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Mini-Review

Liposome-Based Antibacterial Delivery: An Emergent Approach to Combat Bacterial Infections

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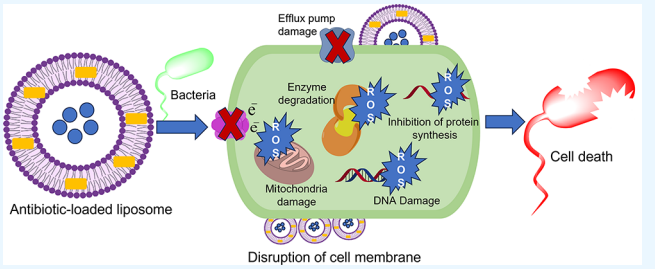
ABSTRACT: The continued emergence and spread of drug-resistant pathogens and the decline in the approval of new antimicrobial drugs pose a major threat to managing infectious diseases, resulting in high morbidity and mortality. Even though a significant variety of antibiotics can effectively cure many bacterial infectious diseases, microbial infections remain one of the biggest global health problems, which may be due to the traditional drug delivery system's shortcomings which lead to poor therapeutic index, low drug absorption, and numerous other drawbacks. Further, the use of traditional antibiotics to treat infectious diseases has always been accompanied by the emergence of multidrug resistance and adverse side effects. Despite developing numerous new antibiotics, nanomaterials, and various techniques to combat infectious diseases, they have persisted as major global health issues. Improving the current antibiotic delivery systems is a promising approach to solving many life-threatening infections. In this context, nanoliposomal systems have recently attracted much attention. Herein, we attempt to provide a concise summary of recent studies that have used liposomal nanoparticles as delivery systems for antibacterial medicines. The minireview also highlights the enormous potential of liposomal nanoparticles as antibiotic delivery systems. The future of these promising approaches lies in developing more efficient delivery systems by precisely targeting bacterial cells with antibiotics with minimum cytotoxicity and high bacterial combating efficacy.



INTRODUCTION

Over the last few decades, bacterial infections have led to a new major threat to human health, resulting in high morbidity and mortality globally. In the early 1900s, when antibiotics were first developed, it was believed that mankind had defeated bacteria. However, it soon became clear that bacteria may become resistant to any treatment being utilized. It seems that most pathogenic bacteria can become somewhat resistant to antibiotics. According to the World Health Organization (WHO), antibiotic resistance is one of the worst health risks in the present day.[1] It can be overcome by chemically developing new medicines and modifying existing medications. However, the creation of new antibiotics does not guarantee that they will defeat bacterial infection quickly enough to prevent the emergence of resistance in the future. There are a few factors that are mainly responsible for antibacterial resistance. They include inhibiting cell wall synthesis, depolarizing the cell membrane, inhibiting protein synthesis, inhibiting nucleic acid synthesis, and inhibiting metabolic pathways in bacteria. Inadequate antibacterial therapy and an increased usage of antibiotics by humans and animals are two factors that have majorly contributed to the problem of rising drug resistance. Further, the widespread dissemination of resistance and the sharing of resistance genes among many types of bacteria causes multidrug-resistant bacteria. This issue worsens when bacteria form biofilms (extracellular polymeric substances), accelerating the onset of multidrug-resistant infections and increasing bacterial resistance by up to 1000-fold.[2a]

Therefore, we urgently need alternative antibacterial therapy. In this regard, using nanotechnology is one of the promising ways to deal with these infectious diseases. Nanotechnology in medicine has given rise to an entirely new branch, generally termed "nanoparticles" (NPs). Due to their narrow size range, NPs possess unique qualities such as large surface area and enhanced reactivity.[2b] These NPs fight the pathogens in various antibacterial ways; they may break the cell membrane or form free radicals. NPs use multiple biological pathways to exert their antibacterial mechanisms, such as cell wall disruption, inhibition of DNA, protein, or enzyme synthesis, photocatalytic reactive oxygen species (ROS) production, damaging cellular and viral components, and disrupting biofilms (Figure 2).[2c−e] NPs help lower the cost, overcome

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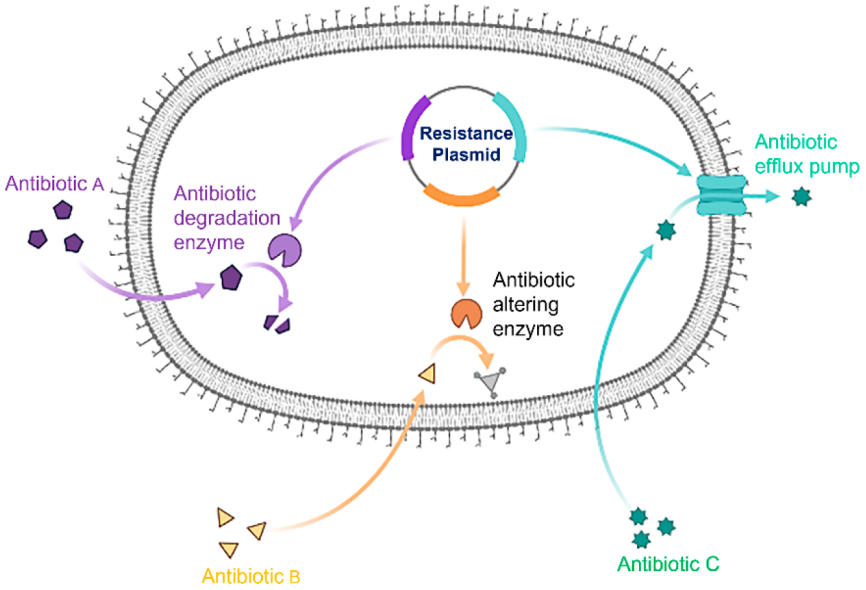

