International Journal of Nanomedicine

Dovepress
Taylor & Francis Group

Open Access Full Text Article

REVIEW

Surfactant-Engineered Niosomal Antibiotic Systems for Biofilm-Associated Infections: Design Principles and Translational Perspectives

Siti Rahma[1,2], Eri Amalia[3], Sri Agung Fitri Kusuma[2]

[1]Master Program of Pharmacy, Faculty of Pharmacy, Padjadjaran University, Sumedang, West Java, Indonesia; [2]Department of Pharmaceutical Biology, Faculty of Pharmacy, Padjadjaran University, Sumedang, West Java, Indonesia; [3]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Padjadjaran University, Sumedang, West Java, Indonesia

Correspondence: Sri Agung Fitri Kusuma, Department of Pharmaceutical Biology, Faculty of Pharmacy, Padjadjaran University, Sumedang, West Java, Indonesia, Email s.a.f.kusuma@unpad.ac.id

Abstract: Persistent microbial biofilm infections remain a major obstacle to effective antimicrobial therapy due to restricted drug diffusion, metabolic heterogeneity, and the presence of tolerant bacterial subpopulations. In device-associated infections, biofilms substantially reduce antibiotic efficacy and contribute to chronic relapse despite adequate systemic exposure. Although nanocarrier-based delivery systems have been widely investigated, many formulations remain empirically developed with insufficient consideration of biofilm-specific physicochemical and biological barriers. This review examines surfactant-engineered niosomal antibiotic systems from a rational design perspective. Key formulation parameters, including surfactant type, hydrophile–lipophile balance (HLB), cholesterol content, surface charge, and microenvironment-responsive behavior, critically influence bilayer rigidity, permeability, encapsulation efficiency, intrabiofilm transport, and release kinetics. In particular, electrostatic interactions with the negatively charged extracellular polymeric substance (EPS) matrix and pH-responsive destabilization strategies are discussed as important determinants of localized antibiotic delivery within heterogeneous biofilm environments. Despite promising antibiofilm activity in vitro, translational progress remains limited by variability in formulation characterization, insufficient in vivo validation, and incomplete alignment between carrier responsiveness and biofilm microenvironmental conditions. By integrating insights from pharmaceutics, materials science, and microbial pathophysiology, this review proposes a structured framework for the rational design of surfactant-engineered niosomes and highlights key considerations for advancing antibiofilm nanomedicine.

Keywords: niosomes, surfactant engineering, biofilm-associated infections, antibiotic delivery, vesicular nanocarriers, translational nanomedicine

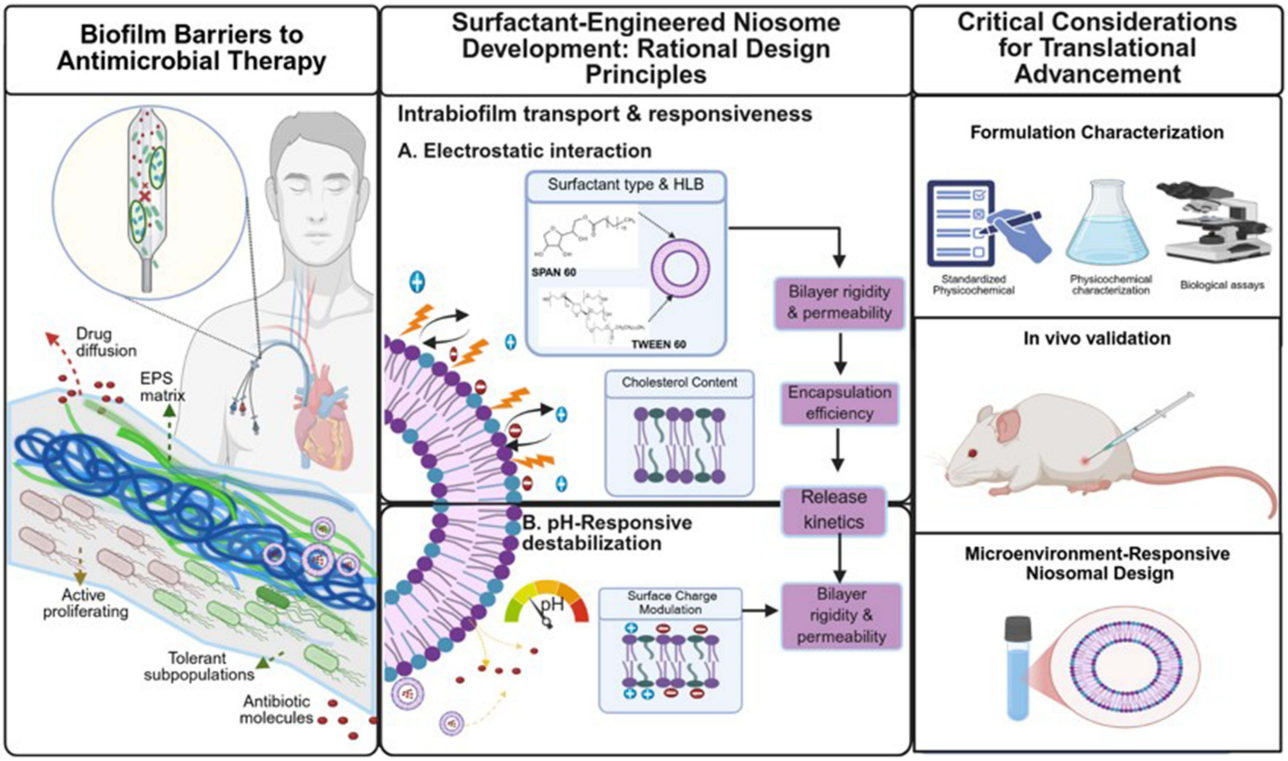
Introduction

Antimicrobial resistance (AMR) continues to compromise the effectiveness of conventional antibiotic therapy, with biofilm-associated infections representing a major driver of chronic and treatment-refractory disease.[1,2] Clinically, biofilms are implicated in a substantial proportion of persistent infections, particularly those involving indwelling medical devices, where antibiotic tolerance may exceed that of planktonic bacteria by several orders of magnitude.[3,4] In addition to device-associated infections, biofilm persistence also contributes to chronic non-device-related conditions such as *Pseudomonas aeruginosa* lung infections in patients with cystic fibrosis. The recalcitrance of these infections is rooted not only in genetic resistance but also in the structural and physiological organization of biofilms.[5] In addition to increasing morbidity and therapeutic failure, biofilm-associated infections also impose substantial healthcare and economic burdens because of prolonged hospitalization, recurrent treatment, and device replacement requirements.[3,5]

Biofilms consist of spatially organized microbial communities embedded within a self-produced extracellular polymeric substance (EPS) matrix that anchors them to biotic and abiotic surfaces.[4,6] This hydrated polymeric network functions as a diffusion-modifying barrier, restricting antibiotic penetration and generating subinhibitory intrabiofilm

https://doi.org/10.2147/IJN.S607567
Received: 7 March 2026
Accepted: 12 June 2026
Published: 25 June 2026

Graphical Abstract



concentrations. Concurrent nutrient and oxygen gradients promote metabolic heterogeneity, including dormant or slow-growing subpopulations that exhibit transient antibiotic tolerance.[7] Stress response activation, efflux systems, enzymatic drug inactivation, and horizontal gene transfer further reinforce survival within dense microbial consortia.[3,8] Together, these features create a localized microenvironment that is insufficiently addressed by conventional systemic antibiotic therapy. Among emerging nanocarrier systems, surfactant-engineered niosomes provide structurally adaptable vesicular platforms that can modulate membrane rigidity, drug retention, and interfacial transport behavior relevant to biofilm-targeted delivery.[3]

Within this framework, surfactant selection represents a critical design variable rather than a purely formulation-driven choice. Parameters such as hydrophile–lipophile balance, alkyl chain characteristics, and cholesterol incorporation influence bilayer organization and drug retention within biofilm microenvironments. However, despite increasing reports of niosomal antibiofilm efficacy, a systematic synthesis linking surfactant physicochemical attributes to transport performance and therapeutic outcomes remains limited. Peptide-based antibacterial nanomaterials have emerged as promising alternatives to conventional antibiotics because of their membrane-disruptive activity, broad-spectrum efficacy, and reduced susceptibility to classical resistance mechanisms. Recent advances in peptide–nanohybrid systems have further demonstrated enhanced antibacterial and antibiofilm performance through improved membrane interactions, structural self-assembly, and synergistic integration of nanomaterials.[9,10] However, many peptide-based systems remain constrained by proteolytic instability, rapid degradation, limited loading flexibility, high production cost, and challenges in maintaining sustained localized delivery within complex biofilm microenvironments.[11] In contrast, surfactant-engineered niosomes provide a compositionally tunable platform capable of encapsulating both hydrophilic and lipophilic agents while modulating bilayer rigidity, release kinetics, colloidal stability, and microenvironment-responsive transport behavior. Rather than competing technologies, peptide-based nanomaterials and surfactant-engineered vesicular systems represent complementary strategies in the broader development of precision antibiofilm nanomedicine.

This review critically evaluates recent advances in niosomal antibiotic delivery, with an emphasis on surfactant engineering as a mechanistic determinant of biofilm penetration, drug-release kinetics, and antibacterial efficacy. Particular attention is given to rational surfactant engineering principles, including hydrophile–lipophile balance (HLB) modulation, bilayer stabilization, electrostatic interactions, vesicle surface charge, and microenvironment-responsive behavior, which are key determinants of antibiofilm delivery performance. By integrating insights from pharmaceutics, materials science, and microbiology, the review proposes a structured framework for aligning niosomal design with biofilm physiology and therapeutic requirements. Such integration is essential to support the rational and clinically relevant development of precision nanomedicine strategies for persistent and device-associated infections.

Microbial Biofilms: A Challenge to Antibiotic Therapy

Conventional antibiotic regimens frequently fail against biofilm-associated infections despite adequate systemic exposure. This discrepancy reflects limited drug access to bacteria embedded within the EPS, rather than insufficient intrinsic antimicrobial activity.[4] The EPS matrix operates as a hydrated, polymer-rich scaffold composed of polysaccharides, proteins, extracellular DNA, and associated biomolecules that collectively impede molecular diffusion. Antibiotics entering the biofilm experience delayed penetration and uneven spatial distribution, resulting in concentration gradients across the biofilm depth.[4] Within this structured environment, bacterial subpopulations adopt distinct metabolic states shaped by local nutrient and oxygen availability. Reduced growth rates and altered physiology diminish antibiotic susceptibility without requiring stable genetic resistance.

As a consequence, bacteria residing in protected niches withstand antibiotic levels substantially exceeding those required to inhibit planktonic cells. Surviving subpopulations maintain the biofilm reservoir, allowing persistence under therapy and recurrence after treatment withdrawal. The resulting tolerance complicates eradication and prolongs infection. Therapeutic failure in this context is therefore governed primarily by transport constraints within the biofilm architecture. Effective intervention requires delivery strategies that improve intrabiofilm drug distribution and overcome diffusion barriers, rather than relying solely on increasing systemic dosage.

Biofilms are particularly problematic in catheter- and prosthesis-related infections, where microbial communities establish on abiotic surfaces and exhibit marked tolerance to both antimicrobial therapy and host immune clearance.[5,6] Surface physicochemical properties, implantation duration, and interactions with the host material facilitate initial adhesion and subsequent EPS accumulation.[6] As biofilms transition from attachment to maturation, their three-dimensional architecture becomes increasingly organized, reinforcing diffusion limitation and physiological heterogeneity.[7] Dispersal events may further seed adjacent sites, perpetuating infection cycles.

Clinically, this progression translates into chronicity, recurrent inflammation, and frequent need for device removal. Standard antimicrobial regimens often suppress but do not eliminate the structured community, contributing to repeated therapeutic failure. The absence of diagnostic and delivery systems capable of achieving sufficient intrabiofilm concentrations further constrains clinical management.[8] Taken together, these findings indicate that biofilm-associated treatment failure is driven predominantly by impaired drug delivery within structured microbial communities. This recognition provides a rational basis for nanocarrier-based approaches, including surfactant-engineered vesicular systems, designed to enhance matrix penetration and optimize local drug availability while maintaining systemic safety.

Mechanisms of Biofilm Formation

Biofilm development proceeds through four sequential yet interdependent stages, each presenting distinct therapeutic vulnerabilities that conventional antibiotics struggle to overcome. Below, we dissect these phases using a structured analytical lens tailored to nanomedicine applications.

Initial Adhesion

Surface colonization by planktonic bacteria marks the onset of biofilm infection, yet conventional antimicrobial strategies rarely intervene at this reversible stage, allowing progression to irreversible attachment. Pioneering cells exploit host-derived conditioning films formed by adsorbed proteins and glycoproteins on implant surfaces to anchor via adhesins, fimbriae, and pili, transitioning from transient contact to firm binding that resists shear forces.[12,13] Established adhesion

creates a receptive substrate for latecomer species, amplifying biofilm biomass and elevating device-related infection risk by orders of magnitude, as seen in 65–80% of catheter and prosthetic failures.[14,15] Nanocarriers must prioritize anti-adhesive coatings or receptor-mimicking surfactants to disrupt this foundational step, forestalling the cascade toward mature biofilms where eradication becomes exponentially costlier.

EPS Production and Microcolony Formation

The nascent biofilm lacks structural cohesion until EPS precipitates, rendering early microcolonies impervious to systemic drugs despite their limited mass. Proliferating cells secrete polysaccharides, proteins, and eDNA that self-assemble into a gel-like matrix, nucleating three-dimensional microcolonies through phase separation and interfacial polymerization.[2,4] This scaffold imposes a diffusion gradient that restricts antibiotic access while fostering metabolic cooperation within microcolonies, resident cells thus endure up to 128-fold higher minimum inhibitory concentrations (MIC) compared to planktonic counterparts.[16] Targeted delivery systems bearing matrix-degrading enzymes or penetrative moieties offer a window to fragment these nascent structures before consolidation, circumventing the tolerance amplification inherent to later phases.

Maturation

Fully differentiated biofilms evade clearance through architectural sophistication, confounding even high-dose regimens that achieve extracellular lethality but spare the core. Interconnected water channels mature alongside upregulated efflux and stress genes, stabilizing a heterogeneous milieu where persister subpopulations thrive amid nutrient gradients and quorum sensing (QS) orchestrated consolidation.[14,17] Tolerance escalates to >10,000-fold MIC elevation, driving 80–90% recurrence in indwelling device infections and necessitating explantation in chronic cases like orthopedic hardware failures.[13] Vesicular platforms engineered for deep-tissue partitioning and stimuli-responsive payload release address this recalcitrance, rationally aligning pharmacokinetics with the biofilm's spatiotemporal defenses.

Dispersal

Therapeutic gains prove fleeting as viable cells detach, disseminate to distal sites, and undermine source control despite apparent resolution. Environmental cues, such as nutrient depletion, nitric oxide surges, or quorum signal decay, trigger enzymatic matrix dissolution and activation of motility genes, reverting cells to a planktonic, hypervirulent phenotype.[2,18] Dispersed cells establish secondary infection sites, accounting for 40–60% relapse rates in ventilator-associated pneumonia and recurrent UTIs following catheter removal.[13] Prophylactic nanotherapeutics incorporating dispersal inhibitors alongside bactericides could trap cells in vulnerable sessile states, converting transient suppression into durable eradication.

Biofilm Characteristics and Regulatory Mechanisms

Physical Barriers

Antimicrobial agents often exhibit limited penetration beyond the biofilm periphery because the EPS matrix restricts drug diffusion despite adequate systemic exposure. Exopolysaccharides, proteins, and extracellular DNA interweave into a viscoelastic lattice permeated by tortuous nanochannels, selectively retarding antimicrobial penetration through steric occlusion and charge-mediated sequestration. This configuration has been documented in staphylococcal biofilms formed on abiotic substrates.[19,20]

This scaffold confines drug gradients to peripheral laminae, sparing internal populations, as evidenced by vancomycin's limited disruption of staphylococcal biofilms, with free formulations yielding markedly inferior inhibition relative to encapsulant counterparts.[15] Niosomal carriers may improve antibiotic transport across biofilm matrices through surfactant-mediated modulation of interfacial interactions and controlled drug release.[21,22]

Cell-to-Cell Communication (Quorum Sensing)

Microbial collectives orchestrate resistance beyond structural fortification; their synchronized physiology reduces the efficacy of antimicrobial agents designed to target planktonic cells. Autoinducers N-acyl homoserine lactones among

Gram-negatives, competence-stimulating peptides in Gram-positives, attain threshold concentrations that trigger transcriptional shifts toward matrix augmentation, efflux escalation, and virulence coordination.[17,23]

QS circuitry amplifies tolerance by coordinating key resistance mechanisms, including EPS production, metabolic adaptation, and efflux-associated antimicrobial tolerance, thereby sustaining biofilm persistence.[24] Vesicular anti-quorum payloads disrupt this regulatory confederacy at inception, restoring susceptibility windows prior to architectural entrenchment, a strategy meriting niosomal adaptation.[22,25]

Key Reasons for the Failure of Conventional Therapies: Limited Penetration and Drug Inactivation

Low Antibiotic Penetration

Circulating antibiotics frequently establish subtherapeutic concentration gradients within biofilms despite adequate vascular levels. The EPS forms a viscoelastic matrix characterized by tortuous diffusion pathways that restrict molecular transport through steric hindrance, electrostatic repulsion of charged antibiotics, and transient binding to polymeric constituents.[3,25] As a consequence, antimicrobial concentrations decline progressively toward deeper biofilm layers, allowing embedded bacterial populations to survive therapeutic exposure. This restricted transport sustains microbial viability within device-associated staphylococcal and pseudomonal communities, contributing to incomplete clinical eradication.[26] Strategies aimed at modifying interfacial interactions, including surface-engineered nanovesicles, seek to improve intrabiofilm distribution; however, effective penetration requires alignment between carrier physicochemical properties and the structural heterogeneity of the EPS.

