	[89]
		DMSO soln	0.49		[88]
Monocaprin	C10	LNCs	0.12–0.23	0.45	[89]
Capmul MCM	C8, C10	LNCs	0.08	-	[93]
Monolaurin	C12	LNCs	0.015	1.8	[89]
		Non aqueous gel	0.5	-	[91]
Myristic acid	C14, saturated	DMSO soln	1.0	NA	[88]
Linolenic acid	C18, 3 unsaturated	Liposomes	0.5	NA	[88, 94, 95]
		DMSO soln	0.50	-	
Oleic acid	C18, 1 unsaturated	Liposomes	0.010 (MRSA)	NA	[96]
		DMSO soln	NI	-	

For example, combination therapies are necessary for tuberculosis and *Helicobacter pylori* infections [101]. An additive effect is defined as the sum of two or more compound's effects, whereas synergy is greater than the effect of the two compounds added together [102]. Clinically, a synergistic antimicrobial effect is most beneficial when using two compounds with different modes of action. The significant advantage of this phenomena is the ability to reduce the dosage requirement of the antibiotic which would have otherwise not been achievable due to toxicity concerns; this is explicitly relevant in biofilm infections where up to 1000-fold higher concentrations of antibiotics may be required for efficacy.

Glycerolipids are commercially available in pharmaceutical grades from various sources (e.g. Capmul MCM and Imwitor) and frequently used for multiple purposes in a variety of formulations [93]. These commercial lipids have demonstrated synergistic effects with antibiotics, by increasing their potency against bacteria in both planktonic and biofilm states. For example, the susceptibility of gentamicin to clinical isolates of *S. aureus* increased by tenfold in the planktonic state by the addition of 0.25 mg/mL Capmul MCM and 320-fold in the biofilm state by the addition of 1 mg/mL Capmul MCM. The antibiotic classes that benefited from the glycerolipids included; aminoglycosides, carbapenems, cephalosporins, glycopeptides, macrolides, phenicols and quinolones. The increased potency occurs across important clinically relevant bacteria, including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp., otherwise known as ESKAPE pathogens [93]. As these glycerolipids are frequently used in a variety of LBDDS, exploiting the synergistic antimicrobial combination has clear advantages in clinical development.

While the mechanism behind the antimicrobial effect of fatty acids and monoglycerides is non-specific [13], the specific molecular mechanism behind the synergistic effect between each glycerolipid and antibiotics combination is not entirely understood. Synergistic effects have also been described with monolaurin LNCs and antimicrobial peptides, including the plectasin derivatives AP114 and AP138. The monolaurin LNCs synergistically increased the antimicrobial effect against methicillin-resistant *S. aureus*, decreasing the effective concentration required of the two antimicrobial peptides [12]. However, contrary to in vitro results, in a murine incision wound model, the antimicrobial performance of the antimicrobial peptide DPK-060 did not differ between monolaurin-LNCs and lecithin LNCs [103]. The discrepancies between in vitro and in vivo results highlights the need for rigorous characterisation and understanding of the molecular basis behind the synergistic effects occurring, which may drive more fruitful clinical development.

Furthermore, the non-specific antimicrobial effect of glycerolipids has primarily been investigated in the planktonic state, whereas little is reported on the effect in biofilms. Moreover, as fatty acids are generally more active against Gram-positive bacteria, they are rarely further investigated in Gram-negative bacteria. Conversely, linolenic acid is a fatty acid that can disrupt quorum sensing in *P. aeruginosa*, inhibiting biofilm formation. Combining the action of linolenic acid and tobramycin has been a beneficial strategy to improve the total antimicrobial effect in *P. aeruginosa* biofilms. The disruption of the *P. aeruginosa* biofilm communication by linolenic acid facilitated the inhibition of bacterial protein synthesis by tobramycin and helped to eradicate *P. aeruginosa* biofilms [94]. The linolenic acid and tobramycin combination showcases the advantages of understanding the molecular mechanisms and combining multiple antimicrobial targets in *P. aeruginosa* biofilms. Combinations of glyceryl palmitostearate, glyceryl behenate and tristearin in SLNs have shown a sevenfold increase in the anti-virulence activity of pyocyanin quorum-sensing inhibitors [104]. This effect was further modulated by the surfactants poloxamer 407 and polysorbate 80 used for the preparation of SLNs, inhibiting pyocyanin without the quorum-sensing inhibitors by an unknown molecular function. Together, the synergistic effects of glycerolipid-based LBDDS can be highly beneficial for antimicrobial therapies, and rigorous characterisation and evaluation are warranted for the future development of smart, synergistic systems.

Biomimetic properties: fusion with the bacterial cell membranes

The quintessential biological properties of lipids are an unequivocal benefit of LBDDS. In a biological environment under conditions such as acidic pH or the presence of cations, lipids can undergo a phase transition that facilitates their interaction with cell membranes, promoting reciprocal mixing and membrane destabilisation. Cell membranes are composed of phospholipids as a lamellar bilayer and supplemented with cholesterol and various (lipo)-proteins. Liposomes drive increased cellular uptake of drugs through fusing with cell membranes, which results in the release of the cargo inside the cell's cytoplasm [105]. This form of directed and targeted therapy is a widely known advantage of liposomal systems.

Liposomes are known to interact with the cell membrane and increase the intracellular uptake of drugs. There are four different types of interactions between liposomes and cell membranes that enable this, including (1) adsorption, (2) endocytosis clathrin-(in)-dependent, (3) lipid exchange and transfer across the cell membrane and (4) fusion with intracellular membranes [106–108]. Enhanced uptake of antibiotics has been observed following the fusion of mammalian cells and liposomes based on 2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC)

and 1,2-dimyristoyl-rac-glycero-3-methylpolyoxyethylene (DMG-PEG$_{2000}$) and penicillin G conjugated to the phospholipid chain. As a result of enhanced endocytosis of the liposomes, the uptake of penicillin G increased by 8–10% and this translated to a significantly improved antimicrobial effect against an *S. aureus*-infected alveolar epithelial cell line (A549), eliminating 99.9998% of the bacteria [109]. In intracellular infections, liposomes are widely regarded as advantageous to deliver antibiotics across the hydrophobic cell membrane and other host-related barriers, as previously described [110, 111].

The bacterial cell membrane is similar in composition to the mammalian cell membrane, but a peptidoglycan-based cell wall surrounds it, forming the cell envelope. For Gram-negative bacteria, the additional outer membrane comprises phospholipids and lipopolysaccharides. Despite the concealed cell membrane, lipids destabilize the bacteria membrane via insertion of their hydrocarbon chain into the bacterial cell membrane to produce the bactericidal effect [13]. Similarly, liposome fusion with the cell membrane can occur with reciprocal mixing of lipid-based membranes [112].

Fluidosomes^{TM} were conceived on the discovery that liposomes with decreased membrane fluidity undergo fusion with bacterial cells, potentiating the effect of antibiotics. Fluidosomes^{TM} are negatively charged liposomes composed of dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphatidylglycerol (DMPG) [113]. In Sprague-Dawley rats with a respiratory tract infection, tobramycin Fluidosomes^{TM} eradicated all *P. aeruginosa* (inocoluum: 1×10^6 CFU/mL). *P. aeruginosa* eradication was not achieved with tobramycin in uncharged distearoylphosphatidylcholine (DSPC) and dimyristoylphosphatidylcholine (DMPC) liposomes or as an unformulated solution [114]. The DPPC/DMPG Fluidosomes^{TM} displayed a lower degree of crystallinity and rigidity compared with DSPC/DMPC liposomes, where this increased fluidity promoted fusion with the outer bacterial cell membrane of Gram-negative *P. aeruginosa* and increased the concentration of tobramycin in the bacterial cytoplasm (Fig. 8a) [112, 114, 115]. The Fluidosomes^{TM} technology also enhanced the concentration of tobramycin in the lung following pulmonary administration, unlike unformulated tobramycin. The Fluidosomes^{TM} tobramycin formulation was granted orphan drug status in Europe in 2006 and was licensed to Axentis Pharma in 2008, although since 2013, the sponsor withdrew this status [116]. Analogous liposomes are under development to enhance the anchoring and fusion to bacterial cell membranes by the inclusion of fatty acids such as lauric, oleic and linolenic acid into the lipid bilayer [79, 95, 96]. The targeted antibiotic delivery by bacterial membrane fusion is an enormous benefit of liposomes that is driving clinical development.
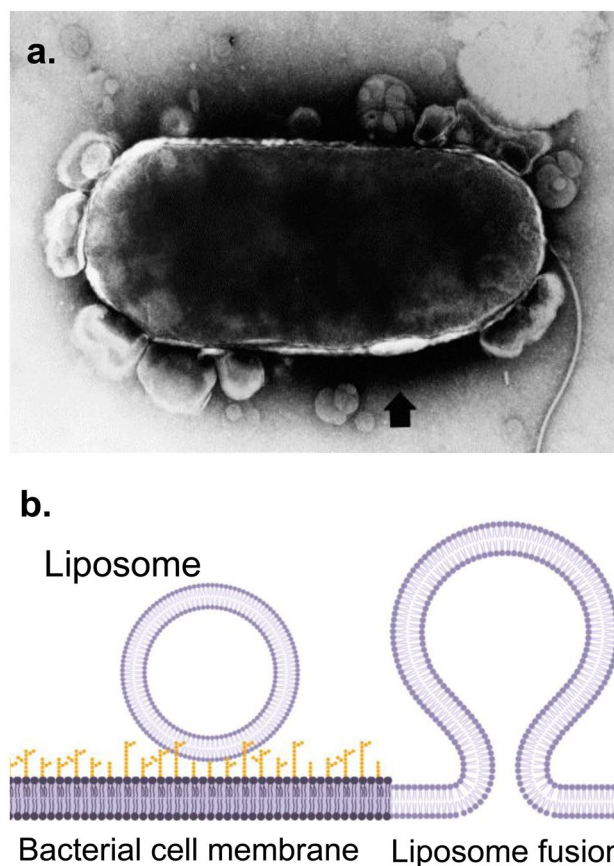
Fig. 8 **a** Interaction of Fluidosomes^{TM} with *Pseudomonas aeruginosa* 429 cells as observed by negative staining with phosphotungstic acid. Magnification: ×50 561, reprinted from Sachetelli et al. [112], Copyright 2000, with permission from Elsevier. **b** Schematic of liposomes fusing to the bacterial membrane

In comparison with the extensive body of literature on liposomes to enhance the antimicrobial effect, less work has been reported on LCNPs. Cubic phase LCNPs (i.e. cubosomes) have bilayers organized in space based on infinite periodic minimal surfaces providing a highly tortuous structure and greater surface area for interaction, which provides fusogenic properties similar to liposomes [117–119]. Cubosomes have emerged as fusogenic nanoparticles to facilitate higher drug uptake into mammalian cells with the cubic LC phase as the potential intermediate in the membrane fusion mechanism [120–122]. While phytantriol-based LCNPs have demonstrated enhanced fusion with membranes compared with monoolein-based LCNPs, the former have shown increased haemolysis of healthy cells and propensity to disrupt the cell membrane [118, 123]. LCNPs have also demonstrated superior skin retention compared with liposomes due to the greater surface area of the lipid bilayer, facilitating interaction with the hydrophobic dermis layer of the skin [124].

