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Soft Nanocarriers as Antimicrobial Co-Delivery Systems to Combat Resistant Infections

*Anamarija Nikoletic, Evangelos Natsaridis, Timon Flathmann, Daiane P. C. Quadros, Nubia Gogniat, and Oya Tagit**

The rise of antimicrobial resistance (AMR) poses a global health challenge, driving the need for innovative solutions to effective treatment and prevention. The advancing field of nanotechnology has facilitated the development of nanocarriers based on polymer and lipid excipients with precisely controlled compositions, release mechanisms, and targeted biological interactions. These "soft" nanocarriers can be tailored to deliver multiple antimicrobial drugs with complementary mechanisms of action, offering an effective strategy to prevent and combat AMR. This review highlights the design and applications of nanocarriers as co-delivery systems, emphasizing their potential for optimized drug loading, spatio-temporal control of release, and pathogen-specific targeting. The unique benefits of diverse nanocarrier types, including lipid-based, polymer-based, and hybrid systems, are covered with a comprehensive set of examples from recent literature in multidrug encapsulation and synergistic antimicrobial therapies. The review also delves into the key challenges in achieving precise control over the sequence and rate of release, ensuring pathogen specificity, and overcoming biological barriers such as biofilms, and provides perspectives on future research efforts to advance nanocarrier engineering and optimization strategies to develop robust, targeted treatments capable of addressing the urgent threat of resistant infections.

1. Introduction

The alarming rise in antimicrobial resistance (AMR) presents a global risk to public health. The emergence and spread of resistance to conventional antimicrobials due to incorrect, prolonged, and widespread use of antibiotics poses a significant challenge to treat a growing list of infections.[1] As the development of new antimicrobials languishes, clinically effective alternative treatment strategies are urgently needed.

Several mechanisms acquired by pan-resistant bacteria to evade the effects of widely used antibiotics have rendered commonly available antibiotics ineffective (**Figure 1**).[2] A key resistance mechanism involves the production of enzymes that inactivate antibiotics either by breaking them down, such as β-lactamases, or by chemically modifying them, including aminoglycoside-modifying enzymes and chloramphenicol acetyltransferases.[3,4] Another mechanism involves changes to the drug's target inside the bacterial cell. For example, in methicillin-resistant *Staphylococcus aureus* (MRSA), a modified protein PBP2a prevents β-lactam antibiotics action, and in macrolide resistance, certain genes modify the ribosomal RNA to block drug binding. Bacteria also use efflux pumps to remove antibiotics from the cytosol before they can act, which is seen in multidrug-resistant strains such as *Acinetobacter baumannii*.[3] Some bacteria reduce the amount of antibiotic that enters the cell by changing the structure or number of porins, which are proteins that help drugs pass through the outer membrane. This mechanism is especially common in Gram-negative bacteria

A. Nikoletic, E. Natsaridis, T. Flathmann, D. P. C. Quadros, N. Gogniat, O. Tagit
Group of Biointerfaces
Institute of Chemistry and Bioanalytics
University of Applied Sciences and Arts Northwestern Switzerland
Hofackerstrasse 30, Muttenz 4132, Switzerland
E-mail: oya.tagit@fhnw.ch

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A. Nikoletic
Department of Chemistry
University of Basel
Mattenstrasse 24a, Basel 4058, Switzerland
A. Nikoletic, T. Flathmann, O. Tagit
Swiss Nanoscience Institute
University of Basel
Klingelbergstrasse 82, Basel 4056, Switzerland
D. P. C. Quadros
Department of Chemistry
Federal Institute Catarinense
Schlosser 605, Brusque-SC, Hugo, Brazil

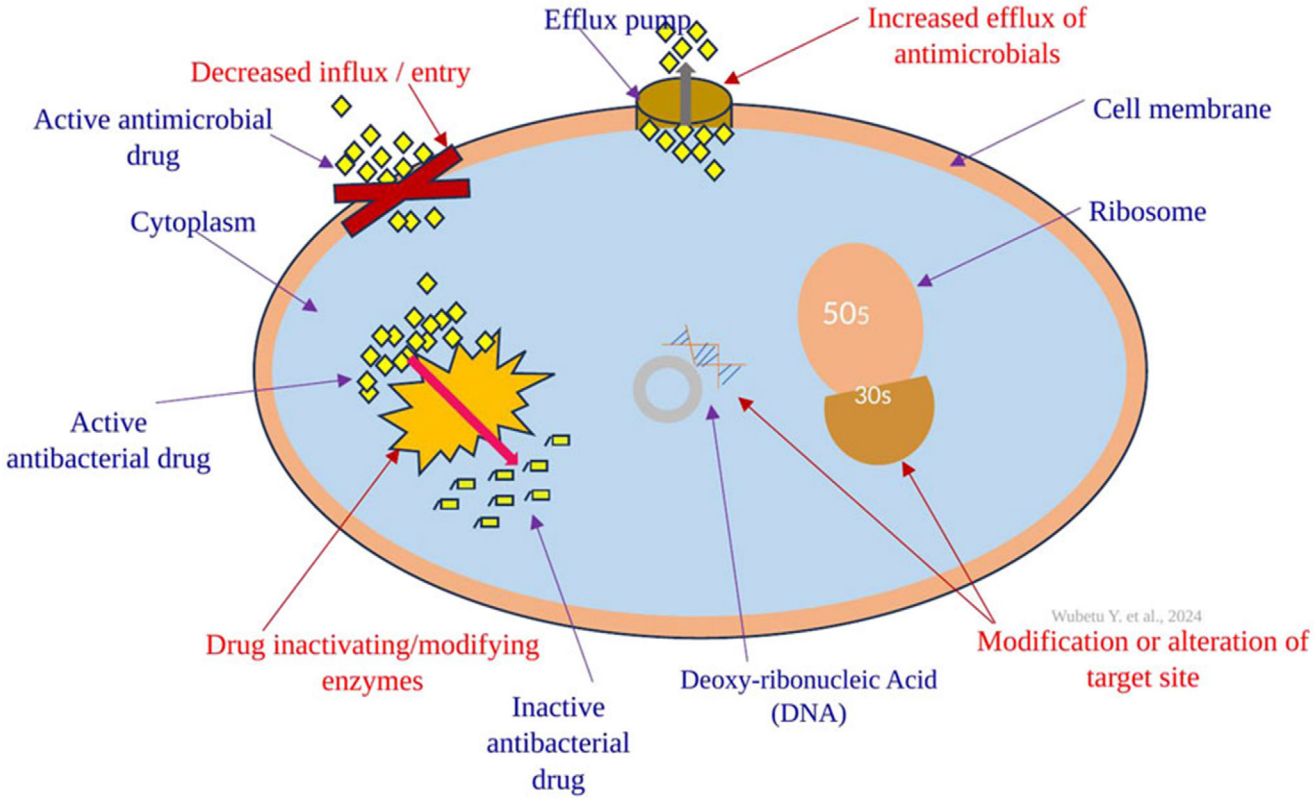

Figure 1. The primary mechanisms of antibacterial drug resistance include target modification (alteration) through, for example, ribosome alteration; restricting drug access using lipopolysaccharides (LPS) and porins; enhancing efflux via various efflux pumps (transporters); and modifying and inactivating the drug with different bacterial enzymes. Reprinted from,[3] CC BY Copyright 2024, Belay et al., Frontiers.

such as *Klebsiella pneumoniae*.[4] In other cases, bacteria can bypass the drug's effect entirely by using alternative pathways or altered target molecules. For instance, in vancomycin-resistant enterococci (VRE) the cell wall precursors are changed so the drug can no longer bind. Biofilms also play a role by forming protective layers that make it difficult for antibiotics to reach the bacteria and allow resistance genes to spread more easily between cells.[3] Horizontal gene transfer through plasmids or other mobile elements is another key mechanism, via which bacteria can acquire resistance.[4] Lastly, bacteria can temporarily and reversibly become resistant through adaptive changes triggered by environmental stress, such as low drug concentrations or changes in pH.[3] Together, these intrinsic and acquired resistance mechanisms make bacterial infections increasingly difficult to treat, and contribute to the global challenge of AMR.

One promising approach to overcome antibiotic resistance is combination therapy.[5] By using multiple drugs with complementary modes of action, combination therapy can target different pathways in bacterial cells to efficiently destroy infections. Such combinations can also prevent the development of resistance by making it more difficult for bacteria to adapt to multiple agents simultaneously. Some antimicrobial drug combinations, such as trimethoprim (TMP) and sulfamethoxazole (SMX),[6] are well-known for their synergistic activity, where the combined effect exceeds the sum of the individual drugs.[7] Such synergistic combinations can also broaden the antimicrobial spectrum, restore the efficacy of otherwise ineffective antibiotics, and counteract bacterial defense mechanisms such as efflux pumps and biofilms.[8] For instance, studies show that combining antibiotics with antimicrobial peptides (AMPs) can significantly enhance bacterial killing by promoting biofilm disruption and improved cellular uptake.[9] In addition to therapeutic benefits, effective synergistic therapies may lead to shorter treatment durations, potentially reducing hospitalization times and healthcare costs.

To achieve synergistic antimicrobial effects in patients, it is essential to first evaluate the synergistic potential of antibiotic combinations in vitro. To this end, several established methods have been developed to assess drug interactions and identify effective combinations.[10] However, it is not straightforward to reach synergistic dose ratios in vivo due to differences in solubility parameters and pharmacokinetics of different antibiotics. In this context, nanocarrier-mediated co-delivery of antibiotics can provide an efficient solution. Nanoencapsulation strategies allow for the controlled delivery of antibacterial agents in defined dose ratios directly at the infection site, improving bioavailability of drugs while minimizing systemic side effects.[11] The nanocarriers protect the drugs from hydrolytic or enzymatic degradation and thereby help reduce the overall administered dose. Furthermore, by precisely engineering the nanocarriers in terms of their size, surface, composition, and degradation profiles; targeted, sustained, and on-demand release can be achieved.[12] This approach could represent a transformative advancement in the fight against multidrug-resistant infections, where novel, adaptable treatments are urgently needed.

Over the past decades, "soft" nanocarriers made of polymer and lipid excipients and combinations thereof have emerged as promising vehicles for potentiating antimicrobial therapies. These versatile nanosystems can encapsulate and deliver multiple therapeutic agents, targeting both bacterial cells and biofilms, which are often more resilient to conventional treatments. Compared to "hard" nanocarriers that are often associated with poor degradability, potential toxicity, and limited flexibility in drug encapsulation, soft nanocarriers offer several distinct advantages that make them particularly well-suited for co-delivery applications. Their structural versatility allows for fine-tuning of drug release kinetics, size, surface charge, and responsiveness to external stimuli.[13] Such responsive behavior enables controlled and targeted release, which is crucial for maximizing antimicrobial efficacy and minimizing resistance development. Additionally, soft nanocarriers are typically composed of biodegradable and biocompatible materials,[14] minimizing systemic toxicity and the risk of long-term accumulation in vivo. They possess flexible membranes or matrices that can adapt to biological environments, improving circulation and tissue penetration.[15] Finally, soft nanocarriers can controllably interact with hard nanocarriers and develop new hybrid delivery systems with different properties.[13] Overall, these characteristics uniquely position soft nanocarriers as effective tools in treating resistant infections.

By leveraging co-delivery strategies, soft nanocarriers can simultaneously deliver antimicrobial drugs and adjuvants, boosting their efficacy and addressing bacterial resistance mechanisms such as efflux pumps and enzymatic degradation.[16] In this review, we highlight the potential of soft nanocarriers co-delivering antimicrobial agents to effectively eradicate infections. First, we elaborate on critical design parameters that need to be considered when engineering such nanocarriers, from drug loading ratios to spatial and temporal control of the release. Next, we give a comprehensive overview of nanocarriers for antimicrobial co-delivery based on different soft materials with recent examples from literature. Finally, we provide our perspective on the key challenges that need to be addressed to realize the enormous potential of these nanocarriers in combatting AMR.

2. Nanocarrier Design Considerations for Antimicrobial Co-Delivery

When designing nanocarriers for the delivery of drugs, several general properties of the delivery system must be carefully evaluated. Key factors typically include size of nanoparticles (NPs), their surface chemistry and charge, and the materials used for NP formation, all of which are extensively discussed in the literature.[17,18] However, when engineering nanocarriers specifically for antimicrobial co-delivery, there are other elements that need to be considered. Foremost, drugs will often have distinct physicochemical properties such as hydrophilicity, stability, and solubility, making their co-encapsulation within a single carrier challenging in many ways. Nevertheless, distinct therapeutics can be confined within the same nanocarrier through careful choice of materials comprising the delivery system and co-encapsulation approach.[19] Another important factor is the drug-to-drug ratio, as well as the concentration of each drug necessary to achieve the synergistic effects at the infected site. The ability to control the drug ratios is particularly important in combination therapies, where synergistic effects can be harnessed to enhance antimicrobial potency and reduce the likelihood of resistance development.[20] Additionally, the specific microenvironment of infection can be used as a stimulus to trigger the nanocarrier, giving control over the drug release. The infection microenvironment cues can also be exploited to appropriately functionalize the surface of NPs and achieve targeting.[21] These various considerations play a critical role when engineering a co-delivery nanocarrier for bacterial infections (**Figure 2**) and are discussed in detail in this section.

2.1. Drug Loading

The efficient loading of therapeutics is a crucial factor determining the efficacy of nanodelivery systems. One of the most straightforward and widely utilized methods for drug loading is physical encapsulation utilizing noncovalent interactions or the physical entrapment of drugs during the formation of the nanocarrier. Loading of hydrophobic drugs relies on the hydrophobic effect, enabling efficient encapsulation to spaces such as a hydrophobic core of a polymeric NP or within a bilayer membrane of a vesicle. On the other hand, hydrophilic drugs are usually encapsulated within hydrophilic environments including the aqueous core of vesicles, or in hydrophilic brushes of a polymeric micelle.[22] Co-encapsulation of both hydrophilic and hydrophobic therapeutics commonly utilizes nanocarriers based on amphiphilic building blocks such as lipids and amphiphilic block copolymers.[23] More precision over drug loading can be achieved through supramolecular interactions such as H-bonding, electrostatic and host–guest interactions.[24] For example, nanocarriers based on macrocyclic host cyclodextrin could form inclusion complexes with different hydrophobic antibiotics and were further used as platforms to treat resistant bacteria.[25]

In addition to physical encapsulation, drugs can be covalently conjugated to the surface or within the core of the NP. This approach requires both the drug and NP to be appropriately functionalized to facilitate the formation of covalent bonds and is predominantly utilized with polymer-based nanocarriers, given the chemical versatility that polymers offer. A key consideration when using this approach is that the covalent bond should preferably be cleaved at the delivery site, typically in response to a specific stimulus, limiting the choice of linkages. Although click chemistry has emerged as an exceptionally versatile tool, the stability of the formed bonds in these reactions generally precludes their use for covalent drug encapsulation.[26] Instead, the most commonly employed covalent linkages for drug-NP conjugation include amide, ester, hydrazone, Schiff base, carbonate, and disulfide bonds, providing a balance between stability during the systemic circulation and the drug release at the delivery site.[26] In most cases, therapeutics are covalently attached to the NP itself, but there are also instances where drugs are conjugated to each other to maintain a specific drug ratio or to enhance drug loading. In terms of co-encapsulation, the covalent conjugation approach can therefore be used to load one or more distinct drugs and is predominantly combined with physical encapsulation.