Antimicrobial Agent Inactivation

Even when antibiotics successfully penetrate the biofilm matrix, their activity may be reduced prior to reaching intracellular targets. Enzymes embedded within the EPS, including β-lactamases, esterases, and nucleases, contribute to extracellular drug degradation, while upregulated efflux systems further limit intracellular accumulation under hypoxic microenvironmental conditions.[27,28] These matrix-associated and cellular mechanisms have been linked to treatment failure in biofilm-related implant infections.[29] Encapsulation within vesicular systems may reduce premature enzymatic exposure during matrix traversal and enable more controlled drug release in regions with less pronounced degradative activity.

Phenotypic Resistance in Microorganisms in Biofilms

Biofilm-associated treatment failure is attributable not only to restricted transport or enzymatic degradation but also to phenotypic adaptation within microbial subpopulations. Nutrient and oxygen gradients promote the emergence of persister phenotypes characterized by metabolic dormancy, activation of stress-response pathways, and increased efflux activity, particularly in anoxic regions of mature biofilms.[30,31] These transient physiological states contribute to persistent bacteremia in catheter-associated infections despite susceptibility under planktonic conditions.[14,32] Delivery systems capable of sustaining effective local drug concentrations over extended periods may help counteract dormancy-associated tolerance without relying on dose escalation.

Representative Examples of Biofilms on Medical Devices

Urinary Catheters

Catheter-associated urinary tract infections (CAUTIs) remain among the most prevalent healthcare-associated infections, largely driven by biofilm formation on indwelling urinary catheters.[26,28] Once established, these biofilms act as persistent reservoirs that are difficult to eradicate with systemic antibiotics.[27] Microorganisms initially adhere to catheter surfaces and subsequently secrete EPS, forming multilayered communities that progressively encase bacterial cells.[26] The matrix not only stabilizes colonization but also facilitates biomineralization and encrustation, phenomena documented in prospective clinical evaluations of polymer-coated catheters.[28] Coagulase-negative staphylococci and other uropathogens exploit this structured niche to sustain long-term persistence.

Matrix-embedded communities promote recurrent infection, necessitate repeated catheter replacement, and prolong hospitalization. Even when antimicrobial therapy is appropriately selected, eradication is limited because the device surface itself remains colonized.[26,28] Preventive strategies must prioritize interference with early adhesion and matrix establishment. Surface-engineered or polymer-modified catheters that reduce biomass accumulation provide preliminary clinical signals of benefit.[28] These observations support the integration of surface-directed and nanocarrier-based approaches to enhance local antimicrobial activity and extend device functionality.

Central Venous Catheters (CVCs)

Central venous catheters constitute a major source of catheter-related bloodstream infections (CRBSIs), particularly in critically ill populations.[29,31] Methicillin-resistant *S. aureus* and coagulase-negative staphylococci predominate in these device-associated infections.[30,32] Microbial attachment to luminal and extraluminal catheter surfaces initiates EPS matrix assembly, enabling persistent colonization.[33] Staphylococcal species form structured biofilms that resist mechanical clearance and systemic antibiotics, particularly following breaches in aseptic handling or hematogenous seeding.[29,30]

Biofilm-mediated colonization precipitates bloodstream dissemination, prolongs intensive care stays, and increases mortality risk.[31] Isolates from catheter surfaces frequently exhibit antibiotic resistance patterns that complicate therapy.[32] Conventional prophylaxis alone is insufficient once biofilms have formed on device surfaces. Targeted antimicrobial delivery systems capable of penetrating the EPS matrix, including vesicular nanocarriers, have been proposed to enhance local drug exposure and disrupt staphylococcal biofilms.[30,34] Such approaches align with the recognized limitations of systemic therapy in CRBSIs.

Cardiac Implantable Electronic Devices (CIEDs)

Infections involving pacemakers and implantable cardioverter-defibrillators are frequently biofilm-associated and are associated with substantial morbidity.[35,36] Pathogens, particularly *S. aureus*, adhere to device pockets and transvenous leads, where EPS-embedded communities develop during implantation or subsequent bacteremia.[35] Meta-analytic data identify biofilm persistence as a central contributor to infection risk beyond baseline microbial virulence.[36]

Once established, these biofilms are difficult to eradicate without device explantation. Clinical consequences include systemic infection, repeated interventions, and significant healthcare expenditure.[35] Preventive strategies should emphasize anti-adhesive or antimicrobial surface modifications at the time of implantation. Recognition of biofilm formation as a mechanistic driver of CIED failure supports the development of device-integrated or locally delivered antimicrobial technologies.[36]

Respiratory Devices

Endotracheal and tracheostomy tubes are rapidly colonized by microbial biofilms, contributing to ventilator-associated pneumonia (VAP) in intensive care settings.[37,38] Microorganisms adhere to the internal lumen of respiratory tubing and initiate EPS production, forming structured microcolonies. Systematic analyses report culture positivity rates exceeding 50%, depending on sampling methodology.[37] Device material properties and antimicrobial coatings may reduce, but do not eliminate, biofilm colonization.[39]

Biofilm-laden tubes increase the risk of pneumonia, prolong mechanical ventilation, and increase mortality.[38] Persistent colonization despite the use of coated devices indicates that material modification alone remains insufficient. Advanced surface engineering and localized antimicrobial delivery strategies are therefore required to better limit intraluminal colonization and reduce VAP incidence.[37,39]

Chronic Wounds

Chronic wounds, including diabetic foot ulcers and pressure injuries, frequently harbor polymicrobial biofilms that impede healing and complicate clinical assessment.[13,27] Surface contamination progresses to EPS-embedded microbial aggregates dominated by *Staphylococcus* and *Pseudomonas* species, which sustain chronic inflammation and resist host clearance.[40,41] Matrix-imposed diffusion constraints limit topical antimicrobial penetration to the superficial wound layers, often to depths of less than one-fifth of the tissue depth.[40]

Persistent biofilms are associated with high relapse rates after therapy, delayed wound closure, and increased risk of limb loss. Therapeutic failure frequently reflects inadequate delivery rather than intrinsic antimicrobial inefficacy.[40] Effective management requires strategies capable of traversing the EPS barrier and sustaining local drug concentrations within wound tissue. Surfactant-based nanocarriers, including niosomal systems, have demonstrated enhanced antibacterial and antibiofilm activity in experimental models, supporting their consideration as rational adjuncts to conventional wound care in biofilm-dominated lesions.[15,42]

Niosomes: Surfactant-Based Drug Delivery Systems

Conventional drug delivery systems frequently fail to reconcile drug instability, rapid systemic clearance, limited tissue accumulation, and dose-related toxicity. Although liposomes have addressed some of these limitations, issues related to cost, oxidative instability of phospholipids, and large-scale manufacturing remain relevant. Consequently, alternative vesicular systems with improved physicochemical robustness and formulation flexibility have been sought.[1,43]

Niosomes are self-assembled bilayer vesicles formed from non-ionic surfactants, typically stabilized with cholesterol. Their amphiphilic architecture enables spontaneous organization in aqueous environments into closed bilayers that encapsulate both hydrophilic compounds in the aqueous core and lipophilic agents in the hydrophobic membrane domain.[1,44] Surfactant molecular geometry, HLB, alkyl chain length, and phase transition behavior collectively determine bilayer rigidity, permeability, and drug retention.[45,46] Cholesterol incorporation further modulates membrane packing and mechanical stability, reducing leakage and improving structural integrity during storage and biological exposure.[1]

This structural adaptability allows niosomes to improve drug encapsulation efficiency, modulate release kinetics, and enhance physicochemical stability compared with several conventional formulations.[44,47] Their compositional versatility has supported applications across anticancer, antimicrobial, and transdermal delivery contexts, where controlled release and enhanced tissue interaction are required.[48,49] The bilayer-forming capacity of non-ionic surfactants establishes niosomes as a rational platform for precision drug delivery. However, formulation performance is contingent upon careful surfactant selection and structural optimization rather than generic vesicle preparation.

Controlled fabrication improves drug loading, prolongs release, and enhances storage stability. Empirical optimization studies demonstrate that rational adjustment of formulation variables significantly influences antibacterial and anticancer efficacy, reflecting the tight coupling between structure and biological performance.[45,49] Manufacturing strategy is not merely procedural but mechanistically determinative. Standardization of preparation conditions and systematic characterization are essential to ensure reproducibility and clinical translatability.

Many therapeutic agents exhibit suboptimal bioavailability, rapid degradation, or inadequate penetration into pathological tissues, limiting clinical effectiveness. Encapsulation within niosomal bilayers protects labile drugs from premature degradation while enabling sustained or controlled release through diffusion across the surfactant membrane.[1,45] Surface modification and compositional tuning can further influence interaction with biological membranes and barrier tissues, including the stratum corneum in transdermal applications.[48]

Vesicular delivery systems enhance localized drug retention and controlled release within structurally complex biological microenvironments.[46,49] Improved pharmacokinetic behavior and localized drug concentration contribute to enhanced therapeutic efficacy across multiple delivery settings. Optimized niosomal formulations have demonstrated significant antibacterial and antibiofilm activity compared with free-drug counterparts, underscoring the advantages of vesicular encapsulation in resistant infections.[42] Similar improvements in sustained release behavior and localized drug retention have also been reported in other vesicular delivery applications.[46,49] Niosomes, therefore, function not merely as passive carriers but as modulators of drug disposition and release dynamics. Their therapeutic performance depends on rational alignment between vesicle composition and the physicochemical characteristics of the target biofilm microenvironment.

Despite expanding interest, the positioning of niosomes within the broader nanocarrier landscape remains subject to critical evaluation regarding stability, scalability, and comparative advantage. Non-ionic surfactants confer chemical stability and lower susceptibility to oxidative degradation relative to phospholipid-based systems.[1,43] However, vesicle aggregation, potential leakage during long-term storage, and batch-to-batch variability can occur depending on surfactant chemistry and preparation conditions.[44] Reproducibility, scale-up feasibility, and physicochemical consistency remain

central challenges in the development of vesicular nanocarriers. Variability in vesicle size distribution, entrapment efficiency, and membrane stability can compromise translational relevance. Niosomes can be prepared using thin-film hydration, reverse-phase evaporation, ether injection, microfluidization, and other solvent-based or solvent-free approaches.[1,47] Process parameters, including hydration temperature relative to surfactant phase transition, agitation intensity, and cholesterol-to-surfactant ratio, directly influence vesicle size, lamellarity, and polydispersity.[44,46] Optimization strategies frequently aim to balance entrapment efficiency with membrane stability, as excessive fluidity may enhance release but compromise retention.[45]

While many preclinical studies demonstrate enhanced therapeutic outcomes, translating these findings into standardized clinical products requires addressing stability and manufacturing constraints. Evidence from recent evaluations in nanomedicine indicates promising functional performance but underscores the necessity of rigorous characterization frameworks.[45,47] The future development of niosomal systems depends on integrating physicochemical optimization with scalable fabrication and regulatory-oriented characterization. Within the context of rational nanomedicine design, surfactant-based vesicles represent a viable but formulation-sensitive platform rather than a universal solution.

Basic Structure and Working Mechanism of Niosomes

Biofilm-associated infections exhibit limited intrabiofilm penetration of antibiotics due to hydrated EPS matrices and metabolically heterogeneous bacterial populations.[50] In methicillin-resistant *S. aureus* (MRSA) biofilms, more than 95% of administered antibiotics may fail to reach embedded bacterial populations, allowing persistence of dormant subpopulations that constitute a minor fraction of biomass but dominate post-treatment viability.[42]

Niosomes are self-assembled vesicular systems whose bilayer organization and stability are governed by surfactant composition and cholesterol incorporation.[47,51] Span 60 (sorbitan monostearate), characterized by saturated C18 alkyl chains, undergoes hydrophobic aggregation supported by van der Waals interactions, while polyoxyethylene headgroups orient toward the aqueous phase, enabling bilayer formation. Cholesterol intercalation increases membrane rigidity, enhances chain ordering, and reduces permeability near the phase transition temperature (Tm ≈ 53°C), thereby limiting premature drug leakage.[45,52]

This vesicular architecture enables compartmentalized incorporation of drugs with differing physicochemical properties. Hydrophilic molecules such as meropenem (MW 383.45 Da) are predominantly entrapped within aqueous cores (entrapment efficiency 84.86 ± 3.14%), whereas amphiphilic or larger molecules such as vancomycin (MW 1449.25 Da) associate with bilayer domains through hydrogen bonding interactions.[34,42] Surfactant composition and cholesterol ratio further regulate membrane stability and release behavior.[46,49,53]

In the context of biofilms, nanoscale vesicles facilitate diffusion through hydrated polymeric channels. Size-dependent penetration behavior reported in niosomal systems underscores the importance of optimized particle dimensions in structured biological matrices.[54] Figure 1 conceptually integrates vesicular stability during systemic transit with progressive diffusion and localized drug release within EPS networks.

Meropenem-loaded niosomes demonstrated 4–6-fold reductions in MIC values against MRSA isolates compared with free drug and achieved 92% biofilm eradication after 72 hours versus 38% for non-encapsulated meropenem.[42] Confocal microscopy indicated approximately threefold higher intramatrix accumulation. Similarly, vancomycin-loaded niosomes exhibited superior antibiofilm activity relative to free vancomycin.[15] Ciprofloxacin-loaded niosomes also reduced biofilm formation and established biofilms more effectively than free ciprofloxacin.[55] These data indicate that bilayer compartmentalization modifies intrabiofilm pharmacodynamics by improving spatial drug distribution and retention. Enhanced efficacy therefore reflects improved intrabiofilm drug distribution and retention under diffusion-limited conditions.

Biofilms possess a net negative surface charge attributable to extracellular DNA phosphodiesters, uronic acid residues, and other polyanionic EPS constituents.[50] This electrostatic environment limits interaction with neutral vesicles and reduces drug accumulation within deeper biofilm layers. Surface modification of niosomes with cationic lipids such as stearylamine alters zeta potential and interfacial behavior. Unmodified Span 60-based niosomes exhibit a zeta potential of −8.2 ± 2.1 mV, whereas stearylamine incorporation shifts this value to +32.4 ± 1.8 mV.[15] This charge reversal promotes electrostatic attraction between positively charged vesicles and negatively charged EPS components.

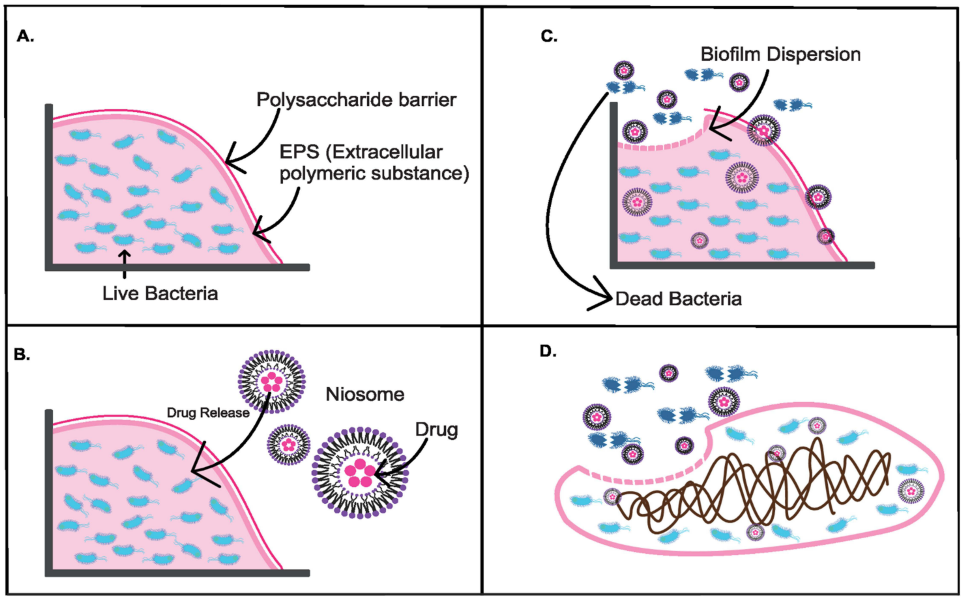


Figure 1 Proposed mechanism of niosomal penetration and antibiotic release within microbial biofilms. (**A**) Biofilm formation on surfaces protected by EPS and polysaccharide barriers. (**B**) Interaction of niosomal vesicles with the biofilm matrix and localized drug release. (**C**) Biofilm disruption and bacterial killing following intrabiofilm antibiotic delivery. (**D**) Penetration of niosomal carriers into deeper biofilm-associated tissue regions. The schematic illustrates how surfactant-engineered niosomes facilitate targeted antibiotic delivery by overcoming physical and biochemical barriers within microbial biofilms.

Beyond surface charge modification, incorporation of charged amphiphiles influences bilayer structural organization and membrane dynamics. Structural analyses of cationic and anionic niosomes demonstrate that electrostatic interactions influence packing density, intermolecular forces, and diffusion within the bilayer interface.[56] Electrostatic complex formation may reduce hydration around surfactant headgroups and disrupt hydrogen-bonded EPS networks. These interactions may increase local matrix permeability. Within biofilm microenvironments, partial bilayer destabilization has been associated with enhanced meropenem diffusion relative to aqueous conditions.[15] Figure 2 schematically illustrates this electrostatic recruitment and localized release process.

Cationic niosomal formulations achieved a 4.2 $\log_{10}$ CFU reduction in mature MRSA biofilms compared with 1.8 $\log_{10}$ for neutral vesicles, corresponding to approximately 2.3-fold greater antibiofilm efficacy.[34] Cationic lipid-modified quercetin-loaded niosomes similarly demonstrated improved antibacterial performance against *P. aeruginosa* compared with non-modified systems.[34] Surface charge engineering directly influences biological performance under biofilm conditions. Enhanced antibiofilm efficacy appears to be attributable to improved interfacial adhesion and intramatrix retention rather than to changes in intrinsic antibacterial potency. Electrostatic modulation, therefore, represents a rational design parameter for optimizing vesicle matrix interactions.