Boge et al. [125] visualized the interaction of LCNPs loaded with antimicrobial peptide (LL-37) and Gram-negative

bacteria using super-resolution laser scanning microscopy and cryogenic electron tomography. Furthermore, greater adsorption of the LCNPs loaded with LL-37 into the supported lipid bilayer was identified by a larger drop in frequency and a corresponding increase in dissipation via quartz crystal microbalance with dissipation monitoring compared with LL-37 and LCNPs individually. Neutron reflectivity further demonstrated direct interaction of LCNPs loaded with LL-37 with the lipid bilayer membrane of *E. coli* cells with a Bragg peak occurrence, which did not occur for LL-37 alone, suggesting that the LCNPs act as an adjuvant-like antimicrobial carrier for LL-37 [125]. In Fig. 9b, the scattering density length profiles show a more dense lipid bilayer when LCNPs loaded with LL-37 interacted with a lipid bilayer membrane compared with unloaded LL-37 and LCNPs alone. This was indicative of the LCNPs and the antimicrobial peptide acting as an 'antimicrobial unit' against *E. coli.*

The extended benefits of the antimicrobial peptide and LCNPs 'acting as an 'antimicrobial unit'' remain relatively unknown. Perhaps, the unit can alter the antimicrobial's structure and therefore, the bacteria's recognition of antibiotics, which could overcome antimicrobial resistance mechanisms. Presently, there is no clear evidence of LCNPs enhancing the efficacy of antibiotics. In planktonic *S. aureus*, LCNPs slightly decreased the MIC of rifampicin from 0.1 to 0.05 μg/mL [126]. However, as the clinical breakpoint for rifampicin against *S. aureus* is 0.1 μg/mL, this effect was not clinically significant. LCNPs are underexplored in terms of the benefits they could provide antimicrobial therapies in overcoming the barriers of bacteria. Nevertheless, harnessing the potential of LCNPs to interact with the bacteria should guide future evaluation and characterization as an effective LBDDS for antimicrobial therapies.

Nanoparticle characteristics that target bacteria and overcome barriers

Nanoparticulate-based LBDDS harbour functional characteristics that seemingly go unrecognized in their effect on potentiating the effects of antimicrobial compounds. These include the nanoparticle size and surface charge and useful surface stabilizers that enable higher permeation through the bacterial and host barriers. Specifically, electrostatic interactions between the LBDDS and bacteria can direct drug delivery systems to a target site of infection with highly specific attractive forces [127]. Strategic selection of surface stabilizers permits penetration across physical and chemical barriers such as mucus or the EPS matrix of biofilms [128]. Fine control over the particle size and surface properties of drug delivery systems is a feature that broadly applies to other carriers, including polymeric or inorganic drug delivery systems [80]. However, unlike these other carriers, the particle size and surface characteristics of liposomes and LCNPs are

complementary to their biomimetic features. Similarly, the size and surface properties of SLNs, NLCs and LNCs are complimentary with the inherent antimicrobial properties of the lipids. Table 3 highlights the different attractive features of LBDDS that are discussed in the following sections.

Using electrostatic interactions for targeting bacteria

The cell envelope of bacteria generally imparts a negative charge, prominently from the teichoic acids attached to the cell membrane in Gram-positive cells and the lipopolysaccharides in Gram-negative cells [135, 136]. For this reason, cationic drugs or delivery systems are usually employed to target bacteria [136]. For example, antimicrobial peptides, with a positive charge, are heralded as a new generation of targeted antibiotics that attract and insert into the bacterial cell envelope with additional immunomodulating effects [137]. However, while bacteria are negatively charged, the environment surrounding the bacteria can also contribute and affect these electrostatic interactions occurring during the targeted antibiotic delivery. When bacteria cluster into biofilms, the extracellular DNA and various polysaccharides in the EPS matrix including alginate impart an additional overall negative charge [138]. In this scenario, cationic antibiotics (e.g. aminoglycosides and colistin sulphate) bind to the negatively charged EPS matrix, immobilising the antibiotic and limiting access to the encased bacteria [49]. The negative charges within the EPS matrix hinder the effects of antibiotics and drug delivery systems, increasing the complexity of using simple electrostatic interactions for targeting bacteria.

The effect of cationic drug delivery systems interacting with the EPS matrix is multifarious. Liposomes have been a prominent formulation approach for various aminoglycoside antibiotics in biofilm-related infections. The lipid bilayer core provides an excellent shield to conceal the cationic antibiotics in the aqueous core, enabling higher permeation across the EPS matrix of biofilms. In addition to fusion with the bacterial cell membrane, as previously discussed [139], employing different lipid combinations enables tailoring of the surface charge and size of liposomes, where multiple combinations have been examined and widely reviewed by Forier et al. [6]. Interestingly, both positively and negatively charged liposomal systems have been trialled, each with a unique targeting argument.

Cationic, uni-lamellar liposomes have demonstrated higher innate antimicrobial activity compared with 1000 nm multilamellar vesicles/liposomes, due to the combined effects of electrostatic attraction and small particle size (~150 nm) [131]. However, in a different study, larger (~250 nm) cationic (+30 mV) liposomes were immobilized in the EPS matrix, thereby not reaching the encased bacteria and relied on the loaded antibiotic to reach the inner
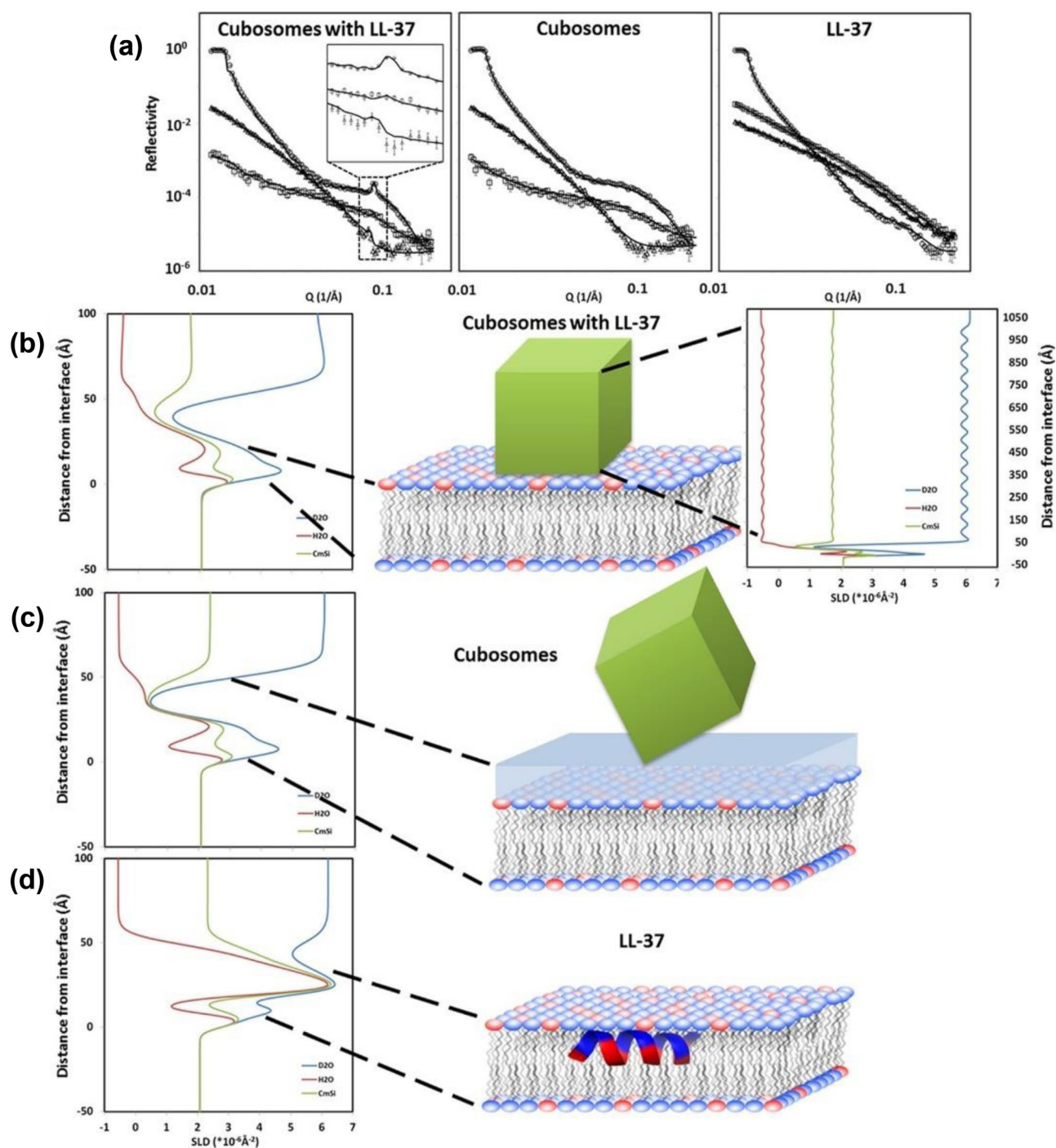
Fig. 9 (a) Experimental (markers) and fitted (solid lines) neutron reflectivity profiles of lipid (dDMPC/dDMPG) bilayers after exposure to cubosomes with LL-37 (the inset shows magnification around the Bragg peak), cubosomes without LL-37, and pure LL-37, experimental data were collected in three contrasts: d-buffer (circles), CmSi-buffer (squares) and h-buffer (triangles). Scattering length density profiles and schematical models of the bilayers are presented in (b–d). The cubosomes in illustrations (b) and (c) are not to scale. Reprinted with permission from Boge et al. [118]. Copyright, 2019, American Chemical Society

bacterial community. In comparison, negatively charged (−22 mV) liposomes of similar size were more likely to penetrate the biofilms EPS matrix and were detected closer to the bacterial cells [132]. Interestingly, the anionic liposomes did not potentiate the antimicrobial effect of tobramycin

compared with free drug, which may be because of electrostatic repulsion from the negatively charged bacterial cell membrane and low level of antibiotic loading.

As a consequence of non-ionic surfactants being used to stabilize LCNPs, the particles generally have a slightly

Table 3 Notable LBDDS that demonstrate greater biofilm penetration or targeting of bacteria

Effect	Attractive surface characteristic	LBDDS	Composition	Ref
Increased penetration and anti-biofilm effect	Low negative surface charge/membrane fluidity	Liposomes	DPPC/DMPG	[112, 114, 129]
Increased penetration and anti-biofilm effect	Neutral surface charge/300 nm particle size	Liposomes	DPPC/cholesterol	[130]
Biofilm targeting	+60 mV surface charge/130 nm particle size	Liposomes	DSCP/DOTAP	[131]
Increased penetration	−22 mV surface charge/250 nm particle size	Liposomes	DOPC/DPPG	[132]
Bacterial targeting and increased anti-bacterial (planktonic) effect	+30 mV surface charge/135 nm particle size	LCNPs	DOTAP-monoolein	[126]
Increased penetration in artificial mucus	Poloxamer 407/polysorbate 80 surface coating. Slightly negative surface charge/50–150 nm particle size	SLNs	Glyceryl palmitostearate, glyceryl behenate and tristearin with either poloxamer 407 or polysorbate 80	[104, 133]
Increased mucus penetration and anti-biofilm formation	Polysorbate 80 surface coating suggesting mucus penetration. −20 mV surface charge/180 nm particle size	LNCs	Poly(ε-caprolactone), sorbitan monostearate and oleic acid	[134]

negative or near-neutral surface charge, which can also be controlled by loading different antibiotics or other additives into the structures [140]. The addition of cationic dioleoyl-3-trimethylammonium propane (DOTAP) to rifampicin-loaded monoolein-LCNPs decreased the MIC against planktonic *S. aureus* from 0.1 to 0.03 μg/mL [126]. The electrostatic interaction permitted more co-localisation events between the cationic nanoparticles and negatively charged *S. aureus*. Reductions in fluorescence resonance energy transfer (FRET) confirmed that the cationic nanoparticles fused with the bacteria cells, which facilitated the enhanced antimicrobial effect [126]. In this case, electrostatic interactions lead to an enhanced fusion of the nanoparticle with bacterial cell membranes. Comparatively, the interaction between the bacterial cells and the unmodified monoolein LCNPs was not explored. Moreover, understanding the specific electrostatic targeting modalities in the clinical situation, where bacteria primarily survive in biofilms rather than single colonies, is of extreme importance. In the case of biofilm infections, the positive charge may adversely affect the action of LCNPs, as aforementioned with the liposomes [132] and is yet to be examined.