Figure 1. Mechanisms of development of antibiotic resistance. It develops by a variety of methods, including the breakdown of medications through the development of an enzyme that renders them inactive, a reduction in the uptake of antibiotics, a degrading enzyme, a shift in typical metabolic pathways, the extrusion of pharmaceuticals outside the cell by an efflux pump, and a change and alteration in antibiotic target. Created with BioRender.

resistance, and reduce toxicity compared to conventional antibiotics. In addition, the efficacy of the NPs can be improved manifold by encapsulating them in a safe drug carrier. Hence, the need of this hour is to protect the nanomaterials or the antibiotics inside safe carriers to improve their therapeutic and pharmacokinetic profiles by limiting drug degradation, increasing accumulation at infection sites, and reducing toxicity.

Recent developments in this area have made it possible to create drug delivery vehicles with improved pharmacokinetic and antibacterial properties.[3a−c] These biomedical nanotechnology systems offer high potential to significantly enhance the efficiency of currently available antibiotics and can dramatically increase the therapeutic efficacy of traditional metallic NPs.[3d] Among the wide array of drug delivery systems currently being researched within the diverse range of nanoplatforms, liposomes are one of the most promising antibiotic delivery systems.[3e,f] These lipid-based nanosystems were first used as drug carriers in 1970s; since then significant advances in liposome technology have increased interest in their application as effective antibacterial drug delivery systems.[4] The liposome-based system provides the most effective method of addressing multidrug-resistant bacteria since they act as carriers for natural antibiotics to actively combat bacterial infections. Moreover, loaded liposomes have the capacity to safely and effectively deliver a wide variety of cargos to the site of infection when they are bonded to their surface or enclosed within a structure.[5] Thus, antibiotic-encapsulated liposomes or nanoparticle-encapsulated liposomes (lipid nanoparticle) could be a promising alternative to combat multidrug-resistant infections and eradicate biofilms.

The main objective of this minireview is to emphasize the benefits of NPs over conventional antibiotics and highlight the advantages of using liposome-based antibiotic/nanomaterial delivery systems and their potential to combat antibiotic resistance, for selectivity toward diseased cells over normal cells, for limiting drug degradation, and for minimizing toxicity. Hence, in the first part of this minireview, we discuss antibacterial resistance and how NPs are potent candidates to combat infectious diseases. Next, we focus on liposomes and liposome-based antibiotic delivery. At the end, we present the future outlook on liposome-based NPs delivery, an emerging field in biomedical research.

MECHANISM OF ANTIBACTERIAL RESISTANCE

The success of preventing and treating infectious diseases as well as other diseases like cancer has been threatened by antibacterial resistance. Due to multidrug resistance several medical procedures, including major surgeries and organ implants, are also facing uncertain outcomes. Numerous bacteria, including *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae*, and *Staphylococcus aureus* (*S. aureus*), have acquired multidrug resistance over time, making it difficult to treat them with over-the-counter antibiotics.[1b−e,2d] The fact that some bacterial strains can develop resistance to cutting-edge drugs like vancomycin worsens the healthcare burden caused by drug-resistant bacteria.[1b] Antibiotics can be rendered inactive by bacteria via a variety of molecular mechanisms. They have been schematically represented in Figure 1. Some of the mechanistic pathways have been discussed below:

1. Inactivation by an enzyme. Bacteria produce specific enzymes that specifically inactivate the antibiotic, rendering it incapable of performing its biological role. For instance, this happens when lactamases break down lactam medications. Some bacteria develop extended-spectrum lactamases (ESBLs), which have the same inactivating activity and make them challenging to eliminate. Additional enzymes that can render certain antibiotics inactive include acetyltransferase, phosphotransferase, and adenyl transferase.[6a,b]

2. Alteration in the antibiotic target. Methylation of an adenine residue in the peptidyl transferase of r-RNA 23S diminishes the enzyme's affinity for the antibiotic without affecting protein production in the case of erythromycin resistance. Another important case involves the modification of