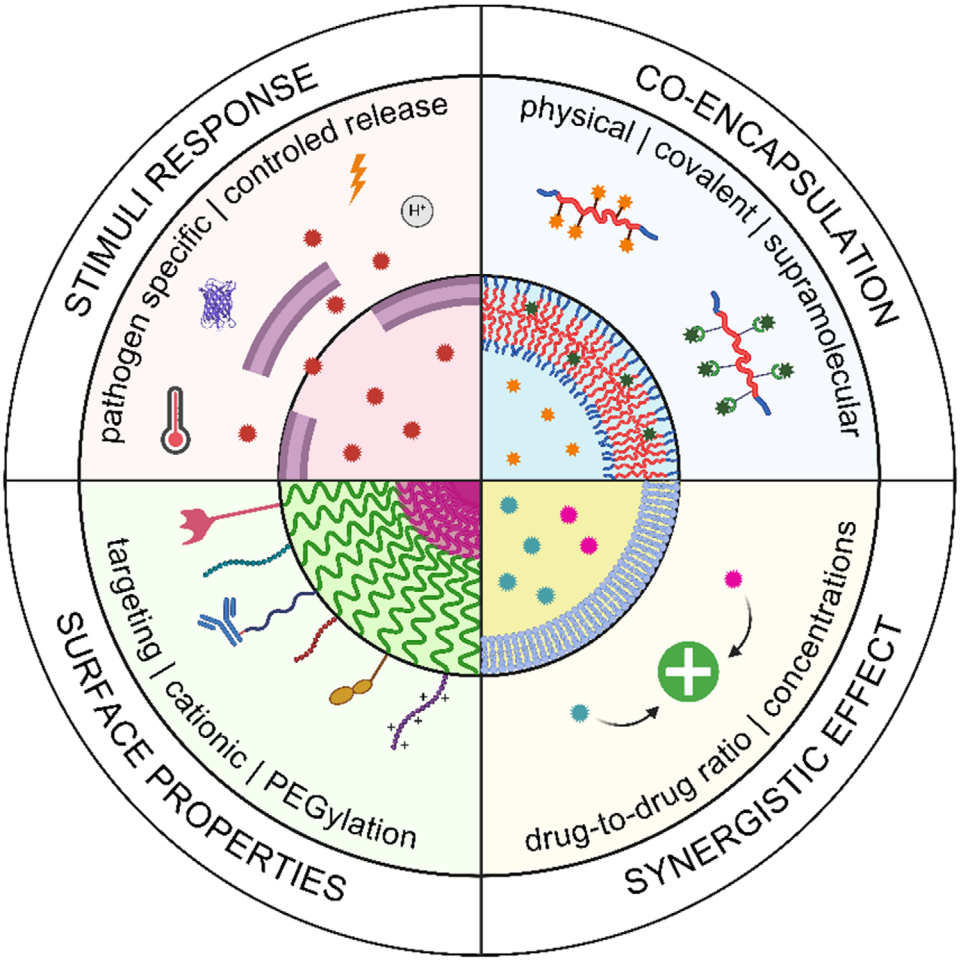

Figure 2. Key considerations for the design of nanocarriers for antimicrobial co-delivery. (Top left) Stimuli-responsive nanocarriers can utilize the infection microenvironment to trigger controlled drug release. (Top right) Different drugs can be co-encapsulated based on physical, covalent or supramolecular interactions. (Bottom left) Surface modification can improve targeting, cellular uptake, and circulation times. (Bottom right) Special attention needs to be given to drug concentrations and drug-to-drug ratios to ensure a synergistic effect.

2.2. Temporal Control of Release

Antimicrobial co-delivery systems leveraging nanocarriers can be designed to deliver multiple drugs simultaneously or sequentially to enhance the therapeutic outcomes. Temporal control design within these systems—encompassing the specific drug-to-drug ratio, the order of release, and timing—is essential for maximizing the efficacy and reducing resistance potential. These nanocarriers can be engineered to encapsulate and protect multiple drugs, releasing them in a controlled manner that aligns with the pharmacokinetic and pharmacodynamic profiles necessary for an effective treatment.[27–29]

The order in which drugs are released also plays a key role in determining the success of antimicrobial therapy, which signifies the temporal control in drug release as another important aspect of nanocarrier design. For instance, certain infections may require an initial release of one drug to disrupt bacterial defenses, followed by a delayed release of a second drug to kill the bacteria effectively. This strategy can be especially useful in treating biofilm-associated infections, which are notoriously difficult to eradicate. By timing the release of each drug, these systems ensure that the bacterial population is exposed to the optimal concentration of each agent at the most effective time, thereby maximizing therapeutic outcomes.[22–24]

2.3. Spatial Control of Release

Nanocarriers provide a sophisticated platform for delivering multiple drugs in a controlled and targeted manner directly at the site of infection, which can greatly enhance the treatment specificity and efficacy while minimizing side effects and dose requirement. The versatility of nanocarrier platforms allows for both passive and active targeting approaches, which can be exploited to achieve pathogen-specificity.[28]

Passive targeting leverages the natural physiological and pathological characteristics of the disease site. The enhanced permeability and retention (EPR) effect, widely observed in cancer, is also applicable to infectious diseases. Pathogens often cause inflammation, leading to increased vascular permeability and re-

tention of macromolecules at the site of infection. Nanocarriers designed within an optimal size range (typically 10–200 nm) can exploit the EPR effect, allowing them to accumulate preferentially in infected tissues.[27] In addition to size, the morphology and surface characteristics of nanocarriers are also crucial determinants of their efficacy in passive targeting.[31] Smaller NPs can evade clearance by the reticuloendothelial system (RES) and have prolonged circulation times, allowing more time for accumulation at the target site. Surface modification with hydrophilic polymers, such as poly(ethyleneglycol) (PEG), can further increase their circulation time by reducing opsonization and subsequent phagocytosis.[32]

While passive targeting is effective, active targeting provides an additional layer of specificity by decorating the surface of nanocarriers with ligands that recognize and bind to specific receptors on the pathogen or infected cells. These ligands include antibodies, peptides, or small molecules that can bind to pathogen-specific markers or to the receptors overexpressed in infected tissues. For example, mannose receptors are overexpressed on macrophages infected with *Mycobacterium tuberculosis*. Nanocarriers conjugated with mannose ligands have been shown to specifically target alveolar macrophages, enhancing therapeutic efficacy.[33] Moreover, certain pathogens alter the expression of specific receptors on the host cells. For instance, the expression of transferrin receptors is upregulated in cells infected with the malaria parasite *Plasmodium falciparum*. Nanocarriers functionalized with transferrin can thus selectively deliver antimalarial drugs to infected erythrocytes, reducing the parasite load while sparing uninfected cells.[34]

2.4. Stimuli-Responsiveness

Stimuli-responsive nanocarriers offer a promising approach for enhancing the specificity and efficiency of drug delivery systems in treating infections caused by resistant bacterial infections. These advanced nanostructures can be designed to respond to various external stimuli such as temperature, pH, light, enzyme activity, and tothe unique microenvironment created by the pathogens. For instance, the acidification of the local microenvironment of certain bacterial infections[35] can be exploited by pH-sensitive nanocarriers that release their payload only in the low pH environment of the infection site. In addition, bacterial phosphatase and phospholipase enzymes can be exploited to trigger the drug release from polyphosphoester-based nanocarriers.[36]

Another promising strategy relies on the utilization of temperature-responsive nanocarriers that remain stable at physiological temperatures and only release the encapsulated drugs when the local temperature exceeds a critical temperature threshold.[37] This approach is particularly effective in conjunction with photothermal therapy (PTT), which uses near-infrared (NIR) light to trigger a targeted response. Photothermal heating enhances the effectiveness of the drug delivery system by not only triggering drug release but also directly damaging bacterial cell membranes, thereby increasing antibiotic susceptibility. NIR radiation can also be used to activate photosensitizers, which generate reactive oxygen species (ROS) that effectively destroy bacterial cells by interacting with their proteins, DNA, and phospholipids.[38] This so-called photodynamic therapy (PDT) is typically used in combination with PTT and chemotherapies to augment the treatment efficacy toward eradicating infections, particularly those involving multidrug-resistant bacteria.[39]

Overall, stimuli-responsive nanocarriers provide the unique opportunity to combine multimodal treatment approaches, as they not only deliver the antibiotics more effectively but also disrupt the bacterial defenses, enhancing the overall antibacterial activity. By localizing the external stimuli at the infection site, these systems can deliver drugs more effectively, reduce the risk of systemic side effects, and prevent the development of further drug resistance. Detailed discussions of selected examples are presented in the following sections.

3. Types of Nanocarriers and Applications in Antimicrobial Co-Delivery

Nanocarriers designed for antimicrobial delivery can be effectively constructed using lipid and polymer excipients, and combinations thereof. Lipid-based delivery systems such as liposomes interact with bacterial cells through distinct mechanistic pathways, including adsorption, fusion, and endocytosis, each playing a critical role in enhancing antibacterial efficacy.[40] Adsorption occurs when electrostatic or ligand-receptor interactions drive liposomes to adhere to the bacterial membrane, promoting localized drug release. The surface of most bacterial cells is negatively charged due to the presence of lipopolysaccharides in Gram-negative species and teichoic acids in Gram-positive strains. This makes them highly susceptible to interaction with cationic nanocarriers.[41] Lipid-based NPs, including liposomes modified with cationic lipids such as 1,2-Dioleoyl-3-trimethylammonium propane (DOTAP), exploit this charge differential to adhere to bacterial membranes and promote localized drug delivery. For instance, the incorporation of DOTAP into rifampicin-loaded monoolein liquid crystalline NPs significantly enhanced bacterial binding and reduced the minimum inhibitory concentration (MIC) values for *Staphylococcus aureus* via fusing with and disrupting the bacterial membrane.[42]

Fusion involves the direct merging of the liposomal bilayer with the bacterial membrane, facilitating antibiotic delivery into the cytoplasm. Liposomes composed of 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) or Dipalmitoylphosphatidylcholine:1,2-Dimyristoyl-sn-glycero-3-phosphoglycerol (DPPC: DMPG) mixtures have demonstrated the ability to merge with bacterial envelopes, enhancing antibiotic penetration into both Gram-negative and Gram-positive bacteria. This mechanism bypasses enzymatic degradation and the extracellular biofilm matrix, as demonstrated in studies using fluorescence resonance energy transfer (FRET), flow cytometry, and electron microscopy across a variety of pathogens, including *Pseudomonas aeruginosa, Burkholderia cepacia, E. coli*, and *S. aureus*.[43,44]

Furthermore, endocytosis, which is primarily observed in eukaryotic cells, may also occur in certain bacterial species. Endocytosis leads to intracellular uptake of liposomal contents, where antibiotics or antimicrobial agents can exert their effects. For example, 2-diphytanoyl-sn-glycero-3-phosphocholine/1,2-dimyristoyl-rac-glycero-3-methylpolyoxyethylene (DPhPC/DMG-PEG2000) liposomes enhanced the cellular uptake of encapsulated penicillin G as compared with free penicillin G and

eliminated up to 99.9998% of ≈$10^{8.5}$ intracellular methicillin-sensitive *S. aureus* in infected A549 cells.[45]

Cholesterol content has been identified as a crucial modulator of lipid NP behavior as well. Increasing cholesterol levels promotes toxin-mediated interactions. Xie et al. demonstrated that liposomes containing up to 70 mol% cholesterol formed lipid microdomains that enhanced interaction with *S. aureus* biofilms and improved pore formation triggered by bacterial toxins, resulting in improved drug release and biofilm eradication.[46] Likewise, cholesterol-rich Moxifloxacin liposomes exhibited increased binding to *S. epidermidis* and a more efficient drug release upon exposure to bacterial virulence factors, leading to strong efficacy in both in vitro and in vivo infection models.[47]

These pathways collectively enhance drug bioavailability and improve therapeutic outcomes in combating resistantinfections . Additionally, liposomes can disrupt bacterial biofilms by penetrating the extracellular polymeric matrix, thereby increasing drug availability within resistant microbial communities.[40] The dense extracellular matrix of biofilms, which is rich in negatively charged polymers, can repel free cationic antibiotics. Liposomes, especially those with neutral or tunable surface charges, have shown efficacy in navigating these barriers. Encapsulation improves the delivery of drugs such as rifampicin that would otherwise be prevented from reaching the bacterial membrane.[41] Moreover, lipid-based NPs can be engineered for targeted, stimuli-responsive delivery. Certain formulations, such as 1,2-distearoyl-sn-glycero-3-phosphocholine/1,2-distearoyl-sn-glycero-3-phosphoglycerol (DSPC/DSPG) liposomes, degrade selectively in the presence of phospholipase toxins secreted by *S. aureus* and *Helicobacter pylori*, leading to localized release of antibiotics.[48,49] Similarly, pH-sensitive lipid NPs can respond to acidic microenvironments typical of infected tissues, thereby minimizing off-target effects and enhancing treatment precision.[50]

In contrast to lipid-based systems, the mechanisms by which polymeric NPs interact with bacterial cells remain comparatively underexplored and less defined. While there is mounting evidence that these carriers can effectively deliver antibiotics and disrupt microbial viability, the precise molecular pathways governing their uptake, binding, and intracellular behavior are still being explored. Most known interactions have been attributed to surface charge-dependent effects, where cationic polymers such as chitosan engage with the negatively charged cell wall of Gram-positive bacteria, leading to membrane destabilization and rupture.[51,52] These electrostatic interactions for "passive targeting" between cationic NPs and negatively charged bacterial membranes, along with hydrophobic and van der Waals forces[53] are widely reported, but the downstream consequences—such as pore formation, internalization, or triggering of stress responses—are still not fully characterized. Active targeting enhances specificity by functionalizing NPs with peptides, antibodies, or antimicrobial drugs that bind to bacterial surface structures. Once attached, these NPs disrupt the membrane through pore formation, oxidative stress, or direct membrane degradation, leading to bacterial cell death. Additionally, they can penetrate and break down biofilms, making bacteria more susceptible to treatment.[53]

Additionally, polymeric NPs are often engineered for environmentally responsive release (e.g., pH- or enzyme-triggered release). For example, poly(D,L-lactic-co-glycolic acid)-b-poly(L-histidine)-b-poly(ethylene glycol) (PLGA-PLH-PEG) nanocarriers have been shown to release vancomycin (VAN) selectively in acidic microenvironments associated with bacterial infections.[50] However, whether this also facilitates improved intracellular penetration or bacterial targeting remains a topic of ongoing investigation.

