REVIEW PAPER

Liposomes used in the delivery of various antimicrobials to biofilm-producing infections: a systematic review

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ABSTRACT

Objective(s): Persistent clinical infections have driven extensive research to find effective solutions. Liposomes, known for their biocompatibility, versatility, targetability, and tunability, have emerged as a prominent drug delivery system. They enhance the delivery of contemporary antibiotics to resistant infections and facilitate the introduction of a wide range of novel antimicrobial agents.

Materials and Methods: This review adopts a systematic and thematic approach to encompass all studies involving liposomal antimicrobials for biofilm-producing infections. Original papers were retrieved from NCBI/PubMed using MeSH terms 'liposome', 'antimicrobial', and 'biofilm'. An inductive qualitative thematic analysis was then conducted to identify the main themes and sub-themes. Themes supporting the primary objective and fundamentals were included, while those covered in previous reviews were excluded.

Results: Liposomes are an exceptional delivery system for treating clinical biofilm infections. They improve the delivery of contemporary hydrophobic antibiotics and enable the combinatorial introduction of natural and synthetic antimicrobials. Liposomes also serve as a suitable platform for controlled drug release, physicochemical modification, and surface functionalization with various biological ligands. Additionally, they allow for modifications that enhance adhesion to biotic and abiotic surfaces and support extended, prolonged drug release profiles with implants, scaffolds, and hydrogels.

Conclusion: Given their ease of manipulation and modulation, liposomes are anticipated to remain a long-standing drug delivery platform in future research focused on treating persistent infections with antimicrobials.

Keywords: *Liposome, Anti-infective agents, Biofilm, Anti-bacterial agents*

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INTRODUCTION

The ongoing battle between infectious diseases and antibiotics necessitates continuous innovation to combat the ever-evolving pathogenic microorganisms [1-4]. Despite extensive efforts by scientists from various disciplines, bacterial resistance remains a significant concern, threatening the efficacy of antibiotics and public health [5-8]. However, the extensive knowledge and experience gained in addressing persistent infections, such as pseudomonal pulmonary infections [2, 9, 10], staphylococcal prosthetic and implant-related infections [11, 12], and resistant candidal infections [13-15], offer a glimmer of hope.

Microbial species employ various strategies to evade antibiotics and antimicrobials [1, 9, 16-18]. These defense mechanisms can be broadly categorized into two classes: those that render antibiotics ineffective through chemical modification and enzymatic breakdown [19], and those that sequester antibiotics outside the pathogenic cell core [9, 20]. The latter includes

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challenges such as microbial concealment in macrophages [21], the thick mucosal barrier, especially in cystic fibrosis (CF) patients with impaired biosurfactant systems [22, 23], and the efflux of antibiotics from microbial cells [19, 24]. Additionally, the most notorious defense strategy is the formation of biofilms, where pathogenic species congregate with other microorganisms within a dense extracellular polymeric substance (EPS) matrix [3, 4, 11, 25, 26]. Examples of biofilm-related infections include dental plaques responsible for tooth decay [27, 28], staphylococcal biofilms on biomedical devices [11], and persistent pulmonary infections in CF patients [23]. Biofilms significantly increase bacterial resistance, with microcolonies being up to 1000-fold more resistant to antibiotics than their planktonic counterparts [29, 30]. This resistance allows infections to persist despite high doses of antibiotics [29, 30].

To address biofilm infections, various therapeutic approaches have been developed, with a focus on liposomes in this review [31]. Liposomes exhibit multiple beneficial properties for antibacterial infection therapies [32], which are addressed within the scope of this paper.

Firstly, liposomes are artificial vesicular drug delivery platforms formulated mainly from natural lipid ingredients, enabling them to interact naturally with bacterial cell walls and host cell membranes [32]. Secondly, liposomes offer a versatile platform for modifying their physicochemical properties through composition alteration and conjugation with various synthetic [33-35] and natural moieties [36-38]. By selecting different lipids and emulsifiers, liposomes can achieve a variety of physical and chemical characteristics, including bilayer elasticity, colloidal stability, varied surface charge, and enhanced cell penetrability [11, 39-42].

Thirdly, the liposomal platform allows for variation in drug release kinetics, such as sustained release [23], highly-prolonged release [43], and triggered-release profiles [23, 30, 44]. Innovative sensitive liposomal systems have been developed to control drug release within and outside bacterial microcolonies [23, 30, 44]. These sensitive liposomes release their drug cargo in response to exogenous stimuli (e.g., temperature [30, 44], ultrasound [45, 46], radiation [47, 48], electromagnetic fields [49]) or endogenous factors (e.g., protonation capability of the infection microenvironment [50]). Complex liposome-impregnated scaffolds also offer highly prolonged, spatiotemporally-controlled drug release [51, 52].

Fourthly, liposomes serve as an excellent platform for incorporating a wide range of antimicrobial agents [24, 53-58], particularly those with poor pharmacokinetic features [47, 59-61], reviving them as promising antibiotic candidates for biomedical applications. Lastly, different targeting moieties can be conjugated onto the surface of liposomes to enhance their targeting efficacy [36-38].

Given the vast diversity of combinatorial innovations employed by researchers, this paper aims to provide a comprehensive overview of the main liposomal modifications used in the treatment of biofilm infections with antimicrobials. The author has avoided the repetition of concepts covered in previous reviews, integrating only those that enhance the understanding of the principal concept under discussion. Furthermore, the key players involved in the improvement of liposomal antimicrobial biofilm therapy are addressed in their relevant subsections.

Data gathering and thematic analysis

This review focuses on the principal theme of "key players in liposomal antimicrobial formulations for biofilm infections," based on a collection of original reports retrieved from NCBI/PubMed. An inductive, qualitative thematic analysis was conducted to identify major themes and subthemes in the literature.

The main concepts of 'liposome,' 'antimicrobial,' and 'biofilm' were explored using MeSH terms and Boolean logic. The search terms included "liposomalization," "liposomalized," "liposomally," "liposome," "liposomic," "phytosome," "virosome," "anti-infective agents," "antimicrobial," "antimicrobials," "antimicrobially," "biofilm," and "Extracellular Polymeric Substance Matrix." Review papers were filtered, and terms were primarily constrained to [Title/Abstract] to retrieve the most relevant papers. Boolean logic was applied with "OR" between terms within a concept and "AND" between concepts. A total of 134 reports were retrieved, of which 105 were selected for this review.

Qualitative thematic analysis was performed using Microsoft Word to identify main themes and subthemes, which were then organized into major sections and subsections of the paper. Various keywords were searched within the

titles and abstracts of the papers using the Ctrl + F shortcut. These keywords included 'alginate,' 'cationic liposome,' 'cell-penetrating peptide,' 'chitosan,' 'copper,' 'gallium,' 'biomineral,' 'DNase,' 'hydrogel,' 'lectin,' 'niosome,' 'nebulized,' 'pH-*,' 'photodynamic,' 'photothermal,' 'temperature-sensitive,' 'ultrasound,' and 'quorum sensing.' Additional terms were identified through in-depth reading and further searches to expand the keyword-related concepts. For example, 'inhalable' and 'inhaled' were used to expand the concept of "liposomes administered via the respiratory route," and 'positively-charged' for "any liposome with a positive surface charge."

Accordingly, this paper distills the principal theme into seven major themes: (i) various liposomes improving the antimicrobial properties of hydrophobic agents; (ii) different combinatorial treatments offered by liposomes, including the use of various natural compounds with antimicrobial properties; (iii) approaches to enhancing the penetration of antimicrobials using liposomes; (iv) the role of key liposomal ingredients, such as cholesterol (Chol), ionic, and non-ionic surfactants; (v) infection site-specific delivery using various sensitive liposomes; (vi) approaches to modify liposome-bacteria interactions, including modulation of liposomal surface charge; liposomal surface hydration; and targeted drug delivery through liposomal functionalization with different biological ligands; (vii) modulations to tailor the adhesion ability of liposomes and control antibiotic delivery for long-term protection against infections.

Whenever further information was necessary, the entire paper was searched. Reports that did not relate to the major themes and subthemes, or those confined to concepts already covered in published reviews, were excluded to avoid repetition for the readers.

In addition to the papers retrieved through the mentioned procedure, some additional citations were manually added to explain the concepts addressed in the paper.

Why have liposomes gained popularity in treating biofilm infections?

Reviewing the retrieved original reports showed that liposomal formulations have been employed with diverse groups of antibiotics and antimicrobials against resistant and often biofilm-producing pathogens [17, 55, 62, 63]. Liposomes also provide a medium for numerous combinatorial therapies [26, 45, 62, 64, 65], some of which are addressed throughout this review. They are highly biocompatible and can be functionalized with various biological [36-38] and synthetic moieties [33-35], offering high versatility for physicochemical and biological modification [35, 66].

Liposomes enhance the antibacterial activity of contemporary antibiotics, particularly improving the properties of hydrophobic antibiotics with poor pharmacokinetic and pharmacological profiles [3, 47, 59-61]. Liposomal formulations can be manipulated to optimize the delivery of these antibiotics.

Moreover, liposomal formulations have been used for a wide range of natural antimicrobial compounds, many of which cannot be used for clinical infections without liposomal encapsulation [53, 58, 67]. Several natural products [68, 69], herbal compounds [5, 58, 64, 70], oxidizing agents [47, 57, 71, 72], antivirulent agents, and bioactive agents [9, 20, 73] have been encapsulated in liposomes and tested against biofilm-producing infections.

Liposomes offer a highly adaptable platform for the biological and physicochemical modulation of their properties [33, 36, 40, 74, 75]. Incorporating antivirulent agents [16, 20], emulsifiers [10, 76], and biosurfactants [24, 76, 77] into liposomal formulations can significantly enhance the penetrability of encapsulated antimicrobial agents. Various liposomal formulations have been developed with selective drug-release characteristics. These sensitive liposomes can release their drug payload in response to external stimuli applied locally to the infection site [44, 45, 48, 49], or internal stimuli observed exclusively at the site of infection [50, 78, 79]. Additionally, attributes such as particle surface charge, hydrophobicity, and fluidity have been optimized through composition adjustments and functionalization of the liposomes [33, 35, 36, 75].

Liposomes enhance antimicrobial properties of hydrophobic agents

Liposomes provide a promising platform for the application of hydrophobic photosensitizing agents and antimicrobial natural compounds with poor bioavailability (Fig. 1).