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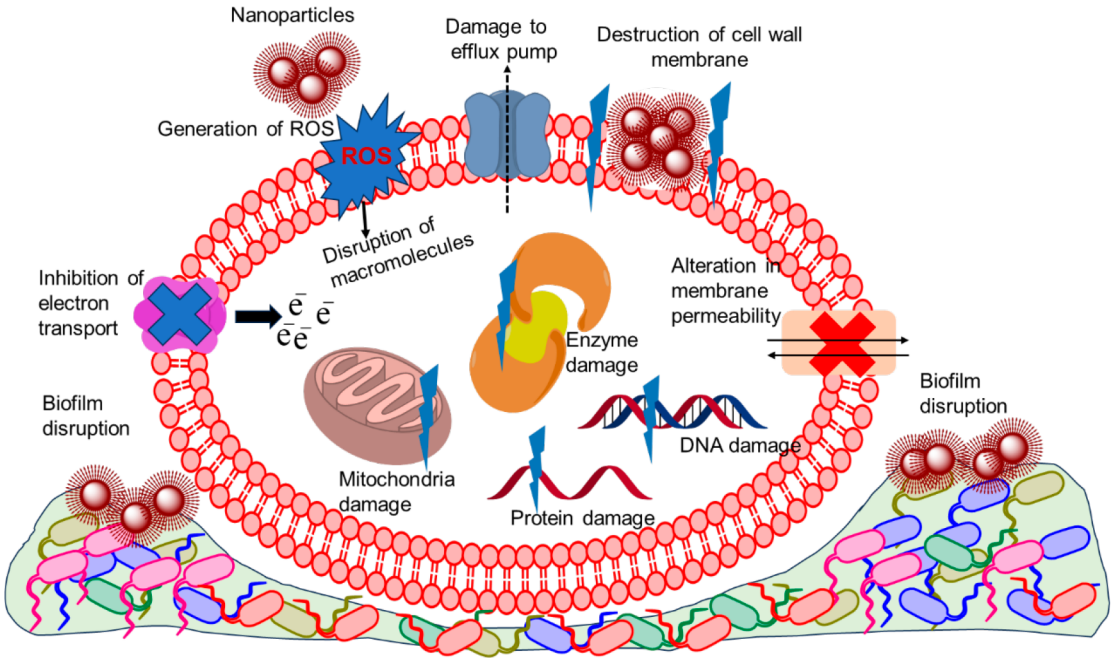

Figure 2. Mechanism action of NPs. They function by destroying the bacterial cell wall and membrane, producing excessive levels of reactive oxygen species (ROS), which cause oxidative stress and harm important intracellular macromolecules. They also alter membrane permeability; damage proteins, enzymes, mitochondria, and DNA; prevent drug extrusion by damaging efflux pumps; inhibit the electron transport chain; and prevent and disrupt biofilm.

penicillin-binding proteins (PBPs) by *methicilin resistant staphylococcus aureus* (*MRSA*).[7]

3. Efflux of drug. Energy-driven drug efflux mechanisms remove antibiotics that have been absorbed by bacterial cells through the extrusion of the drug outside the cell. Antibiotic-resistant bacteria frequently exhibit increased efflux pumps.[8a,b] Zhang et al. described a novel efflux pump in *Pseudomonas aeruginosa* named PA1874-1877, the expression of which was higher in biofilm than during planktonic growth. This pump appears to be involved in biofilm resistance to ciprofloxacin, gentamicin, and tobramycin.[8c]

4. Decrease in antibiotic uptake. Activation of alternative metabolic pathway via changing in structural architecture in cell surfaces can hinder antibiotic entry.[9a] For instance, a change or reduction in the number of porins or porin gene mutation may cause the resistance in Gram-negative bacteria.[9b]

5. Resistance gene transfer. Resistance genes spread among bacterial populations as a result of bacterial communication and genetic information sharing.[10]

6. Biofilm formation. Some antibiotics, such as aminoglycosides, are unable to penetrate the protective extracellular polymeric components of bacterial biofilms because of electrostatic repulsion.[11a,b] There are many causes of drug resistance in biofilms, including decreased drug absorption across the extracellular polymeric matrix, lower intracellular drug level, decreased bacterial metabolism, and the transfer of resistant genes.[11c,d]

NPS AS ANTIBACTERIAL AGENTS

Antibiotics based on nanomaterials have been shown to be a very effective alternative to prevent antibiotic resistance.[1] Small size, vast surface area, and the highly reactive nature of the nanoparticles allow them to pass through biological barriers like biofilm and have a high selectivity for bacterial cells.[2b] NPs have a larger surface area, which facilitates drug loading, and their small size enables them to pass through biofilms and microbial cell walls. Additionally, NPs have fast kidney excretion and lengthy plasma half-lives.[12a,b] Moreover, the methods used to synthesize NPs and the conditions under which they are synthesized, such as reducing agents, temperature, concentration, and solvent, essentially determine their biological behaviors.[12c] Due to the development of antibiotic resistance by various life-threatening bacteria, metal and metal oxide nanoparticles provide a new avenue for research in the fight against infectious diseases. Naturally existing bacteria have not yet evolved resistance to these nanoantibiotics, which is a great benefit. Human cells are not exposed to any immediate and severe side effects from them.[12d]

The most common NPs involve metallic NPs including silver, gold, copper and copper oxide, iron oxide, nitric oxide, aluminum oxide, titanium oxide, zinc oxide, magnesium oxide, molybdenum oxide, and molybdenum sulfide.[13a−c] Of all the NPs, Ag NPs are the most researched and used for antibacterial efficacy.[13a] Further, two-dimensional (2D) materials have proved to be highly beneficial in this area due to the distinct physical and chemical characteristics that result from their two-dimensionality.[13d] Layered material exhibits novel properties different from its bulk counterpart when thinned to its physical limits. Therefore, these materials are considered 2D materials at the physical limit. Graphene is the most highly studied 2D material because of its exceptional electronic, optoelectronic, electrochemical, and biomedical applications. Beyond graphene, there is a wide spectrum of 2D electronic materials including molybdenum disulfide (MoS_2).[13e] Two-dimensional materials, such as graphene oxide, various graphene derivatives, ce-MoS_2, and $Ti_3C_2T_x$, have been proven to have greater antibacterial characteristics than the small-molecule-based antibiotics currently in use.[13f] De and co-workers have extensively worked on various 2D NPs such as graphene oxide, MoS_2, Fe_2O_3, etc. to enhance