While a sub-micron particle size is desirable, there is little consensus on what surface charge best targets biofilm infections. Seemingly, either highly positive or negative charges tend to increase restrictions on the nanoparticle reaching the site of bacteria. Moreover, cationic nanoparticles are typically more toxic than anionic particles due to their strong interaction with the negatively charged mammalian cell membrane. However, different cell types may internalize different particle morphologies to different degrees. In general, non-phagocytic cells preferentially take up cationic particles [141]. In contrast, anionic particles are more likely to be phagocytosed by macrophages due to resembling negatively charged bacteria cells [142]. Correspondingly, anionic liposomes may be more beneficial for intracellular infections that reside inside phagocytic cell types. For example, a threefold increase in the uptake of rifampicin in the lungs of albino rats resulted from rifampicin-loaded anionic liposomes compared with the free antibiotic and neutral liposomes [143, 144]. Undoubtedly, every nanoparticulate system has its own advantages and disadvantages.

In (commercially) successful liposomal systems, the negatively charged DMPG in Fluidosomes™ was fundamental in providing decreased fluidity and bacterial cell membrane fusion [112]. While in comparison, a neutral surface charge of the sub-micron DPPC and cholesterol liposomes was essential to produce the enhanced antimicrobial effect of amikacin, which was not achievable with a highly negatively charged liposomes (i.e. −25 to −40 mV) [130]. The amikacin liposomal formulation has later been the first commercialized liposomal antibiotic product, called Arikayce®.

Overall, for targeting bacteria in the clinical environment, understanding the surrounding environment is crucial to the design of the targeting modality. For intracellular infections, specific cellular uptake is essential and can be guided by intricate electrostatic interactions, such as macrophages recognising foreign anionic particles. In comparison for biofilm targeting, cationic lipid nanoparticles can stick to the EPS matrix as a strategy that requires the antibiotic to do the rest of the work. While to overcome the EPS matrix, a slightly negative or near-neutral charged lipid nanoparticle is proposed to facilitate greater antibiotic penetration and effect.

Surface functionalisation for mucus penetration to reach bacterial communities

The upregulated inflammatory response and increased mucus production in response to bacterial infections result in significant biological and physical barriers to antimicrobial therapies [145]. Mucus is a viscous substance primarily composed of mucins which sterically hinder the diffusion of many compounds and delivery systems [133]. Mucus-penetrating particles reduce the steric hindrance experienced and can diffuse through mucus. These particles most commonly have a diameter less than 300 nm and when coated with polyethylene glycol (PEG) they diffuse through the pores of mucus, regardless of the composition of the inner core [128, 146]. Contrary to mucus penetration are mucus-adhering particles, which do not penetrate but rather attach to the mucus, decreasing particulate removal following mucociliary clearance [147], as schematically represented in Fig. 10. While mucus-adhering particles were considered to be more beneficial, mucus-penetrating particles are now considered to provide greater therapeutic effects.

Pluronic® F-127/poloxamer 407 is a commercially available triblock copolymer of PEG-polypropylene glycol-PEG that has been used as a biocompatible surface coating of PLGA nanoparticles to form mucus-penetrating particles [148]. These mucus-penetrating particles can reach the underlining epithelium coated with native cystic fibrosis sputum in the trachea following pulmonary administration to mice. In contrast, uncoated PLGA nanoparticles remained on top of the mucus [148].

Pluronic® F-127 is usually the surfactant of choice to stabilize SLNs and LCNPs [149, 150]. The mucus-penetrating properties of SLNs are dependent on the surfactant used; SLNs possessing a core prepared from glyceryl palmitostearate (Precirol), glyceryl behenate (Compritol) and tristearin, stabilized using either

poloxamer 407 or polysorbate 80 as the surfactant, were more likely to penetrate across artificial sputum mucus compared with polyvinyl alcohol-coated SLNs [104, 133]. Chemically, artificial sputum mucus (ASM) mimics the composition of pulmonary mucus, particularly the concentration of mucins, which act as a significant chemical barrier in native mucus [151]. However, differences arise in the mechanical and rheological properties between ASM and native mucus, where ASM has a considerably lower viscosity and viscoelasticity [152]. In a later study, the same group found discrepancies in the diffusion of SLNs through ASM, cystic fibrosis-patient sputum and a Calu-3 cell line producing mucus. The diffusion of the SLNs stabilized using polysorbate 80 through cystic fibrosis sputum was considerably restricted, while the poloxamer 407 or Pluronic® F-127 stabilizers led to greater mucus penetration [150]. While mucus surrogates are economical and plentiful, it is imperative that in vitro models resemble the in vivo environment as closely as possible. For this reason, ASM can be modified with gel-forming polymers such as polyacrylic acid to form more bio-relevant viscoelastic models of mucus [152]. Furthermore, it is not yet evident whether enhanced penetration through mucus will lead to an improved antimicrobial effect. There are limitations to the studies investigating mucus-penetrating SLNs for antibiotics, where most do not include mucus in the bacterial infection model [104, 134, 153], highlighting the importance of understanding the models used and correlating the effect to the physiological and clinically relevant environment.

In contrast, LC phases have been described with mucoadhesive properties [64, 154–156]. The cubic phase structure was essential for mucoadhesion of mono-olein LCs within porcine's stomach, intestine and buccal mucosa [154], although phytantriol and oleic acid LCNPs with hexagonal phase structure demonstrated greater mucoadhesion to porcine buccal tissue compared with vesicle structures [64]. The mucoadhesive hexasomes described were formed with various surfactants, including Pluronic® F-127, Pluronic® F-68 and Pluronic® F-108. Pluronic® F108 showed similar mucus-penetrating properties to Pluronic® F127 [157]; however, Pluronic® F-68 did not sufficiently coat the particle surface and was more mucoadhesive [128]. The LC cubic and hexagonal phase structure may provide mucoadhesive properties, regardless of the surface stabilizer present, suggesting that the LC phase is powerful to control the interaction with mucus. Further controlled studies in (infection-associated) mucus-rich environments are required to understand the mucus interaction of LCNPs, in which adhesion may act as a reservoir to increase antibiotic concentration across the mucosal barrier.
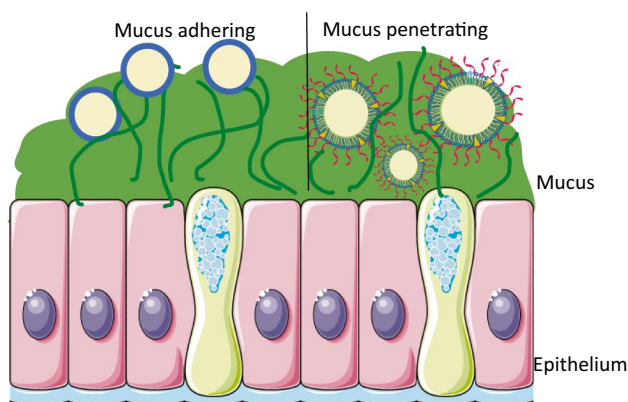

Fig. 10 Comparison between mucus-adhering and mucus-penetrating nanoparticles

Triggered antibiotic release from lipid particles in the presence of bacteria

Drug delivery systems can further revolutionize antibiotic therapy through either passively or actively targeting a specific area of the body where the antibiotic is required. The specific surface chemistries and size parameters of drug delivery systems facilitate passive accumulation without relying on a stimulus. These drug delivery systems have been extensively reviewed elsewhere in association with the enhanced permeability and retention (EPR) effect [158, 159]. A significant shortcoming of these passive targeting systems is the lack of absolute specificity in where they may accumulate, which leads to unwarranted side effects [159]. In comparison, active targeting drug delivery systems rely on an explicit tissue functionality or condition to either achieve particle accumulation at a highly specific site or selective release of drug from the carrier at the site of action [158]. Systems that respond to an external stimulus are termed 'triggered drug delivery systems' (TDDS), designed to deliver an appropriate amount of drug directly to the intended site and minimize off-target side effects [160].

Bacteria change the physiological microenvironment through the release of a range of compounds, as represented in the schematic in Fig. 11. These specific environmental changes can be harnessed to design a TDDS to respond to a bacterial infection specifically. For example, *P. aeruginosa* produces biosurfactants (i.e. mono- and di-rhamnolipids) that increase virulence, motility and stimulate biofilm formation. The release of amikacin from Arikayce® is triggered by the biosurfactants solubilising the liposomes [130, 161]. There are numerous secretion products of bacteria, including toxins that promote pathogenicity of the organism, and hydrolases that obtain nutrients and invade the host organism [162]. Other secretion products can change the microenvironment of the host, e.g. the local pH or temperature [163, 164]. These factors, which depend on the presence of an infection, provide multiple opportunities for targeted and triggered release of antimicrobials for a specific bacterial TDDS.

Digestion by bacterial toxins as a mechanism for triggered release

Bacterial toxins elicit damage to the host, exerting dominance of the bacteria and permitting survival [165]. There are an array of toxins, such as exotoxins secreted by the bacteria and endotoxins (otherwise known as lipopolysaccharides), that constitute the bacterial cell outer membrane and are secreted upon lysis of bacterial cells [166]. Exotoxins are composed of type I cell surface-active toxins (e.g. super-antigens and neurotoxins), type II membrane damaging toxins (e.g. pore-forming toxins and lipases) [167] and type III intracellular and extracellular matrix toxins (e.g. hyaluronidase and elastase) [162]. During the early stages of infection, toxins inform the host of the presence of bacteria by raising inflammatory markers, causing tissue damage and disabling the host immune systems [164]. If the immune system does not clear the bacteria (and toxins), the toxins cause fatal pathologies, such as toxic shock syndrome and sepsis [166].