Conventional burst-release kinetics may result in premature drug depletion before adequate intrabiofilm accumulation occurs. Consequently, antibiotic exposure may not coincide with the metabolic reactivation phases of persister cells, thereby reducing bactericidal efficacy. Release kinetics of niosomes are governed by surfactant composition, cholesterol content, and bilayer phase behavior.[45,47] Span 60-based bilayers exhibit metastable structural properties that maintain integrity during systemic circulation but may gradually destabilize under biofilm-associated conditions, including mildly acidic pH (<6.2) and altered hydrophobic microenvironments. Local pH variation can influence cholesterol desorption and membrane permeability. Interactions between divalent cations and surfactant ether groups may further affect bilayer stability within complex biological matrices.[45]

In meropenem-loaded systems, sustained release profiles were observed, indicating controlled diffusion across the vesicular membrane.[42] Within biofilms, this matrix-responsive behavior may shift drug exposure from rapid systemic release to localized diffusion in proximity to bacterial cells. Meropenem-loaded niosomes achieved 92% biofilm eradication at 72 hours compared with 38% for the free drug. Time-kill analyses demonstrated prolonged bactericidal activity beyond 48 hours, associated with sustained intramatrix concentrations above the MIC_{90} threshold.[42] These

https://doi.org/10.2147/IJN.S607567

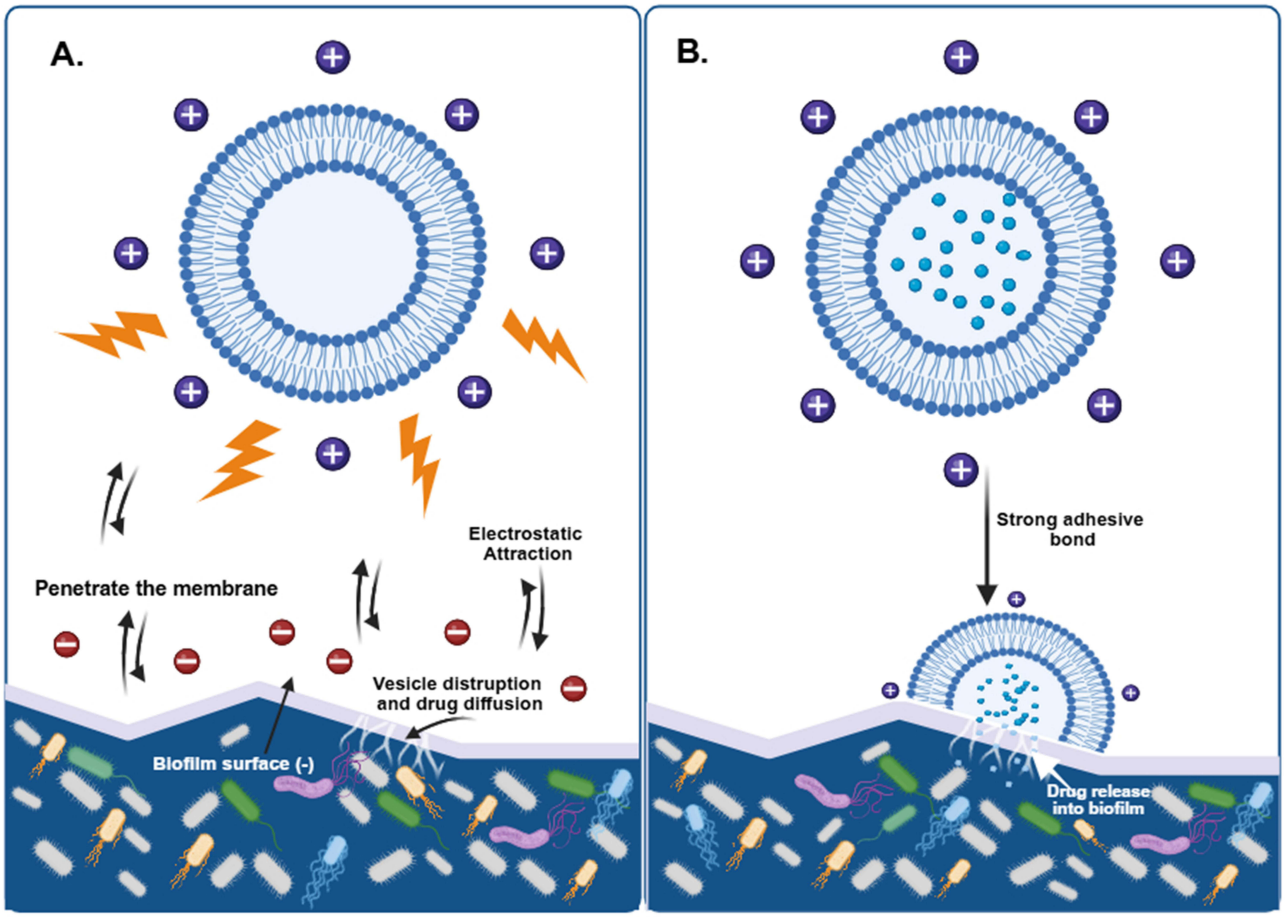


Figure 2 Proposed electrostatic interaction and localized drug release mechanism of cationic niosomes within negatively charged biofilm matrices. (**A**) Positively charged niosomes interact electrostatically with anionic extracellular polymeric substance (EPS) components, promoting vesicle adhesion, membrane destabilization, and localized drug diffusion within the biofilm matrix. (**B**) Strong adhesive interactions between cationic vesicles and the biofilm surface facilitate sustained intrabiofilm drug release and enhanced retention within deeper biofilm layers. The schematic illustrates the role of surface charge engineering in improving niosomal transport behavior and antibiofilm delivery efficiency.

findings suggest improved pharmacokinetic-pharmacodynamic coupling under biofilm conditions. Therapeutic persistence in biofilm-associated infections may partially reflect a temporal mismatch between drug exposure and windows of bacterial susceptibility. Environment-responsive niosomal systems may improve the alignment between intrabiofilm drug exposure and microbial susceptibility. This effect may enhance antibiofilm performance while remaining consistent with currently reported data.

Essential Components: Non-Ionic Surfactants and Cholesterol

Vesicular drug delivery systems based on phospholipids are inherently vulnerable to oxidative degradation, hydrolysis, and physicochemical instability during storage and processing. Such liabilities compromise membrane integrity, reduce drug retention, and limit scalability. Moreover, variability in lipid purity and susceptibility to environmental stressors pose translational constraints for long-term pharmaceutical development.[3,43] Niosomes replace phospholipids with synthetic or semi-synthetic non-ionic surfactants capable of spontaneous bilayer self-assembly in aqueous media. Amphiphilic molecules such as sorbitan esters (eg, Span series) and polysorbates organize into closed vesicular structures through hydrophobic association of alkyl chains and stabilization of hydrophilic headgroups at the aqueous interface.[44,47] The absence of charged headgroups reduces electrostatic repulsion within the bilayer, contributing to cohesive membrane packing and compositional adaptability.

Comprehensive structural analyses indicate that alkyl chain length, degree of saturation, and HLB critically determine vesicle size, lamellarity, transition temperature, and drug encapsulation efficiency.[3,47] Surfactants with longer saturated chains, such as Span 60, generate membranes with higher phase transition temperatures and reduced permeability, thereby improving drug retention. Experimental investigations in anti-inflammatory, anticancer, and antimicrobial applications confirm that surfactant-based vesicles maintain structural integrity while encapsulating both hydrophilic and lipophilic compounds.[46,52,57]

Non-ionic surfactants are not merely substitutes for phospholipids but constitute the primary structural determinant of niosomal performance. Their chemical stability, tunable bilayer characteristics, and compatibility with diverse therapeutic agents provide a rational platform for controlled delivery. The structural predictability afforded by surfactant selection supports reproducible formulation development and aligns vesicle architecture with intended pharmacokinetic objectives.

Surfactant-only bilayers may exhibit excessive membrane fluidity and permeability, leading to premature drug leakage and reduced in vivo persistence. Such instability undermines sustained-release objectives and compromises therapeutic efficiency.[44,47] Cholesterol is incorporated into the surfactant bilayer to regulate membrane order and mechanical strength. By intercalating between adjacent alkyl chains, cholesterol decreases membrane mobility, increases packing density, and modulates phase transition behavior.[3,46]

Empirical studies consistently demonstrate that increasing cholesterol content reduces bilayer permeability and enhances entrapment efficiency by limiting drug diffusion across the membrane.[3] Structural characterizations confirm that cholesterol broadens the gel-to-liquid crystalline transition and stabilizes vesicle morphology during storage.[47] Formulations combining Span 60 and cholesterol have shown improved physicochemical stability and enhanced biological performance, including sustained drug release and improved therapeutic activity.[52,57] Cholesterol functions as a structural regulator that fine-tunes bilayer permeability without impairing vesicle formation. The interplay between surfactants and cholesterol defines membrane rigidity, drug retention capacity, and release behavior. Optimizing this ratio is therefore central to rational niosomal design rather than an auxiliary compositional adjustment.

Advantages Over Other Drug Delivery Systems

Liposomes remain clinically established; however, phospholipid oxidation and hydrolytic degradation can shorten shelf life and compromise structural integrity. These limitations affect formulation reproducibility and large-scale manufacturing.[3,43] Niosomes employ chemically stable non-ionic surfactants that exhibit greater resistance to oxidative stress. Their preparation methods such as thin-film hydration, reverse-phase evaporation, and microfluidic techniques are adaptable and reproducible, facilitating scalable production.[44,47]

Comparative studies further position niosomes as viable alternatives to liposomes, highlighting comparable vesicular organization with improved physicochemical stability and formulation robustness.[51] Niosomes address key stability-related limitations of liposomal carriers without sacrificing bilayer architecture. Their compositional resilience enhances translational feasibility and may reduce manufacturing-related variability, supporting broader pharmaceutical applicability.

Many nanoparticulate systems exhibit limited flexibility in accommodating drugs with divergent physicochemical properties, as single-domain carriers may preferentially retain either hydrophilic or lipophilic molecules.[3] Niosomes enable compartmentalized incorporation of drugs with differing physicochemical properties while allowing modulation of release kinetics and transport behavior through surfactant composition, cholesterol proportion, and vesicle size.[44,47]

Experimental findings demonstrate enhanced encapsulation efficiency and adaptable release behavior across compounds with diverse physicochemical properties.[46,57] Size-dependent penetration studies further show that vesicle dimensions influence drug permeation across biological barriers, underscoring structural adaptability.[54] These observations confirm that membrane composition and vesicle size can be rationally tailored to modify release and distribution profiles. These properties enable adaptable drug loading and controlled-release behavior across diverse antimicrobial formulations. This adaptability distinguishes niosomes from rigid polymeric systems and compositionally fragile lipid vesicles, supporting their potential for localized and controlled antimicrobial delivery applications.

Critical Formulation Parameters

The therapeutic performance of niosomal systems is governed by interconnected physicochemical parameters, including vesicle size, entrapment efficiency (EE%), zeta potential, and pH-responsive behavior. These factors collectively influence bilayer stability, intrabiofilm transport, release kinetics, and interactions with heterogeneous biofilm microenvironments.

Particle Size

Niosome vesicle size, typically ranging from 50–500 nm, strongly influences biodistribution, tissue penetration, and formulation stability. Large multilamellar vesicles (>250 nm) often resist deformation and exhibit limited vascular extravasation, whereas sub-100 nm particles are susceptible to rapid renal clearance and reduced payload retention.[58–61]

Distinct size ranges govern different transport pathways. Small unilamellar vesicles (SUVs, 10–100 nm) favor osmotic and paracellular diffusion across mucosal and transdermal barriers, whereas larger unilamellar vesicles (LUVs, 100–250 nm) exploit endothelial fenestrations and enhanced permeability and retention (EPR)-mediated accumulation. In contrast, multilamellar vesicles (MLVs, >250 nm) primarily serve as local depots, but exhibit reduced systemic persistence due to reticuloendothelial system (RES) uptake.[57,62,63]

These size-dependent properties create inherent trade-offs. SUVs enhance biofilm penetration and transdermal transport but undergo rapid systemic filtration, whereas vesicles in the 100–200 nm range strike a balance between circulation time and localization efficiency. Uncontrolled polydispersity further contributes to erratic release kinetics and therapeutic variability.[59] Accordingly, strategic size engineering through selection of preparation methods, control of cholesterol-mediated rigidity, and minimization of polydispersity (PDI < 0.3) remains essential for optimizing localization efficiency and therapeutic performance.[58,60]

Entrapment Efficiency (EE%)

Insufficient drug sequestration remains a major limitation of vesicular systems because low entrapment efficiency (EE%) accelerates payload leakage and reduces sustained drug exposure at pathological sites. In niosomes, EE% reflects the fraction of active pharmaceutical ingredient retained within vesicles and directly influences therapeutic persistence, particularly in biofilm-associated and chronic infections.[64]

Mechanistically, EE% is governed by surfactant architecture, cholesterol content, hydration strategy, and drug physicochemical properties. Long-chain surfactants with high phase-transition temperatures, such as Span 60, form densely packed bilayers with reduced permeability and consistently achieve EE% values above 70%.[60,65] However, excessive bilayer rigidity may impair vesicle deformability and biofilm penetration.[61] Conversely, Tween 80 increases membrane fluidity and transport permissiveness but often reduces EE% through enhanced diffusional leakage.[65,66] Optimization of EE% improves pharmacokinetic performance and sustained drug release. Azithromycin-loaded niosomes with EE% around 75% maintained localized drug levels for up to 72 hours, whereas chitosan-coated ursolic acid niosomes (EE% 78%) achieved fourfold greater hepatic accumulation than conventional dispersions.[67,68] High-EE% niosomes produced via vortex-reactor fabrication further prolonged systemic exposure compared with standard hydration methods.[69]

Similar benefits were observed in anti-inflammatory niosomes with EE% values of 85–92%, which preserved therapeutic activity following single-dose administration and reduced dosing intensity.[57] Collectively, EE% functions as a mechanistic integrator linking formulation design with pharmacodynamic stability and therapeutic durability in niosomal drug delivery.

Zeta Potential

Zeta potential functions as a dynamic determinant of niosomal stability and biological interaction rather than a static physicochemical descriptor. Although values within ±20–40 mV are generally considered stable under simplified buffer conditions, physiological exposure frequently induces protein adsorption, surface charge neutralization, aggregation, and opsonin-mediated clearance.[67,70] Such charge reprogramming compromises transdermal transport and reduces electrostatic interaction with negatively charged biofilm matrices.[61,68]

Mechanistically, biomolecular corona formation strongly influences charge behavior. Albumin adsorption attenuates electrostatic barriers in Tween-based niosomes, whereas polysaccharides such as chitosan may invert surface charge through shielding effects. Acidic microenvironments can further protonate surfactant headgroups, inducing curvature stress and transient zeta oscillations.[70] These effects create transport trade-offs, as cationic niosomes enhance bacterial membrane interactions but may accelerate RES sequestration, whereas anionic systems reduce macrophage uptake but exhibit weaker affinity for anionic biofilms.[69,71]

These charge-dependent interactions significantly influence in vivo performance. Zeta neutralization may reduce circulation stability through splenic trapping, whereas acidic environments transiently increase fusogenicity and cytotoxicity before corona formation attenuates activity.[66,68] Accordingly, adaptive charge engineering using pH-responsive surfactants and antifouling strategies remains necessary to align in vitro stability with in vivo biofate and targeted antimicrobial delivery.[70,72]

pH

pH critically influences the chemical and physical stability of niosomal systems, as deviations from the optimal range can induce aggregation, particle enlargement, a reduction in zeta potential, and drug degradation.[73,74] Conventional formulations optimized for physiological pH (~7.4) often fail to exploit the acidic gradients characteristic of bacterial biofilms and other pathological microenvironments (pH 5.5–6.5), resulting in non-selective release and suboptimal target accumulation despite high initial entrapment efficiency.[74,75]

Buffering systems are commonly used to preserve vesicle integrity and reduce premature leakage during storage but provide limited spatial control of drug release.[65,76] In contrast, pH-responsive engineering introduces protonation-triggered bilayer reorganization through modifications such as hexadecyl–poly(acrylic acid) grafting, mPEG oleic acid incorporation, or gelatin–alginate coatings.[74,75,77] These modifications increase membrane permeability and accelerate release kinetics under acidic conditions. Because mature biofilms frequently exhibit mildly acidic microenvironments, pH-responsive niosomes may improve localized intrabiofilm drug accumulation while minimizing premature systemic leakage. Thus, pH-responsive systems enable conditionally activated drug release aligned with disease-specific microenvironments. In antimicrobial and oncologic applications, pH modulation, together with surfactant composition, cholesterol ratio, vesicle size, and surface charge, collectively govern therapeutic performance and targeted drug delivery.[1,77,78]

Role of Surfactants in Niosome Design and Therapeutic Efficacy

A major challenge in niosomal delivery arises from the hydrophile–lipophile balance (HLB)-dependent trade-off between circulatory stability and penetration across complex biological barriers. High-HLB surfactants such as Tween 60 (HLB 15.9) promote prolonged systemic stability through rigid, hydrogen-bond-stabilized bilayers but may limit antibiotic diffusion within dense biofilm matrices despite high entrapment efficiency. In contrast, low-HLB Span 60 (HLB 4.7) enhances vesicle deformability and penetration into *Klebsiella pneumoniae* biofilms but is associated with faster payload leakage and reduced retention at infection sites.[79,80] As illustrated in Figure 3, surfactant HLB directly influences bilayer organization, vesicle stability, and intrabiofilm transport behavior.