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bactericidal activity.[14a−d] They also demonstrated that ce-MoS_2 can be functionalized with several thiol ligands to vary the surface's charge and hydrophobicity and examined its effectiveness against various Gram-positive and Gram-negative bacteria.[14e]

MECHANISTIC ACTION OF NPS TO COMBAT ANTIBACTERIAL RESISTANCE

Nanomaterials can use a number of bactericidal strategies to fight bacteria, including the generation of reactive oxygen species (ROS), the rupturing of cell walls and membranes, the distribution of drugs via membrane fusion, and interacting with intracellular components (such as DNA and ribosomes) (Figure 2). It has been found that the distinctive physicochemical properties of nanomaterials, particularly their interaction with bacterial cells, depend on a variety of factors, including van der Waals forces, receptor−ligand interactions, hydrophobic interactions, and electrostatic attractions. The various approaches to combating antibacterial resistance have been discussed below.

1. Disruption of cell wall and cell membrane. Microorganisms have evolved a physical defense against antibiotics in the form of their cell membranes. Both lipopolysaccharides, present in the outer membrane of Gram-negative bacteria, and teichoic acids, present in the cell walls of Gram-positive bacteria, include phosphate or carboxyl groups that make the surfaces of the bacteria negatively charged. The capacity of hydrophobic antimicrobials to enter membrane is hampered in this highly polar environment, which reduces their effectiveness against bacteria.

Further, electrostatic adsorption on the cell wall causes membrane depolarization, a reduction in membrane permeability, and a loss of membrane fluidity, which disrupts energy transfer and causes cell death.[1]

In addition, an accumulation of NPs results in the formation of "pits" in the bacterial cell wall. As a result, they have the ability to enter cells, alter cell membranes, and result in structural damage and cell death.[2a,b] By interacting with the negative charges on the surfaces of the bacteria (via carboxyl or phosphate groups), the positively charged NPs exhibit a stronger bactericidal effect.[2a,b] However, due to the strong peptidoglycan coating on Gram-positive bacteria, it is more difficult for NPs to penetrate them.[1b]

2. Generation of reactive oxygen species (ROS). ROS are byproducts of oxidative metabolism. They affect the growth, signaling, survival, and demise of cells.[15a] Additionally, they possess a high positive redox potential. Superoxide radicals ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), hydroxyl radicals ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2) are examples of ROS. Various NPs produce different combinations of ROS, each with a unique antibacterial property. ROS are produced when the respiratory chain is disrupted or when NPs are present.[15b,c]

3. Damage of intracellular components. For bacteria to survive and function, cellular homeostasis and intracellular signaling pathways are crucial. Cell death may occur when NPs are created to block these pathways. Modifications to protein synthesis, DNA damage, and changed gene expression are a few of these disruptions.[2]

4. Disruption of biofilms. NPs can destroy biofilms due to their powerful penetrating ability. They penetrate thick biofilm (extracellular polysaccharide matrix) layers, resulting in bacterial death. Only cationic-charged NPs can penetrate a biofilm and interact with the microorganisms inside.[13a,15d] NPs have the ability to interact with bacteria and have an antibacterial effect when they enter biofilms. As demonstrated in earlier studies, a high local drug concentration at the infection site, controlled drug release, and negligible drug degradation were all made possible by liposomal delivery systems, in which the antibiotic is encapsulated into the liposome.[15e]

Even though metal-based NPs (inorganic) are much more advantageous over conventional antibiotics in terms of developing antibiotic resistance, there are a few challenges to be taken care of while handling these metal-based NPs.[13a−c] NPs must overcome some obstacles, such as interactions between NPs and cells, tissues, and organs; the ideal dose; the choice of the best administration routes; the toxicity of both short- and long-term exposures; and the precise mechanism of cellular uptake. Furthermore, NPs can accumulate in the spleen, lung, and bone marrow when given intravenously. Therapeutic NP administration may result in multiorgan nanotoxicity. In all harmful circumstances, NPs are connected to oxidative stress, which in turn produces liver and lung toxicities as well as metabolic changes such as oxidation of fatty acids, decreased ketogenesis, and glycolysis, which happen through ROS and may be connected to hepatotoxicity and nephrotoxicity.[1b,e,15d]

LIPOSOMES AS THE MOST PROMISING ALTERNATIVE

The activity of NPs can be significantly enhanced by coating or coupling them with additional substances. Using NPs with antibiotics can aid in reducing bacterial resistance. NPs may encapsulate antibiotics or be coated with them to keep antibiotics from being broken down by chemicals and enzymes.[3d,5b] The therapeutic potency of a drug can be increased dramatically when it is trapped inside NPs. Dosage must be decreased to enhance therapeutic results and lessen host hazard.[15f] By tailoring cargo release and delivering drugs with several modes of action, nanocarriers can lessen the selection of resistance in bacteria. This prevents bacteria from being exposed to less-than-minimal inhibitory levels of the drug. Additionally, bacterial cell wall penetration is made simpler when they act as antibiotic carriers. The antibiotic subsequently weakens the cell wall, making it simpler for the NPs and their complex to enter the body.