One class of toxins are digestive enzymes that catalyse the hydrolysis of esters or amides. Many excipients utilized as drug delivery systems contain the same functional groups [168, 169]. Enzymatic digestion of the excipient by the secreted enzymes can facilitate a triggered drug release

Fig. 11 Bacteria represented in a biofilm with potential triggers for a drug delivery system, including toxins produced by the bacteria, alternations in the environment pH surrounding the infection, and productions of pyrogens increasing the (local) body temperature
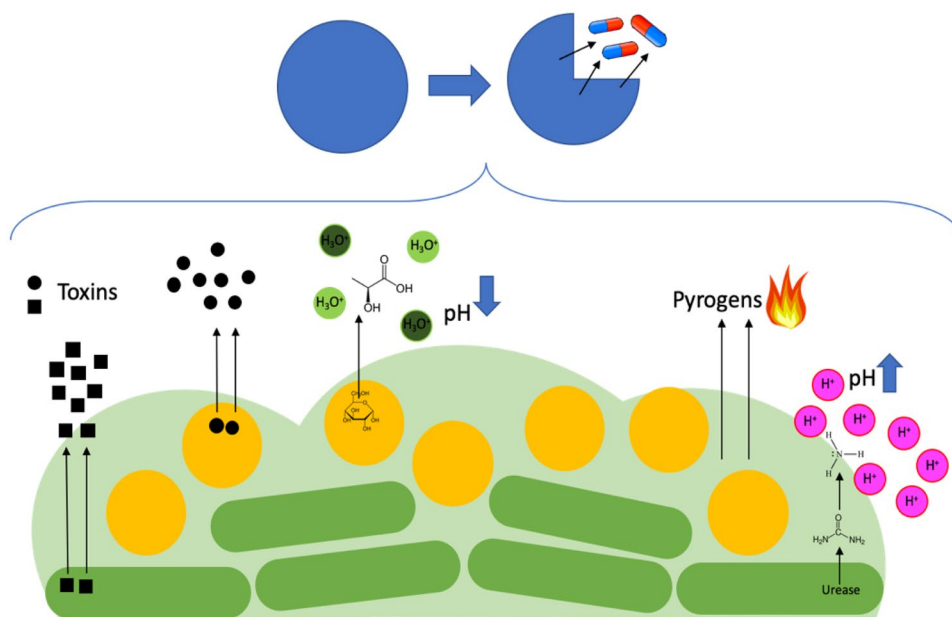
and is a prime example of a bacterial TDDS. Table 4 details different drug delivery systems triggered by bacterial toxins, described for antibiotics.

The lipases produced by bacteria are extracellular enzymes fundamental for nutrient supply and pathogenicity [168]. Critically important organisms of *Staphylococcus* and *Pseudomonas* produce lipases that block and disarm the host immune system, thereby enhancing the disease pathogenesis [181]. Lipases are hydrolytic enzymes that target the carboxyl ester bonds found in acyl-glycerols to liberate fatty acids and glycerol. The hydrolytic activity occurs at the lipid-water interface and is stereo-selective for the carbon position on the glycerol substrate (denoted as *sn*-1, 2, or 3), as represented in Fig. 12 [182]. Consecutively, *sn*-1/3 lipases liberate fatty acids positioned at the primary or tertiary hydroxyl position on the glycerol backbone and *sn*-2 at the secondary hydroxyl position. Bacterial lipases are denoted by either having no specific stereo-selectivity (e.g. *S. aureus)* or hydrolysing primary ester bonds (i.e. *sn*-1 and *sn*-3 selectivity) (e.g. *P. fluorescens)* [168, 183].

Assembling LBDDS from glycerolipids therefore offers an opportunity for the development of bacterial lipase-triggered drug delivery systems. *Pseudomonas* lipase mediated digestion of a monoolein-based lyotropic LC gel and enhanced the release of the encapsulated gentamicin and biofilm-dispersing enzyme, alginate lyase [172]. The monoolein-based LCs were evaluated in vitro against *P. aeruginosa* biofilms and maintained the activity of alginate lyase with a 100-fold reduction in bacterial load [172].

Nanoparticle-based LC systems (i.e. LCNPs) have a greater surface area, enabling an opportunity for increased rate and extent of digestion and therefore release of cargo. Monoolein-based particles were more digestible than the bulk LCs due
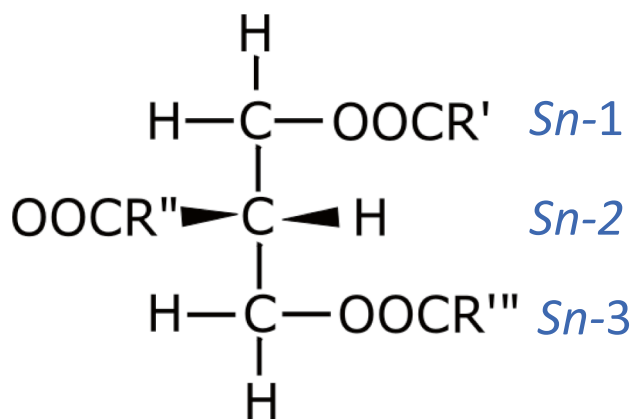

Fig. 12 Fischer projection of triacylglycerol with the designated stereo-selectivity

to the substantial increase in surface area, promoting higher activity of the interfacial enzyme [173]. As lipases digested monoolein, the liquid crystalline structure was degraded, triggering the release of alginate lyase and small molecular weight hydrophobic antibiotics, such as rifampicin, which are otherwise confined in the LC structure. For hydrophilic compounds such as gentamicin and ciprofloxacin, the release is already rapid from the LC nanostructure and cannot be further enhanced by a trigger mechanism [173]. Moreover, non-stereoselective enzymes resulted in complete digestion of the LC structure, unlike *sn*-1,3 stereo-selective lipases [173]. The monoolein-LCNPs were susceptible to degradation by gastric and pancreatic lipases limiting their use to the local administration at an infection site. Since lipases cannot digest phytantriol-based LCNPs, the release of antibiotics could not be triggered in the presence of bacteria. However, others have

Table 4 Different bacterial triggered lipid-based delivery systems explored

Trigger	LBDDS	Trigger mechanism	Ref
Alpha toxin	Liposomes stabilized by chitosan gold nanoparticles	Bacterial toxin forms pores in liposomes membrane	[170]
Lipase	Lipid coated mesoporous silica nanoparticle	The shell of lipid-coated mesoporous silica nanoparticles is digested by lipase, opening the pores for antibiotic release	[171]
Lipase	LCNPs	Lipases digest monoolein-based LCNPs, degrading the LC structure	[172, 173]
Phospholipase A2 toxin	Liposomes stabilized by gold chitosan nanoparticles	Bacterial toxin forms pores in liposomes membrane	[174]
Switch from pH 7.4 to 6.5	Liposomes	The collapse of electrostatic interaction between pH-sensitive lipids in liposomes bilayer	[175, 176]
Switch from pH 7.4 to 6.5	Solid lipid nanoparticles	The collapse of electrostatic interaction between lipid in SLNs	[177]
Switch from pH 7.5 to 5.5	Liquid crystals	LC phase change between the hexagonal phase (pH 7.5) and cubic phase (pH 5.5)	[178]
Switch from pH 5 to 7	Liquid crystals	LC phase change between the hexagonal phase (pH 5) and cubic phase (pH 7.4)	[179, 180]

shown that the addition of triglycerides in phytantriol-based LCNPs can create alternative digestion-triggered changes in the liquid crystalline structure and could be implemented for other antimicrobial compounds [184, 185].

Phospholipids used to construct liposomes, such as DSPC, are more susceptible to lipase-mediated hydrolysis than ethylphosphocholine building blocks [186], presenting opportunities for tailored liposome-based triggered systems that are responsive to bacterial phospholipase [187]. Phospholipases specifically target phospholipids in the cell membrane, thereby compromising the integrity of host cell membranes [181]. Alpha-toxin is a phospholipase produced by *S. aureus* that causes haemolysis and tissue damage through pore formation [188]. The toxin is a 293 protein monomer that significantly increases the virulence of *S. aureus* by insertion into the phospholipid-based cell membranes, causing apoptosis [189]. The alpha-toxin-triggered system is more specific to bacterial infections than lipase-triggered systems, which have similarities to human lipases [168]. By pore formation in the liposome bilayer, alpha-toxin triggered the release of vancomycin in vitro as a methicillin-resistant *S. aureus* (MRSA) bacterial TDDS [170]. Liposomes prepared from DSPC and 1,2-dioctadecanoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (DSPG) were stabilized by gold nanoparticles functionalized with chitosan to prevent fusion and vancomycin leakage. The stabilized liposomal system maintained the antibacterial activity of vancomycin against MRSA in vitro. Prominently, the release of vancomycin from the nanoparticles was also triggered by phospholipase A toxin produced by *Helicobacter pylori*, which digested the DSPG [174].

Bacterial environment effects—pH triggered release

Although less specific than the enzymatic environment at the site of infection, differences in environmental conditions such as pH can also be utilized in the development of TDDSs. The pH of blood and plasma is tightly regulated at pH 7.4, while the pH of the skin, stomach and bladder is acidic (i.e. pH 2–5.5) facilitating protection against microorganisms [190]. Consequentially, the pH surrounding an infection site, specifically with bacterial colonisation, is variable. The bacterial species, site of colonisation and the host inflammatory response all contribute to the pH surrounding a bacterial infection [166, 191]. Common pathogenic bacteria (e.g. *P. aeruginosa*, *E. coli* and *S. aureus*) are more likely to colonize wounds at pH values higher than physiological (i.e. 8.5, 8 and 7.5, respectively) [192]. While once an infection is established, the bacteria may decrease the pH through by-products or via the increased immune cell activity, as observed for urinary tract and respiratory tract infections [193–195]. Bacterial biofilms further introduce another layer of variability due to pH gradients, resulting in difficulties

in obtaining accurate measurements leading to ill-defined environmental pHs. In tissue-related biofilm infections, the insult to the host tissue results in inflammation and anaerobic metabolism, where an acidic environment predominates [196, 197]. Due to the variable pH surrounding an infection, that is highly site and organism-specific, there is no generalized TDDS that fits all infection sites and organism, and variable pH triggers have been explored.

pH-induced changes in the protonation state of acids or bases can result in electrostatic bond breakage, disrupting the structure of the TDDS and triggering the release of the loaded cargo. While many studies have investigated polymeric or organic-based delivery systems [198–201], LBDDS containing fatty acids or other glycerolipids present an opportunity for triggered release at high pH values by deprotonating the acid or hydrolysing an ester group. Govender and co-workers synthesized fatty acids with secondary amines that become protonated in a slightly acidic environment (pH 6). In liposomes [175] and SLNs [177], incorporation of the functionalized fatty acids resulted in a structural change at pH 6 that triggered vancomycin release. These systems furthermore decreased the minimum inhibitory concentration in vitro and in vivo in mice with an *S. aureus* skin infection [176, 177].

Multiple other LBDDS systems have demonstrated pH-triggered release in non-antimicrobial applications which could potentially be applied in this area. For example, phytantriol, sucrose stearate and oleic acid formed LC bulk phases that switched between the inverse hexagonal with limited drug release to inverse bicontinuous cubic phase (fast release) structures upon a change in pH from 5.5 to 7.4. The pH switch triggered the release of different sized macromolecules using FITC-dextrans as a model [179]. Others have made similar systems responsive to the switch between pH 2 (slow release) to pH 7 (fast release) by the addition of linoleic acid to monoolein LC systems due to the lower pK_a of the acid [180]. An oppositely triggered system (i.e. fast release at low pH) can furthermore be achieved by incorporation of a weak base, such as pyridinylmethyl linoleate, providing a slow (inverse hexagonal phase) to fast (inverse bicontinuous cubic phase) transition from pH 7.4 to pH < 5.5 [178]. Tailoring the chemical composition of LC systems to respond to the infective environment presents opportunities to produce smart systems and requires further exploration in the field of antimicrobial therapy.