Similar to conjugating a hydrophobic agent with cyclodextrin to achieve a highly stable suspension

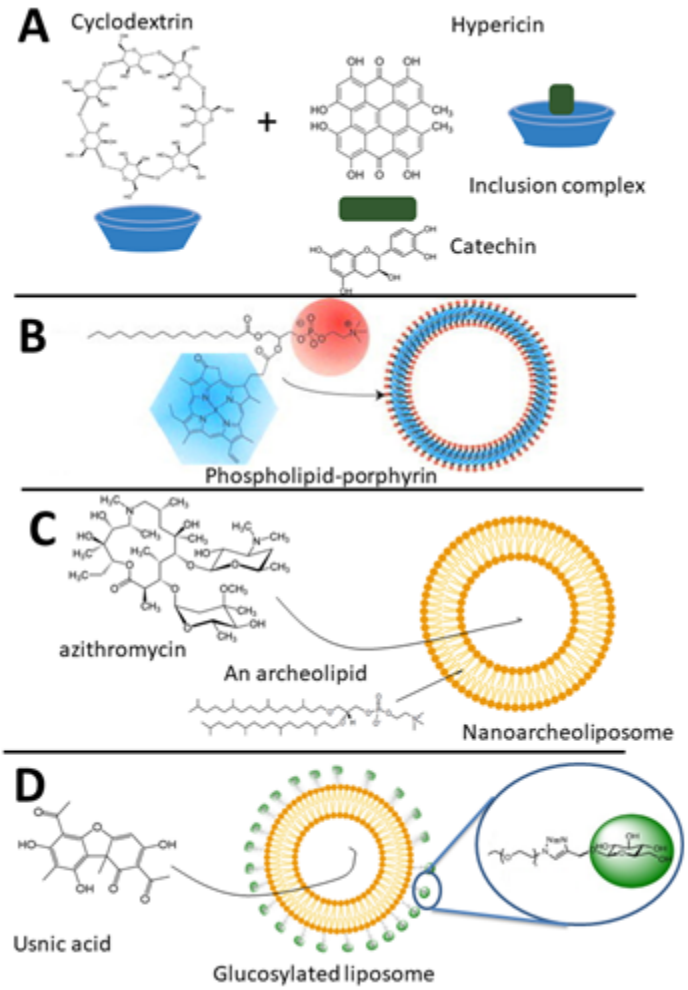

Fig. 1 Various hydrophobic antimicrobials benefited from liposomal formulation: hypericin [47] and catechin [61] are molecularly sequestered inside the cyclodextrin as inclusion complex, which is then entrapped in liposome (A); porphyrin (60) is conjugated to phospholipids in liposome (B); azithromycin is entrapped in nanoarcheoliposome [3] composed of archaeolipids with high capacity for the entrapment of hydrophobic agents (C); usnic acid is entrapped in glucosylated liposome [75] with high liposomal suspension stability in aqueous media and also biofilm targetability (D).

in aqueous physiological media, conjugating it to a lipid and incorporating it into liposomes can enhance colloidal stability. For instance, the cyclodextrin inclusion complex with hypericin (Fig. 1A) prevents hypericin from assimilating in aqueous biological media [47]. Likewise, studies by Pourhajibagher and Partoazar [59] demonstrated that certain phospholipid-porphyrin (PL-Por) conjugates form highly stable liposomes when combined with other phospholipids (Fig. 1B). Incorporating PL-pheophorbide into a lipid matrix such as dipalmitoylphosphatidylcholine (DPPC) enabled the formation of stable liposomes. These liposomes allowed photosensitizing conjugates to exhibit photothermal and photodynamic activity against planktonic cultures and biofilms of *Staphylococcus aureus and Pseudomonas aeruginosa*.

Encapsulation of catechin in cyclodextrin, followed by incorporation into liposomes (Fig. 1A), exemplifies how liposomal formulations can enhance the therapeutic efficiency of hydrophobic phytochemical agents [61]. The catechin-in-cyclodextrin-in-phospholipid liposome significantly inhibited the production and activity of various anti-virulent factors involved in biofilm formation and bacterial motility, outperforming free catechin. These factors include EPS production (reduced by 1.71-2.25-fold), slime production (reduced by 0.40-0.50-fold), proteolytic production (reduced by 14.65-18.04%), hemolytic activity (reduced

by 28.08-49.07%), DNase production, lipase production, autolysis, and cell auto-aggregation in methicillin-resistant *S. aureus* (MRSA

Altube and Martínez [3] leveraged total polar archaeolipids (TPA) to create a highly stable liposomal formulation of azithromycin (Fig. 1C). Azithromycin, a hydrophobic antibiotic, typically faces challenges in delivering to CF-associated intraluminal lung infections due to its rapid clearance. However, azithromycin nebulized liposomes, made from the total polar lipid extract of Halorubrum tebenquichense archaebacteria, demonstrated promising results. These included a high-loading capacity of azithromycin (0.28 w/w of azithromycin/TPA), physical stability of the formulation, and efficient deep intraluminal delivery of the lipophilic antibiotic to *P. aeruginosa biofilms*.

Similarly, Francolini and Giansanti [75] encapsulated the water-insoluble natural antimicrobial compound usnic acid into glucosylated liposomes (Fig. 1D), achieving a highly stable formulation with a high usnic acid loading capacity. They observed improved biofilm delivery with the liposomal usnic acid.

These findings indicate that liposomal formulations significantly advance antibiotic therapy for poorly water-soluble antibiotics.

Liposomes and combinatorial antimicrobial treatment

In addition to advancing antibiotic therapy with hydrophobic antibiotics, liposomal formulations offer significant potential for combinatorial treatment with various emerging antimicrobial agents.

Use of metal particles with antimicrobial activity in liposomal formulations

Liposomal antimicrobials have been explored in combination with metal nano- and macro-particles possessing antimicrobial properties (Fig. 2).

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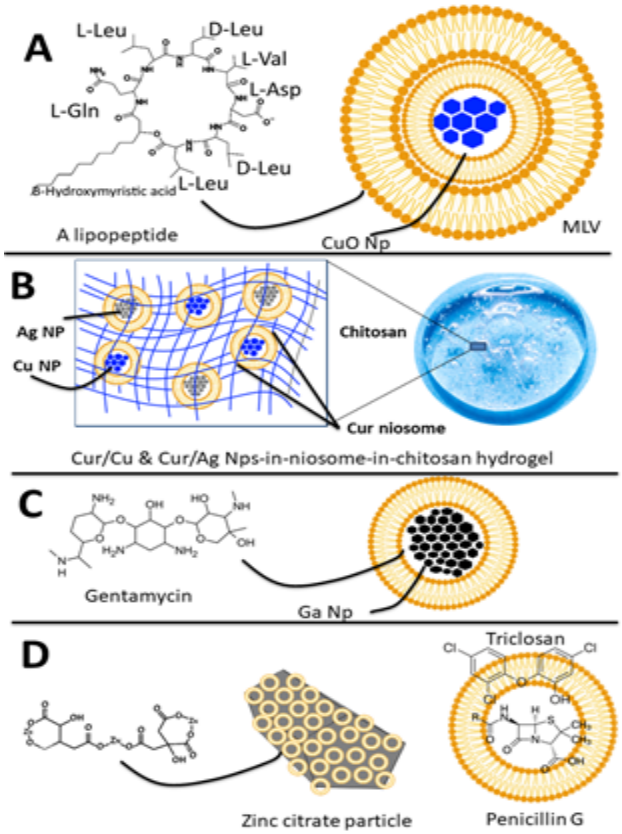

Fig. 2 Different metal particles with antimicrobial activity that are used together with other active agents in liposome: CuO NPs along with a lipopeptide surfactant in MLV (A, [24]); Cu- and Ag- NPs along with curcumin entrapped in liposomes and chitosan hydrogel (B, [54]); Ga NP entrapped along with gentamycin in liposome (C, [80]) are metal NPs that are entrapped in liposome. Vice versa, liposomes entrapping triclosan and penicillin G in their respective lipid membrane and internal aqueous space coat the surface of size citrate particles (D, [32]).

Agency (EPA) has identified copper as the primary metal with antimicrobial activity. Combinatorial therapy using copper and anti-biofilm agents within liposomal platforms has demonstrated superior antimicrobial efficacy against resistant pathogens.

In a study by Kannan and Solomon [24], researchers utilized a lipopeptide biosurfactant—purified from the human skin bacterium *Paenibacillus thiaminolyticus*—along with copper oxide nanoparticles (CuO NPs) to prepare multilamellar liposomes (Fig. 2A). This combination exhibited remarkable antivirulence and antibacterial properties. The liposomes significantly reduced secreted virulence factors such as staphyloxanthin, pyocyanin, and extracellular polysaccharides, and notably inhibited the growth of clinically-resistant *P. aeruginosa* and MRSA in both planktonic and biofilm forms.

Similarly, Targhi and Moammeri [54] reported synergistic antibacterial activity against *S. aureus* and *P. aeruginosa* using curcumin-Cu and curcumin-Ag NPs entrapped in a chitosan niosomal hydrogel (Fig. 2B).

Gallium is another mineral agent with notable antimicrobial properties. It exhibits photosensitizing activity in photothermal therapy [55]. Due to its similarity to iron (Fe), gallium competes with Fe ions in the bacterial microenvironment. This competition interferes with quorum-sensing (QS) bacterial communication for Fe assimilation and disrupts Fe metabolism, making bacteria more vulnerable [55].

Gallium has proven to be an effective inhibitory agent against *P. aeruginosa* growth and biofilm formation when combined with gentamicin [80]. The co-encapsulation of gentamicin with gallium in liposomes (Fig. 2C) significantly increased antibacterial efficacy against a *P. aeruginosa* (PA-48913) isolate from the sputum of CF patients, reducing the required concentration from 256 mg/L to 2 mg/L. While unformulated gentamicin failed, the liposomal formulation completely eradicated biofilms by blocking the production of quorum-sensing molecule (QSM) N-acyl homoserine lactone (AHL) at a very low dose (0.94 mg/L gentamicin).

However, not all combinatorial trials with liposomes are successful. Catuogno and Jones [32] coated zinc citrate particles with phosphatidylinositol (PI)/DPPC anionic, dodecyl-dimethylammonium bromide (DDAB)/DPPC cationic liposomes, and DPPC/Chol liposomes (Fig. 2D). These liposomes carried the bactericides triclosan, an oil-soluble agent in their membrane, and the aqueous-soluble penicillin-G in their internal aqueous milieu. Zinc citrate, a bactericide used in toothpaste formulations, was expected to gain additional therapeutic properties against the immobilized biofilms of the oral bacterium *Streptococcus oralis* when coated with drug-carrying liposomes. However, the presence of liposomal drugs inhibited the bactericidal effect of the particles.

While some metals can interfere with the quorum-sensing mechanisms of pathogens, thereby enhancing the therapeutic benefits of liposomal antimicrobials, combining liposomal antimicrobials with metals does not always lead to improved antimicrobial efficacy.

Use of QSM interference as antimicrobial agents in liposomes

QSM are chemical compounds produced by microorganisms that facilitate intra- and inter-species communication. The production of QSM directs microbial colonization and biofilm formation, leading to antibiotic-resistant infections (Fig. 3). Several studies have explored the co-encapsulation of QS-blocking agents as novel bioactive antimicrobials alongside antibiotics (Fig. 3 A-C).

The co-encapsulation of bismuth-ethanedithiol with tobramycin in liposomes has been shown to enhance antibacterial efficacy by blocking QS and biofilm production [81]. These liposomes significantly inhibited the secretion of AHL from *P. aeruginosa*. Similar findings were reported by other researchers [56, 62]. Mahdiun and Mansouri [56] demonstrated that their niosomal tobramycin, combined with bismuth-ethanedithiol, significantly lowered the minimum inhibitory concentration (MIC) of tobramycin, reduced biofilm formation, and effectively inhibited AHL secretion. Likewise, Alipour and Suntres [62] reported superior inhibitory properties for liposomal formulations delivering both tobramycin and bismuth. They observed further reductions in AHL, pyoverdine, pyocyanin, elastase, protease, chitinase, bacterial attachment, and biofilm formation *in vitro*.