Numerous nanosized delivery systems have been developed with improved therapeutic properties due to having their characteristic features such as controlled release, decreased systemic toxicity, drug targeting, and higher efficiency.[4] Examples include liposomes, dendrimers, polymeric NPs, carbon nanotubes, etc. Lipid-based nanosystems like liposomes have shown particularly appealing features in terms of physicochemical properties and safety issues among these nanotechnology based techniques. Liposomes are vesicular structures with concentric bilayers that are mostly made of hydrophilic and hydrophobic layers. Due to having their unique abilities to encapsulate both hydrophilic and hydrophobic drugs, added biocompatibility, biodegradability, low toxicity, and absence of immune system activation, they have a number of benefits over the other available delivery systems.[3d,5a,b] Additionally, liposomes can be easily conjugated with targeting platforms, such as antibodies, proteins, or enzymes, enabling the administration of a particular substance for targeted delivery enabling them as potential candidates for targeted antibiotic delivery (Figure 3).

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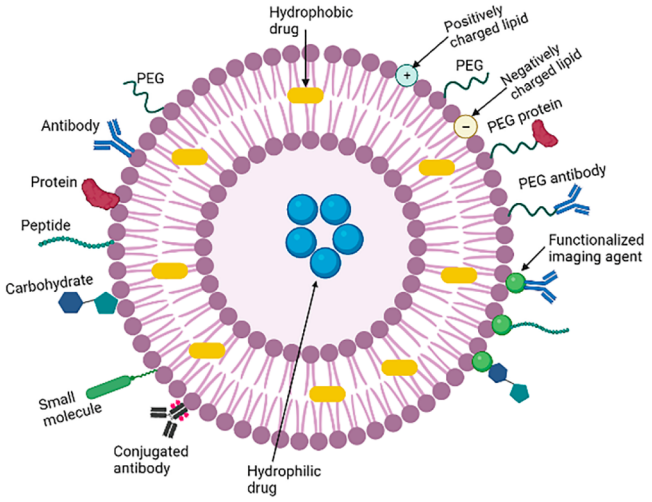

Figure 3. Schematic representation of different types of liposomes and their various functions. A traditional liposome is made up of a lipid bilayer with an aqueous core, and the surface can be anionic, cationic, or neutral. Hydrophobic or hydrophilic drugs can be entrapped in the lipid bilayer and the aqueous core, respectively. Liposome properties and behavior can be changed by adding a hydrophilic polymer coating, such as PEG, to the liposome surface. Ligand-targeted liposomes can be made by conjugating various ligands, such as antibodies, peptides, carbohydrates, protein, etc., to the liposome's surface or to the end of the connected PEG chains. Created with BioRender.

STRUCTURE AND PROPERTIES OF LIPOSOMES

Liposomes are small, spherical vesicles composed of one or more phospholipid bilayers surrounding aqueous compartments or units.[5b] Liposomes are commonly categorized according to their size, lamellae, and manufacturing process. They may have one lipid bilayer (multilamellar vesicles, MLVs) or a single lipid bilayer (unilamellar). Unilamellar vesicles can also be divided into three different sizes: small unilamellar vesicles (SUVs), which have diameters of 25−100 nm; large unilamellar vesicles (LUVs), which have diameters of 100−400 nm; and giant unilamellar vesicles (GUVs), which have diameters of more than 1 μm.[5c] Additionally, there are multivesicular vesicles that house smaller vesicles.[5c] Different applications benefit from different liposome types. The charge and composition of the lipids are essential features since they determine the fluidity and stability of the liposomal membrane and impact the liposome−bacteria interaction. The versatility of liposomes for surface modification is a key quality that elevates them to enhance antibacterial efficacy dramatically. Surface functionalization with ligands, such as polymers (such as PEGylated liposomes) and molecules (such as antibodies, proteins/peptides, and carbohydrates), is used for specific targeting (ligand-targeted liposomes) and is crucial for effective delivery and therapeutic efficacy (Figure 3).[16a,b] Liposomes have been used as antimicrobial agents since 1995 when the FDA approved Doxil (doxorubicin liposomes) as the first liposomal delivery system to treat AIDS associated Kaposi's sarcoma.[16a]

ADVANTAGES OF LIPOSOMES AS ANTIBIOTIC CARRIERS

Recent advancements in liposomal formulations have made it possible to establish viable platforms for the administration of antibiotics, potentially resolving important problems in the treatment of infectious diseases. As previously mentioned, liposomes have a number of benefits as antibiotic delivery nanosystems, resolving issues with drug efficacy or the choice of resistant strains.[5] Several studies have demonstrated that liposomal encapsulation enhances the stability and safety of antibiotics, resulting in more appropriate pharmacokinetic and pharmacodynamic profiles by prolonging the bloodstream's circulation time and enabling targeted administration to specific infection sites via various routes (Figure 4).[5f,16b] Indeed, a number of investigations have demonstrated liposomal antibiotic formulations that have increased efficacy even against resistant bacteria.[5d] Here lies the added advantage of using liposome-based NPs (organic NPs) over free metal or metal oxide NPs (inorganic NPs). Liposome can bypass the resistance mechanism of decreased uptake of antibiotic/drug. A larger intracellular concentration of the drug and faster delivery are additional benefits of liposomes.[16a] This drug