LBDDS comparisons and clinical translations

Head-to-head comparison between LBDDS

There has been little head to head studies with different LBDDS in the antimicrobial field. One study has compared

LNCs and LCNPs loaded with the antimicrobial peptide DPK-060 in a poloxamer gel [103]. The LNCs were either composed of lecithin or monolaurin with medium-chain triglycerides and macrogol (15)-hydroxystearate, as mentioned before [89] while the LCNPs were formed with monoolein, as previously described by Boge et al. [202]. In a murine superficial incision wound infected with *S. aureus*, the antimicrobial peptide alone in the poloxamer gel performed better than the LNCs and LCNPs [103]. Both lipid nanoparticles were applied to the wound bed in a poloxamer gel for ease of administration. The limited release (30%) of DPK-060 from the LCNPs in poloxamer gel in 24 h could explain the limited activity; however, all DPK-060 was released from the LNCs in the poloxamer gel. The in vitro antimicrobial activity of DPK-060 was also reduced following the loading of both the lipid nanoparticles and in the poloxamer gel. While previous studies with the LNCs and LCNPs demonstrated activity maintenance or enhancement with the antimicrobial peptide, it is possible the poloxamer gel limited the release and activity of the peptide. Longer incubation or more frequent doses may be required to achieve the desired concentration and effect of the antimicrobial peptide from those formulations.

Liposomes and LCNPs have the potential for fusion with the cell membrane of bacteria [108, 112]. In contrast, while SLNs, NLCs and LNCs can be made of lipids that are inherently active antimicrobial compounds, they have not demonstrated the ability to fuse with the lipid bilayer of mammalian or bacterial cell membranes. SLNs, NLCs and LNCs systems are usually employed for the delivery of lipophilic drugs and used to improve the biopharmaceutical characteristics of drugs with low solubility and low permeability. Given that few antibiotics fit into this category, SLNs, NLCs and LNCs likely will have a limited role in enhancing the delivery and efficacy of antibiotics. Conversely, liposomes and LCNPs are suitable for a broader range of antimicrobial therapies, including hydrophilic, hydrophobic and amphiphilic drugs [79, 95, 96, 99]. While liposomes suffer from low drug loading capacities or potential drug leakage, LCNPs further enable loading of a higher proportion of each drug due to the larger internal surface area of the lipid structure and great potential to fuse with lipid bilayer membranes. Liposomes and LCNPs have also demonstrated the ability to incorporate lipids into the structure with inherent antimicrobial properties. However, given the lack of vigorous preclinical and clinical studies, it is premature to assign particular importance to one class of lipid nanoparticle to tackle infectious diseases.

Challenges and limitations in available models

Recently, the Food and Drug Administration (FDA) approved the liposomal amikacin formulation, Arikayce®,

for *Mycobacterium avium* complex (MAC) lung infections. Arikayce® improved the penetration of amikacin in biofilms, enhanced the activity against intracellular infections of *M. avium* [203] and overall improved the odds of patients providing uninfected sputum cultures in the phase III clinical trial (CONVERT) [111]. The liposomal formulation of amikacin is composed of DPPC and cholesterol. Using proprietary technology, the amikacin load in Arikayce® (up to 40% *w/w*) also exceeded typical drug loads in liposomes (max 20%) by twofold [111, 204].

While considering the breadth of studies performed on lipid nanoparticles, besides Arikayce®, there are no other LBDDS that have translated into the antimicrobial market. The bottleneck arises from a multitude of factors, including discontinuation of research programs due to limitations in funding, limited correlations between in vitro experimentations and (pre)clinical studies and limitations in well-planned studies. As demonstrated by the comparison between LNCs and LCNPs [103], the lipid nanoparticles in a topical gel provided no additional benefit compared with the gel containing the antimicrobial alone. In-depth analysis of the underlying molecular mechanisms that promote or prevent the antimicrobial activity is critical to developing the field further. In the head to head study with LNCs and LCNPs, there are many unanswered questions to why the LNCs and LCNPs did not perform. How well did the animal model correlate to human infection? Did longer or more frequent doses need to be administered? Did the gel hinder the lipid nanoparticles? Interdisciplinary and collaborative initiatives involving experts in drug delivery, material sciences and clinical microbiology might be able to address these fundamental question and drive innovation collectively.

Moreover, adequate evaluation of the system in an appropriate and biologically relevant system is critical for future development. Without broader in vitro and preclinical understanding, and careful design of clinical trials, translation into clinical efficaciousness may suffer, as exemplified by Apulmiq (also known as Lipoquin and previously known as Pulmaquin). Apulmiq® (Aradigm Corporation) is a liposomal formulation of ciprofloxacin [205]. In a phase II clinical trial (ORBIT-2), the dual release of ciprofloxacin from the inhaled Apulmiq® resulted in a 4.2-log reduction in *P. aeruginosa* and a 76-day delay to first pulmonary exacerbation compared with placebo in non-cystic fibrosis bronchiectasis patients [206]. However, the delay in pulmonary exacerbation in non-cystic fibrosis bronchiectasis patients was non-significantly different compared with placebo in a phase III clinical trial (ORBIT-3 and -4) [207]. Due to the discrepancies between phase III clinical trials, the FDA and the European Medicine Agency (EMA) have presently declined the market approval application for Apulmiq® for non-cystic fibrosis bronchiectasis.

The paucity of well characterized, biologically relevant infectious disease models is a limiting factor for clinical development. No animal model completely replicates the human response to infection, and often, a combination of models is required to understand the full pathogenesis or treatment response following infection. Mouse models are routinely employed; however, due to physiological and genetic differences in mice infectious disease models, they can significantly differ to the infection in humans [208]. For example, a cystic fibrosis murine model exists with the cystic fibrosis transmembrane conductance regulator gene mutation, but different clinical syndromes manifest compared with humans, particularly in the pancreas and lungs [209]. There is a growing interest in using organ-on-a-chip and 3D co-cultures of bacteria and humans' cells as a more biologically relevant model while decreasing the reliance on animal models [210]. However, there is a long road to travel ahead before organ-on-a-chip and cellular co-cultures dominate over animal studies that lead into human, clinical trials.

Overview and future perspectives

Biomimetic and antimicrobial lipids are an excellent solution and tool to improve antimicrobial therapies against infectious diseases. Liposomes are, by far, the most well-known and studied lipid nanoparticle for antimicrobial therapies. Besides targeted delivery via fusing with the bacterial cell membrane, liposomes can also be tailored as a bacterial triggered release drug delivery system. However, low drug loading and drug leakage are the primary issues of liposomes, ultimately leading to decreased shelf-life stability and incorrect or sub-therapeutic dosages in patients. In comparison, SLNs, NLCs and LNCs have improved shelf-life stability and can have intrinsic antimicrobial properties. Nevertheless, similar to liposomes, these lipid nanoparticles also have a limited ability to load large amounts of hydrophilic compounds, which includes most antibiotics. Alternative methods have been developed, such as active loading of hydrophilic compounds onto surfactant capsules or utilizing lipophilic pro-drugs, and hydrophobic ion-pairing, which may be viable for future developments of SLNs, NLCs and LNCs as antimicrobial drug delivery systems [77, 78]. Yet, there is little evidence to suggest that SLNs, NLCs and LNCs are a better approach than liposomes.

The modulating effects of lipids and LBDDS on antibiotics are promising for the development of advanced, novel antimicrobial therapies. By producing enhanced synergistic antimicrobial effects with the loaded antibiotics, innately antimicrobial active LBDDSs are advantageous when high concentrations of actives are required (i.e. biofilm infections). By simultaneously employing two or more antibacterial principles against pathogens, the development of antimicrobial resistance may also be reduced. The perfect lipid that produces a synergistic effect with antibiotics has yet to be confirmed; however, monolaurin is a prominent front runner undergoing pre-clinical and clinical trials. From the current literature, it furthermore appears that LBDDS with a submicron particle size with near-neutral surface charge and comprised lipids with low crystallinity or medium-chain fatty acids/monoglycerides are suitable for antimicrobial therapy to target the most notorious biofilm bacterial infection, fuse with bacteria and elicit an antimicrobial effect. While liposomes have had commercial and clinical success, development of the next generation systems could improve the range of effective antimicrobial therapies.

In this context, LCNPs have emerged as a potentially promising type of LBDDS for antimicrobials [117]. However, LCNPs have yet to be further validated mechanistically and clinically before becoming a primary strategy to improve the efficacy of antimicrobials. The unique nanostructure and functions of LCNPs can ideally combat the chemical and physical barriers of bacteria. While the fusion of LCNP has been modelled with lipid bilayers and mammalian cells [119], direct quantification of the fusion mechanics with bacteria is required. The fusion dynamics of LCNPs may also lead to increased biofilm penetration, as demonstrated with fusogenic liposomes [211]. The limitation in understanding this phenomenon involves the complexity and skill of directly quantifying the interaction between a single bacterial cell and the complex structure of LCNPs. The responsiveness of LCNPs to bacterial stimuli and the ability to incorporate antimicrobial monoglycerides might lead to the development of an "all-rounder" LBDDS. So far, these LBDDS are underexplored in the field of antimicrobials, but with advanced laboratory, preclinical and clinical investigations, they could further innovate antimicrobial therapies. The "all-rounder" LBDDS can therefore ultimately lead to an advanced antimicrobial therapy, which targets, interacts and synergistically increases the effects of loaded antibiotics, overcoming the multiple biological, chemical and physical barriers associated with bacterial infections.

Declarations

Ethics approval No ethics approval was required for this work.

Research involving human participants and/or Animals No animal or human studies were carried out by the authors for this article.