Mechanistically, HLB regulates bilayer phase behavior, membrane packing, and transport adaptability. These effects are mediated through hydrophobic interactions, hydrogen bonding, and electrostatic interactions among surfactants, cholesterol, and biological interfaces. Span 60-dominant systems undergo gel-to-liquid crystalline transitions near physiological temperatures, facilitating deformation within EPS matrices, whereas Tween 60 enrichment generates tightly packed bilayers with higher zeta potential but lower barrier permeability. Cholesterol further stabilizes Span-based bilayers and prolongs shelf life but may reduce biofilm penetration. These physicochemical differences directly influence antibiofilm transport and therapeutic performance. Span-rich niosomes generally enhance diffusion within biofilm matrices but may exhibit increased payload leakage, whereas Tween-rich systems preserve vesicle integrity but show lower penetration efficiency.[80,81]

These limitations support the use of hybrid surfactant strategies rather than single-component systems. Intermediate HLB formulations, such as Span 60: Tween 60 (2:1; HLB ~9), balance deformability and structural stability, enabling

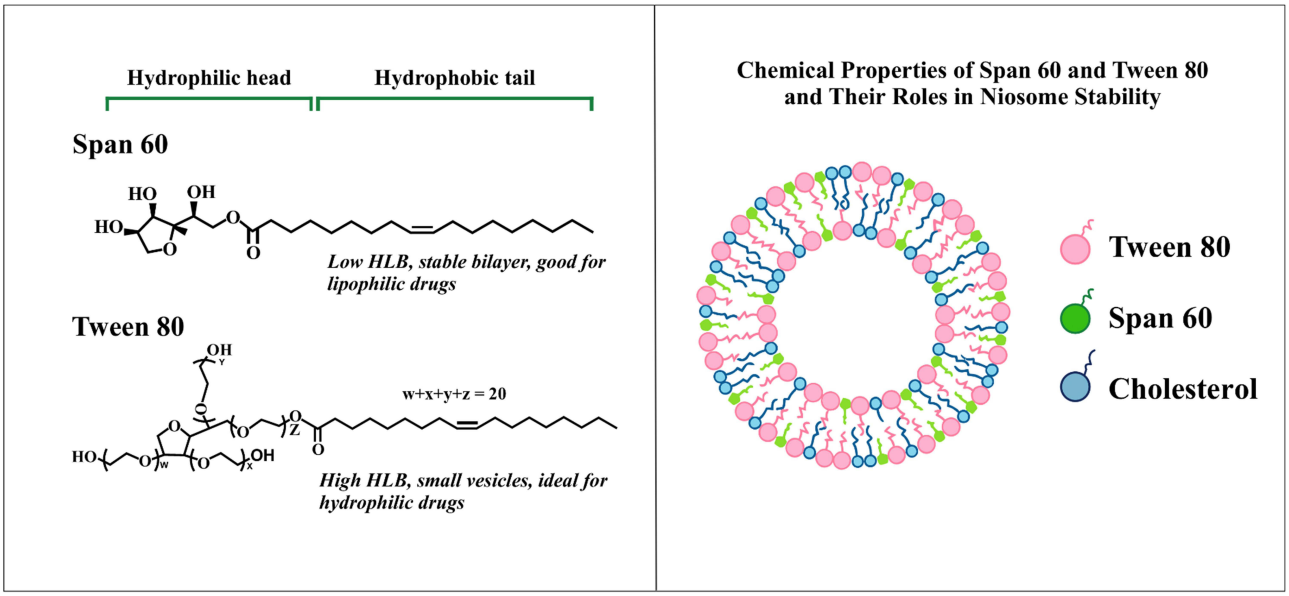


Figure 3 Structural and physicochemical characteristics of Span 60 and Tween 80 and their influence on niosome stability and transport behavior. The schematic compares the organization of surfactant headgroups, the hydrophile–lipophile balance (HLB), and bilayer assembly in niosomal vesicles. Low-HLB Span 60 promotes rigid bilayer formation and enhanced drug retention, whereas high-HLB Tween 80 increases membrane fluidity and transport adaptability. Cholesterol incorporation further modulates bilayer stability, permeability, and vesicle integrity. The figure illustrates how surfactant composition governs physicochemical properties relevant to antibiofilm drug delivery.

controlled penetration without excessive payload loss. In addition to bilayer structure, surfactant composition directly affects zeta potential, colloidal stability, and antibiofilm efficacy, with Span-based niosomes loaded with imipenem or ciprofloxacin demonstrating greater activity against *S. aureus* and *E. coli* than Tween-based formulations.[55,81] Overall, surfactant–cholesterol engineering enables niosomes to serve as adaptable, microenvironment-responsive delivery systems to overcome transport limitations in infectious and biofilm-associated applications.[3,57,79] The following section further discusses how surfactant type and HLB influence the physicochemical properties governing therapeutic performance.

Influence of Surfactant Type on the Physicochemical Properties of Niosomes

A major limitation in niosomal formulations is the tendency to prioritize colloidal stability and high drug EE% over transport competence across biological barriers. Although surfactants govern bilayer assembly and vesicle physicochemical behavior, many formulations remain optimized for structural stability rather than efficient intrabiofilm transport and therapeutic performance.[82,83]

Mechanistically, surfactant HLB regulates bilayer packing density and membrane phase behavior. Low-HLB surfactants such as Span 60, characterized by long saturated alkyl chains, form rigid bilayers that enhance vesicle stability and drug retention. In contrast, higher-HLB surfactants such as Tween 80 generate more hydrated and flexible membranes with increased bilayer fluidity but lower long-term stability.[65] Cationic surfactants improve electrostatic interactions with negatively charged membranes but are often limited by aggregation and cytotoxicity, whereas anionic surfactants may destabilize bilayers and induce irritation.[82,84] Amphoteric surfactants exhibit pH-dependent interfacial behavior yet remain underexplored in niosomal systems.

These differences directly affect permeability, retention, and intrabiofilm transport efficiency, as formulations optimized primarily for stability and EE% may not achieve effective penetration across complex biological barriers. Accordingly, surfactant type functions not only as a structural component but also as a key regulator of permeability, retention, and biological interaction.[84,85] However, many studies still emphasize entrapment efficiency, particle size, and short-term antibacterial activity as primary performance indicators, whereas direct evaluation of intrabiofilm transport competence remains limited. Consequently, improved physicochemical stability does not always translate into enhanced antibiofilm efficacy in structured microbial environments.

A formulation-oriented strategy should therefore align surfactant composition with the dominant biological barrier within the target biofilm. In diffusion-limited matrices, bilayer flexibility and surface charge influence penetration across dense EPS networks. In enzyme-rich microenvironments, vesicular shielding and controlled release are required to minimize premature degradation, whereas persister-dominated biofilms may require sustained local drug exposure rather than rapid burst release. Collectively, these barrier-matched design principles provide a framework for optimizing niosomal systems beyond conventional formulation metrics.

Interaction Between Surfactants, Antibiotics, and the Biofilm Matrix

Surfactants and biosurfactants modulate this barrier through multiple interfacial mechanisms. At the matrix level, surfactants interact with EPS components, reducing surface tension and disrupting hydrophobic and electrostatic interactions, thereby loosening matrix architecture and increasing porosity.[86–89] Simultaneously, surfactant-based vesicular systems modify surface charge and lipophilicity, enhancing interaction with microbial membranes and facilitating membrane insertion or fusion.[8,85] Cationic surfactants impose combined electrostatic and hydrophobic destabilization of bacterial membranes, partially overcoming diffusion constraints imposed by the EPS.[90] During early biofilm development, biosurfactants also inhibit initial adhesion by altering cell surface hydrophobicity and charge, a mechanism not addressed by conventional antibiotics targeting planktonic populations.[91,92]

These physicochemical alterations enhance intrabiofilm diffusion, increase the matrix's mechanical compliance, and improve antibiotic penetration, thereby enabling deeper drug distribution and greater antibiofilm activity in dense and mature biofilms.[61,93] Surface charge modulation at biomaterial interfaces has been shown to reduce *P. aeruginosa* adhesion in both in vitro and in vivo settings, confirming that controlled electrostatic interference can suppress biofilm persistence rather than enhance retention.[93,94] Lipid-based delivery systems, including peptide-conjugated liposomes and lipid-coated hybrid nanoparticles, achieve near-complete eradication of *P. aeruginosa* and *S. aureus* biofilms through membrane fusion and hydrophobic insertion, particularly when combined with antibiotics such as vancomycin.[95,96] In contrast, bacterial outer membrane vesicles generally reinforce biofilm stability and resistance, underscoring the mechanistic distinction between therapeutic nanovesicles and endogenous vesiculation pathways.[97] As illustrated in Figure 4, niosomal systems enhance intrabiofilm transport by improving EPS penetration, protecting encapsulated antibiotics from degradation, and enabling controlled drug release within structured biofilm matrices.

Collectively, these findings position surfactant-assisted and vesicular antibiotic delivery as a transport-oriented strategy that directly addresses EPS-imposed diffusion failure rather than relying solely on antimicrobial potency. Niosome-loaded antibiotics, including ciprofloxacin, streptomycin sulfate, and imipenem, consistently demonstrate enhanced antibiofilm efficacy against clinically relevant pathogens by improving EPS penetration and controlled release, supporting their translational potential in chronic and device-associated biofilm infections.[62,81,97]

Comparative Studies on the Efficacy of Span vs. Tween-Based Formulations

The performance of niosomal systems is strongly influenced by surfactant composition, which governs the balance among vesicle stability, drug retention, and release kinetics. Comparative studies consistently show that Span- and Tween-based formulations exhibit distinct physicochemical behaviors, particularly in applications requiring sustained drug exposure and efficient transport across diffusion-limited biofilm environments.

These divergent behaviors originate from fundamental physicochemical differences between Span and Tween surfactants. Variations in HLB, alkyl chain saturation, and headgroup hydration govern bilayer packing density and phase behavior. Low-HLB surfactants, particularly Span 60, preferentially form densely packed gel-phase bilayers with high phase-transition temperatures and limited free volume. These properties generate rigid membranes with restricted transbilayer diffusion.[98,99] In contrast, high-HLB Tweens form more hydrated, liquid-crystalline bilayers with increased molecular mobility, thereby facilitating faster drug diffusion but concomitantly increasing susceptibility to leakage and colloidal instability.[100]

Cholesterol further modulates bilayer rigidity and permeability in a surfactant-dependent manner. In Span-rich bilayers, cholesterol intercalates efficiently between surfactant molecules, forming stabilizing interactions that markedly reduce membrane fluidity and payload leakage while maintaining vesicle integrity. Conversely, in Tween-dominant

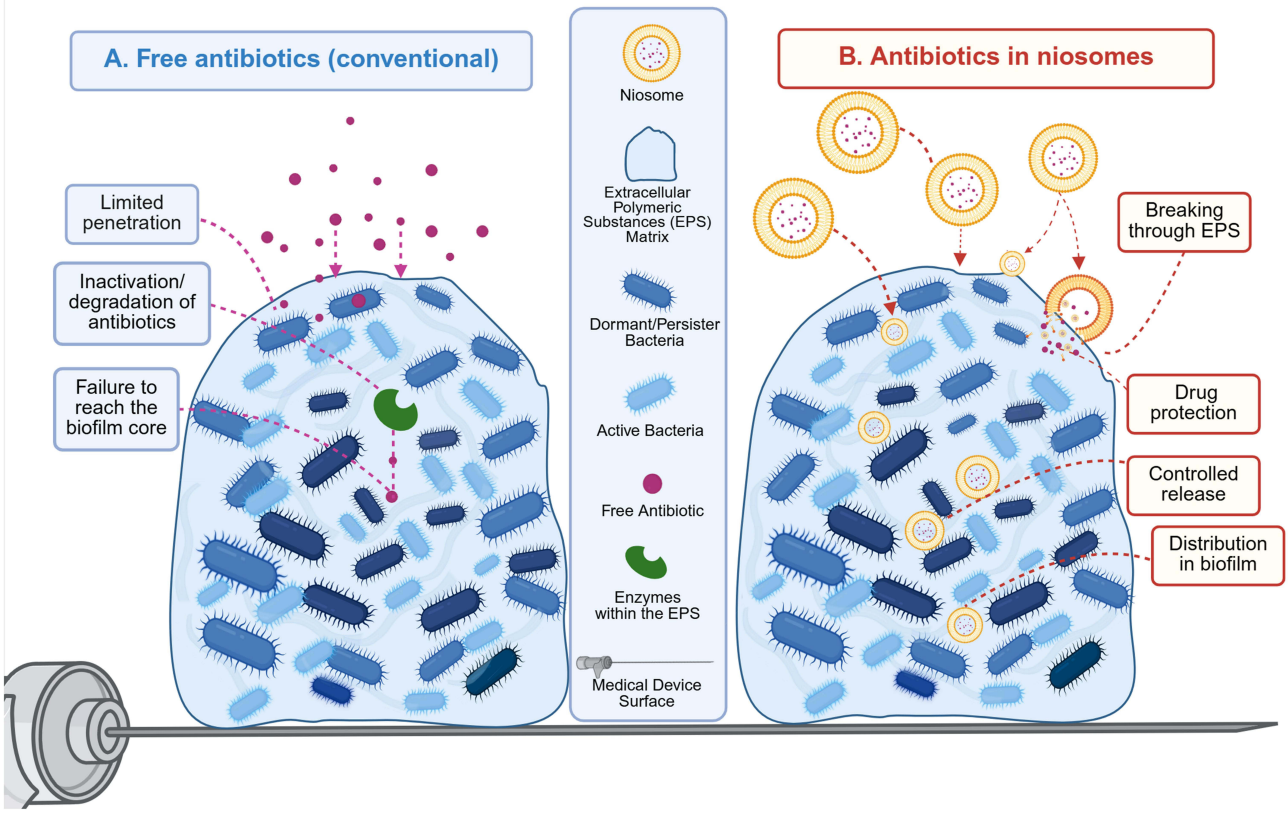


Figure 4 Comparative schematic illustration of free-antibiotic diffusion (**A**) versus niosomal antibiotic delivery (**B**) within microbial biofilms. Conventional antibiotics exhibit limited penetration, enzymatic degradation, and uneven distribution within EPS-rich biofilm matrices, resulting in reduced antibacterial activity in deeper biofilm layers. In contrast, niosomal systems enhance intrabiofilm transport by improving EPS penetration, protecting encapsulated antibiotics from degradation, enabling controlled drug release, and promoting localized drug retention, thereby improving antibacterial distribution within structured biofilm environments.
Abbreviation: EPS, extracellular polymeric substance.

systems, extensive headgroup hydration limits cholesterol's ability to induce comparable rigidification.[98,101,102] As a result, mixed Span/Tween formulations provide a compositional continuum in which bilayer order, rigidity, and permeability can be systematically modulated through ratio-dependent HLB adjustment.[103,104]

These differences directly influence vesicle stability, drug retention, and release behavior. Span-based niosomes generally exhibit greater colloidal stability, narrower particle size distributions, higher entrapment efficiency, and reduced drug leakage because of dense bilayer packing and cholesterol-induced microviscosity.[88,99] Their release behavior commonly follows diffusion-controlled or biphasic kinetics, supporting prolonged drug availability. In contrast, Tween-rich systems exhibit faster release profiles and lower drug retention due to increased bilayer fluidity and permeability.[89,103]

In antibiofilm applications, these formulation-dependent properties translate into measurable differences in therapeutic efficacy. Vancomycin-loaded niosomes have been shown to reduce the minimum biofilm inhibitory and eradication concentrations by severalfold against *S. aureus* and *S. epidermidis*, enabling effective inhibition at substantially lower drug doses compared with free vancomycin.[15,19] Similarly, imipenem-loaded niosomes significantly decreased minimum biofilm inhibitory concentrations (MBIC) values and suppressed biofilm growth in methicillin-resistant *S. epidermidis*, accompanied by downregulation of key biofilm-associated genes, including icaD, fnbA, and ebpS.[87]

Collectively, these findings indicate that Span- and Tween-based niosomes differ primarily in bilayer organization and transport behavior. Accordingly, surfactant composition and ratio should be considered critical design parameters to balance vesicle stability and permeability in line with antibiofilm delivery requirements. Accordingly, surfactant type and compositional ratio should be regarded as mechanistic design parameters, selected to balance stability and permeability in accordance with the specific therapeutic context rather than as interchangeable formulation components.

Active Pharmaceutical Ingredient (API)-Loaded Niosomal Formulations for Biofilm-Associated Infections

Niosomal vesicles have been employed as modular carriers for diverse active pharmaceutical ingredients (APIs) targeting biofilm-forming bacteria. Biofilm-associated pathogens, including MRSA and multidrug-resistant Gram-negative species, exhibit intrinsic tolerance mechanisms that limit the efficacy of conventional antibiotics.[15,42,81,86,90] Niosomal platforms allow precise tuning of bilayer composition, surfactant HLB balance, cholesterol content, and vesicle surface charge to optimize penetration into the EPS and to regulate drug release. Surfactant combinations of Span and Tween with cholesterol yield mechanically robust bilayers that maintain high encapsulation efficiency (>70%) while controlling permeability and drug leakage. Surface modifications, including cationic or bioactive co-loads, facilitate electrostatic interactions with negatively charged biofilm matrices, promoting localized drug accumulation. Representative studies evaluating API-loaded niosomal systems for biofilm-associated infections, including their physicochemical characteristics and antibiofilm performance, are summarized in Table 1.

Physicochemical characterization correlates with functional outcomes. Niosomal formulations consistently exhibit nanoscale particle sizes (<200 nm), sufficiently negative zeta potentials (≥ |25| mV), and high encapsulation efficiencies, collectively supporting deep EPS penetration and controlled antibiotic release. As summarized in Table 2, these

Table 1 Niosomal Formulations Targeting Bacterial Biofilms

Active Pharmaceutical Ingredient (API)	Target Microorganism/ Biofilm	Surfactant Type	Preparation Method	Author
Ciprofloxacin	*E. coli, S. aureus* (biofilm)	Tween 85: Span 80 (1:1)	Thin Film Hydration	[55]
Cefazolin	*S. aureus, E. coli*	Span 60 + Cholesterol	Film hydration	[66]
Tobramycin	MDR *P. aeruginosa*	Span 60: Tween 60:Chol	Thin-film hydration + sonication	[65]
Azithromycin	Gram positive and negative (topical)	Span 60 + Tween 80	Thin Film Hydration	[68]
Cephalexin	*S. aureus, E. coli, P. aeruginosa*	Span 60, Span 80, Tween 60, Tween 80 cholesterol	Thin Film Hydration	[71]
Meropenem	MRSA (Methicillin-resistant *S. aureus*) clinical isolates biofilm	Span 60 + Tween60+ Cholesterol	Thin Film Hydration + Sonication	[42]
Itraconazole	Candida spp. (skin)	Span 60 + Tween 80 + Carbopol gel	Thin Film Hydration	[72]
Naproxen	Not biofilm	Span 60 + Tween 60	Ether Injection Method	[78]
Doxorubicin	*S. aureus, P. aeruginosa* biofilms	Span 60 + Cholesterol	Thin film hydration + 3D printing	[74]
Streptomycin sulfate	*S. aureus, P. aeruginosa* biofilms	Span 60 +Tween80 Cholesterol	Thin film hydration	[73]
Florfenicol	*E. coli* ATCC35218, *S. aureus* ATCC29213	Span 60 + Cholesterol + Dihexadecyl phosphate (DDP)	Thin-film hydration	[84]
Azithromycin	Methicillin-resistant *S. aureus*	Span/Tween mixtures	Thin-film hydration	[68]
Imipenem	Methicillin-resistant *S. epidermidis* biofilm	Span/Tween + cholesterol	Thin-film hydration	[87]
Tetracycline	*Klebsiella pneumoniae* biofilm	Span/Tween (not explained)	Thin-layer hydration	[80]
Tiamulin	*Mycoplasma gallisepticum* (broilers)	Span 60 + Cholesterol	Thin film hydration	[84]

(*Continued*)

https://doi.org/10.2147/IJN.S607567

Table 1 (Continued).