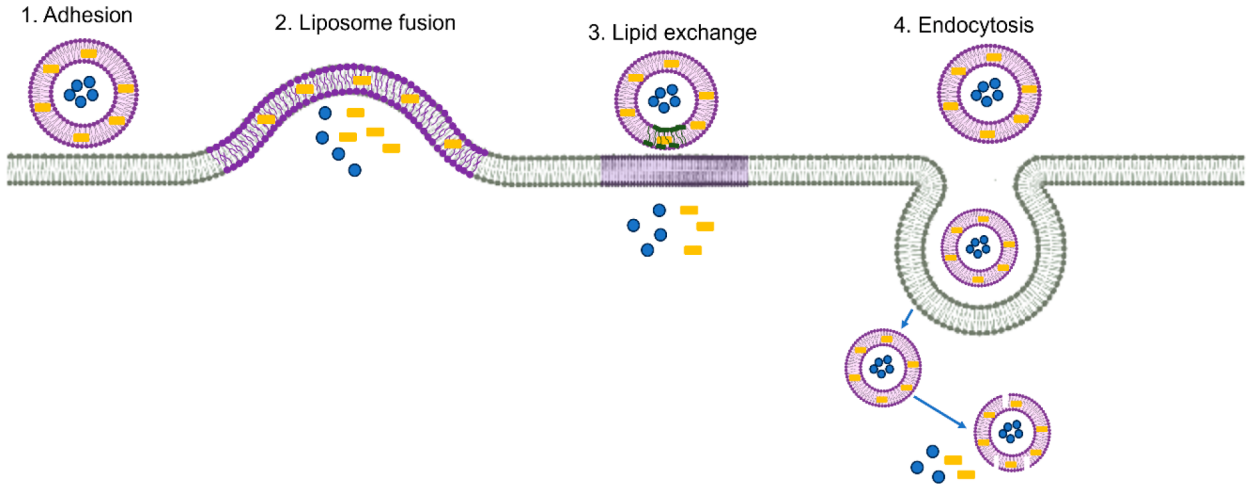

Figure 4. Schematic representation of antibacterial resistance mechanisms by antibiotic-loaded liposomes. Interaction of liposomes with the lipid bilayer of eukaryotic cells. It can happen via adhesion when electrostatic forces cause the liposomes to be drawn to the cell membrane, ultimately encouraging the release of the cargo inside. It can also occur via fusion, which combines the lipids in the liposome with those in the cell membrane. Next, lipid exchange takes place when the lipids from the liposome and those from the cell membrane transfer exchange among them.

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Table 1. FDA-Approved Liposome-Based Antibiotic Delivery Systems[a]

drug name	targeted pathogen	delivery system	ref
amikacin	*P. aeruginosa*, *S. aureus*, MAC, *M. tuberculosis*	DPPC, Chol	19a
gentamicin	highly gentamicin-resistant mucoid and nonmucoid clinical strains of *P. aeruginosa*	DMPC, DPPC, DSPC, Chol	19b
meropenem	*P. aeruginosa*	DMPC, Chol	19c
ampicillin	*L. monocytogenes*, *Helicobacter pylori*, *Salmonella enterica* serovar Typhimurium	PCT-LC, DDAB, Chol	19d
ciprofloxacin	*Francisella tularensis*, *S. aureus*, *P. aeruginosa*	SM, Chol	19e
aminoglycosides	*Burkholderia cenocepacia*	DSPC, Chol	20a
rifampicin	resorcinomycin A *Mycobacterium* spp.	SPC, Chol	20b
resorcinomycin A	*Mycobacterium* spp.	DMPC, PI, P-NBD-PC	20c
clarithromycin	*P. aeruginosa*, MAC, *H. pylori*	DPPC, Chol, NBD-PC	20d
polymyxin B	*P. aeruginosa*	DPPC, Chol	20e
		POPC, Chol	
ticarcillin	*P. aeruginosa*	egg lecithin, Chol	20f
daptomycin	*S. aureus*	SPC, sodium cholate	21a
doxycycline	*H. pylori*	egg PC	21b
epigallocatechin gallate	MRSA	egg lecithin, Chol	21c
isoniazid	*M. tuberculosis*	SLPC, POPC, Chol	21d
azithromycin	*Mycobacterium avium*−*Mycobacterium intracellulare* complex (MAC)	EPC, EPG, HSPC-3	21e
sparfloxacin	MAC	DPPG, DPPC, Chol	22a
ofloxacin	MAC	DMPC, Chol, DP	22b
gentamicin	*Salmonella* spp., *Listeria monocytogenes*, *P. aeruginosa*, *Brucella abortus*	DMPC, Chol	22c
tobramycin	*Burkholderia cepacia* complex, *P. aeruginosa*	DPPC, DOPG, DOPC	22d
vancomycin	MRSA	DOPE, DPPC, CHEMS, DSPE-PEG	22e
meropenem	*P. aeruginosa*	PC, DOTAP, Chol	23a
tetracycline	*Chlamydia trachomatis*	DPPC, DSPC, Chol	23b
colistin	*Mycobacterium* spp.	DOPC, Chol	23c
levofloxacin	pulmonary infection	DPPC, Chol	23d
streptomycin	*Salmonella enteritidis*, *M. avium* complex	DPPC, Chol	23e
norfloxacin	Enterobacteriacea	PCT2−EPC, Chol, tocopherol	24a
piperacillin	*S. aureus*	PC, Chol	24b
cefepime	Enterobacteriacea	EPC, Chol	24c