References

1. WHO. The top 10 causes of death. 2018.
2. Wolf DM, et al. Memory in microbes: quantifying history-dependent behavior in a bacterium. PLoS One. 2008;3(2):e1700.
3. Beceiro A, Tomás M, Bou G. Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? Clin Microbiol Rev. 2013;26(2):185–230.
4. Ciofu O, et al. Antibiotic treatment of biofilm infections. APMIS. 2017;125(4):304–19.
5. Thakur A, Mikkelsen H, Jungersen G. Intracellular pathogens: host immunity and microbial persistence strategies. J Immunol Res. 2019.
6. Forier K, et al. Lipid and polymer nanoparticles for drug delivery to bacterial biofilms. J Control Release. 2014;190:607–23.
7. Canaparo R, et al. Recent Developments in Antibacterial Therapy: Focus on stimuli-responsive drug-delivery systems and therapeutic nanoparticles. Molecules. 2019;24(10)
8. Dua K, et al. Whether a novel drug delivery system can overcome the problem of biofilms in respiratory diseases? Drug Deliv Transl Res. 2017;7(1):179–87.
9. Lee NY, Ko WC, Hsueh PR. Nanoparticles in the Treatment of infections caused by multidrug-resistant Organisms. Front Pharmacol. 2019;0(1153).
10. Bongers S, et al. Intracellular penetration and effects of antibiotics on Staphylococcus aureus inside human neutrophils: a comprehensive review. Antibiotics. 2019;8(2):54.
11. Baptista PV, et al. Nano-Strategies to Fight Multidrug Resistant Bacteria—"A Battle of the Titans". Frontiers in Microbiology. 2018;9(1441).
12. Umerska A, et al. Synergistic interactions between antimicrobial peptides derived from plectasin and lipid nanocapsules containing monolaurin as a cosurfactant against *Staphylococcus aureus*. Int J Nanomed. 2017;12:5687–99.
13. Desbois AP, Smith VJ. Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Appl Microbiol Biotechnol. 2010;85(6):1629–42.
14. Flemming HC, et al. Biofilms: an emergent form of bacterial life. 2016;14: p. 563.
15. Mansour SC, et al. Bacterial abscess formation is controlled by the stringent stress response and can be targeted therapeutically. EBioMedicine. 2016;12:219–26.
16. Costerton JW, et al. Microbial biofilms. Annu Rev Microbiol. 1995;49:711–45.
17. Brauner A, et al. Distinguishing between resistance, tolerance and persistence to antibiotic treatment. Nat Rev Micro. 2016;14(5):320–30.
18. Silva VO, et al. Biofilm formation on biotic and abiotic surfaces in the presence of antimicrobials by *Escherichia coli* Isolates from cases of bovine mastitis. Appl Environ Microbiol. 2014;80(19):6136–45.
19. World Health Organisation: Geneva. 2017.
20. Davies JC. Pseudomonas aeruginosa in cystic fibrosis: pathogenesis and persistence. Paediatr Respir Rev. 2002;3(2):128–34.
21. de la Fuente-Nunez C, et al. D-enantiomeric peptides that eradicate wild-type and multidrug-resistant biofilms and protect against lethal Pseudomonas aeruginosa infections. Chem Biol. 2015;22(2):196–205.
22. de la Fuente-Núñez C, et al. Broad-spectrum anti-biofilm peptide that targets a cellular stress response. PLoS Pathog. 2014;10(5):e1004152.
23. Pletzer D, Hancock REW. Antibiofilm peptides: potential as broad-spectrum agents. J Bacteriol. 2016;198(19):2572–8.
24. van Delden C, et al. Stringent response activates quorum sensing and modulates cell density-dependent gene expression *Pseudomonas aeruginosa*. J Bacteriol. 2001;183(18):5376.
25. Pesci EC, et al. Regulation of las and rhl quorum sensing in Pseudomonas aeruginosa. J Bacteriol. 1997;179(10):3127–32.
26. Papenfort K, Bassler BL. Quorum sensing signal-response systems in Gram-negative bacteria. Nat Rev Microbiol. 2016;14(9):576–88.
27. Miller MB, Bassler BL. Quorum sensing in bacteria. Annu Rev Microbiol. 2001;55:165–99.
28. Ferrand J, Ferrero RL. Recognition of extracellular bacteria by NLRs and its role in the development of adaptive immunity. Front immunol. 2013;4:344–344.
29. Fraunholz M, Sinha B. Intracellular Staphylococcus aureus: live-in and let die. Front Cell Infec Microbiol. 2012;2:43.
30. Löffler B, et al. Staphylococcus aureus persistence in non-professional phagocytes. Int J Med Microbiol. 2014;304(2):170–6.
31. Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. J Infect Dis. 2008;197(8):1079–81.
32. Santajit S, Indrawattana N. Mechanisms of antimicrobial resistance in ESKAPE pathogens. Biomed Res Int. 2016;2016:2475067.
33. Munita JM, Arias CA. Mechanisms of Antibiotic Resistance. Microbiology Spectrum. 2016;4(2): p. https://doi.org/10.1128/microbiolspec.VMBF-0016-2015.
34. Blair JMA, et al. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol. 2015;13(1):42–51.
35. O'Neill J. Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations, in the review on antimicrobial resistance. 2014; p. 1–16.
36. Thambavita D, et al. Biowaiver monograph for immediate-release solid oral dosage forms: amoxicillin trihydrate. J Pharm Sci. 2017;106(10):2930–45.
37. Jafari Ozumchelouei E, et al. Physicochemical properties of antibiotics: a review with an emphasis on detection in the aquatic environment. Water Environ Res. 2020;92(2):177–88.
38. delMoral-Sanchez JM, et al. Classification of WHO essential oral medicines for children applying a provisional pediatric biopharmaceutics classification system. Pharmaceutics. 2019;11(11).
39. Hancock REW, Bell A, Uptakeinto Gram-Negative Bacteria. A. Berlin. Heidelberg: Springer, Berlin Heidelberg; 1989.
40. Graef F, Gordon S, Lehr CM. Anti-infectives in drug delivery—overcoming the gram-negative bacterial cell envelope. In: Stadler M, Dersch P, editors. How to overcome the antibiotic crisis : Facts, challenges, technologies and future perspectives. Cham: Springer International Publishing; 2016. p. 475–96.
41. Nakae T. Outer-membrane permeability of bacteria. CRC Crit Rev Microbiol. 1986;13(1):1–62.
42. Dever LA, Dermody TS. Mechanisms of bacterial resistance to antibiotics. Arch Intern Med. 1991;151(5):886–95.
43. Pagès JM, James CE, Winterhalter M. The porin and the permeating antibiotic: a selective diffusion barrier in Gram-negative bacteria. Nat Rev Microbiol. 2008;6(12):893–903.
44. Cowan T. Biofilms and their management: from concept to clinical reality. J Wound Care. 2011;20(5): p. 220, 222–6.
45. Lewis R. The rise of antibiotic-resistant infections. FDA consumer magazine. 1995;29(1).
46. Hastedt JE, et al. Scope and relevance of a pulmonary biopharmaceutical classification system AAPS/FDA/USP Workshop March 16–17th, 2015 in Baltimore, MD. AAPS Open. 2016;2(1):1.
47. Shah VP, et al. A science based approach to topical drug classification system (TCS). Int J Pharm. 2015;491(1):21–5.

48. Fahy JV, Dickey BF. Airway mucus function and dysfunction. N Engl J Med. 2010;363(23):2233–47.

49. Tseng BS, et al. The extracellular matrix protects Pseudomonas aeruginosa biofilms by limiting the penetration of tobramycin. Environ Microbiol. 2013;15(10):2865–78.

50. Alipour M, Suntres ZE, Omri A. Importance of DNase and alginate lyase for enhancing free and liposome encapsulated aminoglycoside activity against Pseudomonas aeruginosa. J Antimicrob Chemother. 2009;64(2):317–25.

51. Justo JA, Bookstaver B. Antibiotic lock therapy: review of technique and logistical challenges. Infection and Drug Resistance. 2014;7:343–63.

52. Singh R, et al. Penetration of antibiotics through *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. J Antimicrob Chemother. 2010;65(9):1955–8.

53. Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol. 2010;8(9):623–33.

54. Tanford C. The hydrophobic effect: formation of micelles and biological membranes 2d ed. 1980: J. Wiley.

55. Hyde ST. Identification of lyotropic liquid crystalline mesophases. Handbook of Applied Surface and Colloid Chemistry. 2001;2:299–332.

56. Cronan Jr, JE, Gelmann EP. Physical properties of membrane lipids: biological relevance and regulation. Bacteriol Rev. 1975;39(3): p. 232.

57. Small DM. A classification of biologic lipids based upon their interaction in aqueous systems. J Am Oil Chem Soc. 1968;45(3):108.

58. Israelachvili JN, Mitchell DJ, Ninham BW. Theory of self-assembly of hydrocarbon amphiphiles into micelles and bilayers. J Chem Soc Faraday Trans 2. 1976;72(0): p. 1525–1568.

59. Israelachvili JN, Mitchell DJ, Ninham BW. Theory of self-assembly of lipid bilayers and vesicles. Biochim Biophys Acta Biomembr. 1977;470(2): p. 185–201.

60. Khalil RA, Al-hakam AZ. Theoretical estimation of the critical packing parameter of amphiphilic self-assembled aggregates. App Surf Sci. 2014;318: p. 85–89.

61. Tan A, et al. Self-assembled nanostructured lipid systems: is there a link between structure and cytotoxicity? Adv Sci. 2019;6(3):1801223.

62. Dong Y-D, et al. Impurities in commercial phytantriol significantly alter its lyotropic liquid-crystalline phase behavior. Langmuir. 2008;24(13):6998–7003.

63. Dong Y-D, et al. Bulk and dispersed aqueous phase behavior of phytantriol: effect of vitamin E acetate and F127 polymer on liquid crystal nanostructure. Langmuir. 2006;22(23):9512–8.

64. Du JD, et al. A novel approach to enhance the mucoadhesion of lipid drug nanocarriers for improved drug delivery to the buccal mucosa. Int J Pharm. 2014;471(1–2):358–65.

65. Kaasgaard T, Drummond CJ. Ordered 2-D and 3-D nanostructured amphiphile self-assembly materials stable in excess solvent. Phys Chem Chem Phys. 2006;8(43):4957–75.

66. Bulbake U, et al. Liposomal formulations in clinical use: an updated review. Pharmaceutics. 2017;9(2):12.

67. Sharma A, Sharma US. Liposomes in drug delivery: progress and limitations. Int J Pharm. 1997;154(2):123–40.

68. Rizwan SB, et al. Bicontinuous cubic liquid crystals as sustained delivery systems for peptides and proteins. Expert Opin Drug Deliv. 2010;7(10):1133–44.

69. Ghasemiyeh P, Mohammadi-Samani S. Solid lipid nanoparticles and nanostructured lipid carriers as novel drug delivery systems: applications, advantages and disadvantages. Research in Pharmaceutical Sciences. 2018;13(4):288–303.

70. Mukherjee S, Ray S, Thakur RS. Solid lipid nanoparticles: a modern formulation approach in drug delivery system. Indian J Pharm Sci. 2009;71(4):349–58.

71. Naseri N, Valizadeh H, Zakeri-Milani P. Solid lipid nanoparticles and nanostructured lipid carriers: structure. Preparation and Application Advance Pharmaceutical Bulletin. 2015;5(3):305–13.

72. Joyce P, et al. Solidification to improve the biopharmaceutical performance of SEDDS: opportunities and challenges. Adv Drug Deliv Rev. 2019;142:102–17.

73. Dening TJ, et al. Novel nanostructured solid materials for modulating oral drug delivery from solid-state lipid-based drug delivery systems. AAPS J. 2016;18(1):23–40.

74. Chakraborty S, et al. Lipid–an emerging platform for oral delivery of drugs with poor bioavailability. Eur J Pharm Biopharm. 2009;73(1):1–15.

75. Williams HD, et al. Strategies to address low drug solubility in discovery and development. Pharmacol Rev. 2013;65(1):315–499.

76. Thomas N, Rades T, Müllertz A. Recent developments in oral lipid-based drug delivery. J Drug Delivery Sci Technol. 2013;23(4):375–82.

77. Islan GA, et al. Smart lipid nanoparticles containing levofloxacin and DNase for lung delivery. Design and Characterization Colloids Surf B. 2016;143:168–76.

78. Phan TNQ, Shahzadi I, Bernkop-Schnürch A. Hydrophobic ion-pairs and lipid-based nanocarrier systems: the perfect match for delivery of BCS class 3 drugs. J Control Release. 2019;304:146–55.

79. Zhang Q, et al. Antimicrobial lipids in nano-carriers for antibacterial delivery. J Drug Target. 2020;28(3):271–81.

80. Eleraky NE, et al. Nanomedicine fight against antibacterial resistance: an overview of the recent pharmaceutical innovations. Pharmaceutics. 2020;12(2):142.

81. Wang DY, et al. Lipid-based antimicrobial delivery-systems for the treatment of bacterial infections. Front Chem. 2020;7:872–872.

82. Natan M, Banin E. From Nano to Micro: using nanotechnology to combat microorganisms and their multidrug resistance. FEMS Microbiol Rev. 2017;41(3):302–22.

83. Koo H, et al. Targeting microbial biofilms: current and prospective therapeutic strategies. Nat Rev Microbiol. 2017.

84. Thormar H. Antibacterial effects of lipids: Historical review (1881 to 1960), in lipids and essential oils as antimicrobial agents. p. 25–45.

85. Brogden NK, et al. The emerging role of peptides and lipids as antimicrobial epidermal barriers and modulators of local inflammation. Skin Pharmacol Physiol. 2012;25(4):167–81.