Active Pharmaceutical Ingredient (API)	Target Microorganism/ Biofilm	Surfactant Type	Preparation Method	Author
Meropenem/ Ertapenem/ Tigecycline	CRE & ESBL (*E. coli, K. pneumoniae*)	Span 60 + Cholesterol	Thin film hydration	[79]
Vancomycin	*S. aureus* biofilm (abiotic surfaces)	Span 60: Tween 60 (1:1)	Thin-film hydration + sonication	[19]
Vancomycin	MRSA clinical isolates biofilm	Span 60: Chol (1:1)	Ethanol injection + sonication	[15]
Quercetin	*P. aeruginosa* biofilm	Span 60: DOTAP (cationic)	Thin-film hydration	[34]
Selenium NPs (not antibiotic)	*S. aureus, E. coli, P. aeruginosa* biofilm	Span 60: Chol: Tween80	Ethanol injection	[105]
Ciprofloxacin	CR-MRSA biofilm	Span 60: Chol (3:1)	Ethanol injection	[106]
Meropenem	MRSA clinical isolates biofilm	Span 60: Tween 60	Thin-film hydration + sonication	[42]
Anti-inflammatory compounds (curcumin, etc).	Not specified (in vitro inflammation models)	Span 60: Chol (1:1, 1:2 ratios)	Thin film hydration	[57]
Stavudine	Not specified (HIV therapy)	Span 60: Tween 80: Chol	Ethanol injection	[107]
Tretinoin	Not specified (skin delivery)	Span 60: Chol: Tween80	Microfluidic mixing	[108]
Benzalkonium chloride	Wound healing (not biofilm specific)	Span 60 (niosomes); Span 60: TPGS (spanlastics)	Ethanol injection	[109]

Table 2 Niosomal Physicochemical Properties and Antibiofilm Efficacy

Active Pharmaceutical Ingredient (API)	Encapsulation Efficiency (%)	Particle Size (nm)	Zeta Potential (mV)	Effectiveness (MIC/MBEC/Zone of Inhibition)	Author
Ciprofloxacin	20.1 ± 2.3	~180 (PDI 0.2)	−22.4 ± 1.8	Biofilm biomass ↓ 65% (*E. coli*), 52% (*S. aureus*)	[55]
Cefazolin	83.2 ± 2.1	212 ± 15	–45.1	MIC ↓ 2–4×; zone ↑ 16 mm (*S. aureus*)	[66]
Tobramycin	67.4 ± 3.8	189 ± 12	−42.5 ± 2.9	MIC ↓ 4–8×; resistance ↓ 64%	[65]
Azithromycin	79.3%	165–185	–34.6	Increased permeation and topical release	[68]
Cephalexin	55–80% depending on surfactant	150–300	–25 to –40	MIC ↓ 4× (*E. coli*), 8× (*S. aureus*)	[71]
Meropenem	84.5%	187.6	−31.4	8x reduction in MBEC vs free meropenem	[42]
Itraconazole	~72%	200–250 nm	–30 mV	Enhanced antifungal activity, delayed release time	[72]
Naproxen	~68%	~150 nm	–26 mV	Controlled release, potential topical anti-inflammatory activity	[78]
Doxorubicin	65–83%	~160	–33.7	Enhanced antibiofilm	[74]
Streptomycin sulfate	~77%	~175	–30.5	Reduced biofilm biomass and enhanced antibacterial effect	[73]

(*Continued*)

Table 2 (Continued).

Active Pharmaceutical Ingredient (API)	Encapsulation Efficiency (%)	Particle Size (nm)	Zeta Potential (mV)	Effectiveness (MIC/MBEC/Zone of Inhibition)	Author
Florfenicol	≈ 75–85%	~150 nm	–30 mV	MIC and MBEC significantly reduced vs free drug	[84]
Azithromycin	~80–85%	~150–200 nm	–20 to –30 mV	Significantly reduced MIC/MBIC values compared to free drug	[68]
Imipenem	79.4 ± 1.1%	192.3 ± 5.8 nm	Not explicitly stated	4–6-fold reduction in MIC/MBIC; 69% biofilm growth inhibition; > 90% cell viability	[87]
Tetracycline	75.3 ± 1.5	169.5 ± 9.6	–24.6 ± 1.6	Significant biofilm inhibition and decreased mrkA gene expression compared to unbound antibiotics.	[80]
Tiamulin	71.25%	198.4 ± 1.6	–28.7 ± 0.9	MIC = 0.5 μg/mL (niosome) vs 2 μg/mL (free drug)	[84]
Meropenem/ Ertapenem/ Tigecycline	~70–85	~120–140	~12.5–13.5	MIC ↓ significant; wound healing ↑	[79]
Vancomycin	82.4 ± 3.2	145 ± 12	−28.5 ± 2.1	Biofilm biomass ↓ 78%; MBEC ↓ 4× vs free VAN	[19]
Vancomycin	91.3 ± 2.8	135 ± 10	−22.1 ± 1.5	MIC ↓ 2–4×; MBC ↓ 2–4×; biofilm eradication ↑	[15]
Quercetin	76.5 ± 5.2	162 ± 18	+32.4 ± 3.2 (QCT-SA)	MIC ↓ 2–32×; MBEC-1day ↓ vs free QCT; zone ↑ 15 mm	[34]
Selenium NPs (not antibiotic)	89.2 ± 4.1	128 ± 15	−25.3 ± 1.8	MIC ↓ 2–4×; biofilm inhibition 2–4× vs free SeNPs	[105]
Ciprofloxacin	84.2 ± 4.5	142 ± 16	−24.7 ± 1.9	MIC ↓ 4×; biofilm ↓ 80%; icaB gene ↓	[106]
Meropenem	88.7 ± 3.1	148 ± 12	−27.2 ± 2.3	MIC/MBEC ↓ significant vs free MRP	[42]
Anti-inflammatory compounds (curcumin, etc).	85-92 (curcumin optimal F3)	150-250 (optimized)	−20 to −35	Edema inhibition 64.1% (24h); carrageenan model	[57]
Stavudine	78.5 ± 3.2 (optimized)	185 ± 22	−26.4 ± 2.1	Sustained release 72h; EE↑ vs free drug	[107]
Tretinoin	81-87 (temperature dependent)	120-200	−22 to −30	Controlled release pH 5.5; viscosity optimized	[108]
Benzalkonium chloride	72.4 ± 4.1 (niosomes); 88.3 ± 2.8 (spanlastics)	156 ± 18 (niosomes)	−24.7 ± 1.9	Wound closure ↑2×; zone inhibition ↑18 mm	[109]

Notes: ↑ indicates increase/enhancement; ↓ indicates reduction/decrease; × indicates fold change relative to free drug formulations; ~ and ≈ indicate approximate values.

parameters underpin reproducible antibiofilm activity across both Gram-positive and Gram-negative biofilms. Representative formulations include ciprofloxacin-loaded niosomes,[81] meropenem-encapsulated vesicles,[42] vancomycin niosomes,[15] zingerone niosomes,[86] and amikacin-loaded niosomes,[90] demonstrating the platform's adaptability across multiple API classes and microbial targets. Collectively, the tables provide a concise framework linking formulation composition, physicochemical attributes, and antibiofilm potential, illustrating the modularity and applicability of API-loaded niosomes for biofilm-associated infections.

https://doi.org/10.2147/IJN.S607567

Mechanisms of Drug Release from Niosomal Vesicles

A persistent limitation in niosomal drug delivery is the difficulty of achieving reproducible, application-specific release profiles without compromising vesicle stability. Current evidence indicates that release behavior cannot be sufficiently rationalized by generic processes such as passive diffusion, membrane disruption, or vesicle fusion in isolation, but is primarily governed by the HLB of the surfactant and the cholesterol-induced phase state of the bilayer.[82,83]

From a mechanistic standpoint, low-HLB surfactants exemplified by Span 60 (HLB 4.7), particularly at equimolar ratios with cholesterol, generate densely packed gel-phase bilayers with reduced molecular mobility, resulting in diffusion-controlled release profiles consistent with Higuchi kinetics, as demonstrated by tiamulin-loaded systems showing a limited early burst (~25% within 1 h) followed by sustained release up to 48 h.[82] In contrast, high-HLB surfactants such as Tween 80 (HLB 14.9) form liquid-crystalline bilayers with lower packing constraints, thereby facilitating rapid Fickian diffusion and accelerating drug release to exceed 80% within 6 h.[83]

Cholesterol functions as a regulatory component rather than a purely stabilizing additive; its intercalation increases bilayer packing density and microviscosity while preserving vesicle integrity, thereby shifting the rigidity permeability balance without suppressing release altogether.[84] These physicochemical distinctions have measurable biological consequences, as HLB-dependent release patterns correlate with antibacterial performance: biphasic release from vancomycin-loaded niosomes is associated with 2–8-fold reductions in MBIC and MBEC values against MRSA relative to the free drug.[15] Whereas sustained release from imipenem-loaded niosomes supports 4–6-fold MBIC reduction and up to 69% inhibition of MRSE biofilm growth.[87] Collectively, these observations position bilayer phase behavior, dictated by surfactant HLB and cholesterol content, as a controllable determinant of niosomal release kinetics, with clear implications for formulation design in which Span-based systems are favored for prolonged, antibiofilm-oriented delivery and Tween-based systems for applications requiring rapid drug availability and penetration.[103]

Comparative Efficacy Against Planktonic Bacteria versus Biofilm-Embedded Cells

The therapeutic failure of conventional antibiotics against biofilm-associated infections is largely attributable to profound phenotypic differences between planktonic and biofilm-embedded bacteria. Biofilm-resident populations typically require antibiotic concentrations that are 100–1,000-fold higher than planktonic MICs, owing to the protective EPS, matrix, metabolic dormancy of persister cells, and active efflux mechanisms.[90] These factors collectively limit drug penetration and sustain sublethal exposure within the biofilm core.

API-loaded niosomal systems have consistently demonstrated a disproportionate enhancement of antibiofilm activity relative to their effects on planktonic cells. For instance, vancomycin-loaded niosomes achieved two- to eight-fold reductions in MBIC/MBEC compared with planktonic MIC values against MRSA, indicating preferential efficacy within structured biofilms.[15] Similarly, imipenem-loaded niosomes produced four to six-fold MBIC reductions and approximately 69% biofilm growth inhibition in methicillin-resistant *S. epidermidis* models.[87] Amikacin niosomes likewise improved antibacterial activity against both planktonic and biofilm-associated *K. pneumoniae*, although the magnitude of enhancement was more pronounced in biofilm settings.[85,90]

The enhanced antibiofilm activity of niosomes stems from their structural and kinetic properties. Sub-200 nm vesicles penetrate the EPS matrix, while biphasic release sustains inhibitory concentrations in deeper layers. An initial burst induces bacterial stress, followed by prolonged exposure that suppresses regrowth and tolerance. Rather than merely increasing exposure, this system aligns drug availability with biofilm-specific barriers, thereby narrowing the susceptibility gap between planktonic and sessile cells. Such characteristics make niosomal delivery particularly relevant for persistent biofilm infections unresponsive to conventional dosing.

Targeting Strategies and Advanced Modifications for Biofilm Eradication

While passive niosomal delivery improves antibiofilm efficacy, biofilm eradication remains constrained by heterogeneity in EPS composition, bacterial physiology, and host-pathogen interactions. To address these challenges, advanced niosomal systems have incorporated targeting and functional modifications to enhance specificity and disrupt biofilms through complementary mechanisms.

One widely explored strategy involves surface charge modulation to promote electrostatic targeting. Biofilms typically possess a net negative charge due to the presence of polysaccharides and extracellular DNA within the EPS. Cationic surface modification, therefore, enhances vesicle adhesion and penetration. Hemmati et al (2024) demonstrated that stearylamine-modified, quercetin-loaded niosomes produced approximately four-fold reductions in MBIC and MBEC values against *P. aeruginosa* compared with neutral counterparts, an effect attributed to strengthened electrostatic interactions with the anionic matrix.[34] Consistently, vancomycin-eluting niosomes optimized for surface adhesion achieved up to 92% inhibition of *S. aureus* and *S. epidermidis* biofilm formation on abiotic surfaces, markedly outperforming free drug.[19]

Beyond physicochemical targeting, immunological strategies have been explored to disrupt biofilm-associated virulence. Targeting the PcrV protein, a key component of the type III secretion system in *P. aeruginosa*, interferes with bacterial pathogenicity rather than directly increasing antibiotic concentration. Inhibition of PcrV has been shown to impair biofilm development by modulating host immune responses, including promoting M1 macrophage polarization.[92] Anti-PcrV monoclonal antibodies achieved approximately 80% survival in pneumonia and burn wound infection models, highlighting the therapeutic potential of immunologically guided targeting.[91,93] Although not inherently niosomal, such strategies are conceptually compatible with vesicle-based delivery platforms.

A third approach exploits biochemical disruption of biofilm architecture using polyphenolic payloads. Polyphenols such as tannic acid exert antibiofilm effects by interfering with EPS integrity and QS pathways. Heidari et al (2020) reported that tannic acid-loaded niosomes achieved up to 85% biofilm eradication, underscoring the value of combining carrier-mediated delivery with biofilm-active compounds that extend beyond conventional antibiotics.[94] Collectively, these targeting strategies (including cationic surface modification for enhanced physical penetration, immunological targeting to attenuate virulence, and polyphenol-mediated biochemical disruption) illustrate the modularity of niosomal platforms. Their integration enables multifunctional interventions tailored to the structural and biological complexity of biofilms. Such adaptability supports the continued development of advanced niosomal systems as rational, versatile tools for eradicating persistent, treatment-resistant biofilm infections.

Ligand-Targeted Delivery Systems

Active ligand functionalization is frequently proposed as a solution to nonspecific drug distribution and limited penetration across biological barriers; however, its therapeutic contribution is often overstated when decoupled from vesicle mechanics. Accumulating evidence from lipid and vesicular nanocarriers demonstrates that ligand receptor affinity alone cannot compensate for unfavorable bilayer properties, as vesicle transport, deformation, and residence time are fundamentally governed by membrane rigidity, permeability, and surface architecture.[51,110] This limitation is particularly relevant in niosomal systems, where surfactant chemistry dictates bilayer organization and, consequently, the functional availability of surface ligands.

At the mechanistic level, bilayer behavior in niosomes is primarily controlled by the HLB of the constituent surfactants and their interaction with cholesterol. Low-HLB Span surfactants form densely packed, ordered bilayers with reduced molecular mobility, conferring high structural stability and prolonged drug retention, whereas high-HLB Tween surfactants generate hydrated, liquid-crystalline membranes characterized by enhanced deformability and permeability.[45,101] Cholesterol further modulates this balance by increasing bilayer order and microviscosity, stabilizing ligand-decorated vesicles while constraining excessive membrane fluidity when present within an optimal range.[95,102] Surface modifications such as PEGylation introduce an additional layer of control by regulating steric spacing, interfacial hydration, and ligand exposure, thereby influencing productive receptor engagement during circulation.[96,97]

These interdependent mechanisms explain why enhanced ligand density or affinity does not consistently translate into superior therapeutic outcomes. Ligand-mediated targeting remains effective only when bilayer mechanics permit sufficient structural integrity, controlled permeability, and adequate residence time at the target interface. Over-functionalization or mismatched surfactant composition can destabilize vesicles, induce aggregation or leakage, and obscure ligand accessibility, ultimately diminishing functional targeting despite increased surface complexity.[45] The so-called "PEG dilemma" further illustrates this constraint, as dense stealth coatings may protect against protein adsorption while simultaneously masking ligands and impairing receptor recognition.[111]

The impact of integrating surfactant selection with ligand engineering is therefore evident in the differential performance of Span- versus Tween-based systems. Span-rich bilayers provide a mechanically robust platform that preserves ligand orientation and stability under shear and confinement, favoring sustained interaction with biological targets. In contrast, Tween-containing formulations enhance vesicle deformability and matrix penetration but require careful optimization of cholesterol and PEG to prevent premature leakage and loss of targeting efficiency.[97,101,110] These findings underscore that bilayer mechanics, rather than ligand identity alone, determine whether active targeting yields measurable therapeutic gains.

Taken together, ligand targeting should be regarded as a secondary specificity layer whose functional efficacy is constrained by surfactant-driven bilayer design. In rational niosomal engineering, ligand density and chemistry must be optimized in concert with surfactant selection, cholesterol content, and surface architecture. Only through this integrated approach can active targeting translate from nominal increases in uptake to robust and reproducible therapeutic performance in complex biological environments.