Overall, polymeric soft NPs offer clear therapeutic potential, particularly in drug protection and controlled release. Their mechanistic interactions with bacterial membranes and biofilms warrant further detailed study, especially in comparison to the better-understood lipid-based platforms.

While each type of nanocarrier offers unique advantages in combatting bacterial infections, they are also associated with certain drawbacks. For instance, lipid-based nanocarriers, such as liposomes and solid lipid nanoparticles (SLNs), provide excellent biocompatibility and the ability to encapsulate both hydrophilic and hydrophobic drugs, but may suffer from poor storage stability. On the other hand, polymer-based nanocarriers, including polymeric micelles and dendrimers, offer precise control over drug release profiles and enhanced stability in biological environments, however, drug co-loading capacity may be limited. In this section, we provide an in-depth discussion on antimicrobial co-delivery with lipid, polymer, and polymer-lipid hybrid nanocarriers (**Figure 3**). Various nanocarriers that have been reported for their synergistic effects in bacterial infection treatment are summarized in **Table 1**.

3.1. Lipid-Based Nanocarriers

Lipid-based nanocarriers can be divided into several categories depending on the preparation method and composition. The main categories include: (1) liposomes, (2) lipid NPs, (3) transferosomes, (4) solid lipid nanoparticles (SLNs), (5) nanostructured lipid carriers, and (6) niosomes.[54] Among those, we will focus on liposomes and SLNs, which are primarily utilized for the co-delivery of antimicrobial substances to treat resistant bacterial infections.

3.1.1. Liposomes

Liposomes are spherical, amphiphilic vesicles made of natural or synthetic diacyl chain phospholipids and cholesterol. They form in aqueous solutions through the self-assembly of phospholipids, with polar groups positioned at the interface of the vesicle's interior and exterior, allowing them to encapsulate both hydrophobic and hydrophilic drugs within the lipid bilayer and aqueous core, respectively.[55] Due to their unique properties, such as prolonged retention and sustained release of encapsulated drugs at the site of action, decreased side-effects and toxicity, and increased drug protection from possible harsh environments, they represent excellent carriers for the delivery of various therapeutics, including antimicrobial agents.[56]

Numerous studies have highlighted the benefits of co-encapsulating antimicrobial agents in conventional liposomes, which enhance efficacy against various bacterial strains. Li and colleagues demonstrated that co-encapsulating daptomycin and

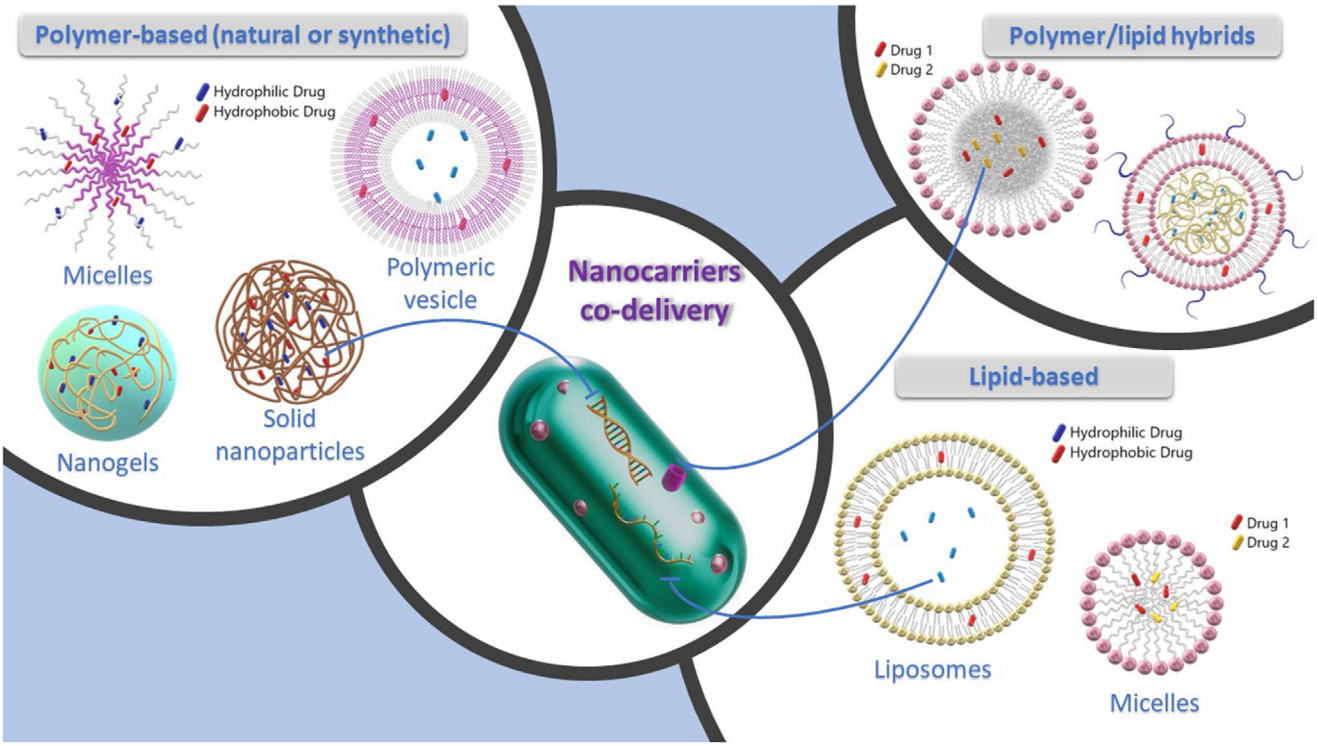

Figure 3. Main types of soft nanocarriers used for antimicrobial co-delivery according to their composition: polymer-based, lipid-based, or polymer/lipid hybrids. These materials can form different architectures such as solid nanoparticles, liposomes, vesicles, micelles, or cross-linked nanogels.

clarithromycin in hydrogenated soybean phosphatidylcholine (HSPC)/cholesterol/mPEG2000-DSPE liposomes (15:10:1 molar ratio, with a 1:32 drug ratio) significantly enhanced antibacterial activity against MRSA, including both planktonic and biofilm-embedded forms. This improvement was evidenced by reduced bacterial counts compared to both free drugs and drugs individually encapsulated into liposomes. In addition, the intravenous administration of the formulation into a murine model of systemic infection demonstrated prolongation of the survival rates and enhanced therapeutic efficacy with a sustained release profile.[57] Of note, although the exact mechanism underlying the synergistic action between daptomycin and clarithromycin remains unclear, it has been suggested that clarithromycin may disrupt MRSA biofilm formation, thereby enhancing daptomycin's availability and efficacy.[58] Indeed, sustained release of the encapsulated antimicrobial agents can play a pivotal role in enhancing the antibacterial effect,[59] and allowing for a lower antibacterial agent dose, which, in turn, reduces potential toxicity risks. The enhanced efficacy with liposomal co-delivery systems can also partly result from hydrophobic interactions that facilitate fusion between bacterial membranes and liposomes.[60,61]

A recent study has reported HSPC, cholesterol (Chol), and DSPE-PEG2000 (HSPC:Chol:DSPE-PEG) liposomes co-loaded with colistin and curcumin aiming to address multidrug-resistant (MDR) Gram-negative bacterial infections.[62] The dual drug loaded liposomal system demonstrated substantial stability, effective drug-loading, and sustained release. In vitro antibacterial assays revealed synergistic effects against clinically isolated colistin-resistant bacteria, outperforming the free drugs in reducing bacterial counts. Specifically, encapsulation of colistin/curcumin into liposomes allowed for increased interaction with the bacterial cell membrane. Subsequently, the membrane could be more efficiently disrupted by colistin, possibly enhancing further uptake of curcumin, which in turn inhibited bacterial efflux pumps. In vivo studies using a mouse intestinal infection model revealed that the liposomal formulation effectively reduced bacterial load and inflammation, compared to free drugs while demonstrating reduced toxicity. Overall, the formulation enhanced the therapeutic outcomes and reduced the frequency of required injections compared to free colistin and curcumin.[62]

The synergistic effects of antibiotics and essential oils have also been demonstrated through co-encapsulation in liposomes. Ben Khalifa and colleagues have demonstrated that combining antibiotics with essential oils, such as carvacrol (CAR)—a phenolic compound in thyme, oregano, and marjoram oils—could enhance their antibacterial efficacy.[63] This synergy likely results from increased antibiotic diffusion across bacterial membranes, possibly mediated by the affinity of CAR with membrane lipids, and fusion of the liposomes to the membrane. In vitro experiments using multiple lipid NPs co-loaded with CAR and VAN showed significantly enhanced antibacterial effects against *S. aureus*, reducing bacterial counts more effectively than free VAN.[63]

Anionic liposomes, which contain negatively charged lipids in their bilayer, have also been explored for the co-delivery of two distinct antimicrobial agents.[42] In a study aimed at treating lung infections caused by *P. aeruginosa*, colistin was co-encapsulated with ciprofloxacin (CPX) within anionic liposomes.[64] These liposomes were composed of HSPC, DMPG/DSPG, and cholesterol, and drug incorporation within these liposomes was shown to be beneficial for addressing infections caused by multidrug-

Table 1. Overview of antimicrobial co-delivery systems based on soft nanocarriers.

NP platform	Antimicrobial agents	External Stimuli	Bacterial strains	Refs.
Liposomes	Daptomycin and Clarithromycin	–	*MRSA*	[57]
	Colistin and Curcumin	–	*MDR E. coli, MDR K. pneumoniae*	[62]
	Resolvin D1and Ceftazidime	–	*P. aeruginosa*	[66]
	Carvacrolan and VAN	–	*S. aureus*	[63]
	Colistin and CPX	–	*P. aeruginosa*	[64]
	Colistin and CPX	–	*P. aeruginosa H131300444 and P. aeruginosa H133880624*	[68]
	Colistin and CPX	–	*P. aeruginosa*	[65]
	Tobramycin (Tob), Cyanine dye (cypate)	Light	*P. aeruginosa PA14, PA68, PAK, and PAOI*	[69]
	AMPs EKKRLLKWWR (AMP1)/ KWWKLLRKKR (AMP2) and VAN	pH	*MRSA*	[70]
	DP7-C and Azithromycin (AZT)	–	*S. aureus ATCC 25923, MRSA ATCC 33591*	[71]
	ID93 and mGLA-LSQ	–	*M. tuberculosis*	[72]
	Turmeric and Cinnamon extracts	–	*P. aeruginosa*	[78]
	Colistin and Isostevic acid	–	*P. aeruginosa*	[79]
Lipid nanoparticles	Doxycycline and HCQ	–	*B. melitensis*	[75]
	STR and HCQ	–	*B. melitensis*	[76]
Polymeric micelles	CPX and EGCG dispersant	pH	*S. aureus*	[80]
	CPX and NO	Light	*P. aeruginosa*	[81]
	RIF and Colistin	–	*E. coli*, *P. aeruginosa*, and *A. baumannii*	[82]
	VAN and Curcumin	pH	*S. aureus* Xen36	[83]
	CPX and VAN	pH and enzyme	*P. aeruginosa*	[84]
	Amikacin and CO	pH and thiol	MRSA and ESBL- *E. coli*	[85]
	NO and CO	Light	*S. aureus*	[86]
	Erythromycin and salicylate ion	–	*E. coli*	[87]
	Quercetin and RIF	pH	MRSA	[88]
	Protoporphyrin IX and AgNPs	Light	*S. aureus*	[38]
	STR and Rapamycin	pH	*S. typhimurium and S. aureus*	[89]
	MagII and Azithromycin and luteolin	pH	MRSA	[90]
	MagII and Azithromycin and Curcumin	pH	MRSA	[91]
	Piperacillin, Tazobactam	–	*P. aeruginosa*	[92]
	Glabrol and Colistin	–	*E. coli B2*	[93]
	Ce6 and Curcumin	Light	*S. aureus*	[94]
Polymeric vesicles	Quinupristin and Dalfopristin	Enzyme	MRSA	[95]
	CPX and Ceria NPs	–	*E. coli* and *S. aureus*	[96]
	AMP and AgNPs	–	MRSA	[97]
	Methicillin and SPIONs	–	*S. epidermidis* and *E. coli*	[98]
	Ampicillin and AgNPs	–	*E. coli*	[99]
	RIF and MOX	–	*S. aureus*	[100]
Polymeric nanoparticles	Ag+ and Simvastatin	–	*E. faecalis*	[101]
	Tobramycin and AgTob-NCs	–	*P. aeruginosa*	[102]
	OSEO (oregano essential oils), Zn(II) salen, CSNPs (chitosan nanoparticles)	–	*E. coli* RCMB 000103 and *P. aeruginosa* RCMB 000102	[103]
	Caffeic acid phenethyl ester (CAPE) and juglone	–	*S. aureus and E. coli*	[104]
	CPX and functionalized the NP surface with DNase I	–	*P. aeruginosa*	[105]
Nanogels	Triclosan and quaternary ammonium compounds (QACs) functionalized with alkyl chains Qc1-NG and Qc12-NGs.	Temperature and pH	*S. epidermidis* ATCC122282. *S. epidermidis* HBH 453. *S. aureus* 52984.*S. aureus* ATCC12600	[106]
	Nitric Oxide NO and AMPs	Temperature and pH	Biofilms formed by MRSA and *P. aeruginosa*	[107]
	Triclosan and Nisin	pH and Light	*S. aureus* and *E. coli*	[108]

(*Continued*)

Table 1. (Continued)

NP platform	Antimicrobial agents	External Stimuli	Bacterial strains	Refs.
Lipid-polymer hybrids	VAN and 18β-glycyrrhetinic acid	pH	MRSA and *S. aureus*	[109]
	Isotretinoin, Clindamycin phosphate	–	*P. acnes*	[110]
	Curcumin, Gentamicin	–	*P. aeruginosa*	[30]

resistant *P. aeruginosa*. This may be attributed to increased uptake of CPX due to the bacterial membrane disruptive effect of colistin molecules that were presented on the surface of the anionic liposomes. In vitro studies revealed that the liposomal formulations exhibited enhanced antimicrobial activity against resistant strains compared to monotherapy treatments. Furthermore, cytotoxicity assessments using A549 human lung epithelial cells demonstrated the safety of these formulations for pulmonary delivery.[64] Colistin and CPX were also co-encapsulated into HSPC/DSPG-Na)/Chol liposomes to treat respiratory tract infections caused by multidrug-resistant *P. aeruginosa*.[43] In an in vitro human lung epithelial cell model (Calu-3 cell monolayer), the formulations resulted in enhanced antimicrobial activities of the co-delivered drugs compared to individual therapies, with significant drug retention on lung epithelial surfaces and decreased transport across the cell monolayer.[65]

While the anionic liposomes in this study were particularly advantageous for colistin encapsulation—due to the electrostatic interactions between the positively charged colistin and the negatively charged liposomal bilayer—this liposomal system presents challenges for treating bacterial infections caused by e.g. *P. aeruginosa* with a negative surface charge. The negative charge on both the bacterial surface and the liposome leads to electrostatic repulsion, which could reduce the efficacy of the liposomes in facilitating bacterial uptake and treatment delivery.