86. Glassman HN. Surface active agents and their application in bacteriology. Bacteriol Rev. 1948;12(2):105–48.

87. Fischer CL. Antimicrobial Activity of Host-Derived Lipids Antibiotics. 2020;9(2):75.

88. Kabara JJ, Vrable R, Lie Ken Jie MSF. Antimicrobial lipids: Natural and synthetic fatty acids and monoglycerides. Lipids. 1977;12(9): p. 753–759.

89. Umerska A, et al. Antibacterial action of lipid nanocapsules containing fatty acids or monoglycerides as co-surfactants. Eur J Pharm Biopharm. 2016;108:100–10.

90. Schlievert PM, Peterson ML. Glycerol monolaurate antibacterial activity in broth and biofilm cultures. PLoS One. 2012;7(7):e40350.

91. Mueller EA, Schlievert PM. Non-aqueous glycerol monolaurate gel exhibits antibacterial and anti-biofilm activity against Gram-positive and Gram-negative pathogens. PLoS One. 2015;10(3):e0120280.

92. Science, H.L. Pipeline: Human Health Initiatives. 2017;[20 April 2020]. Available from: http://www.hennepinlifesciences.com/human/.

93. Thomas ND, Richter K, Prestidge CA. Antimicrobial compositions and methods of use. Filed 2018, issued 2019.

94. Chanda W, et al. Combined effect of linolenic acid and tobramycin on Pseudomonas aeruginosa biofilm formation and quorum sensing. Exp Ther Med. 2017;14(5):4328–38.

95. Obonyo M, et al. Antibacterial activities of liposomal linolenic acids against antibiotic-resistant Helicobacter pylori. Mol Pharm. 2012;9(9):2677–85.

96. Huang C-M, et al. Eradication of drug resistant Staphylococcus aureus by liposomal oleic acids. Biomaterials. 2011;32(1):214–21.

97. Chanda W, et al. Effectiveness of omega-3 polyunsaturated fatty acids against microbial pathogens. J Zhejiang Univ Sci B. 2018;19(4):253–62.

98. Mendrok-Edinger C, Mongiat SRT, Schlifke-Poschalko A. Use of Phytantriol as an Antimicrobial Agent, DSM, Editor. 2017.

99. Kossena GA, et al. A novel cubic phase of medium chain lipid origin for the delivery of poorly water soluble drugs. J Control Release. 2004;99(2):217–29.

100. Ahmed A, et al. Current concepts in combination antibiotic therapy for critically ill patients. Indian Journal of Critical Care Medicine. 2014;18(5):310–4.

101. Coates ARM, et al. Antibiotic combination therapy against resistant bacterial infections: synergy, rejuvenation and resistance reduction. Expert Rev Anti-Infect Ther. 2020;18(1):5–15.

102. Sueke H, et al. An in vitro investigation of synergy or antagonism between antimicrobial combinations against isolates from bacterial keratitis. Invest Ophthalmol Vis Sci. 2010;51(8):4151–5.

103. Håkansson J, et al. Characterization of the in vitro, ex vivo, and in vivo Efficacy of the Antimicrobial Peptide DPK-060 Used for Topical Treatment. Front Cell Infect Microbiol. 2019;9(174).

104. Nafee N, et al. Antibiotic-free nanotherapeutics: ultra-small, mucus-penetrating solid lipid nanoparticles enhance the pulmonary delivery and anti-virulence efficacy of novel quorum sensing inhibitors. J Control Release. 2014;192:131–40.

105. Nicolosi D, et al. Nanotechnology approaches for antibacterial drug delivery: preparation and microbiological evaluation of fusogenic liposomes carrying fusidic acid. Int J Antimicrob Agents. 2015;45(6):622–6.

106. Ducat E, et al. Cellular uptake of liposomes monitored by confocal microscopy and flow cytometry. J Drug Delivery Sci Technol. 2011;21(6):469–77.

107. Ellens H, Bentz J, Szoka FC. Fusion of phosphatidylethanolamine-containing liposomes and mechanism of L.alpha.-HII phase transition. Biochemistry. 1986;25(14): p. 4141–4147.

108. Jahn R, Lang T, Südhof TC. Membrane fusion. Cell. 2003;112(4):519–33.

109. Zhang C, et al. Antibiotic-derived lipid nanoparticles to treat intracellular Staphylococcus aureus. ACS Appl Bio-Mater. 2019;2(3):1270–7.

110. Couvreur P, Fattal E, Andremont A. Liposomes and nanoparticles in the treatment of intracellular bacterial infections. Pharm Res. 1991;8(9):1079–86.

111. Shirley M. Amikacin liposome inhalation suspension: a review in Mycobacterium avium complex lung disease. Drugs. 2019;79(5):555–62.

112. Sachetelli S, et al. Demonstration of a fusion mechanism between a fluid bactericidal liposomal formulation and bacterial cells. Biochem Biophys Acta. 2000;1463(2):254–66.

113. Jacqueline Lagace CB. Sébastien Clement-Major, Low rigidity liposomal antibacterial composition, and U.D. Montreal, Editors. 1994: World.

114. Beaulac C, et al. Eradication of mucoid Pseudomonas aeruginosa with fluid liposome-encapsulated tobramycin in an animal model of chronic pulmonary infection. Antimicrob Agents Chemother. 1996;40(3):665–9.

115. Omri A, et al. Pulmonary retention of free and liposome-encapsulated tobramycin after intratracheal administration in uninfected rats and rats infected with Pseudomonas aeruginosa. Antimicrob Agents Chemother. 1994;38(5):1090–5.

116. AG, AP. Cystic Fibrosis - Orphan drug designation for innovative treatment against lung infections by Axentis Pharma AG. 2009.

117. Barriga HMG, Holme MN, Stevens MM. Cubosomes; the next generation of smart lipid nanoparticles? Angewandte Chemie. 0(ja).

118. Hinton TM, et al. Bicontinuous cubic phase nanoparticle lipid chemistry affects toxicity in cultured cells. Toxicol Res. 2014;3(1):11–22.

119. Dyett BP, et al. Fusion dynamics of cubosome nanocarriers with model cell membranes. Nat Commun. 2019;10(1):4492.

120. Shen H-H, et al. The influence of dipalmitoyl phosphatidylserine on phase behaviour of and cellular response to lyotropic liquid crystalline dispersions. Biomaterials. 2010;31(36):9473–81.

121. Seddon J. Inverse cubic phases of membrane-lipids, and their relevance to the static and dynamic structure of biomembranes. Acta Pharm. 1992;42(4):255–62.

122. Larsson K. Cubic lipid-water phases: structures and biomembrane aspects. J Phys Chem. 1989;93(21):7304–14.

123. Dong Y-D, et al. Adsorption of nonlamellar nanostructured liquid-crystalline particles to biorelevant surfaces for improved delivery of bioactive compounds. ACS Appl Mater Interfaces. 2011;3(5):1771–80.

124. Rattanapak T, et al. Comparative study of liposomes, transfersomes, ethosomes and cubosomes for transcutaneous immunisation: characterisation and in vitro skin penetration. J Pharm Pharmacol. 2012;64(11):1560–9.

125. Boge L, et al. Peptide-loaded cubosomes functioning as an antimicrobial unit against *Escherichia coli*. ACS Appl Mater Interfaces. 2019;11(24):21314–22.

126. Tran N, et al. Non-lamellar lyotropic liquid crystalline nanoparticles enhance the antibacterial effects of rifampicin against *Staphylococcus aureus*. J Colloid Interface Sci. 2018;519:107–18.

127. Transport of nanoparticles and tobramycin-loaded liposomes in *Burkholderia cepacia* complex biofilms. PLoS One. 2013;8(11): p. no pagination.

128. Yang M, et al. Biodegradable nanoparticles composed entirely of safe materials that rapidly penetrate human mucus. Angew Chem Int Ed Engl. 2011;50(11):2597–600.

129. Beaulac C, Sachetelli S, Lagace J. In-vitro bactericidal efficacy of sub-MIC concentrations of liposome-encapsulated antibiotic against gram-negative and gram-positive bacteria. J Antimicrob Chemother. 1998;41(1):35–41.

130. Meers P, et al. Biofilm penetration, triggered release and in vivo activity of inhaled liposomal amikacin in chronic *Pseudomonas aeruginosa* lung infections. J Antimicrob Chemother. 2008;61(4):859–68.

131. Dong D, et al. Distribution and inhibition of liposomes on *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilm. PLoS One. 2015;10(6):e0131806.

132. Messiaen AS, et al. Transport of nanoparticles and tobramycin-loaded liposomes in *Burkholderia cepacia* complex biofilms. PLoS One. 2013;8(11):e79220.

133. Carlson TL, Lock JY, Carrier RL. Engineering the mucus barrier. Annu Rev Biomed Eng. 2018;20:197–220.

134. Torge A, et al. Ciprofloxacin-loaded lipid-core nanocapsules as mucus penetrating drug delivery system intended for the treatment of bacterial infections in cystic fibrosis. Int J Pharm. 2017;527.

135. Swoboda JG, et al. Wall teichoic acid function, biosynthesis, and inhibition. Chem Bio Chem. 2010;11(1):35–45.

136. Silhavy TJ, Kahne D, Walker S. The bacterial cell envelope. Cold Spring Harbor Perspect Biol. 2010;2(5):a000414–a000414.

137. Hancock REW, Sahl H-G. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotech. 2006;24(12):1551–7.

138. Germoni LA, Bremer PJ, Lamont IL. The effect of alginate lyase on the gentamicin resistance of *Pseudomonas aeruginosa* in mucoid biofilms. J Gen Microbiol. 2016;121(1):126–35.

139. Halwani M, et al. Bactericidal efficacy of liposomal aminoglycosides against *Burkholderia cenocepacia*. J Antimicrob Chemother. 2007;60(4):760–9.

140. Liu Q, et al. Sensitivity of nanostructure in charged cubosomes to phase changes triggered by ionic species in solution. Langmuir. 2013;29(46):14265–73.

141. Fröhlich E. The role of surface charge in cellular uptake and cytotoxicity of medical nanoparticles. Int J Nanomed. 2012;7:5577–91.

142. Lunov O, et al. Differential uptake of functionalized polystyrene nanoparticles by human macrophages and a monocytic cell line. ACS Nano. 2011;5(3):1657–69.

143. Boehm F. Nanomedical device and systems design: challenges, possibilities, visions. 2016: CRC Press.

144. Vyas SP, et al. Design of liposomal aerosols for improved delivery of rifampicin to alveolar macrophages. Int J Pharm. 2004;269(1):37–49.

145. Zanin M, et al. The interaction between respiratory pathogens and mucus. Cell Host Microbe. 2016;19(2):159–68.

146. Yu T, et al. Biodegradable mucus-penetrating nanoparticles composed of diblock copolymers of polyethylene glycol and poly(lactic-co-glycolic acid). Drug Deliv Transl Res. 2012;2(2):124–8.

147. Mucoadhesive buccal drug delivery system. Review article. Int J Curr Pharm Res. 2017;9(4):1–4.

148. Schneider CS, et al. Nanoparticles that do not adhere to mucus provide uniform and long-lasting drug delivery to airways following inhalation. Sci Adv. 2017;3(4):e1601556.

149. Spicer PT, et al. Novel process for producing cubic liquid crystalline nanoparticles (cubosomes). Langmuir. 2001;17(19):5748–56.