Combination Approaches with Anti-EPS Agents, Enzymes, or Quorum-Sensing Inhibitors

Biofilm resilience arises from the functional redundancy and dynamic adaptability of its structural and regulatory networks, rendering single-agent anti-biofilm strategies inherently insufficient. Approaches targeting EPS, enzymatic dispersal, or QS pathways address discrete biofilm components but fail to account for compensatory mechanisms that preserve biofilm integrity. Consequently, monotherapies based on matrix disruption or signaling interference rarely achieve durable biofilm control, particularly in mature or clinically established biofilms.[112,113]

At the mechanistic level, EPS-degrading enzymes such as DNase I and dispersin B induce localized cleavage of extracellular DNA or polysaccharide scaffolds, yet their activity is spatially constrained within dense matrices and rapidly attenuated under physiological conditions. Non-targeted degradation leaves substantial portions of the EPS network intact, allowing residual matrix components to maintain mechanical cohesion and diffusion barriers.[113] Similarly, QS inhibitors suppress regulatory circuits governing virulence and EPS biosynthesis but do not exert direct bactericidal effects. In *P. aeruginosa*, interference with Las, Rhl, and PQS signaling primarily promotes biofilm dispersion rather than elimination, releasing viable cells that retain intrinsic tolerance traits.[114,115]

The clinical impact of these mechanistic limitations is reflected in incomplete and transient biofilm disruption. Enzymatic treatments typically achieve partial biomass reduction, often below thresholds required for irreversible destabilization, while surviving bacterial subpopulations rapidly repopulate the matrix through renewed EPS synthesis within hours of treatment cessation.[112,116] QS inhibition yields moderate reductions in biofilm mass but is markedly less effective against pre-formed biofilms and may impose selective pressure favoring QS-independent or EPS-hyperproducing phenotypes, undermining long-term efficacy.[117,118]

These limitations collectively justify the transition from monotherapeutic interventions toward integrated combination strategies that concurrently dismantle matrix architecture, attenuate regulatory signaling, and deliver bactericidal agents. However, free combinations of enzymes, QS inhibitors, and antibiotics remain constrained by mismatched stability, rapid inactivation, and asynchronous pharmacokinetics.[119,120] Niosomal co-delivery systems may improve the treatment of biofilm-associated infections by simultaneously encapsulating multiple therapeutic agents. Combinations such as antibiotic–inorganic nanoparticle systems and curcumin–metal conjugates can enhance drug protection and support controlled release within biofilm environments. Carboxymethyl chitosan surface modification, coupled with ciprofloxacin entrapment, potentiates adhesion impairment against *E. coli* and *S. aureus*, delivering biofilm eradication that exceeds monotherapy benchmarks and validating niosomes as a mechanistically coherent conduit for polymodal anti-biofilm regimens.[55,121,122]

Development of Responsive Niosomes

pH-Sensitive Niosomes

Biofilm-associated infections exhibit pronounced microenvironmental heterogeneity, particularly in pH distribution. Localized acidic niches (pH ~4.0–6.5), driven by glycolytic metabolism, proton extrusion, and restricted diffusion,

have been documented in oral, wound, and pulmonary biofilms.[123–125] These gradients are spatially resolved within a single biofilm architecture, with acidic cores or interfacial zones coexisting alongside near-neutral regions. Such heterogeneity contributes to antimicrobial tolerance and complicates uniform drug exposure.

Despite this well-characterized variability, most pH-responsive nanocarriers including niosomes have been optimized against generalized acidic thresholds (commonly pH 5.0–6.5) derived primarily from oncology literature. Direct alignment between experimentally measured intra-biofilm pH distributions and the apparent pKa of niosomal formulations remains insufficiently addressed. This disconnect limits rational design for infection-specific applications.

pH-sensitive niosomes rely on protonation of ionizable moieties embedded within the bilayer or associated polymeric coatings. Protonation reduces electrostatic stabilization among surfactant headgroups, decreases packing density, and promotes transient bilayer defects. Protonation of carboxyl-bearing components accelerates the release of all-trans retinoic acid under acidic conditions, consistent with protonation-driven membrane destabilization.[126] This process involves reduced headgroup repulsion, bilayer expansion, and an approximately threefold increase in permeability at pH 5.5 compared with pH 7.4.[75] Protonated surfactants increase bilayer fluidity without compromising structural integrity at physiological pH.[127] In polymer-modified systems, acidic protonation of carboxyl or amine groups induces intrachain electrostatic repulsion, promoting polymer swelling and facilitating diffusion across the vesicular membrane.[128]

Incorporation of pH-responsive polyurethane further enhances selective release within endosomal pH ranges (5.0–6.0).[129] These physicochemical alterations translate into measurable shifts in release kinetics. A 1.6-fold increase in cumulative curcumin release at pH 5.5 relative to pH 7.4 has been reported in HD-PAA-modified systems.[75] Similarly, carfilzomib release increased from 54.55% at pH 7.4 to 74.39% at pH 5.4, consistent with protonation-mediated bilayer destabilization.[130] Chitosan-coated niosomes likewise demonstrate accelerated drug release under acidic conditions while retaining stability at neutral pH.[131] However, these data are derived predominantly from tumor-mimicking buffers rather than from infection-relevant biofilm models. While biofilm-targeted nanoparticles outside the niosomal platform demonstrate substantial reductions in viability when exploiting acidic niches, equivalent validation for pH-responsive niosomes within mature, heterogeneous biofilms remains limited.[132,133]

For translational relevance in infectious settings, pH-responsive niosome design should move beyond fixed thresholds (eg, pH 5.5) and instead integrate quantitative intra-biofilm pH mapping. Species- and site-specific pH distributions must inform the selection of formulation pKa values. Without such alignment, responsiveness risks being either prematurely activated in peripheral regions or insufficiently triggered within metabolically quiescent cores.

Future studies should therefore (i) correlate measured biofilm pH microdomains with niosomal pKa values, (ii) validate release kinetics in mature (≥72–96 h) biofilm architectures, and (iii) evaluate stability in infection-relevant fluids such as saliva, sputum, or wound exudate. Current evidence supports mechanistic feasibility but remains incomplete for infection-specific optimization.

Enzyme-Sensitive Formulations

Biofilms are not only acidic but also enzymatically dynamic. Bacterial communities secrete glycosidases, proteases, and matrix-modifying hydrolases that regulate EPS turnover and nutrient access.[128,134] However, enzyme expression is highly heterogeneous across species, maturation stages, and anatomical sites.[125] Outer layers may be polysaccharide-rich (eg, PNAG or alginate), whereas inner regions contain higher proportions of eDNA and proteins.

Single-enzyme targeting strategies are therefore inherently vulnerable to strain-specific variability. Treatment with single enzymes was insufficient to substantially disrupt most in situ oral biofilms, highlighting the limited applicability and lack of universality of mono-responsive therapeutic strategies.[135] Enzyme-responsive systems typically incorporate cleavable linkers or degradable matrices that undergo hydrolysis in the presence of specific bacterials enzymes. In principle, enzymatic cleavage destabilizes the vesicle or releases conjugated cargo selectively within the biofilm microenvironment.

Yet, biological barriers complicate this mechanism. The EPS matrix significantly restricts diffusion; measured diffusion coefficients within biofilms may be reduced by one to two orders of magnitude compared with free solution.[136,137] This tortuosity limits access of both enzymes and nanocarriers to metabolically active inner layers.

Moreover, mature biofilms exhibit increased crosslinking density, further constraining penetration.[138] A second mechanistic vulnerability arises from non-specific cleavage. Enzyme-responsive linkers may be susceptible to off-target hydrolysis by planktonic bacteria or host-derived proteases, potentially reducing effective payload delivery.[139,140]

These constraints reduce the predictability of enzyme-triggered release. Premature cleavage in peripheral layers dissipates the drug before the tolerant subpopulations.[141] Clinical gaps persist: host proteases degrade enzyme stability, renal clearance limits EPS exposure, and phospholipases destabilize liposomes prior to contact with persisters.[22,128,142] Microdomain gradients cause uneven triggering, preserving 1000-fold MIC.[143] Enzyme-sensitive niosomes require infection-specific validation: (i) enzyme profiling across species/maturity; (ii) diffusion kinetics in intact matrices; (iii) host cross-reactivity; (iv) persister efficacy.[22]

Clinical Applications: Biofilm-Associated Infections on Medical Devices

In vitro and in vivo Studies on Device-Related Infections

Catheter-Associated Infections

Indwelling urinary and vascular catheters provide permissive surfaces for rapid microbial attachment and biofilm maturation, frequently culminating in persistent infection and therapeutic failure. Once established, catheter biofilms act as protected reservoirs that are poorly accessible to systemic antibiotics and difficult to eradicate without device removal.

Surface-directed strategies have therefore focused on limiting initial adhesion and sustaining local antimicrobial activity. Within the Electrospun Nanofibrous Insert Urinary System (ENIUS) framework, polymer-coated urinary stents were shown in in vitro, ex vivo, and in vivo settings to reduce bacterial attachment and subsequent biofilm accumulation.[144] Parallel approaches that integrate antimicrobial agents into catheter materials have demonstrated reduced colonization via localized drug release at the biomaterial interface.[126] These interventions rely on modifying surface physicochemistry and establishing a drug gradient directly at the site of microbial contact. Preclinical and early clinical observations indicate measurable reductions in biofilm biomass and infection incidence when compared with unmodified devices.[126,144] Such findings support the concept that device-centered antimicrobial delivery can complement, rather than replace, systemic therapy. Future development should prioritize durable coatings with predictable release kinetics under urinary or blood flow conditions, alongside validation in clinically representative models that account for long-term implantation and polymicrobial exposure.

Respiratory Device-Associated Infections

Biofilm development on endotracheal tubes represents a central driver of ventilator-associated pneumonia. These biofilms harbor multidrug-resistant organisms and function as persistent sources of lower airway contamination.[145] Antimicrobial coatings applied to endotracheal tube surfaces aim to inhibit colonization at the air-material interface. Concurrently, advanced in vitro and ex vivo respiratory models have been developed to better simulate airway conditions and evaluate targeted delivery systems, including environmentally responsive nanocarriers.[146] Such models enable assessment of penetration, retention, and activity under physiologically relevant airflow and secretion dynamics.

Evidence from experimental systems suggests reduced microbial adherence and delayed biofilm maturation on modified devices.[145,146] These findings reinforce the importance of preventive surface strategies in high-risk critical care settings. Translation requires confirmation of sustained efficacy under prolonged ventilation and compatibility with existing respiratory management protocols.

Translational Challenges for Clinical Implementation

Despite encouraging device-focused data, nanocarrier-based interventions face substantial translational barriers. Demonstration of short-term antibiofilm activity is insufficient to establish safety, pharmacokinetic stability, and long-term biocompatibility.[4] Furthermore, limited in vivo validation and incomplete understanding of nano–bio interactions may obscure long-term safety profiles and therapeutic predictability in complex biological environments.[65,66] Clinical advancement demands rigorous characterization of particle size distribution, surface charge, release kinetics, and physicochemical stability under Good Manufacturing Practice conditions.[75,79] Regulatory heterogeneity and the absence of harmonized standards for nanocarrier evaluation further complicate approval pathways.

Variability in manufacturing reproducibility or critical quality attributes may translate into inconsistent clinical performance, limiting adoption even when preclinical efficacy appears robust. Progress will depend on standardized characterization frameworks, scalable production protocols, and coordinated engagement between academia, industry, and regulatory bodies to align nanocarrier innovation with clinical and regulatory expectations.[64]

Future Opportunities for Managing Chronic and Recurrent Infections

Chronic and relapsing infections are sustained by biofilm-associated limitations in antimicrobial penetration and phenotypic tolerance, which collectively undermine conventional antibiotic therapy. Device-associated infections, chronic wounds, and cystic fibrosis airways share biofilm features such as dense extracellular matrices and metabolic heterogeneity that promote persistent bacterial survival despite prolonged treatment.[4,128,145,147–149] Escalating systemic dosing rarely resolves these reservoirs and instead increases the risk of toxicity, reinforcing the need for localized and biologically adapted delivery strategies.

Niosomal carriers address these constraints through bilayer-based encapsulation that permits modulation of membrane rigidity, permeability, and surface characteristics. Such tunability supports sustained intralesional drug release and reduces premature systemic diffusion.[68,73] Incorporation of pH- or enzyme-responsive elements further aligns drug liberation with infection-associated microenvironmental cues, linking release kinetics to local acidity or enzymatic activity rather than relying on passive diffusion alone.[57] This responsiveness is mechanistically relevant in chronic niches where physicochemical gradients shape antimicrobial efficacy.

Preclinical evidence indicates that niosomal formulations can enhance local pharmacokinetic exposure within biofilms and reduce the antibiotic concentrations required to achieve measurable reductions in biomass or bacterial load.[73,128] In models of device- and wound-associated infection, improved penetration and prolonged retention correlate with more consistent antibiofilm responses.[145] Importantly, the structural flexibility of non-ionic surfactant systems allows conceptual integration with adjunctive approaches, including microbiome-informed or host-directed strategies, reflecting the multifactorial nature of chronic infection pathophysiology.[4,149] Clinical translation demands validation in relevant biofilm models, robust formulation stability, and scalable manufacturing anchored by meaningful efficacy endpoints rather than isolated proof-of-concept antibiofilm activity alone.

Comparison of Niosomes with Other Drug Delivery Systems (DDS)

Conventional drug delivery platforms, particularly liposomes and polymeric nanoparticles, have advanced therapeutic modulation yet remain constrained by physicochemical instability, oxidative susceptibility, cost-intensive manufacturing, and limited scalability. Phospholipid-based vesicles are especially vulnerable to hydrolysis and peroxidation, complicating storage and large-scale production.[51,150] Polymeric and lipid nanoparticles, while structurally robust, often require complex synthesis routes and stringent purification processes that increase translational burden.[49] These limitations have driven continued exploration of alternative vesicular systems capable of retaining encapsulation versatility while mitigating stability and manufacturing constraints.

Niosomes, composed of non-ionic surfactants frequently stabilized with cholesterol, operate through amphiphilic self-assembly into bilayered vesicles analogous to liposomes but devoid of phospholipids.[3,44] The absence of ester-linked phospholipids reduces susceptibility to oxidative degradation and chemical instability, conferring extended shelf stability under standard storage conditions.[43,51] Structurally, niosomes accommodate both hydrophilic and lipophilic cargos within aqueous cores and bilayer domains, paralleling liposomal architecture while permitting modulation through surfactant HLB and chain length selection.[3,47] Compared with polymeric nanoparticles, niosomes rely on relatively mild fabrication techniques including thin-film hydration, reverse-phase evaporation, and microfluidic adaptation reducing the need for organic solvent residues and high-energy processing.[47,150]

These mechanistic distinctions translate into measurable performance differences across therapeutic domains. Experimental comparisons indicate that niosomes can achieve encapsulation efficiencies and release modulation comparable to liposomes, with improved resistance to leakage during storage.[51,57] In inflammatory and oncologic models, niosomal formulations enhance drug bioavailability and pharmacodynamic response relative to free drugs and, in some instances, demonstrate stability advantages over phospholipid vesicles.[49,57] Within transdermal systems, surfactant

flexibility facilitates interaction with stratum corneum lipids, supporting enhanced permeation compared with conventional formulations and certain rigid vesicular carriers.[48] In metabolic and chronic disease applications, niosomes have shown adaptability in modulating release kinetics and improving therapeutic indices, further positioning them as competitive alternatives to established nanocarriers.[151]

Nevertheless, parity with other DDS is not absolute. Batch-to-batch variability, surfactant purity concerns, and scale-dependent structural heterogeneity remain relevant technical challenges.[47] While liposomes benefit from extensive regulatory precedent and clinical validation, niosomes still occupy a comparatively limited translational landscape, with fewer clinically approved products.[43,150] Moreover, despite improved oxidative stability, surfactant-based systems may exhibit leakage or fusion phenomena depending on composition and storage conditions, underscoring the need for formulation-specific optimization.[3]

Collectively, current evidence positions niosomes not as replacements for established DDS but as structurally adaptable, cost-conscious vesicular alternatives whose comparative advantages lie in chemical stability, formulation flexibility, and manufacturing accessibility. Their rational deployment should therefore be indication-driven, leveraging surfactant tunability where liposomal fragility or polymeric complexity impose constraints, while acknowledging that clinical translation will depend on standardized production, reproducibility, and regulatory alignment comparable to other mature delivery platforms.[47,51,150]

Advantages and Limitations in Biofilm-Targeted Therapy

A principal constraint in biofilm-directed nanotherapy is the recurrent inability to achieve therapeutic intrabiofilm concentrations despite demonstrable planktonic efficacy. This limitation is primarily governed by physicochemical sequestration within the EPS, where electrostatic interactions and matrix density impede nanoparticle diffusion and confine carriers to superficial layers. Quantitative analyses indicate that only 15–25% of administered nanoparticles penetrate to viable bacterial clusters in mature *P. aeruginosa* biofilms, while more than 70% remain peripherally entrapped.[152] Complementary evidence shows that metallic nanoparticles undergo irreversible aggregation within alginate-rich matrices, further restricting effective intrabiofilm distribution.[153] Although surface charge modulation and size optimization are mechanistically justified strategies, these approaches incompletely resolve EPS-mediated retention. Consequently, rational surfactant selection becomes a critical determinant of performance; non-ionic vesicular systems may reduce charge-driven trapping that commonly limits cationic carriers, thereby facilitating improved matrix partitioning.[152,153]

A second limitation emerges during scale-up, where laboratory-optimized vesicular formulations frequently exhibit instability under GMP conditions. Variability arises from sensitivity to hydration kinetics, solvent exchange, and process control parameters. Although microfluidic and continuous-flow platforms are proposed to enhance reproducibility, significant physicochemical deviations persist. Batch-to-batch variation exceeding 35% in zeta potential has been documented even under optimized thin-film hydration methods.[154] Moreover, ether injection techniques have produced polydispersity indices above 0.4, values generally unsuitable for clinical-grade filling.[47] The impact extends beyond technical reproducibility to regulatory compliance and manufacturing cost. While Span–Tween hybridization may improve vesicle stability across production scales, extensive lot-release testing and long-term stability requirements increase economic burden relative to conventional small-molecule therapies.[47]

Translational discontinuity further constrains clinical advancement. Preclinical nanomedicine datasets frequently emphasize mechanistic penetration or physicochemical optimization without corresponding alignment to regulatory efficacy endpoints. A scoping review reports that approximately 82% of nanomedicine trials do not progress beyond Phase III, often due to insufficient correlation between experimental outcomes and clinically validated measures.[155] In parallel, long-term stability remains a decisive criterion; niosomal vaccine carriers are expected to demonstrate stability approaching 18 months, yet a substantial proportion of liquid formulations fail to meet this requirement.[150] These observations highlight a structural gap between experimental promise and regulatory validation. Surrogate endpoints reflecting sustained EPS disruption, rather than isolated MIC shifts, may better align mechanistic efficacy with clinical assessment frameworks.[155]

Within these constraints, niosomes exhibit formulation-specific strengths with well-defined boundaries. Conventional phospholipid vesicles are susceptible to mechanical destabilization and oxidative degradation under biofilm-associated conditions, compromising drug retention. In contrast, Span 60-based niosomes retained approximately 87% of encapsulated meropenem after 72 hours of incubation within MRSA biofilms, compared with 42% retention observed in corresponding liposomal systems.[42] Additional data indicate deeper penetration of cholesterol-free niosomes than that of equivalent phospholipid vesicles in biofilm models.[156] The enhanced chemical stability of non-ionic surfactant bilayers, including reduced susceptibility to peroxidation, may support tolerance to sterilization and device-coating applications.[156] Nonetheless, these advantages must be interpreted within the broader context of penetration variability, scale-dependent reproducibility, and regulatory alignment, which collectively determine the translational viability of niosome-based biofilm therapies.