Hybrid strategies are emerging as promising approaches for treating *P. aeruginosa*-associated lung infections. Drawing inspiration from the natural interactions between neutrophils and endothelial cells during infection, Gao and colleagues developed an innovative hybrid system utilizing nanovesicles derived from human neutrophil membranes.[66] These liposome-like vesicles effectively mimic the natural interactions between neutrophils and endothelial cells, enabling targeted delivery to inflamed lung tissues. In their study, the team designed neutrophil-membrane-derived nanovesicles to co-deliver resolvin D1 (RvD1) and the antibiotic ceftazidime (CAZ) to combat lung infections. RvD1, a lipid molecule with potent anti-inflammatory properties, inhibits neutrophil infiltration and promotes macrophage-mediated cellular clearance. However, due to its transient nature and rapid in vivo degradation, maintaining effective levels of RvD1 necessitates a robust delivery system. By harnessing the targeting capabilities of neutrophil-derived nanovesicles, this strategy enables precise delivery of RvD1 directly to inflamed lung tissues. The neutrophil-derived nanovesicles exhibited high targeting efficiency for inflamed lung tissue and facilitated sustained release of both RvD1 and CAZ. In vitro experiments demonstrated that the co-delivery system provided superior anti-inflammatory and antibacterial effects compared to free CAZ alone. Additionally, in vivo studies using a *P. aeruginosa*-induced mouse model of lung infection showed that these nanovesicles significantly reduced bacterial load and inflammation, outperforming free CAZ. The sustained release properties also reduced the dosing frequency, highlighting the potential of this system to enhance therapeutic efficacy and improve patient convenience.[66]

Inhalation technology has proven efficient in the targeted delivery of liposomes in the pulmonary area. A study optimized liposomal powder formulations for inhalation, co-delivering CPX and colistin to treat multidrug-resistant Gram-negative bacterial lung infections.[67] The optimized formulation showed high encapsulation efficiencies and satisfactory aerosol performance, with the liposomes being around 150 nm in size. The in vitro studies using human lung epithelial cells confirmed the efficient transport and cellular uptake of the drugs.[67] A follow-up study developed an inhalable liposomal powder formulation co-loaded with CPX and colistin for the treatment of lung infections caused by multidrug-resistant Gram-negative bacteria, specifically *P. aeruginosa*, with a strong antibacterial efficacy.[68] The liposomes were composed of HSPC, distearoyl phosphatidylglycerol (DSPG), and PEG with cholesterol. Addition of internal mannitol (8% w/v) and sucrose (2% w/v), as cryoprotectants, along with external cytoprotectants/aerosolization enhancers, resulted in formulations with optimal physicochemical characteristics and superior antibacterial activity against clinical isolates of *P. aeruginosa* H131300444 and *P. aeruginosa* H133880624 compared to each antibiotic separately.[68] These results demonstrate that the liposomal powder formulation could potentially be a pulmonary delivery system for antibiotic combination to treat multidrug-resistant Gram-negative lung infections.

In addition to conventional liposomal formulations, stimuli-responsive liposomes have also been explored for enhanced therapeutic effects. NIR-activated thermosensitive liposome system was developed to enable synergistic photothermal and antibiotic therapy (**Figure** 4A), specifically targeting *P. aeruginosa* biofilms. The liposomes were composed of distearoyl phosphatidylcholine (DSPC), cypate (a NIR photothermal agent), and betainylated cholesterol (BC), which impart a positive charge to the liposomes, facilitating effective penetration of biofilm microchannels.[48] Physicochemically, the liposomes remained stable at body temperature (37 °C) and released the encapsulated antibiotic tobramycin upon heating above 45 °C. In in vitro experiments, the liposomes significantly enhanced biofilm dispersion, releasing up to 80% of the antibiotic at elevated temperatures and achieving up to 80% biofilm dispersion (Figure 4B). This was seven to eight-fold greater than the effect of antibiotics alone. In vivo studies using a subcutaneous abscess model in BALB/c mice (Figure 4C) demonstrated remarkable therapeutic outcomes after NIR irradiation (Figure 4D), including substantial bacterial reduction (Figure 4E) and accelerated wound healing (Figure 4F), driven by the combined antibiotic and photothermal effects. These results underscore the potential of this thermosensitive liposome

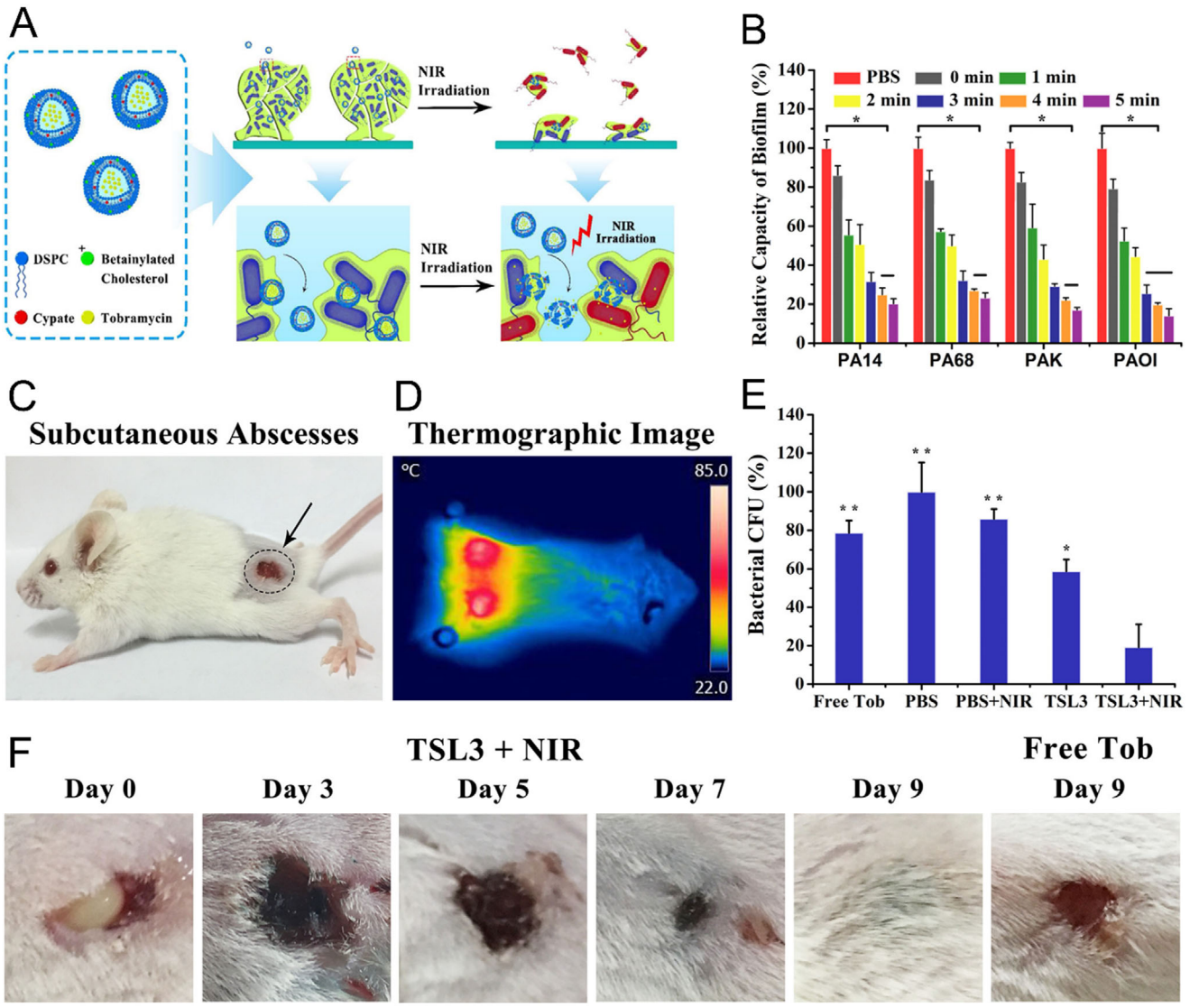

Figure 4. In vivo antibacterial activity of thermally sensitive liposomes (TSL) encapsulating cypate and the antibiotic Tobramycin (Tob). A) Schematic illustration of the synergistic bactericidal effect of cypate-Tob@liposome in vivo under NIR irradiation. B) Dispersion of biofilm by formulation TSL3 under different NIR irradiation times. C) Photograph of mouse model of subcutaneous abscess before treatment. D) Thermographic image of mouse with subcutaneous abscess following treatment by TSL3 under NIR irradiation. E) Quantitative analysis of bacterial colony-forming unit (CFU) obtained from abscess tissues of each group. F) Images of infected skin after treatment with free tobramycin or TSL3 under NIR irradiation. Statistical significance was set at $^{*}p < 0.05$ or $^{**}p < 0.01$. Reprinted with permission[69] Copyright 2018, ACS.

system to improve antibiotic efficacy through thermo-triggered drug release and PTT, offering a promising strategy for treating bacterial biofilms.[69]

Another stimuli-responsive liposomal formulation encapsulating the antimicrobial peptides EKKRLLKWWR (AMP1) and KWWKLLRKKR (AMP2) with VAN was developed against MRSA.[49] The pH-responsive liposomal formulation was composed of phosphatidylcholine (PC), cholesterol, and oleic acid (OA) in 5.5:3.5:0.5 molar ratios. The liposomal formulations were <150 nm in diameter with slight negative surface charge at neutral pH, which shifted to positive values in acidic conditions. In vitro studies demonstrated enhanced antimicrobial activity of the liposomal formulations at pH 6.0, with MICs of 1.95 μg mL^{-1} for AMP1-Lipo-1 and 3.9 μg mL^{-1} for AMP2-Lipo-2, compared to higher MICs at pH 7.4. The encapsulation efficiency (EE) was high, and the liposomes released VAN and AMP1/AMP2 more rapidly at acidic pH, which correlated with their improved antibacterial activity. Mechanistically, it was suggested that the AMPs used in this study dose-dependently increase bacterial membrane permeability and, therefore, improve VAN uptake.[70]

Surface-modified liposomes have been proven efficient in treating resistant bacterial infections. A study by Liu et al. explored the use of antibacterial peptide DP7-C-modified azithromycin (AZT)-loaded liposomes (AZT-D-LPs) as a treatment for MRSA.[71] The liposome composition involved soybean phosphatidylcholine (SPC) and cholesterol, with a lipid-

to-cholesterol mass ratio of 3:1, modified with the antimicrobial peptide DP7-C (Chol-suc-VQWRIRVAVIRK-NH2) to enhance the encapsulation and synergistic antibacterial effect of azithromycin. In vitro results revealed that AZT-D-LPs had lower MICs (0.125 μg mL^{-1} for *S. aureus*, and 0.25 μg mL^{-1} for MRSA strain) than free AZT (0.25 μg mL^{-1} for *S. aureus* and 0.5 μg mL^{-1} for MRSA strain) and DP7-C-modified blank liposomes (D-LPs) (>256 μg mL^{-1} for both *S. aureus* and MRSA strains), showing significant antimicrobial activity against the two strains. Although in vitro experiments showed no significant differences between the AZT-D-LPs and AZT-LPs, the in vivo experiments conducted on a murine model demonstrated significant reduction in bacterial counts for AZT-D-LPs compared to controls, highlighting their potential for clinical treatment of MRSA infections without obvious side effects or toxicity. DP7-C was used here as a modulator of the innate immune response, enabling more effective clearance of the infection site after killing with AZT, an effect which is difficult to investigate without the presence of immune cells.[71]

Another study has explored a strategy involving chemical conjugation to improve the delivery and efficacy of a tuberculosis (TB) subunit vaccine antigen ID93 conjugated to an adjuvant modified liposome (mGLA-LSQ).[72] The liposomes were composed of dipalmitoyl phosphatidylcholine (DOPC), cholesterol, and dioleoyl phosphatidylethanolamine (DOPE). Co-delivery of the antigen and adjuvants enhanced immune responses, elevating the levels of interleukin-2 (IL-2), interferon-gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α) compared to unconjugated mixtures.[72] These results highlight the potential of covalently conjugated liposomal vaccines to enhance Th1-type cytokine responses, which are crucial for protective immunity against TB.

The versatility of liposomal formulations, particularly as co-delivery systems, showcases their potential in combating challenging infections, including multidrug-resistant bacteria. The continued development of these systems, especially with the integration of stimuli-responsive properties and targeted delivery, represents a promising frontier in antimicrobial therapy, potentially overcoming many of the limitations seen with conventional treatments.