The incorporation of farnesol significantly enhanced the antibacterial activity of

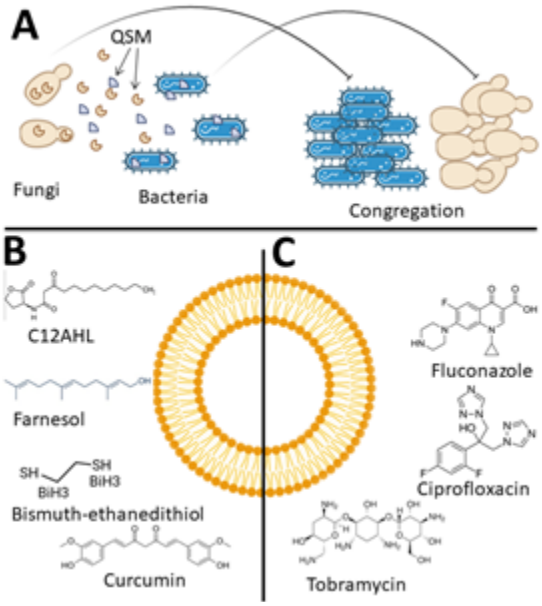

Fig. 3 Various natural and synthetic interfering QSM applied together with common antimicrobials in liposomal formulation. The use of interfering QSM hampers microbial communication and their ensuing congregation, colonization, and biofilm production (A). Bismuth-ethanedithiol [56, 62, 81], C12AHL [17], farnesol [4], and curcumin [53, 82] are compounds with the QSM-interfering properties (B), and fluconazole, ciprofloxacin, and tobramycin are the antibiotics used along in liposome.

liposomal ciprofloxacin. Bandara and Herpin [4] demonstrated that farnesol, a fungal QSM modulated the release rate of ciprofloxacin from liposomes and strongly interfered with the biofilm metabolism of *P. aeruginosa*. The farnesol/ciprofloxacin liposome formulation reduced the required dose of ciprofloxacin for effective biofilm inhibition by 125-fold compared to free ciprofloxacin. Additionally, the incorporation of farnesol synergistically increased the antimicrobial activity by 10-fold compared to the single-agent ciprofloxacin liposomal formulation. This dual-agent liposome enhanced both the extent and depth of bacterial eradication within the biofilm.

Conversely, Bandara and Hewavitharana [17] explored the use of a bacterial QSM to enhance the antifungal effectiveness of fluconazole in a dual-agent liposome. Their investigation revealed that a *P. aeruginosa* QSM, N-3-oxo-dodecanoyl-L-homoserine lactone (C12AHL), inhibited the morphological transition and biofilm formation of *Candida albicans*. Similar to the behavior of farnesol, the addition of this QSM to the liposomal delivery system resulted in an enhanced release of fluconazole (4.27% vs. 0.97%, $P < 0.05$ [4]). The QSM-bearing liposomal formulation demonstrated higher antimicrobial activity against both colonizing and preformed fungal biofilms compared to the single-agent fluconazole liposome, with reductions in viability of over 80% and 60% at a fluconazole concentration of 5.5 µg/mL, compared to 12% and 36%, respectively.

Curcumin, when used in liposomes, also interfered with QSM production by opportunistic pathogens. Curcumin liposomes inhibited the production of extracellular protease and AHL, as well as biofilm formation and bacterial motility in the foodborne pathogens *Aeromonas hydrophila* and *Serratia grimesii* [53, 82].

In addition to natural QSMs, synthetic mimic QSMs have been applied in liposomes to disrupt the QS system of pathogens. Hallan and Marchetti [39] used two synthetic homoserine lactone analogs of N-(3-oxododecanoyl)-L-homoserine lactone (3OC12-HSL) in liposomes to inhibit the synthesis of virulence factors and biofilm formation. These compounds achieved prolonged anti-quorum sensing and anti-biofilm activity against *P. aeruginosa*.

Oxidizing agents as potential antimicrobial agents used with liposomes

Various oxidizing agents have been utilized for their antimicrobial properties in liposomal forms [47, 57, 71, 72]. These oxidizing compounds can be broadly classified into two groups. The first

group consists of phototoxic agents that produce reactive oxygen species (ROS) upon exposure to visible light [12, 47, 48, 55, 59]. This group has gained popularity due to its tunable toxicity, which allows for the restriction of toxicity to the infection site under photodynamic therapy [12, 47, 48, 55, 59].

Other oxidizing agents have also been applied in liposomal forms for their antibacterial and antibiofilm properties. For instance, Zerillo and Polvere [57] reported that a commercial ozonized sunflower oil-in-liposome gel, combined with hypromellose, can prevent biofilm formation on contact lenses by *S. aureus* and *P. aeruginosa*. Additionally, two other research groups [71, 72] employed isosorbide mononitrate (ISMN) in liposomes and achieved effective *S. aureus* biofilm eradication both *in vitro* and in a sheep model of rhinosinusitis following a topical sinus wash with the liposomal formulation.

Herbal products used in liposomes against infections

The lipid membrane of liposomes can incorporate herbal-based compounds, offering multiple beneficial properties against resistant bacterial infections. For instance, the essential oil of *Schinus areira L.* (*Anacardiaceae*) has been used in liposome formulations, demonstrating various antibacterial characteristics [58]. These include alterations in membrane permeability, ROS-inducing activity, and DNA degradation, which collectively enhance the eradication of *S. aureus* in biofilms. Additionally, cinnamon oil-encapsulated liposomes [5] and liposomes co-loaded with cinnamon oil and the antibiotic polymyxin E [67] have been shown to reduce *S. aureus* and MRSA colonies on different abiotic clinical surfaces.

Polyphenolic compounds represent a promising group of phytochemicals used in liposomal formulations to treat persistent biofilm-producing infections. These phytochemicals are employed as active pharmaceutical agents, often in combination with other known active agents, to inhibit bacterial growth and biofilm formation. Bhatia and Sharma [64] reported successful results using curcumin/berberine co-loaded liposomes against intracellular MRSA infections and their biofilm formation.

Interestingly, liposomes offer a solution for phytochemicals with poor pharmacokinetics, positioning them as a promising reservoir of potential antimicrobial agents alongside conventional antibiotics. Notably, the antibacterial activity of liposomal curcumin/berberine surpassed that of clindamycin, with the co-loaded liposome being five times more effective. Furthermore, Angellotti and Di Prima [26] designed a multicomponent lipid nanoparticulate system that enhanced the anti-inflammatory, antioxidant, antibacterial, and antibiofilm properties of resveratrol when encapsulated in liposomes. This formulation, which included PEGylated lipid, glyceryl monoester, 18-β-glycyrrhetinic acid, and menthol, demonstrated improved resveratrol delivery and advanced antibiofilm properties *in vitro*, making it a promising candidate for wound healing applications.

Natural products used in liposomes for preventive medicine

Concerns over the development of resistance to conventional antibiotics, particularly with their prolonged use in regular hygiene and agriculture practices, have driven scientists to seek alternative sources of antimicrobial agents. This has led to the exploration of novel natural antimicrobials. As mentioned previously, liposomal drug delivery offers excellent feasibility and compatibility for physicochemical and biological modifications. This versatility allows for the incorporation of a diverse array of antimicrobial compounds that might otherwise be considered ineffective, harmful, or worthless against pathogens.

In preventive medicine, where the regular use of antimicrobials is essential, it is plausible to shift the focus toward novel surrogate antimicrobials to address concerns related to resistance to contemporary antibiotics. To this end, liposomal formulations containing natural antimicrobials have been suggested for preventive measures. For instance, Pu and Tang [70] employed a natural antilisterial peptide derived from the bromelain hydrolysate of rice bran protein against *Listeria monocytogenes*. They subjected the rice bran protein to bromelain enzymatic hydrolysis, purified the antibacterial peptide (sequence: KVDHFPL), and encapsulated it in a liposome (DPPC/stearylamine (SA)/Chol; 10/3/3 molar ratio). The resulting liposome exhibited long-term storage stability in refrigerators and high adsorption to listerial biofilm, highlighting its preservative properties for the food industry. Additionally, this liposomal formulation shows promise for

treating persistent and recurrent gastrointestinal microflora disturbances and bowel infections.

Nisin is another example of a peptide with broad-spectrum antibacterial activity, particularly effective against a wide range of food-spoilage pathogens. Yamakami and Tsumori [28] demonstrated that a nisin-loaded liposome achieved four times greater effectiveness in inhibiting *Streptococcus mutans* biofilm formation and restricting bacterial growth compared to free nisin. This prolonged effectiveness of liposomal nisin is highly desirable for oral hygiene and the prevention of dental caries.

Platensimycin, a metabolite of *Streptomyces platensis*, is emerging as a promising natural antibiotic, particularly against MRSA [69]. However, its clinical development has been hindered by poor water solubility and undesirable pharmacokinetic properties. Incorporating platensimycin into lipid-based NPs, such as liposomes or micelles, has been shown to address these drawbacks. Pharmacokinetic and survival rate studies in Sprague-Dawley rats and MRSA-infected C57BL/6J mice support the potential of these formulations [69].

Approaches to enhancing the penetrability of antimicrobials

The effective delivery of antimicrobial agents to pathogenic microorganisms is often impeded by multiple physical barriers, leading to resistance [18, 20, 83]. These barriers include the dense mucoid endothelial lining found in the airway tracts of patients with CF and chronic infections, as well as persistent *Helicobacter pylori* infections [83]. Additionally, bacterial cell membranes, cell walls, biofilms, and host extracellular adhesion proteins restrict the delivery of antimicrobials to pathogens [20].

Strategically disrupting these barriers is crucial for enhancing the penetrability of antimicrobials and antibiotics. To date, various mucolytic, enzymatic agents [9, 73, 84], and non-enzymatic agents [3, 85] have been employed in formulating liposomal antimicrobials, demonstrating outstanding and promising therapeutic efficacy.

Biofilm-disrupting agents

Extracellular proteins play a crucial role in the initial adhesion of microbial biofilms to biological surfaces [20]. Proteinase K has been shown to effectively break down these proteinaceous adhesins, preventing the attachment of *Cutibacterium acnes*-related EPS to host cells. Liposomes containing proteinase K, either alone or combined with other active compounds like DNase I [20] or retinoic acid [73], have demonstrated improved penetration of antimicrobials into bacterial biofilms, the epidermis, and hair follicles. These formulations have proven effective in reducing *C. acnes* colonization deep within skin and catheter biofilms due to their enzymatic activity.

Lysozyme is another enzyme used in liposomal formulations to disrupt biofilms and enhance antimicrobial delivery [9, 84]. Naturally part of the innate immune system, lysozyme breaks down bacterial cell wall peptidoglycan through its hydrolytic activity. Its cationic properties aid in bacterial autolysis and enable it to attach to the surface of anionic liposomes via electrostatic attraction [9]. Gentamycin-loaded liposomes utilizing lysozyme have successfully targeted the negatively charged bacterial cell walls in both Gram-positive and Gram-negative bacteria, inhibiting biofilm formation and damaging preformed biofilms [84].

Moreover, alginate lyase is a widely used biofilm-disrupting enzyme in liposomal antimicrobials. Overproduction of the exopolysaccharide alginate is linked to resistant *P. aeruginosa* infections in CF patients. Chan and Burrows [18] noted that alginate acts as an auxiliary bacterial membrane and a diffusion barrier to positively charged antimicrobial peptides. Their research revealed that the anionic alginate polysaccharide obstructs the penetration of these peptides into the bacterial membrane, thereby preventing them from entering bacterial cells.

Consequently, degrading alginate can enhance the penetration of antimicrobial agents into biofilms. Alipour and Suntres [16] demonstrated that alginate lyase, in combination with DNase, boosts the antibacterial activity of aminoglycosides such as gentamicin, amikacin, and tobramycin, particularly in their liposomal forms, against both mucoid and non-mucoid strains of *P. aeruginosa* biofilms. Biofilm formation typically increases the required concentrations of aminoglycosides for bacterial eradication by 8- to 256-fold, but treatment with alginate lyase mitigates these doses.

Similarly, Alipour and Dorval [2] reported that digesting alginate significantly improves

the inhibitory and bactericidal concentrations of tobramycin against clinical mucoid *P. aeruginosa* strains. In their study, bismuth-ethanedithiol effectively disrupted the bacterial biofilm, reduced alginate levels, and enhanced tobramycin penetration, leading to improved antibacterial activity in both free (up to 8-fold) and liposomal forms (up to 32-fold).