[a]DOPC, dioleoylphosphatidyl choline; DSPG, distearoylphosphatidyl glycerol; DOPG, dioleoylphosphatidyl glycerol; DPPC, dipalmitoylphosphatidyl choline; SPC, soybean phosphatidyl choline; DPPC, dipalmitoylphosphatidyl choline; DSPE, distearoylphosphatidyl choline; Chol, cholesterol; DOPE, dioleoylphosphatidyl ethanolamine; POPC, palmitoyloleoylphosphatidyl choline; DMPC, dimyristoylphosphatidyl choline; EPC, egg phosphatidyl choline; PCT, pectin from apple; PCT1, pectin from apple, found in the aqueous phase that surrounds the liposomes; PCT2, pectin from apple, distributed in the water phase inside and outside the liposomes; DPPS, dipalmitoylphosphatidyl serine; DP, dihexadecyl hydrogen phosphate; DPPE, dipalmitoylphosphatidyl ethanolamine; DPPA, dipalmitoyl phosphatidic acid; EPG, egg phosphatidyl glycerol; HSPC-3, hydrogenated soybean phosphatidyl choline; SLPC, monoacyl soybean phosphatidyl choline; PEG, polyethylene glycol; PC, soybean phosphatidyl choline; DSPC, distearoylphosphatidyl choline; SM, egg sphingomyelin; DMPG, dimyristoylphosphatidyl glycerol; DSPE, distearoylphosphatidyl ethanolamine; DODAB, dioctadecyldimethylammonium bromide; DSPE, distearoylphosphatidyl ethanolamine; CHEMS, cholesteryl hemisuccinate.

concentration is sufficient to overwhelm transmembrane pumps that catalyze enhanced drug efflux out of the bacterial cell by saturating them. Liposomes thus also circumvent the resistance mechanism of enhanced drug efflux. Finally, incorporating antibiotics into liposomes increases their antibacterial activity, causing the bacterium to be killed before it can generate resistance toward the antibiotic. For instance, it has been found that the MIC (minimum inhibitory concentration) of liposomal ciprofloxacin and gentamicin is lower than that of the free drug against the majority of common resistant bacteria, including *P. aeruginosa*, *K. pneumoniae*, and *E. coli*.[17] The authors of these investigations proposed that the effective and comprehensive contact of the liposomes with the bacterial cell's outer membrane was the cause of the formulations' improved antibacterial efficacy.

Moreover, liposomes can safely encapsulate antibiotics with various physical and chemical properties, enhancing the stability and solubility of the antibiotic. This is possible due to their distinct structural qualities. Additionally, liposomes can enhance the therapeutic index of the encapsulated medicines by improving their pharmacokinetic characteristics and biodistribution. This means that, compared to a standard formulation, liposomes can carry more antibiotics to the targeted tissues at the same dose, minimizing drug accumulation in healthy tissues, consequently minimizing adverse drug reactions and improving the patient's quality of life.[16b]

MECHANISTIC PATHWAY TO OVERCOMING BACTERIAL RESISTANCE BY ANTIBIOTIC-LOADED LIPOSOMES

The fundamental mechanisms by which liposomes interact with the bacterial cell are adsorption, fusion, lipid exchange, and endocytosis (Figure 4).[16b] For instance, liposomes may bind to the surfaces of cells through ligand/receptor or electrostatic interactions, and the lipid components of cell membranes and liposomes may exchange ions and fusion into the cells. As an alternative, cells can consume liposomes by phagocytosis, and the lysosomes and phagosomes that result from this process can combine to generate secondary

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lysosomes.[18a] The drugs that are entrapped in the liposomes are released as they break down.

Recent research indicates that encapsulating antibiotics into liposomes may help combat various bacterial resistance mechanisms by modifying the interactions between liposomes and bacteria.[2d,4b] Gram-negative bacteria's outer membrane is a complex barrier that can restrict the internalization of antibiotics or alter how they interact with the bacterial wall, serving as a key source of resistance. However, as was already indicated, liposomes have the potential to stimulate fusion with bacterial membranes, hence encouraging structural disturbance and perhaps increasing permeability.[16b] This fusion process can be improved further by increasing the fluidity of liposomes or, as previously mentioned, by adding fusogenic phospholipids to them.[18a] A few illustrations of authorized liposomal compositions to combat various bacterial infections are shown in Table 1.

Another possible strategy to circumvent nonenzymatic drug resistance is liposome−bacteria fusion.[18b] This topic has received special attention since *P. aeruginosa* strains have resistance mechanisms that are primarily characterized by low and nonspecific permeability of their outer membranes and/or efflux pump systems.[18b] Thus, one of the most effective impermeable barriers causing bacterial resistance was overcome using liposomal formulations.

Liposomal antibiotics also effectively overcame bacterial resistance from enzymatic hydrolysis.[18c] Although less research has been done on this strategy. Nacucchio et al.[24b] showed that piperacillin can be protected from staphylococcal lactamases by being enclosed in phosphatidyl choline and cholesterol liposomes. As a result, the antibiotic can retain its antibacterial activity. Since enteric rods' resistance mechanisms are frequently enzymatic, it would be interesting to investigate the construction of liposome-encapsulated antibiotics with special characteristics to avoid enzymatic breakdown.[18b]

The improved liposome impact has also been established for multidrug-resistant intracellular infections like *Mycobacterium tuberculosis*. It is well-known that *M. tuberculosis* can cause a long-lasting infection in people, mostly due to their capacity to infect and survive in macrophages, which makes eliminating this bacteria more difficult. Due to their innate propensity to be ingested by macrophages, liposomes represent a possible treatment for this specific form of infection.