3.1.2. Solid Lipid Nanoparticles

In addition to liposomes, SLNs have also been proven efficient in delivering antimicrobials against resistant bacterial infections. SLNs consist of lipids and surfactants and are formed by emulsification methods. SLNs demonstrate superior properties compared to other lipid-based delivery systems, offering high EE, stability, target-specific release properties, and substance protection.[73] However, SLNs have certain drawbacks, such as low loading capacity, drug expulsion during storage, as well as agglomeration of the particles during the cooling process.[74]

SLNs labeled with Cadmium telluride (CdTe) quantum dots (QDs) have been explored for the co-delivery of doxycycline and hydroxychloroquine (HCQ) for the treatment of acute and chronic brucellosis.[75] A double emulsion-melt dispersion method was used for the preparation of SLNs, which involved the dispersion of antibiotics in palm oil and stearic acid heated above their melting temperatures. The SLNs were stabilized in an aqueous phase containing QDs. In vitro studies evaluating the antimicrobial efficacy of the antibiotics toward *Brucella melitensis*, both in free form and encapsulated within SLNs, demonstrated a significant reduction in colony-forming units (CFUs), particularly for nanoencapsulated formulations. In vivo experiments on rats further showed that the co-encapsulation of doxycycline and HCQ within NPs effectively reduced bacterial colonies in both the spleen and liver during acute and chronic phases of brucellosis. In this study, the use of HCQ is attributed to a pH shift toward more alkaline conditions within bacterial cells, which in turn decreases virulence and potentiates the effectiveness of the antibiotic doxycycline. The physicochemical characteristics of SLN formulation—optimal size, good EE, long-term release, and enhanced stability—appear to be critical to its enhanced antimicrobial efficacy of the formulation.[75] Notably, the NPs exhibited a controlled release of the drugs over 100 h, resulting in the increased accumulation of the drugs in the infection site.

In a follow-up study, QD-labeled SLNs were investigated for the co-delivery of streptomycin (STR) and HCQ.[76] In vitro and in vivo experiments showed that the NP formulation significantly reduced liver enzyme levels and normalized trace element concentrations compared to free drug treatments. In vivo tests also indicated that STR-HCQ-SLN had a superior therapeutic impact on liver function parameters, such as aspartate aminotransferase (AST) and total bilirubin (TBil), reducing them to levels close to those in healthy rats. The results highlight the potential of STR-HCQ-SLN to enhance the treatment efficacy and reduce the recurrence of brucellosis by ensuring targeted and controlled drug release within macrophages, where *Brucella spp*. resides.[76]

These findings highlight the promise of SLNs in improving antimicrobial efficacy through enhanced drug delivery and controlled release. However, challenges such as low loading capacity and particle agglomeration remain, and addressing these issues could further optimize SLN-based co-therapies for resistant infections.

3.2. Polymer-Based Nanocarriers

Nanocarriers derived from polymer building blocks are highly versatile platforms that allow for diverse and sophisticated designs for drug encapsulation and co-delivery. With the feasibility to incorporate various chemical and biological functionalities, polymeric nanocarriers can be tailored to target specific tissues, cell populations, and intracellular compartments, and can respond to external stimuli to release their content. With these attributes, polymeric nanocarriers have gained significant attention in the development of smart drug delivery systems and are expected to play a critical role as new therapeutic agents and treatments emerge.[77] In addition, polymeric nanocarriers display remarkable stability and tunable biodegradation characteristics, which can help overcome limitations associated with lipid-based nanocarriers. Polymer-based nanocarriers can be prepared as self-assembled architectures such as micelles and polymersomes, cross-linked nanogels, and solid polymer NPs, which are discussed in the following sections for the co-delivery of antimicrobials.

3.2.1. Polymeric Self-Assemblies

Amphiphilic block copolymers made up of hydrophilic and hydrophobic segments self-assemble into nanoscopic architectures in an aqueous environment driven by entropy. Relative lengths of hydrophilic and hydrophobic blocks, as well as the type and composition of the solvent, can affect the type of the obtained nanostructures (micelles, vesicles, worms).[111] The main advantage over lipid-based systems is the possibility for chemical modification of building blocks. With proper functionalization, it is possible to conjugate antibodies or cell-penetrating peptides to the surface of nanocarriers. Moreover, when stimuli-responsive polymers are used as blocks, nanostructures capable of triggered drug release can be obtained.

Polymer Micelles: Polymer micelles are colloidal nanostructures with a hydrophobic core and a hydrophilic corona formed via self-assembly of amphiphilic block copolymers. While the hydrophobic drugs can easily be loaded within the core, hydrophilic molecules can interact with the shell via, e.g., electrostatic forces, facilitating the co-loading of cargoes with different physicochemical properties. Polymeric micelles have been demonstrated to be promising for the co-delivery of various antimicrobials, including small molecules, gas molecules, and their combinations,[57–64] with the possibility of achieving triggered release in response to external stimuli.

pH-Responsive Micelles: To harness the specific acidic microenvironment of bacterial infections, pH-responsive antibacterial nanocarriers have been developed. A straightforward strategy involves utilizing pH-responsive block copolymers as building blocks, where changes in pH can lead to destabilization of micelles and release of encapsulated cargo. One commonly used pH-sensitive block is poly(2-(diisopropylamino)ethyl methacrylate) (PDPA), which is hydrophobic at physiological pH but gets protonated and becomes hydrophilic in acidic conditions. PEG-*b*-PDPA micelles co-encapsulating antibiotic azithromycin and flavonoid luteolin were decorated with Magainin II (MagII) AMP on the surface.[90] The surface functionalization with MagII was shown to increase the drug concentration at the MRSA infection site in mice compared to nonfunctionalized micelles, demonstrating improved targeting. Further, combination treatment with luteolin has the potential to reduce the amount of azithromycin necessary for efficient infection eradication, therefore decreasing the risk of resistance development. These targeted, pH-responsive micelles have shown significantly better therapeutic effects in mice with MRSA-induced pneumonia compared to free drugs and micelles without MagII.[90]

Alternatively, pH-responsive release can be facilitated by covalently conjugating drugs through acid-labile linkages, leading to bond cleavage once nanocarriers are internalized. One of such linkages is boronic ester bond, where polymer chains are usually functionalized with phenylboronic acid (PBA) moieties, enabling reversible conjugation of 1,2- and 1,3-diols, which is particularly useful if a drug possesses a carbohydrate moiety. This approach was used to conjugate glycopeptide antibiotic VAN to poly(ethylene glycol)-*block*-poly(lysine-*co*-lysine-phenylboronic acid) (PEG-*b*-P(Lys-*co*-LysPBA)) micelles (**Figure 5A**).[83] The aromatic polyphenol drug curcumin could be co-encapsulated in the micellar core through π–π stacking interaction with VAN, demonstrating synergism against VAN-resistant *S. aureus* both in vitro and in vivo (Figure 5B,C). The rationale behind the use of curcumin in this system was that it broadens the antibiotic treatment spectrum, thus reducing the amount of VAN necessary and the risk of resistance development. This robust strategy therefore could be used for co-encapsulation of any two antibiotics where one has a diol in the structure, and another is aromatic.[83] In another example, (-)-epigallocatechin-3-O-gallate (EGCG) dispersant was conjugated to polymer shell functionalized with PBA, while CPX was loaded in micellar core.[80] Micelles are disassembled under acidic biofilm conditions and EGCG is released, enhancing bacterial killing toward *S. aureus* Xen36 biofilms compared to micelles with only core-loaded antibiotics. Moreover, micelles with in-shell EGCG and core-loaded CPX could self-target, effectively disperse, and eradicate infectious biofilms in mice, causing only mild and fast disappearing septic symptoms due to the sudden increase of bacterial concentrations in blood.[80]

Another acid-cleavable linkage is the hydrazone bond formed between carbonyl and hydrazine functional groups. Antimicrobial hyaluronan-based micelles were developed, with STR conjugated to hyaluronic acid via a dihydrazide linker, and decylamine conjugated via EDC/NHS chemistry to introduce amphiphilic character to the polymer.[89] Micelles were co-loaded with autophagy activator rapamycin during self-assembly. These nanocarriers could effectively eliminate intracellular bacterial infections by promoting STR uptake and rapamycin-initiated activation of autophagy.[89]

Light-Responsive Micelles: Light can be used as exogeneous stimuli, offering spatiotemporal control over drug release. Light-responsive micelles can be obtained by integrating light-responsive moieties, for example by conjugation within the polymer chain. In one such example, N-nitrosamines conjugated with tetraphenylethylene (TPE) antenna were used as monomers to build a hydrophobic block, serving a dual function as nitric oxide (NO)-donor and photoresponsive moiety.[81] Antibiotic CPX was encapsulated within the core of these micelles, while NO gas was released upon irradiation at 419 nm. Photo-mediated co-release of the biofilm dispersant NO and CPX showed a synergistic antibiofilm capacity against *P. aeruginosa*.[81] In another example, NO-donor moiety was grafted onto a carbon monoxide (CO)-donor antenna and conjugated to PMMA hydrophobic block. Upon visible light irradiation, CO and NO are released, demonstrating a superior antimicrobial effect toward Gram-positive bacteria and a better therapeutic capacity to treat MRSA infection in a murine skin in comparison to VAN.[86]

Another strategy for developing light-responsive nanoplatforms is to introduce photothermal agents which can generate localized heating, or photosensitizing agents that can generate ROS, both leading to cell death. Micelles loaded with a photosensitizer protoporphyrin IX (PpIX) in the core and decorated with silver nanoparticles (AgNPs) in the shell were reported as synergistic chemo-photodynamic nanocarriers. Once irradiated at 635 nm, PpIX in the micellar core could generate ROS, while AgNPs contributed to the disruption of the bacterial membrane. Light-activable micelles effectively eradicated subcutaneous infections induced by drug-resistant *S. aureus* in a murine model, benefiting from the synergy of AgNPs and PDT[38]

Dual-Responsive Micelles: Micellar co-delivery systems have also been developed as dual-responsive systems, such as pH and enzyme (lipase)-responsive PEG-*b*-polycaprolacton (PCL)

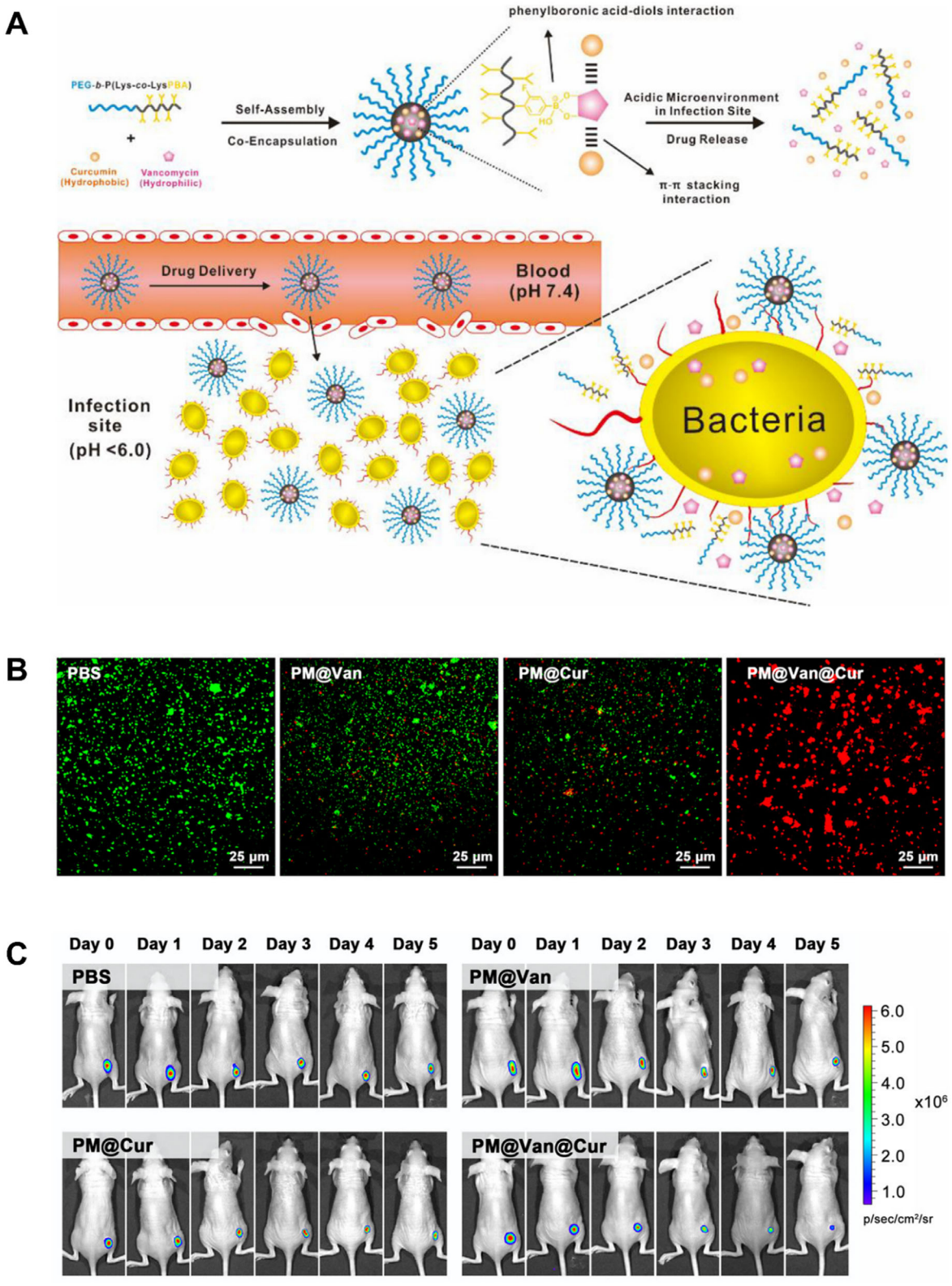

Figure 5. pH-responsive antimicrobial micelles. A) Curcumin and vancomycin (VAN) are coloaded during block copolymer self-assembly. Once nanocarriers reach infected site, bonds between polymer and VAN cleave due to lower pH, and micelles disassemble. B) Fluorescence images of *S. aureus* Xen36 after incubation with micelles encapsulating Curcumin, VAN or both, with PBS as a control. C) Bioluminescence images of *S. aureus* Xen36-infected mice after treatment with micelles encapsulating Curcumin, VAN or both, with PBS as a control, demonstrating synergistic effect. Reprinted with permission,[83] Copyright 2022, Wiley-VCH.