150. Nafee N, et al. Mucus-penetrating solid lipid nanoparticles for the treatment of cystic fibrosis: proof of concept, challenges and pitfalls. Eur J Pharm Biopharm. 2018;124:125–37.

151. Kirchner S, et al. Use of artificial sputum medium to test antibiotic efficacy against *Pseudomonas aeruginosa* in conditions more relevant to the cystic fibrosis lung. J Visualized Exp. 2012;64:e3857.

152. Huck BC, et al. Macro- and microrheological properties of mucus surrogates in comparison to native intestinal and pulmonary mucus. Biomacromol. 2019;20(9):3504–12.

153. Moreno-Sastre M, et al. Pulmonary delivery of tobramycin-loaded nanostructured lipid carriers for *Pseudomonas aeruginosa* infections associated with cystic fibrosis. Int J Pharm. 2016;498(1):263–73.

154. Nielsen LS, Schubert L, Hansen J. Bioadhesive drug delivery systems: I. Characterisation of mucoadhesive properties of systems based on glyceryl mono-oleate and glyceryl monolinoleate. Eur J Pharm Sci. 1998;6(3): p. 231–239.

155. Nguyen TH, et al. Nanostructured reverse hexagonal liquid crystals sustain plasma concentrations for a poorly water-soluble drug after oral administration. Drug Deliv Transl Res. 2011;1(6):429–38.

156. Swarnakar NK, et al. Enhanced oromucosal delivery of progesterone via hexosomes. Pharm Res. 2007;24(12):2223–30.

157. Popov A, Enlow EM. and H. Particles, compositions and methods for ophthalmic and/or other applications: Chen; 2018.

158. Torchilin VP. Passive and active drug targeting: Drug delivery to tumors as an example, in drug delivery, M. Schäfer-Korting, Editor. 2010, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 3–53.

159. Albanese A, Tang PS, Chan WC. The effect of nanoparticle size, shape, and surface chemistry on biological systems. Annu Rev Biomed Eng. 2012;14:1–16.

160. Yu J, et al. Advances in bioresponsive closed-loop drug delivery systems. Int J Pharm. 2018;544(2):350–7.

161. Waters V, Ratjen F. Inhaled liposomal amikacin. Expert Rev Respir Med. 2014;8(4):401–9.

162. Rudkin JK, et al. Bacterial toxins: offensive, defensive, or something else altogether? PLoS Pathog. 2017;13(9):e1006452.

163. Shorrock SM, Kun S, Peura R. The exploration of tissue pH in wounds and its relationship to bacterial contamination. Master Degree Thesis. 2000: p. 20–24.

164. Dinarello CA. Infection, fever, and exogenous and endogenous pyrogens: some concepts have changed. J Endotoxin Res. 2004;10(4):201–22.

165. Actor JK. 11 - Basic Bacteriology. In: Actor JK, editor. Elsevier's Integrated Review Immunology and Microbiology (Second Edition). Philadelphia: W.B. Saunders; 2012. p. 93–103.

166. Cavaillon J-M. Exotoxins and endotoxins: inducers of inflammatory cytokines. Toxicon. 2018;149:45–53.

167. Schmiel DH, Miller VL. Bacterial phospholipases and pathogenesis. Microbes Infect. 1999;1(13):1103–12.

168. Jaeger KE, et al. Bacterial lipases. FEMS Microbiol Rev. 1994;15(1):29–63.

169. Coll Ferrer MC, et al. Designing nanogel carriers for antibacterial applications. Acta Biomater. 2014;10(5):2105–11.

170. Pornpattananangkul D, et al. Bacterial toxin-triggered drug release from gold nanoparticle-stabilized liposomes for the treatment of bacterial infection. J Am Chem Soc. 2011;133(11):4132–9.

171. Yang S, et al. Bacteria-targeting nanoparticles with microenvironment-responsive antibiotic release to eliminate intracellular *Staphylococcus aureus* and associated infection. ACS Appl Mater Interfaces. 2018;10(17):14299–311.

172. Thorn CR, et al. Pseudomonas infection responsive liquid crystals for glycoside hydrolase and antibiotic combination. ACS Applied Bio Mat. 2018;1(2):281–8.

173. Thorn CR, et al. Bacterial lipase triggers the release of antibiotics from digestible liquid crystal nanoparticles. J Control Release. 2020;319:168–82.

174. Thamphiwatana S, et al. Phospholipase A2-responsive antibiotic delivery via nanoparticle-stabilized liposomes for the treatment of bacterial infection. J Mater Chem B. 2014;2(46):8201–7.

175. Jadhav M, et al. Novel lipids with three C18-fatty acid chains and an amino acid head group for pH-responsive and sustained antibiotic delivery. Chem Phys Lipids. 2018;212:12–25.

176. Ahmed S, et al. Identifying the interaction of vancomycin with novel pH-responsive lipids as antibacterial biomaterials via accelerated molecular dynamics and binding free energy calculations. Cell Biochem Biophys. 2018;76(1–2):147–59.

177. Kalhapure RS, et al. Enhancing targeted antibiotic therapy via pH responsive solid lipid nanoparticles from an acid cleavable lipid. Nanomedicine. 2017;13(6):2067–77.

178. Negrini R, et al. pH-responsive lyotropic liquid crystals and their potential therapeutic role in cancer treatment. Chem Commun (Camb). 2015;51(30):6671–4.

179. Bisset NB, Boyd BJ, Dong YD. Tailoring liquid crystalline lipid nanomaterials for controlled release of macromolecules. Int J Pharm Res. 2015;495(1):241–8.

180. Negrini R, Mezzenga R. pH-responsive lyotropic liquid crystals for controlled drug delivery. Langmuir. 2011;27(9):5296–303.

181. Bender J, Flieger A. Lipases as pathogenicity factors of bacterial pathogens of humans, in handbook of hydrocarbon and lipid microbiology, Timmis KN, Editor. 2010. Springer Berlin Heidelberg: Berlin, Heidelberg. p. 3241–3258.

182. Reis P, et al. Lipases at interfaces: a review. Adv Colloid Interface Sci. 2009;147–148:237–50.

183. Alford JA, Pierce DA, Suggs FG. Activity of microbial lipases on natural fats and synthetic triglycerides. J Lipid Res. 1964;5(3):390–4.

184. Hong L, et al. Understanding the mechanism of enzyme-induced formation of lyotropic liquid crystalline nanoparticles. Langmuir. 2015;31(24):6933–41.

185. Pham AC, et al. In vivo formation of cubic phase in situ after oral administration of cubic phase precursor formulation provides long duration gastric retention and absorption for poorly water-soluble drugs. Mol Pharm. 2016;13(1):280–6.

186. Parmentier J, et al. Exploring the fate of liposomes in the intestine by dynamic in vitro lipolysis. Int J Pharm. 2012;437(1):253–63.

187. Drummond CJ, Fong C. Surfactant self-assembly objects as novel drug delivery vehicles. Curr Opin Colloid Interface Sci. 1999;4(6):449–56.

188. Bhakdi S, Tranum-Jensen J. Alpha-toxin of *Staphylococcus aureus*. Microbiol Rev. 1991;55(4):733–51.

189. Wardenburg JB, Schneewind O. Vaccine protection against Staphylococcus aureus pneumonia. J Exp Med. 2008;205(2):287–94.

190. Jones EM, Cochrane CA, Percival SL. The effect of pH on the extracellular matrix and biofilms. Advance in Wound Care. 2015;4(7):431–9.

191. Foulston L, et al. The extracellular matrix of Staphylococcus aureus biofilms comprises cytoplasmic proteins that associate with the cell surface in response to decreasing pH. MBio. 2014;5(5):e01667-e1714.

192. Percival SL, et al. The effects of pH on wound healing, biofilms, and antimicrobial efficacy. Wound Repair Regen. 2014;22(2):174–86.

193. Garrard W, Lascelles J. Regulation of *Staphylococcus aureus* lactate dehydrogenase. J Bacteriol. 1968;95(1):152–6.

194. Ferreira MT, et al. Effect of oxygen on glucose metabolism: utilization of lactate in *Staphylococcus aureus* as revealed by in vivo NMR studies. PLoS One. 2013;8(3):e58277–e58277.

195. Torres IM, et al. Acidosis increases the susceptibility of respiratory epithelial cells to *Pseudomonas aeruginosa*-induced cytotoxicity. Am J Physiol Lung Cell Mol Physiol. 2017;313(1): p. L126-L137.

196. Welbourn C, et al. Pathophysiology of ischaemia reperfusion injury: central role of the neutrophil. Br J Surg. 1991;78(6):651–5.

197. Simmen HP, et al. Analysis of pH, pO2 and pCO2 in drainage fluid allows for rapid detection of infectious complications during the follow-up period after abdominal surgery. Infection. 1994;22(6):386–9.

198. Irwin NJ, et al. Infection-responsive drug delivery from urinary biomaterials controlled by a novel kinetic and thermodynamic approach. Pharm Res. 2013;30(3):857–65.

199. McCoy CP, et al. An infection-responsive approach to reduce bacterial adhesion in urinary biomaterials. Mol Pharm. 2016;13(8):2817–22.

200. Radovic-Moreno AF, et al. Surface charge-switching polymeric nanoparticles for bacterial cell wall-targeted delivery of antibiotics. ACS Nano. 2012;6(5):4279–87.

201. Albayaty Y, et al. pH-Responsive copolymer micelles to enhance itraconazole efficacy against Candida albicans biofilms. J Mater Chemi B. 2020;8.

202. Boge L, et al. Lipid-based liquid crystals as carriers for antimicrobial peptides: phase behavior and antimicrobial effect. Langmuir. 2016;32(17):4217–28.

203. Zhang J, et al. Amikacin liposome inhalation suspension (ALIS) penetrates non-tuberculous mycobacterial biofilms and enhances amikacin uptake into macrophages. Front Microbiol. 2018;9:915–915.

204. Alhariri M, et al. Efficacy of neutral and negatively charged liposome-loaded gentamicin on planktonic bacteria and biofilm communities. Int J Nanomed. 2017;12:6949–61.

205. Cipolla D, Blanchard J, Gonda I. Development of liposomal ciprofloxacin to treat lung infections. Pharmaceutics. 2016;8(1):6.

206. Serisier D, et al. Inhaled, dual release liposomal ciprofloxacin in non-cystic fibrosis bronchiectasis (ORBIT-2): a randomised, double-blind, placebo-controlled trial. Thorax. 2013;68:812–7.

207. Haworth CS, et al. Inhaled liposomal ciprofloxacin in patients with non-cystic fibrosis bronchiectasis and chronic lung infection with *Pseudomonas aeruginosa* (ORBIT-3 and ORBIT-4): two phase 3, randomised controlled trials. Lancet Respir Med. 2019;7(3):213–26.

208. Sarkar S, Heise MT. Mouse models as resources for studying infectious diseases. Clin Ther. 2019;41(10):1912–22.

209. Lavelle GM, et al. Animal models of cystic fibrosis pathology: phenotypic parallels and divergences. Biomed Res Int. 2016;2016:5258727.

210. Artzy-Schnirman A, Lehr CM, Sznitman J. Advancing human in vitro pulmonary disease models in preclinical research: opportunities for lung-on-chips. Expert Opin Drug Deliv. 2020;p. 1–5.

211. Scriboni AB, et al. Fusogenic Liposomes Increase the Antimicrobial Activity of Vancomycin Against Staphylococcus aureus Biofilm. Front Pharmacol. 2019;10(1401).