Digesting alginate enhances antifungal drug delivery to Aspergillus fumigatus biofilm and hyphal structure too. Bugli and Posteraro [86] investigated the effects of amphotericin B (AMB) deoxycholate, both in its free form and as liposomal AMB (AMBL), in combination with alginate lyase. This combination synergistically reduced hyphal thickness and decreased the concentration of antibiotics within biofilm-growing hyphal cells.

Inhalable liposomal antimicrobial delivery with mucolytic ability

P. aeruginosa and *S. aureus* are common culprits in persistent pulmonary infections. *S. aureus* is frequently found in pneumonia cases among hospitalized patients, while *P. aeruginosa* is notably associated with CF conditions. When co-infected with *S. aureus* and other pathogens, *P. aeruginosa* can lead to drug-resistant infections due to extracellular compounds that enhance its virulence. *P. aeruginosa* biofilms are predominantly located in intraluminal spaces and hypoxic mucoid masses. These biofilms also shield *S. aureus* from antibacterial treatments.

Additionally, mucins from damaged lung epithelial cells and leukocytes form a mucoid epithelial lining, especially in CF patients with impaired biosurfactant mucolytic activity. This mucoid layer, along with the biofilm, acts as a diffusion barrier for antibiotics, particularly those with a positive charge. Electrostatic interactions between these antibiotics and the biofilm matrix hinder their effective penetration. As a result, antibiotics reach bacterial cells in insufficient doses, leading to continued biofilm formation and antibiotic-resistant infections.

Given the issues at hand, the optimal approach may involve using an inhalable lipid-based vehicle. Compared to other administration routes, liposomal drug delivery via the respiratory route has been proven more effective in terms of drug concentration delivered and inflamed lung epithelial tissues covered.

Inhalable liposomes typically contain the natural lung biosurfactant DPPC and Chol in their formulations. These lipid components facilitate drug cargo transfer through the mucoid lining and biofilm matrices. For instance, Meers and Neville [23] used nebulized DPPC/Chol liposomes loaded with amikacin, observing a triggered, sustained release of amikacin in response to *P. aeruginosa* glycolipid surfactants (rhamnolipids) in biofilm supernatants. The liposomes significantly improved the delivery efficacy of amikacin compared to inhaled free amikacin and provided effective treatment for chronic lung infections in rats over a 14-day period.

Moreover, Altube and Martínez [3] observed the deep and efficient biofilm penetration of azithromycin using a nebulized azithromycin nanoarchaeosome. The nanoarchaeosome is a type of liposome prepared with TPA extracted from *Halorubrum tebenquichense* archaebacteria. This type of liposome demonstrated enhanced biofilm disruptive activity compared to free azithromycin, and lowered MIC and minimal bactericidal concentration (MBC) of azithromycin.

Inhalable antibiotic-loaded liposomes can include additional mucolytic and biofilm-degrading compounds to enhance the penetration of the antibiotic.

Pinto and Monteiro [83] incorporated multiple mucolytic agents into liposomes. They used N-acetyl-L-cysteine and Tween 80 in the liposomal preparation. They also functionalized the liposomes with three d-amino acids (D-phenylalanine, D-proline, and D-tyrosine) to specifically target biofilms, as these d-amino acids are known to inhibit biofilm formation and disrupt existing biofilms. This formulation enhanced the antibacterial activity of moxifloxacin against *P. aeruginosa* biofilm.

Gupta and Nirwane [29] combined Serratiopeptidase (SRP), a biofilm-disrupting agent, with Levofloxacin liposomes. This approach led to enhanced pulmonary delivery of the antibiotic and improved antibacterial efficacy against *S. aureus* pulmonary infections in rats.

A group of scientists [22, 84] utilized lysozyme in the preparation of inhalable liposomal antibiotics. Gupta and Nirwane [22] used a spray-dried Levofloxacin liposome in combination with lysozyme to treat chronic pulmonary infections caused by *S. aureus*. The researchers observed reduced microbial burden in bronchoalveolar lavage fluid and nasal fluid and enhanced

antibiofilm activity. Similarly, Hou and Wang [84] achieved a triggered, prolonged release of gentamicin using lysozyme, resulting in improved antibacterial and antibiofilm activity.

Role of niosomal preparations against pathogens

Numerous studies have demonstrated enhanced antimicrobial activity through the use of liposomal formulations containing non-ionic surfactants, termed 'niosomes'. These surfactants play a pivotal role in the therapeutic efficacy of antibiotic treatments, especially for biofilm-producing and resistant infections. The observed improvements can be attributed to increased antimicrobial penetrability and other beneficial properties provided by niosomal preparations.

Enhancing biofilm penetration with niosomal preparations

Non-ionic surfactants are crucial excipients in liposomal formulations, facilitating the delivery of antimicrobial agents. When incorporated into liposomes, these surfactants enhance the deep penetration of drugs into biofilms. Liposomes formed with non-ionic surfactants are classified as niosomes, which are used in both topical and parenteral drug delivery of antimicrobial agents. Niosomal formulations have been investigated for their therapeutic benefits against a wide range of resistant, biofilm-producing pathogens.

By varying the surfactant, researchers have aimed to develop effective lipid-based delivery systems to eradicate deeply situated resistant infections. Hallan and Marchetti [39] found that selecting an appropriate non-ionic surfactant, such as SA, is essential for eliciting prolonged antimicrobial activities from QS homoserine lactone analogs. Similarly, Moghadas-Sharif and Fazly Bazzaz [40] achieved effective antibacterial activity against *Staphylococcus epidermidis* biofilm using cationic niosomes containing rifampicin, SA, and dicetyl phosphate (DCP) as emulsifiers with proven anti-biofilm activity.

Other researchers have enhanced antibacterial activity using various Tweens and Spans in niosomal formulations. Piri-Gharaghie and Jegargoshe-Shirin [8] improved the antibiotic activity of imipenem against methicillin-resistant *S. epidermidis* with a niosomal formulation (composed of Tween 60, Span 60, and Chol). Similarly, Mirzaie and Peirovi [41] enhanced the effectiveness of ciprofloxacin treatment in ciprofloxacin-resistant and MRSA infections with optimized ciprofloxacin niosomal formulation (containing Tween 80, Span 60, and Chol). Hedayati Ch and Abolhassani Targhi [10] significantly increased the antibacterial activity of tobramycin using a niosomal formulation (consisting of Chol, Span 60, and Tween 60). Their niosomes reduced tobramycin efflux and down-regulated multiple genes associated with multidrug resistance in *P. aeruginosa* strains. In addition to tobramycin, Mahdiun and Mansouri [56] incorporated bismuth-ethanedithiol into the niosomal preparation (composed of Chol, Span 40, and Tween 40), creating dual-agent niosomes that effectively inhibited further *P. aeruginosa* infection.

Besides the bacteria mentioned, niosomes have been used with natural compounds against other pathogens. Haque and Sajid [76] utilized a sophorolipid—a biosurfactant extracted from the herbaceous plant Sophora japonica—along with DCP emulsifier in a niosomal preparation of amphotericin B. The niosomes reduced the number of hyphae and budding cells of *C. albicans* more than the commercial liposomal amphotericin B (Phosome). Similarly, Kietrungruang and Sookkree [87] reported notable inhibitory activity against *Cryptococcus neoformans* with a niosomal formulation of propolis ethanolic extract. The niosomes (containing Span 60, Tween 80, and Chol) inhibited the replication of phagocytized *Cryptococci* in macrophages and down-regulated virulence factors associated with biofilm formation and development.

Additional advantageous characteristics attributable to niosomal preparations

The enhanced antimicrobial activity of niosomal formulations is not solely due to improved drug penetrability into biofilms but also other beneficial features they provide. Non-ionic surfactants themselves exhibit antimicrobial activity. In addition, niosomes provide a sustained-release profile for antimicrobials, colloidal stability for emerging antimicrobial agents and long-term protection against pathogens by antibiotics.

Niosomes prevent biofilm formation on the surfaces of biomedical devices. Vancomycin-eluting niosomes prepared by Barakat and Kassem [11] demonstrated a strong affinity for hydrophobic implant surfaces and effectively repelled bacterial adhesion to the niosome-coated surfaces. Similar results were observed in the study by Dwivedi and

Mazumder [43], where a layer-by-layer coating of orthopedic implants with vancomycin-loaded niosomes inhibited bacterial colonization and prevented biofilm formation on the implants.

Niosomes offer a sustained drug release, leading to long-term protection against pathogens. The niosomes (Span 60/Chol/vancomycin at a 3/1/1 weight ratio) used by Dwivedi and Mazumder [43] delivered sufficient concentrations of vancomycin for up to two weeks. Similarly, Targhi and Moammeri [54] observed a slow-release profile for curcumin-Cu and curcumin-Ag NPs, where the niosomes were encased in a chitosan hydrogel. The niosomes enhanced antibacterial and anti-biofilm activity against *S. aureus* and *P. aeruginosa*, which could be attributed to the surfactants used.

Niosomes also serve as a suitable platform for delivering emerging antimicrobials. Giordani and Costantini [77] discovered that biosurfactants isolated from *Lactobacillus gasseri* exerted antibiofilm activity against MRSA strains, and can be administered in niosomal form. Moreover, antisense oligonucleotides are considered an outstanding alternative to antibiotics for use in niosomes. By binding to the target mRNA, these synthetic oligonucleotides trigger endonuclease degradation of the mRNA, preventing its expression. Tekintas and Demir-Dora [19] utilized a positively charged transfection niosome (comprising Span 80, Cetyltrimethylammonium bromide or CTAB, and Chol) for the delivery of a synthetic nondegradable antisense oligonucleotide—an LNA-2'-O-methyl hybrid antisense oligonucleotide. These niosomes exhibited high transfection efficiency, leading to effective suppression of the target acyl carrier protein P (acpP) genes, inhibition of *P. aeruginosa* cell wall biosynthesis, biofilm formation inhibition, and bactericidal activity.

Transferosomes for deep skin delivery

Deformable liposomes, also known as flexible liposomes or transferosomes, are a type of liposome suitable for transdermal delivery of drugs, including antimicrobial agents. Like niosomes, transferosomes exhibit a high potential for skin permeation and deep penetration through various layers of the epithelium. However, unlike niosomes, which contain non-ionic surfactants, transferosomes contain ionic surfactants that facilitate drug penetration into the epithelium. These surfactants typically include bile acid salts such as sodium deoxycholate and sodium cholate, although synthetic surfactants are also used. Additionally, transferosomes possess smaller vesicle sizes and higher elasticity due to the ionic surfactants present in their formulation. They also have higher entrapment efficiency for lipophilic antibiotics such as azithromycin.

Compared to liposomes, transferosomes have demonstrated superior microbial eradication on dermal tissues. In the study conducted by Rukavina and Klarić [42], azithromycin-loaded transferosomes were more effective against MRSA strain biofilms than conventional liposomes in terms of skin penetrability, bacterial growth inhibition, and biofilm formation prevention. These transferosomes significantly reduced the minimal biofilm inhibitory concentrations by 32-fold compared to azithromycin solution too. Similarly, Li and Zhang [88] observed efficient dermal permeation of daptomycin in mice following the topical administration of DPPC/sodium cholate transferosomes. These transferosomes provided sufficient therapeutic concentrations of daptomycin and inhibited bacterial growth in injury-induced biofilms for several hours.