micelles.[84] In this study, the PEG block was end-functionalized to conjugate VAN through acid-labile hydrazone bonds, which also serves as a targeting ligand toward the bacterial membrane, while the PCL block is intrinsically lipase-sensitive. CPX was loaded into the core of PEG-*b*-(VAN-hyd-PCL) micelles. Once at the infection site, VAN is cleaved due to the pH-sensitivity of hydrazone bonds, while encapsulated CPX is released due to lipase-catalyzed degradation of PCL. Through careful design, these nanocarriers offer not only dual responsivity, but also temporal control over antibiotics release. The PEG-*b*-(VAN-hyd-PCL)/CPX micelle treatment resulted in higher survival of *P. aeruginosa*-infected mice, fewer bacterial burdens, and lower alveolar injuries in lungs compared with PEG-*b*-PCL/CPX micelles and free drugs.[84]

Furthermore, pH/thiol-responsive, CO-releasing micelles loaded with antibiotics have been reported.[85] CO-releasing catechol modified ruthenium carbonyls were conjugated to the hydrophobic block through pH-cleavable catechol boronic ester bonds, introducing pH-responsivity to the system. The presence of thiol-containing molecules such as cysteine accelerated the release of CO, exploiting the overexpression of thiol-containing biomolecules including glutathione or cysteine in the biofilm formed in the infected sites, making nanocarriers sensitive to both pH and thiol levels of infection microenvironment.[85] These dual-responsive micelles were loaded with four different antibiotics (methicillin, ampicillin, levofloxacin, and amikacin) to investigate their synergism against MRSA and ESBL-*Escherichia coli* as two representative multidrug-resistant bacteria. Combining CO-releasing micelles and β-lactams (Met and Amp) demonstrated antagonistic effects at both neutral and acidic pH, whereas there was no significant difference in antibacterial efficiency when Lev was encapsulated compared to empty micelles. The combination of micelles and Ami showed notable synergistic effects, achieving 4.81 ± 0.21 and 5.72 ± 0.17 log reduction at pH 5.5 against MRSA and ESBL-*E. coli*, respectively.[85] Moreover, micelles encapsulating sub-MIC amikacin exhibited remarkably enhanced in vivo anti-biofilm efficacy, displaying antibacterial effect with a log reduction of 1.42 ± 0.16 and accelerated wound closure in a mouse cutaneous wound model.[85]

Polymersomes: Polymersomes are bilayer vesicles formed by self-assembly of amphiphilic block copolymers. Compared to lipid vesicles, they offer improved mechanical and chemical stability, as well as the possibility to tune thickness, permeability, and stimuli-responsive behavior. Similar to liposomes, polymersomes allow for loading of both hydrophilic drugs in the aqueous core and hydrophobic drugs in the membrane. However, due to the typically thicker membrane, polymersomes have much smaller encapsulation volume, which might affect the EE. Nevertheless, several polymersome architectures have been shown to efficiently co-encapsulate various molecules, including inorganic NPs.

For example, poly(ethelyne glycol)-*block*-poly(D,L)-lactic acid (PEG-*b*-PDLLA) polymersomes were used to co-encapsulate hydrophobic AgNPs in polymer membrane and hydrophilic ampicillin in the core.[99] Furthermore, the same group used PEG-*b*-PDLLA polymersomes to co-encapsulate superparamagnetic iron oxide nanoparticles (SPIONs) and antibiotic methicillin within the membrane bilayer and aqueous core, respectively.[98] Apart from their magnetic properties, SPIONs have also exhibited antimicrobial properties. These magnetically positionable nanocarriers have shown synergistic antimicrobial activity against *E. coli* grown in suspension, as well as complete eradication of 20 µm thick *S. epidermidis* biofilms following the application of an external magnetic field.[98]

In a peculiar example, polymersomes were designed to respond to penicillin G amidase (PGA) and β-lactamase (Bla) enzymes produced by drug-resistant bacterial strains (**Figure 6A**). Enzyme-responsiveness was introduced to polymer vesicles through enzyme-cleavable, self-immolative side linkages in the hydrophobic block.[95] The presence of PGA or Bla could trigger the cleavage of a hydrophobic block with a respective specific terminal group, leading to structural rearrangement and morphological transition, and thereby facilitate the release of encapsulated VAN antibiotics (Figure 6B). A pair of STR antibiotics, quinupristin/dalfopristin (Synercid), co-loaded into these polymersomes demonstrated anti-MRSA activity. Moreover, bacterial strain-selective delivery was evaluated for polymersomes encapsulating only VAN.[95] Enzyme-responsive polymer vesicles loaded with antibiotics only inhibited the growth of MRSA, whereas free drug inhibited the growth of both probiotics and MRSA, demonstrating specificity toward bacteria producing Bla, which probiotics cannot produce (Figure 6C). Interestingly, the vesicles encapsulating VAN could also accelerate the wound healing process in vivo on an infected burn wound model (Figure 6D).[95] Overall, engineering nanocarriers for bacterial strain-specific delivery of antibiotics can provide several advantages to prevent and combat drug resistance.

3.2.2. Polymer Nanoparticles

Polymeric NPs comprise solid matrix nanospheres and nanocapsules that differ in terms of their morphology. While nanocapsules have a distinct core/shell structure, solid polymer nanospheres feature a homogeneous solid matrix.[112] Several techniques have been reported for the preparation of polymer NPs using so-called "top-down" and "bottom-up" approaches. In top-down techniques, NPs are prepared via e.g., emulsion-solvent evaporation, nanoprecipitation, or dialysis using pre-formed polymers. On the other hand, bottom-up techniques rely on chemical synthesis of polymeric NPs from its monomers using e.g. precipitation-, emulsion-, or interfacial-polymerization.[113] In this approach, NPs are obtained in suspension together with many other impurities, including unreacted monomers, initiators, etc., and require additional purification steps. In addition, the cargo with reactive functional groups may display cross-reactivity and interfere with the polymerization process.[114] Therefore, top-down preparation techniques are often preferred over bottom-up approaches, because they also provide a better control over the colloidal NP characteristics.[115]

Polymeric materials based on poly(L-lactide-*co*-glycolide) (PLGA) are notable for their biocompatibility, tunable biodegradation characteristics, and extensive clinical use.[116] The highly versatile PLGA NP platform allows for the preparation of particles in various sizes[117] and large production scales.[118,119] Both hydrophobic and hydrophilic cargo, including biomolecules, can be co-encapsulated[118] by selecting appropriate solvents and preparation methods.

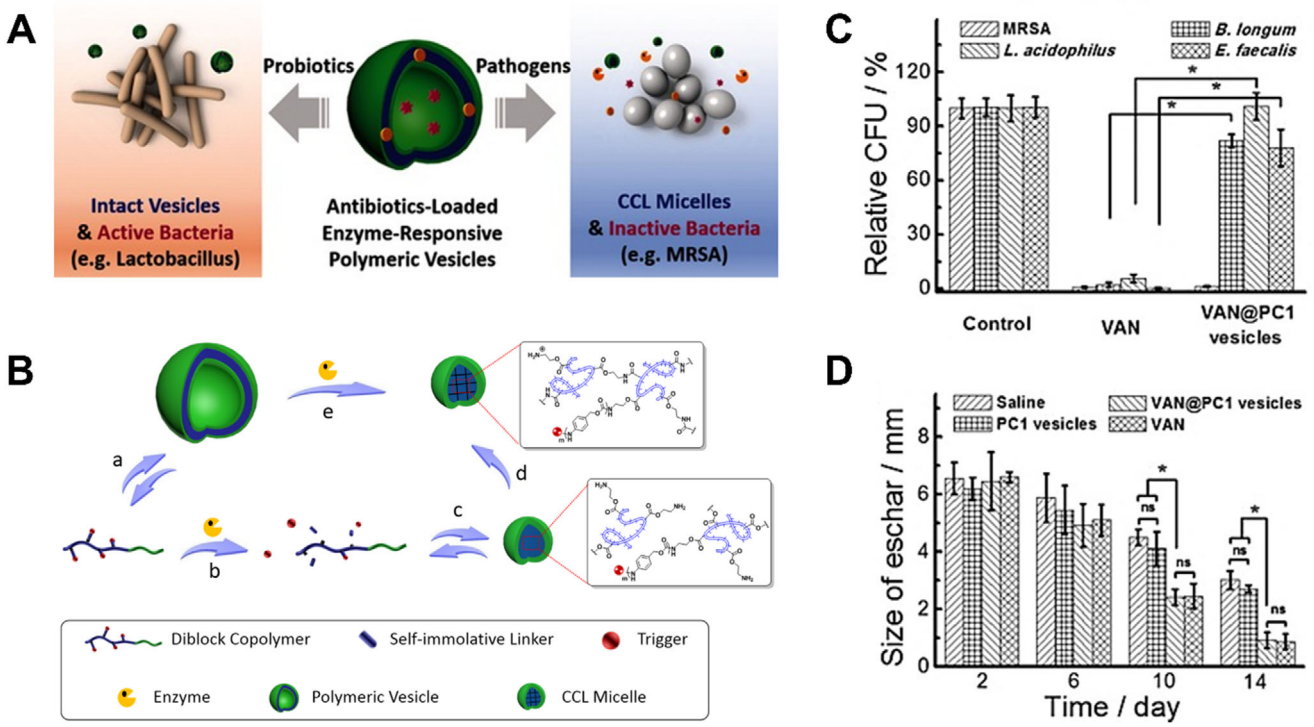

Figure 6. Enzyme-responsive polymersomes for bacterial strain-selective delivery of antibiotics. A) Penicillin G amidase (PGA) and β-lactamase (Bla), enzymes associated with drug-resistant bacteria, cause vesicles to release cargo and change morphology, whereas vesicles stay intact in the presence of probiotics, which lack these enzymes. B) Polymersomes undergo microstructural transformation to core cross-linked (CCL) micelles in response to enzyme triggering self-immolation of the block copolymer. C) Growth inhibition effects of vancomycin (VAN) and VAN-loaded vesicles against methicillin-resistant *Staphylococcus aureus* (MRSA), *B. longum*, *L. acidophilus*, and *E. faecalis*, demonstrating MRSA-selective release. D) Murine-infected burn wound size analysis, showing enhanced wound healing of free antibiotic and antibiotic-loaded vesicles, as compared to the control groups. $^{*}p < 0.05$, student's *t*-test. Reprinted with permission,[95] Copyright 2015, Wiley-VCH.

Among several applications, PLGA NPs have also been explored for the co-delivery of antimicrobial agents. Fan et al. have reported the co-encapsulation of silver ions (Ag^{+}) and simvastatin within sub-micron PLGA particles (AgS-PLGA) to enhance their antimicrobial synergy to inhibit the colonization of *E. faecalis* on dentin slices.[101] By fine-tuning the drug ratios, a sustained and simultaneous release of Ag^{+} and simvastatin have been achieved, ensuring a prolonged antibacterial efficacy. Furthermore, the acidic degradation products of PLGA were proposed to play a role in the observed antibacterial efficacy. Another interesting observation was the anti-inflammatory effect of AgS-PLGA via suppressing the IL-6/IL-1β secretion by RAW macrophages. In this work, synergy is likely due to the simvastatin's inhibitory effects on heavy ion efflux and the synthesis of extracellular polymeric substances, both of which are mechanisms that help bacteria overcome Ag^{+} treatment.[101] Despite the promising anti-bacterial and anti-inflammatory effects in vitro, further studies would be needed to validate these observations on in vivo models.

PLGA chains conjugated to hydrophilic polymers can self-assemble into micelles in aqueous solutions. Morteza et al. have tested the co-delivery of Piperacillin and Tazobactam with PLGA-PEG micelles on clinical isolates of *P. aeruginosa*.[92] The Piperacillin/Tazobactam combination is commonly used as the first-line therapy for nosocomial infections via intravenous administration and sold under different brand names. Tazobactam is used to enhance the efficiency of β-lactam inhibitor piperacillin by targeting the β-lactamase enzyme. The combination has activity against many Gram-positive and Gram-negative bacteria. The nanoformulated Piperacillin/Tazobactam combination displayed more effective inhibition of bacterial motility and biofilm formation with lower MIC values in comparison to free dug combinations.[92]

The antimicrobial effects of biomolecule-antibiotic combinations have also been studied using PLGA NPs.[105] In an elegant approach, Baelo et al. have loaded PLGA NPs with CPX and functionalized the NP surface with DNase I to eradicate *P. aeruginosa* infections. While the DNase I disassembled the biofilm by degrading the extracellular DNA that stabilizes the biofilm matrix, CPX effectively reduced the number of viable bacteria. The repeated administration of this PLGA–CPX–DNase I NP formulation over 3 days resulted in the eradication of more than 99.8% of the established biofilm in comparison to controls (**Figure 7**). The promising results obtained in this study highlight the unique advantages of nanoformulation-assisted co-delivery of not only multiple antibiotics, but also biomolecule-antibiotic combinations.