Research Challenges, Gaps, and Future Directions

Identified Gaps: Rationale for This Review

Niosomal antibiotic systems demonstrate enhanced antibiofilm activity against *S. aureus* and MRSA in vitro, yet face persistent translational barriers including matrix penetration limitations and absence of clinical validation.[34,73] Comprehensive reviews highlight scale-up challenges and insufficient pharmacokinetic data characterizing intrabiofilm drug distribution across diverse infection microenvironments.[152,157]

Surfactant engineering via cholesterol modulation and HLB-optimized non-ionic compositions enables bilayer deformability for EPS matrix traversal, while sequential loading of antibiotics with matrix-disruptors addresses spatio-temporal resistance gradients absent in free-drug regimens.[158,159] Vancomycin-niosomes reduce MRSA biofilm biomass 4-8-fold versus free drug through sustained matrix release and icaR downregulation, yet preclinical confinement persists without Phase I data.[15] Streptomycin-niosomes similarly achieve 2-4-fold MBEC reductions against *S. aureus* via electrostatic interactions with biofilms, confirming mechanistic advantages but underscoring the limited human trial progression.[73,157] Current evidence positions surfactant-engineered niosomes as precision antibiofilm candidates, particularly where matrix recalcitrance limits conventional therapy, provided clinical translation incorporates validated PK/PD modeling and GMP-compliant scale-up to bridge laboratory efficacy to deployable formulations.[152,159]

Areas Requiring Further Research: Targeting and Combination Strategies

Despite encouraging in vitro antibiofilm performance, niosomal antibiotic systems remain largely empirical in their targeting logic.[160,161] Most formulations rely on passive accumulation or nonspecific electrostatic interactions rather than on rationally engineered recognition of biofilm heterogeneity, including EPS density gradients, quorum-sensing-regulated subpopulations, and metabolically dormant cells.[161] Reviews of nanotechnology-based antibiofilm interventions consistently highlight that inadequate spatial targeting and monotherapy-driven selective pressure contribute to incomplete biofilm eradication and the persistence of resistance.[160,161] In parallel, niosomal antibiotic studies demonstrate improved antimicrobial indices but rarely interrogate biofilm-specific pharmacodynamics or microenvironment-adapted delivery parameters, limiting mechanistic precision.[42,162]

Future development should prioritize two convergent axes: (i) microenvironment-responsive or surface-modified targeting, and (ii) rational combination therapy.[34,160] Surface engineering with cationic lipids or functional moieties can enhance adhesion to negatively charged biofilm matrices and promote deeper penetration, as demonstrated in modified quercetin-loaded niosomes.[34] Similarly, antibiotic-loaded niosomes such as Tet-Amp systems indicate that encapsulation may restore activity against multidrug-resistant strains when bilayer composition is optimized for membrane interaction.[80] Beyond encapsulation alone, integration of antibiotics with nanomaterials or adjuvants capable of disrupting EPS architecture or modulating bacterial stress responses has been proposed as a means to overcome tolerance mechanisms.[160] These approaches shift the paradigm from drug carriage to coordinated spatial-temporal intervention.[160,161] Cefazolin-containing niosomes reduced MRSA biofilm viability on chronic wound models more effectively than free drug, supporting the contribution of nanoscale delivery to improved local exposure.[162] Meropenem-loaded niosomes similarly exhibited enhanced antibacterial and antibiofilm activity against MRSA isolates following

formulation optimization.[42] Surface-modified niosomal quercetin demonstrated superior activity against *P. aeruginosa*, attributable to improved interaction with bacterial membranes and biofilm structures.[34] However, these studies predominantly evaluate biomass reduction or CFU decline without dissecting intrabiofilm distribution, resistant subpopulation dynamics, or synergistic indices under clinically relevant conditions.[42,80,160] The broader nanomaterial-antibiotic combination literature underscores potential synergy but also emphasizes variability across pathogen types and biofilm contexts.[160,161]

Advancing surfactant-engineered niosomes requires a transition from formulation-centered optimization to biofilm-informed targeting frameworks.[160,161] Combination strategies should be guided by mechanistic complementarity matrix disruption, membrane permeabilization, and antibiotic action rather than empirical co-loading.[80,160] Moreover, quantitative assessments of penetration depth, time-kill kinetics within structured biofilms, and resistance-suppression metrics must accompany conventional MIC/MBEC endpoints.[161,162] Without such integration of targeting specificity and rational combination design, niosomal systems risk remaining incremental improvements over free antibiotics rather than evolving into precision antibiofilm platforms capable of addressing multidrug-resistant and device-associated infections.[42,160]

Potential of Niosomes in Combination Therapy and Precision Medicine

Biofilm-associated infections remain therapeutically recalcitrant because antibiotic monotherapy fails to adequately address spatial heterogeneity, extracellular matrix shielding, and phenotypic diversification within sessile communities. While niosomal carriers improve drug retention and local concentration, most formulations still rely on single-agent payloads, limiting their ability to target multiple resistance determinants simultaneously. Moreover, the precision dimension aligning formulation architecture with infection-specific microenvironments remains underdeveloped in infectious diseases compared with oncology-oriented nanomedicine.[45] Thus, despite promising in vitro and preclinical findings, the translational value of niosomes will depend on their rational integration into combination regimens and microenvironment-responsive designs.

Combination-oriented niosomal systems offer a structurally adaptable platform for co-delivering antibiotics with adjuvant agents that disrupt biofilm integrity or metabolic resilience. Surfactant composition and bilayer organization can be engineered to enable dual loading, surface functionalization, or stimulus-responsive release, thereby coordinating spatiotemporal drug exposure.[45] Cationic lipid modification enhances electrostatic interaction with negatively charged biofilm matrices, promoting targeted deposition and improved penetration.[34] Surface conjugated inhibitors, such as hyaluronidase-blocking ligands, further refine targeting by interfering with enzymatic pathways that facilitate bacterial dissemination.[163] Co-encapsulation strategies combining antibiotics with non-antibiotic potentiators such as gallium nitrate or phytochemical compounds seek to simultaneously impair quorum signaling, metal metabolism, or membrane integrity while preserving antimicrobial potency.[86,164]

Empirical data substantiate the therapeutic advantage of such integrative approaches. Vancomycin-loaded niosomes demonstrate enhanced antibacterial and antibiofilm activity against MRSA compared with free drug, reflecting sustained release and improved biofilm interaction.[15] Surface-modified quercetin niosomes incorporating cationic lipids significantly reduce *P. aeruginosa* biofilm formation, underscoring the contribution of electrostatic targeting to therapeutic efficacy.[34] Zingerone-loaded niosomes exhibit superior antibiofilm activity relative to the unencapsulated compound, suggesting that vesicular encapsulation enhances phytochemical stability and bioavailability.[86] Importantly, combinatorial wrapping of minocycline and gallium nitrate within niosomes improves anti-biofilm performance in a murine pneumonia model of *Acinetobacter baumannii*, providing rare in vivo validation of dual-agent synergy.[164] Surface-modified niosomes carrying hyaluronidase inhibitors further demonstrate reduced biofilm burden and enhanced antibacterial effects, indicating that adjunctive enzymatic pathway targeting can complement antibiotic delivery.[163]

Collectively, available evidence indicates that niosomes offer a structurally adaptable platform for mechanism-informed combination therapy rather than empirical co-delivery. Surfactant composition and surface engineering permit controlled co-encapsulation aligned with biofilm-specific microenvironments. However, precision application requires systematic pharmacodynamic validation, defined co-loading ratios, and infection-stratified design. In the absence of such a framework, reported synergy remains descriptive rather than translatable.

Conclusion

Biofilm-associated infections remain difficult to eradicate with conventional antibiotic therapy because drug diffusion within the matrix is limited, bacterial populations exist in heterogeneous metabolic states, and persistence phenotypes reduce antimicrobial susceptibility. Increasing the systemic dose rarely compensates for these constraints and may instead elevate systemic exposure without achieving adequate concentrations inside the biofilm. Effective management, therefore, requires delivery systems that enhance local retention and provide controlled drug release within structured microbial communities.

Niosomes offer a formulation-driven strategy to overcome these limitations. Their bilayer architecture, composed of non-ionic surfactants and cholesterol, enables encapsulation of both hydrophilic and lipophilic agents within a single vesicular system. Careful adjustment of surfactant composition, HLB, cholesterol proportion, and surface charge allows modulation of membrane rigidity, permeability, and release behavior. These parameters directly influence intrabiofilm transport and sustained antibacterial activity. In addition, emerging responsive designs may further improve spatial and temporal control of drug delivery within biofilm microenvironments. Collectively, the evidence discussed in this review indicates that surfactant composition, hydrophile–lipophile balance, cholesterol incorporation, vesicle size, surface charge, and microenvironment-responsive behavior function as interconnected determinants governing niosomal transport performance and antibiofilm efficacy.

Although preclinical findings are encouraging, translational progress will depend on standardized physicochemical characterization, reproducible manufacturing processes, and validation in clinically relevant infection models. With rational formulation design and quality-oriented development, niosomes represent a practical and adaptable platform for improving localized antibiotic delivery in chronic and device-associated biofilm infections.

Abbreviations

API, Active pharmaceutical ingredients; Cmax, Maximum concentration; Tmax, Time to reach Cmax; PEG, Polyethylene glycol; FDA, Food and drug administration; QbD, Quality by design; DDS, Drug Delivery Systems; EPS, Extracellular Polymeric Substance; MIC, Minimum Inhibitory Concentration; MBC, Minimum bactericidal concentration; MBEC, Minimum Biofilm Eradication Concentration; CFU, Colony Forming Units; MDR, Multi-Drug Resistant; MRSA, Methicillin-Resistant *Staphylococcus aureus*; MDR, Multi-Drug Resistant; GMP, Good Manufacturing Practice; ICH, International Council for Harmonisation; RP-HPLC, Reverse phase high performance liquid chromatography; HLB, Hydrophilic–Lipophilic Balance; *S. aureus, Staphylococcus aureus; P. aeruginosa, Pseudomonas aeruginosa; A. baumannii, Acinetobacter baumannii*; QS, Quorum Sensing; QbD, Quality by design; PcrV, *Pseudomonas aeruginosa* Type III secretion protein V; EE%, Entrapment Efficiency; CIEDs, Cardiac Implantable Electronic Devices; CVCs, Central Venous Catheters; NPs, Nanoparticles; Candida Sp, Candida Species; SeNPs, Selenium Nanoparticles; CAUTIs, Catheter-Associated Urinary Tract Infections; VAP, Ventilator-associated Pneumonia.

Acknowledgment

This publication charge is funded by Universitas Padjadjaran through the Indonesian Endowment Fund for Education (LPDP) on behalf of the Indonesian Ministry of Higher Education, Science and Technology and managed under the EQUITY Program (Contract No. 4303/B3/DT.03.08/2025 and 3927/UN6.RKT/HK.07.00/2025).

Funding

The article processing charge (APC) was supported by the Indonesian Endowment Fund for Education (LPDP) on behalf of the Indonesian Ministry of Higher Education, Science and Technology, under the EQUITY Program, and administered through Universitas Padjadjaran (Contract No. 4303/B3/DT.03.08/2025 and 3927/UN6.RKT/HK.07.00/2025).

Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this study.

References

1. Abdelkader H, Alani AWG, Alany RG. Recent advances in non-ionic surfactant vesicles (niosomes): self-assembly, fabrication, characterization, drug delivery applications and limitations. *Drug Delivery*. 2014;21(2):87–100. doi:10.3109/10717544.2013.838077
2. Sharma S, Mohler J, Mahajan SD, Schwartz SA, Bruggemann L, Aalinkeel R. Microbial biofilm: a review on formation, infection, antibiotic resistance, control measures, and innovative treatment. *Microorganisms*. 2023;11(6):1614. doi:10.3390/microorganisms11061614
3. Moghassemi S, Hadjizadeh A. Nano-niosomes as nanoscale drug delivery systems: an illustrated review. *J Control Release*. 2014;185:22–36. doi:10.1016/j.jconrel.2014.04.015
4. Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S. Biofilms: an emergent form of bacterial life. *Nat Rev Microbiol*. 2016;14(9):563–575. doi:10.1038/nrmicro.2016.94
5. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. *Nat Rev Microbiol*. 2004;2(2):95–108. doi:10.1038/nrmicro821
6. Stewart PS, Bjarnsholt T. Risk factors for chronic biofilm-related infection associated with implanted medical devices. *Clin Microbiol Infect*. 2020;26(8):1034–1038. doi:10.1016/j.cmi.2020.02.027
7. Sharma S, Kumari N, Garg D, Chauhan S. A compendium of bioavailability enhancement via niosome technology. *PNT*. 2023;11(4):324–338. doi:10.2174/2211738511666230309104323
8. Gebreyohannes G, Nyerere A, Bii C, Sbhatu DB. Challenges of intervention, treatment, and antibiotic resistance of biofilm-forming microorganisms. *Heliyon*. 2019;5(8):e02192. doi:10.1016/j.heliyon.2019.e02192
9. Shao H, Zhou J, Lin X, et al. Bio-inspired peptide-conjugated liposomes for enhanced planktonic bacteria killing and biofilm eradication. *Biomaterials*. 2023;300:122183. doi:10.1016/j.biomaterials.2023.122183
10. Saeed N, Atiq A, Rafiq F, et al. Engineering of self-assembled silver-peptide colloidal nanohybrids with enhanced biocompatibility and antibacterial activity. *Sci Rep*. 2024;14(1):26398. doi:10.1038/s41598-024-78320-7
11. Rahela C, Agota-Katalin B, Anca BK. Smart antibiofilm platforms based on synthetic antimicrobial peptides-engineered hydrogels. *Polymers*. 2026;18(4):471. doi:10.3390/polym18040471
12. Jamal M, Ahmad W, Andleeb S, et al. Bacterial biofilm and associated infections. *J Chin Med Assoc*. 2018;81(1):7–11. doi:10.1016/j.jcma.2017.07.012
13. Sahoo K, Meshram S. Biofilm formation in chronic infections: a comprehensive review of pathogenesis, clinical implications, and novel therapeutic approaches. *Cureus*. 2024. doi:10.7759/cureus.70629
14. Bouhrour N, Nibbering PH, Bendali F. Medical device-associated biofilm infections and multidrug-resistant pathogens. *Pathogens*. 2024;13(5):393. doi:10.3390/pathogens13050393
15. Hemmati J, Chiani M, Asghari B, et al. Antibacterial and antibiofilm potentials of vancomycin-loaded niosomal drug delivery system against methicillin-resistant *Staphylococcus aureus* (MRSA) infections. *BMC Biotechnol*. 2024;24(1):47. doi:10.1186/s12896-024-00874-1
16. Vani S, Vadakkan K, Mani B. A narrative review on bacterial biofilm: its formation, clinical aspects and inhibition strategies. *Futur J Pharm Sci*. 2023;9(1):50. doi:10.1186/s43094-023-00499-9
17. Mirghani R, Saba T, Khaliq H, et al. Biofilms: formation, drug resistance and alternatives to conventional approaches. *AIMSMICRO*. 2022;8(3):239–277. doi:10.3934/microbiol.2022019
18. Chen X, Xu Y, Qu L, et al. Molecular mechanism for ligand recognition and subtype selectivity of α2c adrenergic receptor. *Cell Rep*. 2019;29(10):2936–2943.e4. doi:10.1016/j.celrep.2019.10.112
19. Barakat HS, Kassem MA, El-Khordagui LK, Khalafallah NM. Vancomycin-eluting niosomes: a new approach to the inhibition of Staphylococcal biofilm on abiotic surfaces. *AAPS Pharm Sci Tech*. 2014;15(5):1263–1274. doi:10.1208/s12249-014-0141-8
20. Beloin C, Renard S, Ghigo JM, Lebeaux D. Novel approaches to combat bacterial biofilms. *Curr Opin Pharmacol*. 2014;18:61–68. doi:10.1016/j.coph.2014.09.005
21. Raeiszadeh M, Pardakhty A, Sharififar F, et al. Development, physicochemical characterization, and antimicrobial evaluation of niosomal myrtle essential oil. *Res Pharma Sci*. 2018;13(3):250. doi:10.4103/1735-5362.228955
22. Zhou L, Zhang Y, Ge Y, Zhu X, Pan J. Regulatory mechanisms and promising applications of quorum sensing-inhibiting agents in control of bacterial biofilm formation. *Front Microbiol*. 2020;11:589640. doi:10.3389/fmicb.2020.589640
23. Saxena P, Joshi Y, Rawat K, Bisht R. Biofilms: architecture, resistance, quorum sensing and control mechanisms. *Indian J Microbiol*. 2019;59(1):3–12. doi:10.1007/s12088-018-0757-6
24. Sionov RV, Steinberg D. Targeting the holy triangle of quorum sensing, biofilm formation, and antibiotic resistance in pathogenic bacteria. *Microorganisms*. 2022;10(6):1239. doi:10.3390/microorganisms10061239
25. Paluch E, Rewak-Soroczyńska J, Jędrusik I, Mazurkiewicz E, Jermakow K. Prevention of biofilm formation by quorum quenching. *Appl Microbiol Biotechnol*. 2020;104(5):1871–1881. doi:10.1007/s00253-020-10349-w
26. Trautner BW, Darouiche RO. Role of biofilm in catheter-associated urinary tract infection☆. *Am J Infect Control*. 2004;32(3):177–183. doi:10.1016/j.ajic.2003.08.005
27. Del Pozo JL. Biofilm-related disease. *Exp Rev Anti-Infective Ther*. 2018;16(1):51–65. doi:10.1080/14787210.2018.1417036
28. Kalenderski K, Dubern JF, Lewis-Lloyd C, et al. Polymer-coated urinary catheter reduces biofilm formation and biomineralization: a first-in-man, prospective pilot study. *JU Open Plus*. 2024;2(1). doi:10.1097/JU9.0000000000000097
29. Cangui-Panchi SP, Ñacato-Toapanta AL, Enríquez-Martínez LJ, Reyes J, Garzon-Chavez D, Machado A. Biofilm-forming microorganisms causing hospital-acquired infections from intravenous catheter: a systematic review. *Curr Res Microb Sci*. 2022;3:100175. doi:10.1016/j.crmicr.2022.100175
30. Esposito S, Purrello SM, Bonnet E, et al. Central venous catheter-related biofilm infections: an up-to-date focus on meticillin-resistant *Staphylococcus aureus*. *J Global Antimicrob Resist*. 2013;1(2):71–78. doi:10.1016/j.jgar.2013.03.002
31. Gahlot R, Nigam C, Kumar V, et al. Catheter-related bloodstream infections. *Int J Crit Illn Inj Sci*. 2014;4(2):162. doi:10.4103/2229-5151.134184
32. Medis S, Dissanayake T, Kottahachchi J, et al. Biofilm formation and antibiotic resistance among Coagulase negative Staphylococcus species isolated from central venous catheters of intensive care unit patients. *Indian J Med Microbiol*. 2023;42:71–76. doi:10.1016/j.ijmmb.2022.10.007