Cottenye and Cui [27] employed the ionic surfactant cetylpyridinium chloride, a pyridinium salt known for its potent antiseptic properties, in the development of flexible liposomes. By incorporating this surfactant into two distinct liposomal formulations—phosphatidylcholine/Chol (PC/Chol) and PC/Chol sulfate (PC/Schol)—the researchers successfully targeted and eradicated biofilms of *S. mutans*.

Flexible lipid vesicles are another type of transferosomes used for skin delivery of antimicrobial agents. These vesicles contain propylene glycol (PG) and typically a lipid with a low melting temperature. PG acts as a moistening agent and water-miscible co-solvent, aiding in the aqueous solubilization of hydrophobic drugs like azithromycin, while the main lipids provide varying bilayer elasticity. Vanić and Rukavina [89] demonstrated that PG liposomes of azithromycin were highly effective against *Escherichia coli* biofilm formation and *C. trachomatis* infections. The authors utilized egg PC (EPC) with a very low melting point of 0°C, egg phosphatidylglycerol sodium salt (EPG) with a net negative charge, and monoacyl PC from soybean (Lipoid S LPC 80), all contributing to the flexibility of the liposome. Similarly, Rukavina and Klarić [42] showed that PG flexible liposomes provided deep and efficient

delivery of azithromycin compared to conventional liposomes.

Role of Chol in drug delivery to biofilm

Chol is a widely utilized component in liposomal formulations for treating various biofilm infections [8, 14, 23, 27, 30, 32, 39, 70, 74, 90-92]. It is primarily combined with PC lipids [23, 32, 39, 70, 74] in these formulations, often alongside ionic [27] or non-ionic emulsifiers [39, 70, 74]. Additionally, Chol can be used with a surfactant without the need for additional phospholipids in the drug formulation [8].

Various forms of Chol are also employed in liposomes. As a primary biomolecular substrate, Chol undergoes derivatization and molecular charge modification [14, 27, 30]. It is converted into zwitterionic derivatives for pH-responsive delivery systems (e.g., cholesteryl hemisuccinate or CHEMS [90]), positively charged derivatives (e.g., dimethylaminoethane-carbamoyl-cholesterol or DC-Chol [14]), and negatively charged derivatives (e.g., SChol [27]). These changes allow for adjusting liposomal antibiotic delivery to infected tissues.

Chol is also a versatile molecule for conjugation with other ligands. Different Chol conjugates are used in liposomal preparations to enhance targeting capabilities (e.g., sialo-mannan conjugates [91]), improve binding to dental surfaces (e.g., biomineral-binding conjugates [92]), and achieve tunable, responsive drug release (e.g., betainylate Chol [30]).

Chol plays a crucial role in the effective release of antibiotics near biofilms, where membrane-damaging toxins from microorganisms create pores in the Chol microdomains of liposomal lipid bilayers. In this regard, adjusting the Chol content in liposomal formulations has been shown to enhance bacterial eradication in biofilms, with a positive correlation between Chol content and effectiveness [93].

Site-specific delivery with sensitive liposomes

Sensitive liposomes are a class of liposomal formulations that exhibit tunable, site-specific pharmacological activity in response to unique external and/or internal stimuli (Fig 4). This selective activity is achieved through either a triggered burst release of the liposomal payload or the chemical activation of an inactive prodrug into a biologically active form in response to a stimulus.

For controllable antibiotic release, various

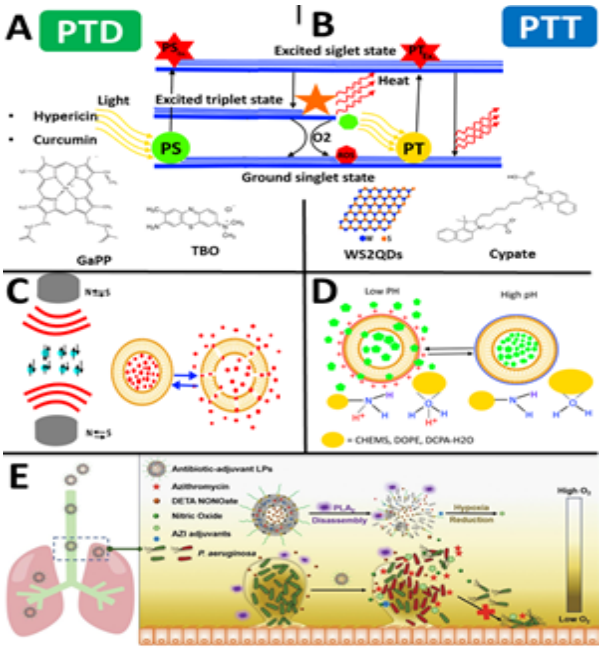

Fig. 4 Various responsive liposomes used for targeted delivery of antimicrobials: photosensitizing agents (hypericin [47], curcumin [59], GaPP [55], and TBO [48]) absorb light energy and are excited, which on return to the excited triplet state and the ground singlet state produce heat and ROS respectively (A); photothermal compounds (WS2QDs [44] and cypate [30]) only produce heat in one-phase decay to the ground singlet state (B). Besides light energy, hyperthermia is also produced via the localized magnetic resonance-guided high-intensity focused ultrasound application [49, 96] which leads to the creation of pores in the lipid bilayer of liposomes and the antimicrobial burst release (C). In addition to intrinsic stimuli, the specific low pH (D, [50, 66, 91]), high PLA2 expression, and reducing activity of the biofilm infection environment [78] are other facts governing the liposomal membrane instability and triggered drug release (E).

temperature-sensitive liposomes have been proposed (Fig 4 A-C). Temperature-sensitive liposomes trigger the burst release of their contents upon increasing temperature above 42°C. This can be achieved via localized application of electromagnetic resonance, ultrasound forces, and photothermal therapy. Additionally, site-specific antimicrobial activity can be achieved by using liposomes containing photoreactive agents that create ROS during photodynamic therapy.

Other sensitive liposomes, which demonstrate burst-release drug profiles, respond to the infection site-specific biochemical characteristics (Fig 4 D & E). These include hypoxia-related high protonating and reducing the capacity of anaerobic growth microcolonies and specific biological changes found in dental plaque and lower respiratory tract infections.

Ultrasound-sensitive liposomes

Ultrasound-sensitive liposomes release their drug cargo upon exposure to pulsed ultrasound. Moderate pulsed ultrasound (frequency = 2.25 MHz at a low intensity of ISATA ≈ 4.4 W/cm²) enhanced gentamicin-liposomal delivery through biofilms [46, 94]. In contrast, higher-intensity ultrasound (ISATA ≈ 90 W/cm²) caused liposomal rupture, resulting in a burst release of gentamicin and improved bacterial eradication.

Fu and Zhang [45] demonstrated the synergistic antibacterial activity of an ultrasound microbubble liposome combined with a chitosan-modified polymyxin B-loaded liposome against *Acinetobacter baumannii*. Upon applying localized ultrasound, the minimal biofilm inhibitory concentration significantly decreased from 32±2 μg/mL to 8±2 μg/mL for polymyxin B. Additionally, treatment with ultrasound microbubbles achieved complete bacterial killing at just 2 μg/mL of polymyxin B.

Temperature-sensitive liposomes

Ultrasound application can induce localized heating too, aiding the eradication of biofilm-producing bacteria through temperature-sensitive liposomes. In a study, Wardlow and Bing [49] achieved a four-fold increase in ciprofloxacin concentration at the infected wound site with low-temperature-sensitive liposomes (LTSL) when subjected to localized magnetic resonance-guided high-intensity focused ultrasound hyperthermia (MR-HIFU). This treatment also caused bacterial membrane deformation, leading to enhanced eradication of *S. aureus* within the biofilm matrix.

Similarly, Munaweera and Shaikh [95] utilized temperature-sensitive liposomes containing ciprofloxacin to treat P. aeruginosa biofilms on metal implants. Under an alternating magnetic field, they observed a 3-log reduction in the colony-forming units of the bacteria.

Light-activated thermosensitive liposomes

Photothermal therapy is another effective method for achieving localized heating at infection sites. When combined with light-absorbing ingredients in liposomes, this approach can trigger a burst release of antibiotics, enhancing microbial biofilm eradication.

In a study conducted by Zhao and Dai [30], near-infrared light-activated thermosensitive liposomes significantly improved tobramycin delivery and treatment efficiency in *P. aeruginosa*-induced abscesses. The liposomes were composed of Distearoylphosphatidylcholine (DSPC), betainylate Chol, and cyanine dye (cypate), a light-absorbing agent that converts light energy into heat. Upon exposure to IR light, the biofilm dispersion rate increased 7- to 8-fold, indicating a localized burst release of tobramycin and effective photothermal co-therapy.

Additionally, Xu and Hu [44] utilized tungsten sulfide quantum dots (WS2QDs) with photothermal sensitivity to develop near-infrared-responsive liposomes. These liposomes produced free radicals and decreased the level of glutathione (GSH) in infected tissues following photothermal therapy as well as targeted vancomycin delivery. This platform demonstrated excellent antibacterial activity against Gram-positive Mu50 (a vancomycin-intermediate *S. aureus* reference strain) and Gram-negative E. coli. It also showed outstanding antibacterial efficacy in mice with Mu50-induced skin abscesses, without noticeable side effects.

Photosensitive liposomes

Unlike light-activated thermosensitive liposomes, which contain a light-absorbing agent to convert light energy into heat, photosensitive liposomes incorporate a photosensitizing (phototoxic) agent to generate ROS upon light exposure. Light-activated thermosensitive liposomes utilize near-infrared light in photothermal therapy, while photosensitive

liposomes employ visible light in photodynamic therapy. Several researchers reported improved photosensitizer delivery and antimicrobial activity with various photosensitizing agents in liposomes.

Plenagl and Seitz [47] achieved an effective suppression of bacterial growth using photosensitive liposomes containing the phototoxic agent, hypericin. As hypericin was insoluble in water, Plenagl and Seitz used it as a water-soluble inclusion complex (hypericin - (2-hydroxypropyl)-beta-cyclodextrin) before encapsulating it in the liposomes. Following photodynamic therapy, these liposomes significantly reduced the number of planktonic and biofilm bacteria, achieving a 4.1 log and 2.6 log reduction *in vitro*, respectively.

Rout and Liu [48] achieved bacterial DNA disruption in multiple bacteria using a transferosome for the photosensitizer toluidine blue O (TBO). The Transferosome enhanced bacterial cellular uptake of TBO, leading to membrane and genomic DNA disruption in *S. aureus*, *E. coli*, *P. aeruginosa*, and even Deinococcus radiodurans, which has a highly effective DNA repair mechanism.

Berti and Dell'Arciprete [12] reported a similar increase in photosensitizer delivery using calcium phosphate-coated 1,2-dioleoyl-sn-glycero-3-phosphate (DOPA) liposomes. The liposomes contained the photosensitizing agent 5,10,15,20-Tetrakis(1-methyl-4-pyridinio) porphyrin (TMP), which led to effective bacterial inactivation in biofilms. By incorporating acridine orange (AO) as a tracking fluorophore in the liposomes, the liposomes offered significant biofilm staining within two hours of incubation, indicating improved biofilm delivery of the photosensitizer too.

The biosafety of photosensitive liposomes was also confirmed by Pourhajibagher and Partoazar [59]. They investigated the photo-disinfecting effect of silver sulfadiazine-curcumin liposomes on burn wound sites infected with *A. baumannii*, and observed no evidence of cytotoxic or hemolytic activity in the histopathological examination of the wound sites related to the liposomes.

pH-sensitive liposomes

Liposomes serve as a viable drug delivery platform, capable of tailoring drug release in response to environmental pH changes. This is achieved by pH-sensitive liposomes incorporating natural and synthetic zwitterionic compounds, such as DPPC lipids, CHEMS, pyridine betaine, and dioleoylphosphatidylethanolamine (DOPE). In contrast to normal tissues with physiological neutral pH, the protonating capacity (low pH) of the infection site promotes supramolecular membrane rearrangement, surface charge alteration, and membrane fusibility in these pH-sensitive liposomes. Consequently, the liposomes interact effectively with infected cells and bacterial colonies in biofilms, resulting in the burst release of antibiotics while avoiding host-cell interaction and systemic antibiotic toxicity.