One of the serious infections where the systemically administered antibiotics are inefficient is osteomyelitis, a bone infection typically caused by *S. aureus*. The blood-bone barrier and the biofilm formed around the infected bone present major obsta-

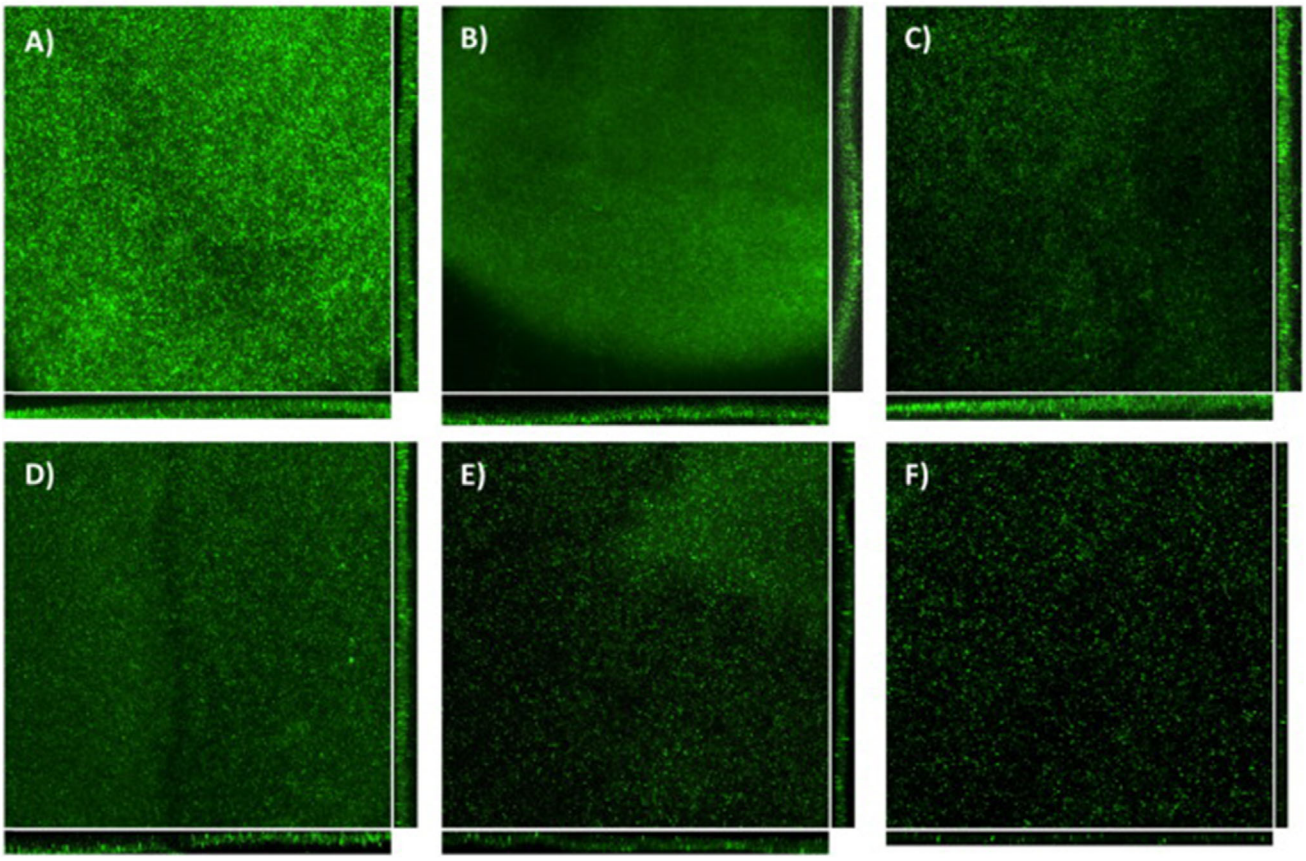

Figure 7. Analysis of *P. aeruginosa* biofilm formation in the absence and presence of PLGA nanoparticle (NP). Live bacteria are stained in green. A) *Pseudomonas aeruginosa (P. aeruginosa)* biofilm without treatment, B) treated with free ciprofloxacin (CPX) (0.5 μg mL^{-1}), C) treated with free CPX (0.5 μg mL^{-1}) + DNase I (10 μg mL^{-1}), D) treated with PLGA–CPX NPs at 0.5 μg mL^{-1}, E) treated with C–CPX NPs at 0.5 μg mL^{-1} + PLGA–DNase I (0.125 mg NP) and F) PLGA–CPX–DNAse I NPs at 0.5 μg mL^{-1}. Field of view: 455 μm X 455 μm. Reprinted with permission,[105] Copyright 2015, Elsevier.

cles for the systemically administered antibiotics to reach the infected area. Therefore, implantable local antibiotic delivery systems are regarded as more efficient treatment alternatives.[120] In this context, PLGA particles have been prepared in micron-sizes to act as drug reservoirs for the co-delivery of rifampicin (RIF) and moxifloxacin (MOX) (RIF-MOX-PLGA) against infections and biofilms caused by *S. aureus*.[100] Parallel investigations of empty, only MOX-loaded, and RIF-MOX-loaded PLGA particles revealed a significantly enhanced anti-biofilm efficacy for the co-loaded formulations. Overall, the high versatility of the PLGA platform makes it an important tool for the delivery of various antimicrobial agents. For a more comprehensive overview of PLGA-based systems for inhibition and destruction of biofilms, readers are referred to the work of Shariati et al.[121]

Polyelectrolyte nanocomplexes (NCs) are an emerging class of polymeric nanocarriers that are formed via electrostatic interactions of oppositely charged polyelectrolytes without the use of organic solvents, chemical cross-linkers, or surfactants.[122] Chitosan is a cationic biopolymer with good biocompatibility, biodegradability, and high abundance. Chitosan-based colloidal polyelectrolyte NCs have been extensively studied in drug delivery applications,[122] including antimicrobial co-delivery. Finbloom et al. have developed antibacterial NCs co-loaded with Tobramycin (Tob) and AgNPs via layer-by-layer (LbL) assembly.[102] The negatively charged dextran sulfate was first used to form complexes with the positively charged Tob. The LbL addition of positively charged chitosan facilitated the co-loading of AgNPs. This approach allowed for high loading efficiency of Tob and AgNPs into the NC formulation with efficient eradication of *P. aeruginosa* biofilm. The LbL deposition of anionic and cationic molecular species could also enable the modulation of NC surface charge via termination of the deposition process after the addition of the anionic or cationic layer. The NCs with positive surface charge were obtained with an extra chitosan coating (AgTob-NC 2b), while the NCs without the extra coating had negative surface charge (AgTob-NC 2a). Treatment of biofilm with both NCs led to observable changes in biofilm morphologies and significant reductions in bacterial biomass with an enhanced antimicrobial activity of NCs bearing positive surface charge (**Figure 8**), underscoring the importance of physicochemical properties of nanocarriers to combat bacterial infections. The leading hypothesis for the mechanisms of synergy in this case is that AgNPs inhibit biofilm formation and permeabilize the bacterial membrane while creating ROS, which improves Tob uptake and efficacy.

Chitosan nanoparticles (CSNPs) were also studied for the co-delivery of *Origanum Syriacum*, an essential oil with antimicrobial properties, and salen complexes of Zn(II) ions.[103] When

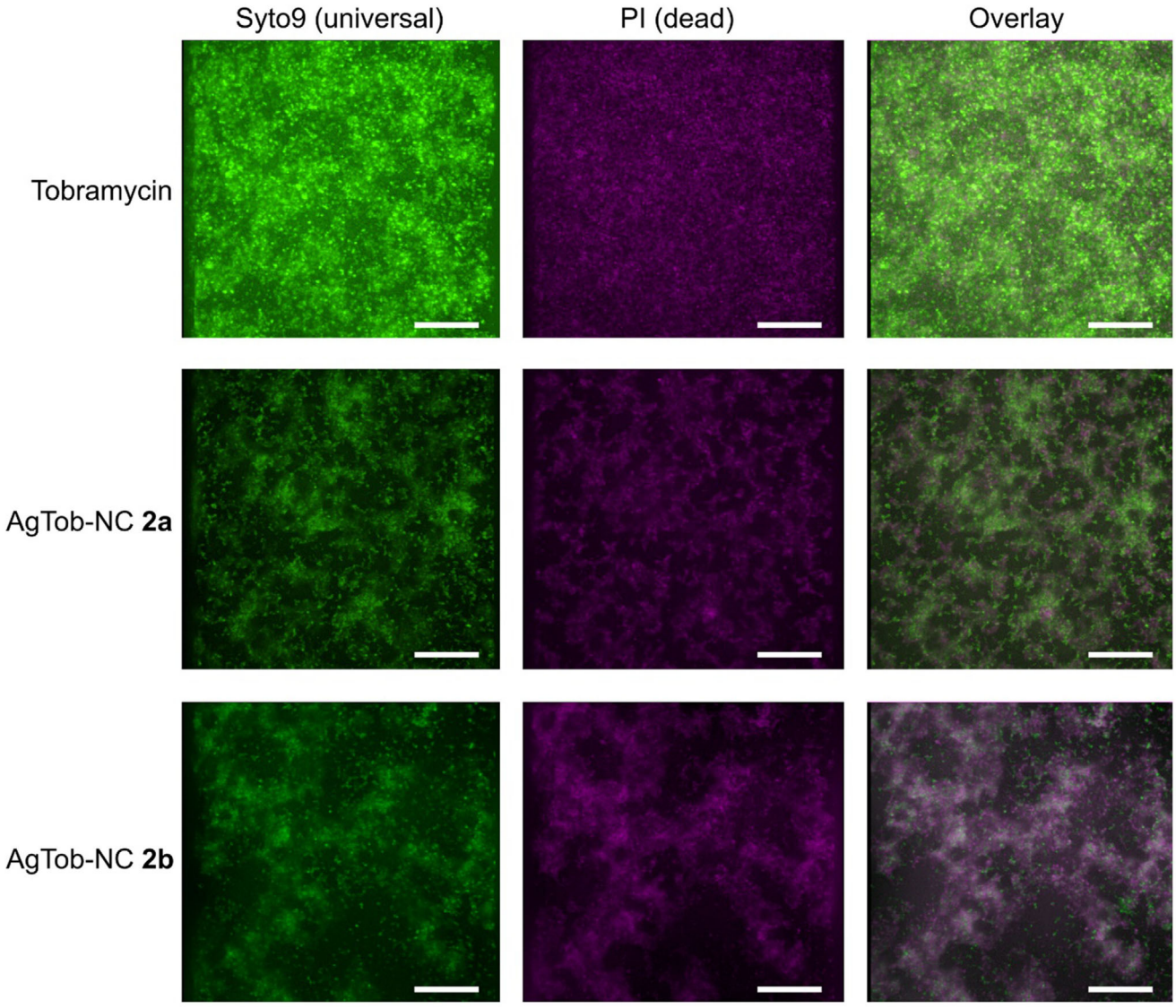

Figure 8. Representative three-dimensional projections of confocal fluorescence microscopy images of *P. aeruginosa* biofilms treated with Tob only, AgTob-NCs with negative (2a) and positive (2b) surface charge. Syto9 (green): stain for both live and dead bacteria, PI (magenta): stains only dead bacteria. Scale bars, 25 µm. Reprinted with permission,[102] Copyright 2023, AAAS.

formulated in CSNPs, each cargo displayed a better efficacy in comparison to their soluble counterparts, with the highest efficacy obtained upon co-encapsulation. The co-loaded CSNPs were effective toward several tested Gram-positive and Gram-negative strains. Similar to other essential oils, synergistic effects here might stem from increased membrane permeability mediated by *Origanum Syriacum*, potentiating the antimicrobial efficacy of Zn(II) salens. While the in vitro studies are promising, the toxicity and immunogenicity of the CSNPs prepared using chitosan obtained from shrimps need to be further evaluated in vivo.

Another class of polymeric nanocarriers emerging as potential alternatives for targeted drug delivery are nanogels made from cross-linked hydrophilic polymers. With their flexible structure and functional diversity, nanogels can be designed to show stimuli responsiveness and volume phase changes in response to external stimuli, offering precise control over drug release.[123] By further engineering the nanogel composition and preparation method, both hydrophilic and hydrophobic cargo[106] can be loaded within and co-delivered by nanogels. Although bulk hydrogels embedded with antimicrobial-loaded nanocarriers have also been developed, they fall outside the scope of this review, which focuses on colloidal nanogels.[28,124,125]

Using a top-down approach, Fasiku et al. developed nanogels co-loaded with AMPs and S-Nitroso-N-acetyl-DL-penicillamine (SNAP), a nitric oxide (NO) donor, from a bulk gel based on cross-linked hyaluronic acid.[107] A broad-spectrum antibacterial and anti-biofilm activity was observed in vitro on *E.coli*, Methicillin-resistant *S. aureus*, and *P. aeruginosa*. NO released by SNAP is believed to have a biofilm-penetrating effect, which facilitates delivery of AMPs to the bacterial membrane. Based on the fractional inhibition concentration (FIC) values, AMP-SNAP combination displayed a synergistic effect against *P. aeruginosa* and

Methicillin-resistant *S. aureus*, while the effect was additive for *E. coli*.[107]

Nanogels have also been explored for combination therapies merging photothermal and chemodynamic treatment of bacterial infections.[108] pH and NIR light-responsive nanogels were prepared by a self-assembly process involving alginate, tannic acid, and Fe^{3+} ions. In the acidic infection microenvironment, Fe^{3+} ions were released and reduced to Fe^{2+} to trigger a Fenton reaction for chemodynamic therapy. When exposed to NIR irradiation, the nanogel exhibited high efficiency in photothermal conversion, enabling synergistic combination therapy. Alginate played a dual role in this antibacterial nanogel formulation: First, it acted as a nanocarrier for the metal-phenolic networks formed between tannic acid and Fe^{3+} ions. Second, it served as an additional source of Fe^{2+} ions to further enhance chemodynamic therapy. Overall, the nanogel demonstrated a strong antibacterial efficacy in vitro on both Gram-positive *S. aureus* and Gram-negative *E. coli*. The findings were consistent for studies conducted on mice with *S. aureus* infection, suggesting that this low-cost and multifunctional nanogel could serve as a promising treatment option for bacterial infections.[108]

3.3. Polymer/Lipid Hybrids

Lipid-polymer hybrid nanoparticles (LPNPs) are innovative co-delivery platforms that merge the beneficial properties of polymeric NPs and lipid-based carriers. These NPs typically feature a polymeric core enveloped by a lipid shell, which enhances the stability and bioavailability of encapsulated therapeutic agents.[126] The structural design of LPNPs can vary, including monolithic matrix NPs where the lipid phase contains a homogeneous polymeric drug complex, core–shell NPs characterized by a polymeric core surrounded by a lipid shell, polymer-decorated liposomes, and lipid-polymer micelles.[20] These hybrid architectures facilitate the encapsulation of a wide range of drugs, providing a versatile platform for drug co-delivery applications, including the antimicrobial agents.