In the end, liposomes may be a disruptive strategy for bacterial biofilms associated with medical devices. Due to conventional antibiotics' low penetration in the extracellular matrix, biofilms alone serve as a resistance mechanism. If the biofilm contains a multidrug-resistant type of bacteria like MRSA, the infection may become chronic and perhaps incurable. However, research conducted in vitro and in vivo has shown that liposomal formulations are more effective against MRSA infections linked to biofilms.[18d,e] In an *S. aureus* osteomyelitis model, a liposomal formulation coloaded with vancomycin and ciprofloxacin enabled full bone sterilization, demonstrating this approach's tremendous therapeutic potential against these fatal infections.[18d]

CHALLENGES ASSOCIATED WITH NANOLIPOSOME-BASED ANTIBIOTIC DELIVERY

Although there are now a large number of liposomal treatments on the market, developing successful liposomal drugs still poses a significant difficulty. To develop an effective liposomal formulation, it is necessary to optimize a number of different factors simultaneously. The following discussion addresses some of the field's unresolved fundamental problems.

It is possible to manufacture liposomes using a variety of techniques, including injection, film dispersion, ultrasonic dispersion, freeze-drying, high-speed shearing, and extrusion.[16,17] The process frequently involves employing various techniques, necessitating a multistep procedure. Maintaining quality control across batches for factors such as antibiotic release behavior, surface charge, and particle size and size distribution is challenging. One of the main challenges is the difficulty of encapsulation of antibiotics. For the encapsulation of a particular antibiotic, many publications claimed that various procedures were most effective; however, the encapsulation efficiencies were determined using multiple formulas, which led to bias in the methodologies, yielding an erroneous outcome.[25a]

Liposomes maintain a more complex fate in comparison to free antibiotics. However, it is still not much clear what happens to liposomes in vivo. The dynamic process of the "three elements" (antibiotic, liposomes, and material) and additional supporting elements including ligands, surfactants, and stabilizers is currently poorly understood.[25b] For instance, there is still no consensus on the crucial issues of when and how antibiotics are released from liposomes in vivo. The design of liposomes will benefit scientifically from a thorough analysis of the biological fate, which can also hasten the clinical transformation process.

Liposomes are often modified with functional molecules (such as sugars, peptides, and antibodies) to get across physiological delivery barriers (such as the blood−brain barrier) via ligand/receptor contact to achieve the active targeting function.[25c] Preclinical research frequently shows encouraging findings, but there is a significant gap between preclinical and clinical applications. Although active targeting liposomal technology has been extensively studied, successful clinical translation has not yet occurred.[25d]

Hence, despite significant progress in liposomal anticancer therapy, liposomal antibiotic delivery systems must optimize various factors to achieve a high therapeutic index.

FUTURE OUTLOOK AND CONCLUSIONS

Due to the advent of microorganisms resistant to the currently available antibiotics, modern medicine is confronted with a significant challenge in treating bacterial infections. To solve this issue, a lot of research is going on to create new NPs and antibiotic delivery methods to increase their antibacterial activity. Liposomes have emerged to be the most promising delivery nanoplatforms. Their broad structural and lipid composition variability enables the possibilities of various liposomal formulations with enhanced pharmacokinetic and pharmacodynamic features. Additionally, they can deliver the antibiotic directly to the infected site, tissue, or pathogen in a controlled and sustained manner, preventing premature enzymatic and immunological inactivation, limiting its distribution to healthy tissues, and minimizing potential side effects. Furthermore, the lipid bilayers of liposomes may provide direct contact or fusion with bacterial cell walls, raising antibiotic concentration within the bacteria and thus enhancing the loaded antibiotic's therapeutic effect. Again, liposome-encapsulated antibiotics have been found to be effective in overcoming enzymatic degradation, efflux mechanisms, and impermeable outer membranes as microorganism

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resistance mechanisms. Thus, in conclusion, liposomes provide an incredibly promising approach to establish therapeutic options against currently multidrug-resistant bacteria. Keeping in mind the challenges associated with liposomal drug delivery systems, the future of this promising approach lies in the developing of more effective methods for creating liposomal nanoparticles, which would have the greatest potential for effective and selective targeting of antibiotics to bacteria that are resistant to currently available drugs for eradication as well as the minimum toxicity for the normal cells. Controlling the antibiotic release rate can also increase the efficacy of liposomal nanoparticles by ensuring that the drug is released from the liposomes quickly enough at the target site. Recently, metallic NP encapsulated liposome systems started gaining significance dramatically because of the synergistic effects of metallic NPs and liposomes. Hence, we believe that small molecule or metallic NP-loaded liposomes could be the best therapeutic options to combat multidrug-resistant bacteria and eradicate biofilms in the coming days.

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The authors declare no competing financial interest.

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Mrinmoy De received his MSc from IIT—Bombay (India) and his Ph.D. from the University of Massachusetts at Amherst under the supervision of Prof. Vincent M. Rotello. He was a CCNE and NSEC postdoctoral fellow at Northwestern University. Since 2014, Prof. De has been at the Indian Institute of Science, Bangalore, where he is now an associate professor in the Department of Organic Chemistry. His research focuses in preparing various nanomaterials including lipid nanoparticles and their biological applications in diverse directions.

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