As an acidic Chol ester and nonionic detergent, CHEMS is commonly employed with DOPE to form pH-sensitive fusogenic liposomes. With a pKa of 5.8, CHEMS loses its self-assembled bilayer configuration through protonation at infection sites, as evidenced by Luo and Lin [90] study. Using the mentioned lipids, these researchers developed pH-sensitive liposomes co-loaded with magnolol and fluconazole. The liposomes rapidly released the antibiotics in dental caries in a rat model, effectively eliminating *C. albicans* and *S. mutans* biofilm-producing pathogens.

Similarly, Wang and Yang [66] demonstrated improved therapeutic efficacy using ciprofloxacin-loaded liposomes containing a pH-sensing lipid - 1,2-dipalmitoyl-sn-glycero-3-phosphatidic acid (DCPA-H_2O). These liposomes enhanced blood circulation times for ciprofloxacin, while triggering drug release in intra-abdominal biofilms with pH < 6.8. This pH-targeted antibiotic delivery resulted in superior therapeutic effectiveness compared to clinically applied ciprofloxacin, neutral DCP liposomes, and cationic dicetyl phosphate methyl ester (DCPM) liposomes in a murine- infected wound model. Wang and Yang [50] further advanced this approach by developing dual-drug-loaded liposomes with bromelain in the core and ciprofloxacin in the membrane, along with the DCPA-H_2O lipid. These liposomes exhibited proton-mediated burst release of bromelain and ciprofloxacin in acidic environments, leading to increased bacterial eradication in *S. aureus* biofilms.

Other sensitive liposomes

Beyond pH, infection sites possess additional specific characteristics that can be leveraged to achieve optimal therapeutic outcomes. This can be accomplished by using liposomal components that trigger antibiotic release and exert antibacterial activity upon reacting within infection sites.

Several factors contribute to the chronicity of pulmonary infections in CF patients caused by opportunistic *P. aeruginosa*. These factors hinder antibiotic delivery and lead to persistent infections. Firstly, the impaired biosurfactant system increases the viscosity of sputum lining the pulmonary airways, which hampers antibiotic penetration. Secondly, chronic infections promote the transition of bacterial growth from planktonic to biofilm formations, creating an additional physical barrier against antibiotics. Thirdly, the viscous sputum layer restricts oxygen penetration into infected regions. Under hypoxic conditions, *P. aeruginosa* induces the secretion of phospholipase A2 (PLA2) from bronchial epithelial cells, facilitating bacterial rearrangement within microcolonies.

To address these specific barriers, Rao and Sun [78] developed an innovative multi-functional liposomal azithromycin formulation capable of penetrating mucus, disrupting biofilms, and eradicating bacteria. The liposomes, being negatively charged and hydrophilic on the surface, effectively traversed the sputum layer. Upon reaching the biofilm beneath the sputum, the liposomes disassembled and released azithromycin in response to elevated PLA2 levels. Additionally, the liposomes contained a hydrophobic compound - 6-nitroimidazole (6-NIH), which can chemically reduce to its hydrophilic counterpart- 6-aminoimidazole (6-AIH) - under hypoxic conditions. This molecular conversion enhanced azithromycin's bacteriostatic activity, demonstrating site-specific reactivity and toxicity. Furthermore, the liposomes incorporated a strong nitric oxide (NO) donor - (Z)-1-[N-(2-aminoethyl)-N-(2-ammonioethyl)amino]diazen-1-ium-1,2-diolate (DETA NONOate), , which was associated with the dispersion of the biofilm. By carefully selecting these excipients, the authors exploited the specific infection conditions to overcome sputum- and biofilm-associated barriers.

Additional enzymatic systems can be exploited in reactive liposomes. For this purpose, enzymes that convert harmless substrates into antibacterial products can be used as ingredients of liposomes. These include lactoperoxidase, myeloperoxidase, and glucose oxidase. Lactoperoxidase and myeloperoxidase are found in milk, tears, and saliva, while glucose oxidase is used as a commercial preservative for oral hygiene. Using reactive DPPC/PI liposomes loaded with glucose oxidase, Hill and Kaszuba [79] achieved antibacterial activity with iodide ions in response to glucose presence. The antibacterial activity was attributed to the generation of hydrogen peroxide and oxyacids, suggesting the potential of reactive liposomes for oral hygiene.

Approaches to modify liposome-bacteria interactions

Modifying the liposomal surface is a key strategy to tailor interactions with the environment, target, and non-target cells. By varying lipid components, formulation scientists can alter the surface characteristics of liposomes. For instance, cationic lipids like dioctadecyl dimethylammonium bromide (DODAB) and DDAB enhance interactions between bacterial cells and liposomal membranes. This enhancement can improve the delivery of antimicrobial agents and facilitate the eradication of bacterial colonies. Additionally, the liposome surface can be functionalized with various chemical and biological ligands to achieve targeted delivery of antimicrobial agents.

Modulation of liposomal surface charge

Altering the surface charge of liposomes is a well-studied parameter for enhancing antibiotic penetration into biofilms and improving antimicrobial efficiency. Numerous studies have demonstrated that cationic liposomes exhibit superior anti-biofilm activity compared to neutral and anionic liposomes, primarily due to the electrostatic attraction between the biofilm matrix and the liposome.

For example, Moghadas-Sharif and Fazly Bazzaz [40] found that among cationic (35 mV), anionic (-35 mV), and PEGylated cationic liposomes (27 mV), cationic liposomal rifampicin was the most effective formulation against *S. epidermidis* biofilm.

Dong and Thomas [96] investigated the biofilm distribution of cationic and anionic liposomes of different sizes. They discovered that cationic liposomes with smaller particle sizes had better penetrability in *S. aureus* and *P. aeruginosa* biofilms.

Similarly, Sugano and Morisaki [97] observed stronger interactions between cationic liposomes and *S. mutans* in both planktonic cells and biofilms compared to conventional liposomes. They found that cationic liposomes penetrated deeply into the biofilm mass and exhibited effective antimicrobial activity.

In another study, Rukavina and Klarić [42] demonstrated that cationic liposomes efficiently inhibited MRSA strain growth, performing similarly to deformable and PG-containing liposomes, and outperforming conventional neutral-charged liposomes. Kim and Jones [98] reported enhanced antibacterial activity of benzylpenicillin (penicillin G) against *S. aureus* when using a cationic liposomal formulation compared to the free antibiotic *in vitro*.

Highly positively charged proliposomes are considered optimal for delivering veterinary antibacterial drugs. Proliposomes are defined as dry, free-flowing granular lipid particles that disperse into a liposomal suspension upon contact with water. Li and Chen [99] developed highly positively charged (65.29 mV) and stably dispersed proliposomes for cefquinome sulfate, a veterinary antibacterial drug against *S. aureus* infection. The proliposomes improved biofilm eradication.

The surface charge of liposomes primarily influences their interaction with *C. albicans*, as demonstrated by Smistad and Nguyen [14]. They found that saturated positively charged liposomes exhibited the best interactions with *C. albicans* biofilm. Their study investigated various main phospholipids (EPC, Dimyristoylphosphatidylcholine or DMPC, DPPC), charged lipids (1,2-diacyl-3-trimethylammonium propane or diacyl-TAP, DC-Chol, phosphatidic acid, phosphatidylglycerol, phosphatidylserine, and PI), and their quantities in a factorial design. The study found the liposomal surface charge as the primary and the saturation of the main lipid as the secondary factor, affecting liposome-bacteria interactions.

In contrast, negatively charged liposomes exhibit the least interaction with bacterial biofilms. Alhariri and Majrashi [25] showed that their negatively charged liposomes (-31.7 mV) offered no advancement in the antibacterial activity of gentamicin compared to the free drug. This was in contrast to their neutral liposomes (-0.22 mV) that exhibited significant antibacterial activity against *P. aeruginosa* and *Klebsiella oxytoca*.

Cottenye and Cui [27] observed different distributions of cationic and anionic liposomes in *S. mutans* biofilm, depending on sterol selection. They used cetylpyridinium chloride (CPC) and Schol to prepare their cationic and anionic liposomes, respectively. While both liposomes showed similar binding ability to *S. mutans* biofilms, the positively charged CPC/Chol liposomes were mainly gathered in the core of the biofilm microcolonies, whereas the negatively charged CPC/Schol liposomes were concentrated at the peripheries.

Occasionally, a positive surface charge does not translate to improved antibiotic delivery to target pathogens. Messiaen and Forier [100] studied the transport of cationic and anionic liposomes into the *Burkholderia cepacia* complex, a consortium of opportunistic species in cystic fibrosis. They found that anionic liposomes reached the vicinity of the bacterial cell clusters, while cationic liposomes were trapped far outside. The authors suggested that cationic liposomes might interact with fiber-like structures in the biofilm, likely the negatively charged extracellular lysed DNA strands from the bacteria.

Cationic liposomes are particularly well-suited for the topical application of antimicrobial agents and antibiotics on clinical abiotic surfaces and skin. Combined with enzymatic biofilm-disrupting compounds, cationic liposomes enhanced the efficacy of eradicating *C. acnes* in both cutaneous and catheter infections [20, 73].

Liposomal surface hydration

Cationic liposomes exhibit remarkable binding capabilities to planktonic bacteria and high penetrability into biofilms. Moreover, they tend to interact with the host's tissues and cells, leading to rapid elimination of the liposomal payload and systemic toxicity. Consequently, the use of cationic liposomes is typically limited to topical or local administration. For other administration routes, where liposomes must travel a significant distance to reach the target tissue, liposomal surface hydration is employed to enhance colloidal stability and achieve cell repulsion.

PEGylation is a method used to hydrate particle surfaces. PEG, or polyethylene glycol, is a long, water-sequestering polymeric chain. PEGylation involves conjugating these polymeric chains to the surface of particles. This can be achieved by conjugating 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) lipid with PEG polymers with varied lengths Ahmed and Gribbon [35] demonstrated that PEGylation reduced the interaction of cationic liposomes with bacterial biofilms. This was evidenced by a decrease in fluorescence within the biofilms as the amount of DPPE-PEG-2000 increased from 0 to 9 mole%.

Therefore, PEGylation of liposomes poses a

problem, as it also diminishes the rate of antibiotic delivery to biofilms. Kluzek and Oppenheimer-Shaanan [101] addressed this issue by employing a different hydration strategy to achieve colloidal stability. Instead of PEGylation, they utilized a zwitterionic membrane-embedded moiety, poly[2-(methacryloyloxy)ethyl phosphorylcholine] (pMPC), for liposome functionalization. This approach enhanced antibiotic delivery to *P. aeruginosa* biofilms, resulting in faster antibiotic release in the cytosol and greater biofilm eradication compared to PEGylated liposomes.

Targeted delivery with biological ligands

Functionalizing liposomes with biological ligands enhances their properties, particularly for antibiotic delivery. Various biological ligands have been employed to functionalize liposomes, providing additional benefits.