LPNPs can indeed combine the best of two worlds: For instance, by incorporating cationic lipid excipients for LPNP nanoformulations with natural antibacterial effects,[127] the treatment efficacy can be significantly improved. In addition, utilizing stimuli-responsive polymers in LPNP formulation can facilitate controlled and targeted delivery of antimicrobials. In a recent study, Jaglal et al. have developed pH-responsive LPNPs for the co-delivery of VAN and a phytochemical compound with antimicrobial properties, 18β-glycyrrhetinic acid (Ga), for the eradication of MRSA infections.[109] The VAN-Ga-LPNPs made up of polyallylamine hydrochloride and oleic acid were prepared via an emulsion method. By varying the lipid:polymer ratio, optimized NPs were obtained with a size of ≈200 nm and a slightly negative zeta potential at neutral pH, which became slightly positive in acidic conditions. Acidic pH values also facilitated the accelerated release of VAN, resulting in a significant reduction of the MRSA biofilm biomass. This dual drug delivery system effectively fought against MRSA by leveraging the synergistic effects of the co-encapsulated drugs and the pH-responsive release mechanism.[109]

In the study reported by Mohammadi et al., LPNPs composed of phospholipid-coated PLGA nanospheres were developed for the co-delivery of curcumin and gentamicin to eradicate *P. aeruginosa* biofilms and intracellular infections.[30] Indeed, *P. aeruginosa* thrives in both biofilm structures and within cells, making it particularly challenging to treat. Therefore, the double-loaded LPNPs were aimed to target both planktonic and biofilm-associated bacteria. Studies showed that LPNPs were nontoxic to mammalian cell lines and could effectively damage established biofilms with strong antibacterial activity. Upon internalization by macrophages, LPNPs could successfully eradicate intracellular bacteria, highlighting their potential for treating persistent and intracellular infections in chronic diseases such as cystic fibrosis.[30]

Lipid-containing hybrid nanocarriers can also be formulated as micelles using surfactants as "non-lipid" components, although they are not technically classified as polymers. In the study conducted by Sharma et al.,[110] a lipid-based hybrid micellar platform was constructed for the treatment of acne by leveraging the synergistic effects of isotretinoin (ITR) and clindamycin phosphate (CLIN) drugs. The mixed micellar nanocarrier system improved the stability, skin permeability, and retention of both drugs with improved skin compatibility. Micelles could retain both drugs effectively in skin layers, enhancing their antimicrobial effects against *Propionibacterium acnes*, a key bacterium involved in acne. Testing on a testosterone-induced acne model revealed that this formulation had superior efficacy compared to commercial products, with evidence supported by cellular uptake, MTT assays, and histopathology.[110]

Overall, the combination of the biomimetic behavior of lipids with long-term stability and mechanical strength of polymers within LPNP formulations offer unique advantages while simultaneously addressing the drawbacks of purely lipid- or polymer-based nanocarriers. As the focus of this review is the applications of LPNPs in co-delivery of antimicrobial agents, readers are referred to the work of Elhassan et al.[20] for an overview of the other types of hybrid nanocarriers combining e.g. organic–hinorganic materials against bacterial infections.

4. Challenges and Perspectives

Nanocarriers co-delivering antimicrobial agents with complementary modes of action can offer unique opportunities both to prevent resistance development and to eradicate resistant infections. Several promising approaches that have been highlighted in this review have achieved success in vitro and in preclinical settings. However, the path to clinical implementation of these co-delivery nanotherapeutics remains long and fraught with significant challenges and demands several considerations.

The first point to consider is the selection of the right antimicrobials. Not all antimicrobials work well together in the same delivery system. Differences in solubility, stability, and release profiles can make it difficult to formulate a stable and effective combination. Ideally, the selected antimicrobials should have complementary modes of action and show synergistic or additive effects rather than antagonizing each other. Therefore, antimicrobials intended for co-delivery should be extensively tested in combination to identify the synergistic dose ratios. Furthermore, the risk

of resistance development should be carefully evaluated. Using antibiotics with different action mechanisms may help reduce this risk.[128] Overall, a strategic approach to antibiotic selection for co-delivery should consider the compatibility, action mechanism, and resistance potential. It is of great importance to gain a deeper insight into the mechanistic interactions between co-delivered drugs. This knowledge will accelerate the rate at which new synergies are discovered.

Another challenge in the co-delivery of antimicrobials is to achieve precise release patterns required for both drugs to display the desired efficacy. The co-administration within the same nanocarrier becomes particularly complex when the drugs have very different solubility profiles, or when a sequential release is needed for the optimal efficacy. The latter could be the scenario to effectively destroy the biofilms, where an initial release of an anti-biofilm agent would facilitate more effective killing of the bacteria by the subsequently released antibiotic.[102] In this context, nanocarriers with precisely engineered structures and "smart" release mechanisms have been developed. For instance, nanocarriers with distinct hydrophilic and hydrophobic compartments, such as liposomes and polymersomes, can enable the co-loading of both hydrophobic and hydrophilic drugs. Incorporating stimuli-responsive polymers into the nanocarrier formulation, on the other hand, can allow for extended, targeted, on-demand, or sequential release of the antimicrobials.[75,84] These systems represent a promising strategy to mimic clinically preferred dosing sequences within a single co-delivery platform. In the realm of stimuli-responsive nanocarriers, one of the primary challenges lies in the variability of the physiological environment. Nanocarriers designed to release drugs in response to pH changes, for example, may not function effectively in the varied pH environments found within different types of infected tissues.[109,129] Additionally, photothermal and photodynamic therapies that rely on external light stimuli require precise control over the administration site, which can be difficult to achieve in deep-seated infections.[39,38,130] To overcome these challenges, it is necessary to develop nanocarriers with multi-stimuli-responsive capabilities that can respond to a combination of internal and external stimuli. However, ensuring the stability and responsiveness of these nanocarriers over time remains a major concern.

In terms of targeted nanocarriers for pathogen-specificity, both passive and active targeting mechanisms offer great promise but are not without their challenges. According to Maeda et al., the primary distinction between the EPR effect caused by infections and that associated with cancer lies in the retention-period's length. In cases of inflammation in normal tissue, retention is shorter compared to cancer, because the lymphatic drainage system is still in operation. Thus, the EPR effect is often unreliable due to variations in the vascular structure of infected tissues, particularly in chronic infections where the EPR effect may be less pronounced. This variation can lead to uneven distribution of nanocarriers, diminishing the efficacy of the co-delivered antimicrobial agents.[84,131] In contrast, active targeting, which utilizes surface ligands to bind specifically to pathogen receptors, faces the challenge of identifying pathogen-specific biomarkers that can be universally targeted. This is particularly problematic in polymicrobial infections or in cases where bacteria develop mutations that affect the expression of surface receptors.[124,132,133] Therefore, the development of nanocarriers equipped with multiple targeting ligands becomes both crucial and indispensable to ensure precise and effective targeting across a diverse range of pathogens.

Achieving pathogen-specificity is further complicated by the presence of biofilms, which protect bacteria from both the immune system and antibiotics. Biofilm eradication requires nanocarriers that can penetrate the biofilm matrix and deliver drugs directly to the bacterial cells within. While certain co-delivery systems have shown potential in targeting biofilm-associated infections,[39,102,124] their effectiveness is often limited by the inability to deliver therapeutics in a controlled manner throughout the biofilm's structure. Moreover, resistance mechanisms such as efflux pumps can expel antibiotics before they reach effective intracellular concentrations.[102,134] Therefore, new delivery strategies are needed to address multi-drug administration to simultaneously inhibit efflux pumps and attack bacteria.

Whether lipid-based or polymer-based, each class of nanocarriers used for antimicrobial co-delivery comes with its own set of challenges. Lipid-based nanocarriers such as liposomes are highly biocompatible and have been widely used in drug delivery. However, their inherent instability and the difficulty in controlling their size and surface characteristics pose challenges in ensuring uniform drug loading and release.[57,62,64,66] Polymer-based nanocarriers, including polymer micelles and nanogels,[82,86,87,106,124] offer greater versatility in terms of drug encapsulation and stimuli-responsiveness, but often suffer from limited biodegradability and potential toxicity due to their synthetic nature. Additionally, solid polymer NPs,[102,103] while robust, may not provide the flexibility required for the co-delivery of drugs with vastly different solubility profiles. As a result, polymer/lipid hybrid systems are being explored to combine the advantages of both materials,[109,110,125] however, synthesis and optimization of these hybrids remain complex.

In recent years, extensive synthetic efforts have been dedicated to developing nanocarriers with tailorable properties. Unlike lipid-based systems, where chemical modifications are inherently limited, polymer-lipid conjugates offer a versatile strategy to introduce functional moieties and change surface properties. Beyond PEG-lipids, various conjugates have been explored, affecting in vitro cellular uptake, protein corona formation, and blood clearance.[135–138] When constructing polymer-based delivery systems, precise control over dispersity and molecular weight is crucial. Reversible-deactivation radical polymerization techniques, such as reversible addition-fragmentation chain transfer (RAFT) polymerization and atom transfer radical polymerization (ATRP), are widely used to achieve this control. These methods also allow for a simple introduction of functional groups such as carboxylic, amine, thiol, azide, and alkyne through either functional monomers and initiators or via post-polymerization modifications.[139] Click chemistry, particularly copper-catalyzed azide-alkyne cycloaddition (CuAAC), has emerged as a powerful and widely used approach for conjugating targeting ligands and drug molecules to appropriately functionalized nanocarriers.[140–142] Additionally, nanocarrier platforms with suitable functional groups offer the possibility to achieve theranostic properties.[143]

Comprehensive safety studies and scalable manufacturing technologies are crucial for clinical translation of antimicro-

bial co-delivery systems. However, scaling up from laboratory to industrial production poses significant challenges, particularly in ensuring batch-to-batch consistency.[144,145] Emerging continuous manufacturing approaches, such as microfluidics and high-pressure homogenization, offer promising solutions for producing large volumes of nanotherapeutics without modifying their physiochemical properties.[119,146] Beyond formulation, attention must also be given to downstream processes such as purification and storage, which should be implemented in compliance with regulatory guidelines to ensure successful clinical translation.[113,118]

Overall, despite the significant challenges associated with the development of nanocarriers for antimicrobial co-delivery, there are several promising avenues for overcoming these hurdles. Advances in nanotechnology coupled with a better understanding of pathogen-specific mechanisms hold the potential to design efficient and safe co-delivery systems, which will benefit from further improvements in drug release control, targeting specificity, and overcoming the biological barriers posed by resistant pathogens and biofilms.

5. Conclusions

As highlighted in this review, soft nanocarriers hold tremendous potential as co-delivery systems to enhance the efficacy of antimicrobial therapies against a rapidly increasing range of resistant infections. By leveraging the synergistic effects of multiple antimicrobial agents with complementary modes of action, these co-delivery systems not only potentiate the elimination of infections but also prevent the emergence of resistance. Despite the substantial progress, challenges such as controlling the sequence and rate of release, achieving pathogen specificity, and overcoming the biological barriers posed by resistant pathogens and biofilms remain to be addressed. Continued research in this field is essential to refine nanocarrier engineering and optimization strategies and translate them into viable, effective treatments that can meet the urgent demands of combatting resistant infections.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

antimicrobials, antimicrobial resistance, co-delivery, hybrids, lipids, nanocarriers, polymers

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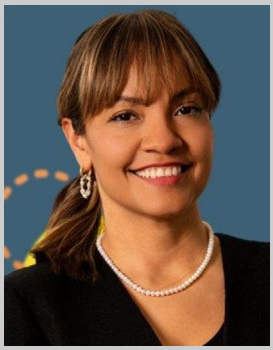
Anamarija Nikoletić is a joint Ph.D. student in the groups of Prof. Dr. Oya Tagit at the University of Applied Sciences and Arts Northwestern Switzerland and Prof. Dr. Cornelia Palivan in the Department of Chemistry at the University of Basel. She received her B.Sc. in Chemistry at the University of Belgrade, Serbia in 2021. In 2023, she obtained M.Sc. in Chemistry at the University of Basel, Switzerland as an Alfred Werner scholar of the Swiss Chemical Society and joined the Swiss Nanoscience Institute Ph.D. School. Her current research focuses on synthesis of stimuli-responsive polymers for advanced drug delivery.

Evangelos Natsaridis is a Postdoctoral Researcher in the Biointerfaces group at FHNW, Switzerland. His current research focuses on the development of mRNA-based nanotherapeutics for the treatment of drug-resistant infections. He earned his Ph.D. in Pharmaceutical Technology from the University of Patras, Greece, during which he obained a fellowship at the Institute of Chemical Engineering Sciences (FORTH/ICE-HT). There, he developed liposomal formulations for the treatment of post-cataract endophthalmitis. His broader research aim is to design advanced drug delivery systems that overcome the limitations of existing therapies for cancer and bacterial infections.

Timon Flathmann is a joint Ph.D. student in the groups of Prof. Dr. Oya Tagit at the University of Applied Sciences and Arts Northwestern Switzerland and Prof. Dr. Nina Khanna at the Department of Biomedicine at University of Basel. After completing his B.Sc. in Nanoscience at the University of Basel in 2021, Timon studied at the European Research Institute for the Biology of Ageing at UMC Groningen for his master's thesis. He obtained his M.Sc. in Nanoscience at the University of Basel in 2024. Current work involves the development of mRNA nanoparticulate delivery systems to tackle resistant bacterial infections.

Daiane P. C. Quadros earned her Ph.D. in Chemistry from the Federal University of Santa Catarina (UFSC), Brazil, in 2015. In 2024, she conducted research as a visiting scholar in the group of Prof. Dr. Oya Tagit (FHNW, Switzerland). She currently serves as a chemistry teaching professor at the Federal Institute of Santa Catarina (IFC), Brusque campus, Brazil. Her academic involvement includes Medicinal Chemistry and Organic Chemistry, and she also supervises student internships and teacher training programs.



Nubia Judith Gogniat holds a Ph.D. in Chemical Sciences from Complutense Madrid University and a Master's in Molecular Nanoscience. Starting as a Chemical Engineer, she's built an international career including postdoctoral research at FHNW's Biointerfaces Group, leading pharmaceutical formulations and managing projects. Her experience spans Switzerland, Spain, Mexico, and Croatia, utilizing expertise in spectroscopy and nanoscience alongside GMP/PMP certifications. As a sales manager at Starlab Schweiz AG, she combines technical knowledge with PMP certification to drive strategic account development – demonstrating commitment to innovative solutions across academia, industry, and pharmaceuticals.

Oya Tagit obtained her Ph.D. at the MESA+ Institute for Nanotechnology, University of Twente, The Netherlands. Following a career in a highly international landscape (France, Singapore, Ireland), she is currently leading the Group of Biointerfaces at FHNW. She is also PI and member of the executive committee at the Swiss Nanoscience Institute. Utilizing an interdisciplinary approach, the group of BioInterfaces aims to address currently unmet biomedical needs in the diagnosis, monitoring, and treatment of complex diseases, such as cancer and resistant infections, with engineered nanoparticles tailored for the desired biological targets and functions.