Liposomal functionalization with peptides: proteoliposomes

Bacterial lectins commonly appear as elongated multisubunit protein appendages, known as fimbriae (hairs) or pili (threads). These structures interact with glycoprotein and glycolipid receptors on host cells, facilitating bacterial adhesion to biotic surfaces and internalization into tissues. Utilizing lectins for liposomal functionalization enables site-specific antibiotic delivery, as demonstrated with lectin-functionalized liposomes (proteoliposomes) used for delivering triclosan, vancomycin, and benzylpenicillin [102].

Functionalizing liposomes with different lectins allows for pathogen-specific delivery [74]. Proteoliposomes conjugated with succinylated concanavalin A preferentially target oral bacteria such as *S. mutans* and *S. gordonii*, as well as the skin-associated bacterium *Coryneform hofmanni*. In contrast, those bearing wheat germ agglutinin (WGA) target *S. epidermidis*. Sudheesh and Jain [38] developed a lectin-functionalized liposomal formulation composed of DPPC/Chol/SA/DPPC-MBS-lectin for topical application against oropharyngeal candidosis. The liposomes conjugated with the lectin N-succinimidyl-S-acetylthioacetate - a derivative of succinyl concanavalin A - exhibited strong interaction with candidal biofilms.

Using LecA and LecB-targeted phospholipids in a liposome, Metelkina and Huck [103] specifically targeted *P. aeruginosa* biofilms. Similarly, Meng and Hou [37] reported enhanced clarithromycin accumulation in the enterocoelia of mice in association with the presence of WGA on the liposomes. They also noted the successful eradication of MRSA colony-forming units in the spleen and kidney through enhanced bacterial uptake by macrophages.

Similar to lectins used for liposomal functionalization, positively charged membrane-active peptides are exploited to target bacterial biofilms. Despite their non-specific toxicity, low bioactivity, and low stability, cationic membrane-active peptides hold promise for liposomal functionalization and as next-generation antimicrobial agents in liposomal form. Shao and Zhou [34] demonstrated that cationic peptide-conjugated liposomes could enhance the binding capability of liposomes to bacterial membranes at optimal peptide density and variety. These liposomes exhibited antibacterial and antibiofilm activity without compromising cytotoxicity. Hemmingsen and Giordani [104] highlighted the antibacterial activity of liposomes conjugated with a synthetic mimic of a small antimicrobial peptide. The peptide-functionalized liposomes inhibited biofilm formation and eradicated preformed biofilms produced by *S. aureus*, *E. coli*, and *P. aeruginosa*.

Conjugation with positively charged cell-penetrating peptides promoted sirtuin inhibitors internalization into bacterial cells too [105]. Inside the bacteria, sirtuin inhibitors target the bacterial genome and inhibit transcription. Conjugation of sirtuin inhibitors with cationic peptides enhanced this antibacterial potency against model Gram-negative and Gram-positive pathogens. The conjugated peptides also exhibited synergistic antibacterial activity in combination with streptomycin and polymyxin B. Given these findings, using these peptides in liposomes is worth investigating.

Immunoliposomes, another type of proteoliposome, enable specific delivery of antimicrobial agents while avoiding non-specific eradication of microflora [36]. Antibody-conjugated liposomes loaded with bactericides such as chlorhexidine and triclosan selectively inhibited *S. oralis* more than beneficial oral bacteria in regrowth assays [36]. This specific bacteria growth inhibition in the dental plaque-associated microbial community could shift the balance toward dental re-mineralization and repair,

potentially more effective than indiscriminate eradication of oral microflora.

Liposomal functionalization with glycolipids: glycoliposomes

Glycolipids that interact with lectins on bacterial pili and bacterial glycoproteins can be employed for liposomal functionalization. Multiple glycolipids with different glycan moieties have been utilized in liposomal platforms, termed glycoliposomes, to target bacterial biofilms.

Francolini and Giansanti [75] utilized positively-charged glucosylated liposomes for the delivery of a hydrophobic lichen-derived antimicrobial agent, usnic acid. These glycoliposomes facilitated the delivery of usnic acid, enhanced its penetration into the biofilm matrix, and improved its antimicrobial activity.

Vyas and Sihorkar [91] investigated the targeting effects of coating the liposomal surface with mannan and sialo-mannan moieties anchored to Chol. The sialo-mannan conjugated liposomes demonstrated superior bacterial targeting and growth inhibition compared to mannan-coated liposomes.

Aptamer-targeted liposomes

Only one study has employed aptamer-targeted liposomes for the delivery of antibiotics. Aptamers are short single-stranded DNA or RNA molecules with a high affinity for specific targets. In the case of *S. aureus*, Ommen and Hansen [33] identified six aptamers capable of targeting bacterial cells and biofilms. The aptamer-conjugated liposomes enhanced the accumulation of liposomes, increased the concentrations of vancomycin and rifampin within the biofilm, and promoted the eradication of *S. aureus*.

Liposomal modulations for controlled antibiotic delivery

Beyond the previously discussed liposomal modifications, liposomes can be applied to various biotic and abiotic surfaces. Additionally, injectable hydrogels and scaffolds can be impregnated with liposomes. These liposome-impregnated hydrogels and scaffolds provide an extra level of control over the drug release rate, enabling prolonged delivery of the drug to the target site.

Approaches to tailor the adhesion capability of liposomes

The ability to modify the binding properties of liposomes has garnered significant interest among researchers. Numerous studies have explored the adhesion of liposomes to both abiotic and biotic surfaces.

Catuogno and Jones [32] investigated the deposition rates of various liposomes on zinc citrate particles. This study aimed to optimize the coating of zinc citrate particles with liposomes carrying bactericides, enhancing the anti-caries properties of zinc citrate particles. The liposomes varied in surface charge and binding affinity (PI/DPPC anionic liposome, DDAB/DPPC cationic liposome, and DPPC/Chol neutral-charge liposome), and were loaded with two bactericides with different solubility profiles: the oil-soluble triclosan and the water-soluble penicillin-G. Zinc citrate particle is commonly used in toothpaste formulations. The authors hypothesized that coating these microparticles with liposomes would enhance their antibacterial activity against immobilized biofilms of the oral bacterium S. oralis. However, the results indicated that the liposome coating reduced the bactericidal effect of the particles.

Liposomes carrying antibiotics that can adhere to osseous tissues offer a protective layer against infections. This approach is particularly suitable for preventing orthopedic implant-associated osteomyelitis. Orthopedic implants are often coated with hydroxyapatite (HA) to ensure compatibility with dental and bone surfaces. Consequently, liposomes that can attach to dental and bone surfaces can also adhere to HA-coated implant surfaces. Liu, Zhang [92] successfully coated implants with HA-targeting liposomes containing oxacillin, a penicillinase-resistant β-lactam antibiotic. The liposomal membrane included a synthesized biomineral-binding agent, alendronate-tri(ethylene glycol)-conjugated cholesterol (ALN-TEG-Chol conjugates). These liposomes demonstrated rapid and strong binding to HA, resulting in significant antibiotic loading on the implant surfaces, which notably reduced bacterial colonization and biofilm formation by *S. aureus*. Similarly, Hu, Zhou [106] achieved effective control of periodontal infections *in vivo* using a liposomal formulation with high binding affinity to dental surfaces. This formulation consisted of liposomes, doxycycline, and N,N,N-trimethyl chitosan (TMC-Lip-DOX NPs), providing strong adhesion to dental surfaces.

Moreover, coating surfaces with liposomes

can effectively prevent microbial colonization. Barakat and Kassem [11] utilized a vancomycin-eluting niosome to inhibit early staphylococcal colonization on clinical abiotic surfaces. The niosome demonstrated a high entrapment capacity for vancomycin, exhibited a strong affinity for implant surfaces, and provided significant repulsion against bacterial adhesion. Similar results were reported by Dwivedi and Mazumder [43] with the layer-by-layer coating of orthopedic implants using vancomycin-loaded niosomes. This coating inhibited bacterial colonization and prevented biofilm formation on implants for up to two weeks. Additionally, coating gold-covered steel surfaces with tobramycin-loaded liposomes inhibited slime-producing *S. epidermidis* colonization and biofilm formation [107].

Conversely, Berti and Dell'Arciprete [12] coated DOPA liposomes with calcium phosphate. The resulting product was a highly stable liposome with negligible fluorophore leakage, making it suitable for high-contrast imaging purposes where precise tissue localization of the fluorescence signal related to liposomal accumulation is desired.

Scaffolds

As previously discussed, cationic liposomes are generally the optimal formulation for eradicating bacteria in biofilms. However, when administered through blood circulation, cationic liposomes are rapidly removed by the reticuloendothelial system, posing a challenge. To avoid systemic toxicity of antibiotics, the use of cationic liposomes containing antibiotics is therefore restricted to local applications. In this context, there is a pressing need to provide a long-term protection against infections that might be posed by prosthetics and tissue implants. This is achieved by infusing liposomes, often cationic liposomes, into tissue scaffolds.

Artificial scaffolds infused with liposomes offer multiple therapeutic benefits for osseous tissues. Biodegradable scaffolds impregnated with antibiotic-loaded liposomes deliver the antibiotic in a highly sustained-release manner, providing prolonged protection against osteomyelitis-associated infections. Additionally, scaffolds serve as a substrate for bone regeneration. Zhou, Su [52] demonstrated that HA combined with β-tricalcium phosphate (β-TCP) offered spatiotemporal control over the delivery of cationic liposomal ceftazidime, eradicating *S. aureus* biofilms. Moreover, the β-TCP/HA scaffold provided a strong mechanical scaffold for bone lesion repair.

Moreover, the rate of antibiotic delivery can be adjusted by varying the scaffolds' components. Ma, Shang [51] tailored the delivery rate of cationic liposomal vancomycin by modulating chitosan and konjac glucomannan, two biodegradable co-polymers with different disintegration rates. Electron microscopy depicted the dispersion of intact cationic liposomal vancomycin in the porous scaffolds, which released the liposomes as the scaffold degraded.

In this regard, using liposomes in scaffolds might improve the antibacterial activity further. The scaffolds designed by Ma, Shang (51) effectively inhibited *S. aureus* biofilm formation, outperforming both non-liposomal vancomycin-impregnated scaffolds and free antibiotics.

Tissue implants also benefit from being impregnated with antibiotic-loaded liposomes, offering prolonged protection against potential infections during implantation. Two weeks post-implantation, X-ray examinations showed that impregnating allogeneic bone tissue with liposomal gentamicin prevented bone tissue disintegration and periosteal reaction to *S. aureus* biofilm infection [108]. Neither *S. aureus* infection nor neutrophil accumulation was observed in the blood and bone marrow cultures of rabbits treated with liposomal gentamicin-impregnated implants.

Similarly, impregnating prosthetic joints with liposomal antibiotics protects against fungal infections. Czuban, Wulsten [15] demonstrated that impregnating polymethylmethacrylate bone cement with liposomal amphotericin B provided antifungal activity against *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. krusei* biofilms

Hydrogels

Liposomal antibiotics are also utilized in combination with hydrogels. Similar to scaffolds, embedding liposomes within a hydrogel matrix provides enhanced spatial-temporal control over the release of antimicrobial agents. For instance, Targhi and Moammeri [54] observed that curcumin-Cu and curcumin-Ag NPs were sustainably released from niosomes encased in a chitosan hydrogel, demonstrating effective antibacterial and anti-biofilm activity against *S. aureus* and *P. aeruginosa*. Likewise, Eroğlu and Aslan [109] found that administering liposomal tetracycline HCl/tretinoin in a carbopol-based gel

resulted in the gradual release of the drugs and prolonged inhibition of biofilm formation by *S. aureus* and *S. epidermidis* strains.

Hydrogel-based liposomal formulations are particularly suitable for dermal wound applications for several reasons. Firstly, the high viscosity of hydrogels makes them ideal for dermal application. Secondly, hydrogels are generally non-toxic to dermal tissues. For example, the liposomal tetracycline HCl/tretinoin in a carbopol-based gel formulation showed no toxicity to mouse fibroblast cells, indicating the safety of hydrogel formulations for dermal use [109]. Similarly, Hurler and Sørensen [110] demonstrated that a mupirocin-liposomes-in-hydrogel formulation was non-cytotoxic to keratinocytes.

Thirdly, hydrogels are excellent platforms for incorporating multiple therapeutic agents. Hemmingsen and Giordani [111] showed that a chitosan hydrogel-based liposomal chlorhexidine/mupirocin formulation possessed both anti-inflammatory and antibacterial properties. This formulation significantly inhibited NO synthesis in lipopolysaccharide (LPS)-induced macrophages, indicating effective anti-inflammatory properties, while simultaneously reducing *S. aureus* and *P. aeruginosa* counts by 64-98% and preventing biofilm formation.

Fourthly, hydrogel/liposomal formulations promote effective wound healing while preventing dermal infections. The mupirocin-liposomes-in-hydrogel formulation reported by Hurler and Sørensen [110] effectively eliminated *S. aureus planktonic bacteria and restricted biofilm formation. Additionally, histological examination of burns in mice showed complete wound healing within a shorter treatment period (28 days) compared to the marketed mupirocin product.*

Hydrogel/liposomal antibiotics are also effective in preventing catheter-related bacterial infections. Coating catheters with hydrogels infused with liposomal antibiotics prevents bacterial adhesion and colonization on their surfaces. Pugliese and Favero [112] cross-linked and immobilized a poly(ethylene glycol)-gelatin hydrogel on the surface of silicone catheters. The hydrogel, impregnated with liposomal ciprofloxacin, completely inhibited *P. aeruginosa* adhesion for seven days by providing sufficient doses of the antibiotic. Similar results were observed in vivo in a rat model with persistent *P. aeruginosa* peritonitis, where the liposomal ciprofloxacin hydrogel-coated dialysis catheter resisted bacterial colonization [6].

CONCLUSION

In conclusion, numerous combinatorial therapies can be explored with liposomes, where different ingredients with specific roles in the formulation are employed (Table 1). These include the co-administration of multiple antibiotics and co-encapsulation of antibiotics with an additional antimicrobial agent. Various emerging natural and synthetic compounds possessing antimicrobial or antivirulent properties can be used with liposomes, and many of them benefit from liposomes in terms of delivery improvement to infection sites. Modulation and functionalization of liposomal formulations allow for facilitating physicochemical and biological interactions with the multiple barriers addressed in this paper, surpassing these obstacles. Moreover, site-specific interactions can be achieved with sensitive liposomes carrying antibiotics by exploiting different ingredients offering antibiotic burst-release profiles and prodrug conversion into bioactive antimicrobial agents. Lastly, artificial tissue implants and prosthetics can also benefit from infusion with liposomes carrying antibiotics and anti-inflammatory medicines. In this regard, hydrogels infused with liposomes also offer long-term protection against infections.

This review aimed to inspire new research and investigations by presenting a comprehensive overview of existing reports on drug formulation development related to infection treatment. The main concepts investigated are summarized in a word cloud in Fig. 5. Future research might explore less investigated areas, such as aptamer-targeted liposomes for antibiotic delivery, a relatively new concept in the field. Given the potential of aptamers for targeted drug delivery, it is expected that more studies will emerge in this area. Other promising concepts include pH-responsive liposomes and reactive liposomes might come into investigations with a broad range of antibiotics and antimicrobial agents. Affibody-targeted liposomes were missed to explore with antibiotics or antimicrobials for biofilm infections, which is worth investigating. The application of hydrogel/scaffold-impregnated liposomal antibiotics was a novel addition to the field of controlled-release antibiotics, and further investigations are anticipated in this area for both existing and new antimicrobials.

Table 1. Common Compounds in Liposomal Formulations and Their Roles

Formulation Components	Role in the Formulation
Antibiotics: amikacin [16, 23], AMB[1] [76, 86], azithromycin [3, 42, 49, 78, 89], ciprofloxacin [4, 41], daptomycin [88], fluconazole [17], gentamicin [16, 46, 80, 84, 94], imipenem [8], levofloxacin [22, 29], moxifloxacin [3], penicillin G [32], polymyxin B [45, 67], rifampicin [39], tobramycin [2, 10, 16, 56, 62, 81]	Serve as the API[2] with antimicrobial activity.
Natural Compounds with Antimicrobial Activity: berberine [64], biosurfactants isolated from *Lactobacillus gasseri* [77], cinnamon oil [67], catechin [61], curcumin [39, 53, 54, 64, 82], essential oil of *Schinus Areira L*. [58], hypericin [47], lipopeptide biosurfactant [24], ozonized sunflower oil [57], porphyrin [59], propolis ethanolic extract [87], resveratrol [26], usnic acid [75]	Serve *per se* or in combination with other antimicrobial agents against infections.
Photosensitizing Agents: cyanine dye (cypate) [30], hypericin [47], porphyrin [59], silver sulfadiazine-curcumin [59], TBO[3] [48], TMP[4] [12]	Upon absorbing light, convert to or produce products with antimicrobial properties.
Water-Miscible Conjugates: cyclodextrin inclusion complex [47, 61], DPPC[5]-porphyrin conjugates [59], sialo-mannan conjugates [92]	Contribute to the delivery of water-immiscible antimicrobials.
QSM[6]: bismuth-ethanedithiol [56, 62, 81], farnesol [4], C12AHL[7][17], synthetic homoserine lactone analogs [39, 53, 82]	Serve in competition with the pathogen's QSM and limit pathogen's growth.
Preventive Medicine Candidates: bromelain hydrolysate of rice bran protein [70], nisin [28], platensimycin [69]	Act as API to prevent food spoilage and infections.
Biofilm-Disrupting Agents: alginate lyase [2, 86], DNase I [16, 20], lysozyme [22, 84], N-acetyl-L-cysteine [83], proteinase K [20], retinoic acid [73], SRP[8] [29], Tween 80 [83], TPA[9] [3], DETA NONOate[10] [78]	Lead to the deep delivery of antimicrobials into biofilm infections.
Nonionic Surfactants: SA[11] [70], DCP[12] [39, 76], sophorolipid [76], Tweens and Spans [8, 10, 41, 56, 83, 87], Lipoid S LPC 80 [49, 89]	Contribute to the niosomal stability in biological media.
Ionic Surfactants: sodium cholate [88], cetylpyridinium chloride [27], DDAB[13] [32], CTAB[14] [19]	Combine with other lipids in transferosomes administered locally, for epidermic and oral infections.
Water-Miscible Co-Solvents: PG[15], EPC[16], EPG[17] [49, 89]	Used in flexible liposomes for local administration.
Positively-Charged Excipients: CTAB [19], DDAB [32], DC-Chol[18] [14], chitosan [45, 54], cell-penetrating peptides [105]	Increase liposome-biological membrane interaction, suitable for local administration of antimicrobials and transfection of antisense oligonucleotides with antimicrobial activity.
pH-Sensitive Agents: CHEMS[19] [66], DCPA-H_2O[20] [50, 66], 6-NIH[21] [78]	Allow for targeted pH-dependent delivery of antimicrobials.
Antiseptics Used in Oral Hygiene: bismuth-ethanedithiol [2, 56, 62, 81], CTAB [19], triclosan [32], zinc citrate [32], cetylpyridinium chloride [27]	Maintain oral hygiene.
Responsive Agents: glucose oxidase [78], 6-NIH [78], betainylate Chol [30]	Facilitate drug delivery in response to specific environments of infection sites.
Biofilm-Targeting Agents: D-phenylalanine [83], D-proline [83], D-tyrosine [83], aptamers [33], WGA[22]- & N-succinimidyl-S-acetylthioacetate and other lectins [37, 74, 75, 91, 103], antibodies [36], cell-penetrating peptides [105]	Target the biofilm in the colonies.

1 amphotericin B, 2 active pharmaceutical ingredient, 3 toluidine blue O, 4 5,10,15,20-tetrakis(1-methyl-4-pyridinio)porphyrin, 5 dipalmitoylphosphatidylcholine, 6 quorum-sensing molecules, 7 N-3-oxo-dodecanoyl-L-homoserine lactone, 8 Serratiopeptidase, 9 total polar archaeolipids, 10 (Z)-1-[N-(2-aminoethyl)-N-(2-ammonioethyl)amino]diazen-1-ium-1,2-diolate, 11 stearylamine, 12 dicetyl phosphate, 13 dodecyl-dimethylammonium bromide, 14 Cetyltrimethylammonium bromide, 15 propylene glycol, 16 egg phosphatidylcholine, 17 egg phosphatidylglycerol, 18 cholesterol, 19 cholesteryl hemisuccinate, 20 1,2-dipalmitoyl-sn-glycero-3-phosphatidic acid, 21 6-nitroimidazole, and 22 wheat germ agglutinin.

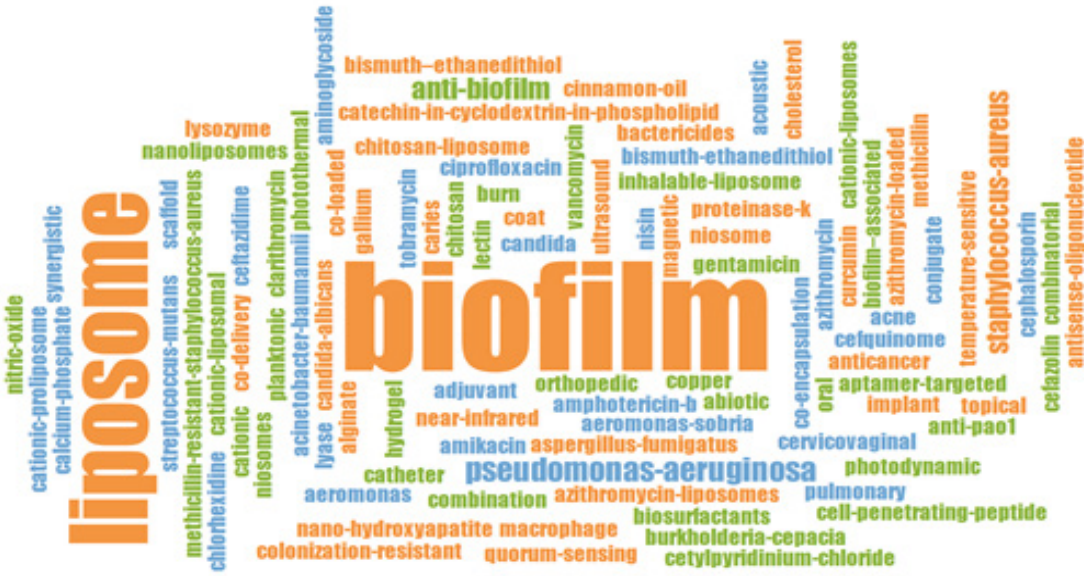

Fig. 5 Wordcloud of concepts and players studied for liposomal antimicrobials used for different biofilm-producing infections. The wordcloud is produced from the title and keywords of the retrieved original papers following the exclusion of the unrelated words.

Despite the comprehensive nature of this review, some valuable concepts may not have been covered. Future original or review papers will likely address these gaps. This review retrieved papers from PubMed/NCBI only. There are also valuable papers on platforms such as Scopus, Wiley, Science Direct, and Springer, which may cover different research scopes, including liposomal antimicrobials for veterinary medicine. Natural antimicrobials, in particular, might be suggested in veterinary medicine and nutrition for cost-effective management of pathogenic infections and for avoiding long-term antibiotic use.

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