33. Ielapi N, Nicoletti E, Lorè C, et al. The role of biofilm in central venous catheter related bloodstream infections: evidence-based nursing and review of the literature. *RRCT*. 2020;15(1):22–27. doi:10.2174/1574887114666191018144739
34. Hemmati J, Chiani M, Chegini Z, Seifalian A, Arabestani MR. Surface modified niosomal quercetin with cationic lipid: an appropriate drug delivery system against Pseudomonas aeruginosa Infections. *Sci Rep*. 2024;14(1):13362. doi:10.1038/s41598-024-64416-7
35. Hussein AA, Baghdy Y, Wazni OM, et al. Microbiology of cardiac implantable electronic device infections. *JACC*. 2016;2(4):498–505. doi:10.1016/j.jacep.2016.01.019
36. Polyzos KA, Konstantelias AA, Falagas ME. Risk factors for cardiac implantable electronic device infection: a systematic review and meta-analysis. *EP Europace*. 2015;17(5):767–777. doi:10.1093/europace/euv053
37. Deshmukh-Reeves E, Shaw M, Bilsby C, Gourlay CW. Biofilm formation on endotracheal and tracheostomy tubing: a systematic review and meta-analysis of culture data and sampling method. *MicrobiologyOpen*. 2025;14(4):e70032. doi:10.1002/mbo3.70032
38. Thorarinsdottir HR, Kander T, Holmberg A, Petronis S, Klarin B. Biofilm formation on three different endotracheal tubes: a prospective clinical trial. *Crit Care*. 2020;24(1):382. doi:10.1186/s13054-020-03092-1
39. Richardson AK, Fuller RG, April MD, et al. Antimicrobial-coated endotracheal tubes: a narrative review. *J Crit Care*. 2026;91:155222. doi:10.1016/j.jcrc.2025.155222
40. Almuhanna Y. Microbial biofilms as barriers to chronic wound healing: diagnostic challenges and therapeutic advances. *JCM*. 2025;14 (22):8121. doi:10.3390/jcm14228121
41. Papadopoulou V, Sidders AE, Lu KY, et al. Overcoming biological barriers to improve treatment of a *Staphylococcus aureus* wound infection. *Cell Chem Biol*. 2023;30(5):513–526.e5. doi:10.1016/j.chembiol.2023.04.009
42. Paseban K, Noroozi S, Gharehcheloo R, et al. Preparation and optimization of niosome encapsulated meropenem for significant antibacterial and anti-biofilm activity against methicillin-resistant *Staphylococcus aureus* isolates. *Heliyon*. 2024;10(16):e35651. doi:10.1016/j.heliyon.2024.e35651
43. Muzzalupo R, Mazzotta E. Do niosomes have a place in the field of drug delivery? *Exp Opin Drug Deliv*. 2019;16(11):1145–1147. doi:10.1080/17425247.2019.1663821
44. Masjedi M, Montahaei T. An illustrated review on nonionic surfactant vesicles (niosomes) as an approach in modern drug delivery: fabrication, characterization, pharmaceutical, and cosmetic applications. *J Drug Delivery Sci Technol*. 2021;61:102234. doi:10.1016/j.jddst.2020.102234
45. Gao S, Sui Z, Jiang Q, Jiang Y. Functional evaluation of niosomes utilizing surfactants in nanomedicine applications. *IJN*. 2024;19:10283–10305. doi:10.2147/IJN.S480639
46. Liga S, Paul C, Moacă EA, Péter F. Niosomes: composition, formulation techniques, and recent progress as delivery systems in cancer therapy. *Pharmaceutics*. 2024;16(2):223. doi:10.3390/pharmaceutics16020223
47. Mawazi SM, Ge Y, Widodo RT. Niosome preparation techniques and structure—an illustrated review. *Pharmaceutics*. 2025;17(1):67. doi:10.3390/pharmaceutics17010067
48. Antonara L, Triantafyllopoulou E, Chountoulesi M, et al. Recent advances in niosome-based transdermal drug delivery systems. *Curr Opin Biomed Eng*. 2025;35:100603. doi:10.1016/j.cobme.2025.100603
49. Moammeri A, Chegeni MM, Sahrayi H, et al. Current advances in niosomes applications for drug delivery and cancer treatment. *Mater Today Bio*. 2023;23:100837. doi:10.1016/j.mtbio.2023.100837
50. Chen R, Du M, Liu C. Strategies for dispersion of cariogenic biofilms: applications and mechanisms. *Front Microbiol*. 2022;13:981203. doi:10.3389/fmicb.2022.981203
51. Bartelds R, Nematollahi MH, Pols T, et al. Niosomes, an alternative for liposomal delivery. *PLoS One*. 2018;13(4):e0194179. doi:10.1371/journal.pone.0194179
52. Zolghadri S, Asad AG, Farzi F, et al. Span 60/cholesterol niosomal formulation as a suitable vehicle for gallic acid delivery with potent in vitro antibacterial, antimelanoma, and anti-tyrosinase activity. *Pharmaceuticals*. 2023;16(12):1680. doi:10.3390/ph16121680
53. Bautista-Solano AA, Dávila-Ortiz G, Perea-Flores MDJ, Martínez-Ayala AL. A comprehensive review of niosomes: composition, structure, formation, characterization, and applications in bioactive molecule delivery systems. *Molecules*. 2025;30(17):3467. doi:10.3390/molecules30173467
54. Soni S, Baghel K, Soni ML, Kashaw SK, Soni V. Size-dependent effects of niosomes on the penetration of methotrexate in skin layers. *Futur J Pharm Sci*. 2024;10(1):48. doi:10.1186/s43094-024-00624-2
55. Maurizi L, Forte J, Ammendolia MG, et al. Effect of ciprofloxacin-loaded niosomes on *Escherichia coli* and *Staphylococcus aureus* biofilm formation. *Pharmaceutics*. 2022;14(12):2662. doi:10.3390/pharmaceutics14122662
56. Poustforoosh A. Investigation on the structural and dynamical properties of cationic, anionic, and catanionic niosomes as multifunctional controlled drug delivery system for cabozantinib. *Colloids Surf A*. 2024;687:133547. doi:10.1016/j.colsurfa.2024.133547
57. Fadaei MS, Fadaei MR, Kheirieh AE, et al. Niosome as a promising tool for increasing the effectiveness of anti-inflammatory compounds. *Excli J*. 2024;23:212. doi:10.17179/EXCLI2023-6868
58. Bashkeran T, Kamaruddin AH, Ngo TX, et al. Niosomes in cancer treatment: a focus on curcumin encapsulation. *Heliyon*. 2023;9(8):e18710. doi:10.1016/j.heliyon.2023.e18710
59. De Silva L, Fu JY, Htar TT, et al. Biodistribution study of niosomes in tumor-implanted BALB/C mice using scintigraphic imaging. *Front Pharmacol*. 2022;12:778396. doi:10.3389/fphar.2021.778396
60. Gugleva V, Michailova V, Mihaylova R, et al. Formulation and evaluation of hybrid niosomal in situ gel for intravesical co-delivery of curcumin and gentamicin sulfate. *Pharmaceutics*. 2022;14(4):747. doi:10.3390/pharmaceutics14040747
61. Yaghoobian M, Haeri A, Bolourchian N, Shahhosseni S, Dadashzadeh S. The impact of surfactant composition and surface charge of niosomes on the oral absorption of repaglinide as a BCS II model drug. *IJN*. 2020;15:8767–8781. doi:10.2147/IJN.S261932
62. Judy E, Lopus M, Kishore N. Mechanistic insights into encapsulation and release of drugs in colloidal niosomal systems: biophysical aspects. *RSC Adv*. 2021;11(56):35110–35126. doi:10.1039/D1RA06057K
63. Pandey P, Pal R, Khadam VKR, Chawra HS, Singh RP. Advancement and characteristics of non-ionic surfactant vesicles (Niosome) and their application for analgesics. *Int J Pharm Invest*. 2024;14(3):616–632. doi:10.5530/ijpi.14.3.74
64. Khaleghian M, Sahrayi H, Hafezi Y, et al. In silico design and mechanistic study of niosome-encapsulated curcumin against multidrug-resistant *Staphylococcus aureus* biofilms. *Front Microbiol*. 2023;14:1277533. doi:10.3389/fmicb.2023.1277533

65. M HC, Abolhassani Targhi A, Shamsi F, et al. Niosome-encapsulated tobramycin reduced antibiotic resistance and enhanced antibacterial activity against multidrug-resistant clinical strains of *Pseudomonas aeruginosa*. *J Biomed Materials Res*. 2021;109(6):966–980. doi:10.1002/jbm.a.37086
66. Shirvany A, Rezayan AH, Alvandi H, Barshan Tashnizi M, Sabahi H. Preparation and evaluation of a niosomal drug delivery system containing cefazolin and study of its antibacterial activity. *Iran J Med Microbiol*. 2021;15(6):638–657. doi:10.30699/ijmm.15.6.638
67. Miatmoko A, Safitri SA, Aquila F, et al. Characterization and distribution of niosomes containing ursolic acid coated with chitosan layer. *Res Pharm Sci*. 2021;16(6):660–673. doi:10.4103/1735-5362.327512
68. Zaid Alkilani A, Hamed R, Abdo H, et al. Formulation and evaluation of azithromycin-loaded niosomal gel: optimization, in vitro studies, rheological characterization, and cytotoxicity study. *ACS Omega*. 2022;7(44):39782–39793. doi:10.1021/acsomega.2c03762
69. Huanbutta K, Suwanpitak K, Ponlakorn P, et al. Development and evaluation of drug-loaded niosomes fabricated by flow chemistry: a novel vortex tube reactor approach. *OpenNano*. 2025;23:100243. doi:10.1016/j.onano.2025.100243
70. Marianecci C, Di Marzio L, Del Favero E, et al. Niosomes as drug nanovectors: multiscale ph-dependent structural response. *Langmuir*. 2016;32(5):1241–1249. doi:10.1021/acs.langmuir.5b04111
71. Ghafelehbashi R, Akbarzadeh I, Tavakkoli Yaraki M, Lajevardi A, Fatemizadeh M, Heidarpoor Saremi L. Preparation, physicochemical properties, in vitro evaluation and release behavior of cephalexin-loaded niosomes. *Int J Pharm*. 2019;569:118580. doi:10.1016/j.ijpharm.2019.118580
72. Kumar A, Dua JS. Formulation and evaluation of itraconazole niosomal gel. *Asian J Pharm Res Dev*. 2018;6(5):76–80. doi:10.22270/ajprd.v6i5.425
73. Mansouri M, Khayam N, Jamshidifar E, et al. Streptomycin sulfate–loaded niosomes enables increased antimicrobial and anti-biofilm activities. *Front Bioeng Biotechnol*. 2021;9:745099. doi:10.3389/fbioe.2021.745099
74. Zaer M, Moeinzadeh A, Abolhassani H, et al. Doxorubicin-loaded Niosomes functionalized with gelatine and alginate as pH-responsive drug delivery system: a 3D printing approach. *Int J Biol Macromol*. 2023;253:126808. doi:10.1016/j.ijbiomac.2023.126808
75. Gugleva V, Mihaylova R, Momekov G, et al. pH-responsive niosome-based nanocarriers of antineoplastic agents. *RSC Adv*. 2024;14(16):11124–11140. doi:10.1039/D4RA01334D
76. De Guzman GQ, Dg Yap CD, Dc Munarriz AP, Dimaano AD, De Guzman LM. Niosomal entrapment improved the bactericidal properties of azithromycin against methicillin-resistant *Staphylococcus aureus*. *Ther Deliv*. 2022;13(8):391–402. doi:10.4155/tde-2022-0034
77. Osman N, Omolo CA, Gafar MA, et al. Niosomes modified with a novel pH-responsive coating (mPEG-OA) enhance the antibacterial and anti-biofilm activity of vancomycin against methicillin-resistant *Staphylococcus aureus*. *Nano Ex*. 2024;5(1):015008. doi:10.1088/2632-959X/ad1d02
78. Sailaja AK, Shreya M. Preparation and characterization of naproxen loaded niosomes by ether injection method. *Nano BioMed ENG*. 2018;10(2):174–180. doi:10.5101/nbe.v10i2.p174-180
79. Abo Kamer AM, Amer NM, Abdelmegeed AA, El Maghraby GM, Gamaleldin NM. Surfactant nanovesicles for augmented antibacterial activity against carbapenemase resistant enterobacteriaceae and extended spectrum beta-lactamases producing bacteria: in vitro and in vivo evaluation. *BMC Microbiol*. 2023;23(1). doi:10.1186/s12866-023-02812-1
80. Bazargan E, Ashrafi F, Elham ST. Antibacteria and antibiofilm properties of tetracycline loaded niosomes against *Klebsiella pneumoniae* isolates: a laboratory study. *JRUMS*. 2024;23(4):207–221. doi:10.61186/jrums.23.4.207
81. Kashef MT, Saleh NM, Assar NH, Ramadan MA. The antimicrobial activity of ciprofloxacin-loaded niosomes against ciprofloxacin-resistant and biofilm-forming *Staphylococcus aureus*. *IDR*. 2020;13:1619–1629. doi:10.2147/IDR.S249628
82. Abonashey SG, Hassan HAFM, Shalaby MA, Fouad AG, Mobarez E, El-Banna HA. Formulation, pharmacokinetics, and antibacterial activity of florfenicol-loaded niosome. *Drug Deliv Transl Res*. 2024;14(4):1077–1092. doi:10.1007/s13346-023-01459-9
83. Durak S, Esmaeili Rad M, Alp Yetisgin A, et al. Niosomal drug delivery systems for ocular disease—recent advances and future prospects. *Nanomaterials*. 2020;10(6):1191. doi:10.3390/nano10061191
84. Abonashey SG, Fouad AG, Hassan HAFM, et al. Preparation and characterization of tiamulin-loaded niosomes for oral bioavailability enhancement in mycoplasma-infected broilers. *Micro*. 2024;4(4):734–750. doi:10.3390/micro4040045
85. Berbel Manaia E, Paiva Abuçafy M, Chiari-Andréo BG, Lallo Silva B, Oshiro-Júnior JA, Chiavacci L. Physicochemical characterization of drug nanocarriers. *IJN*. 2017;12:4991–5011. doi:10.2147/IJN.S133832
86. Larijanian L, Shafiei M, Pirbalouti AG, Ferdousi A, Chiani M. Antibacterial and antibiofilm activities of zingerone and niosomal zingerone against methicillin-resistant *Staphylococcus aureus* (MRSA)s. *IJM*. 2024. doi:10.18502/ijm.v16i3.15794
87. Piri-Gharaghie T, Jegargoshe-Shirin N, Saremi-Nouri S, et al. Effects of imipenem-containing niosome nanoparticles against high prevalence methicillin-resistant Staphylococcus Epidermidis biofilm formed. *Sci Rep*. 2022;12(1):5140. doi:10.1038/s41598-022-09195-9
88. Sezgin-Bayindir Z, Yuksel N. Investigation of formulation variables and excipient interaction on the production of niosomes. *AAPS Pharm Sci Tech*. 2012;13(3):826–835. doi:10.1208/s12249-012-9805-4
89. Shilakari Asthana G, Sharma PK, Asthana A. In vitro and in vivo evaluation of niosomal formulation for controlled delivery of clarithromycin. *Scientifica*. 2016;2016:1–10. doi:10.1155/2016/6492953
90. Rahmati M, Babapoor E, Dezfulian M. Amikacin-loaded niosome nanoparticles improve amikacin activity against antibiotic-resistant *Klebsiella pneumoniae* strains. *World J Microbiol Biotechnol*. 2022;38(12):230. doi:10.1007/s11274-022-03405-2
91. Cui H, Wu M, Wan J, et al. A novel anti-PcrV monoclonal antibody inhibits toxin-mediated cytotoxicity and enhances survival in mouse models of *Pseudomonas aeruginosa* infection. *Hum Vaccines Immunother*. 2025;21(1):2567042. doi:10.1080/21645515.2025.2567042
92. Yu H, Xiong J, Qiu J, et al. Type III secretion protein, PcrV, impairs pseudomonas aeruginosa biofilm formation by increasing M1 macrophage-mediated anti-bacterial activities. *Front Microbiol*. 2020;11:1971. doi:10.3389/fmicb.2020.01971
93. Warrener P, Varkey R, Bonnell JC, et al. A novel anti-PcrV antibody providing enhanced protection against Pseudomonas aeruginosa in multiple animal infection models. *Antimicrob Agents Chemother*. 2014;58(8):4384–4391. doi:10.1128/AAC.02643-14
94. Heidari F, Akbarzadeh I, Nourouzian D, Mirzaie A, Bakhshandeh H. Optimization and characterization of tannic acid loaded niosomes for enhanced antibacterial and anti-biofilm activities. *Adv Powder Technol*. 2020;31(12):4768–4781. doi:10.1016/j.apt.2020.11.008
95. Cevc G, Blume G. New, highly efficient formulation of diclofenac for the topical, transdermal administration in ultradeformable drug carriers, transfersomes. *Biochimica Biophysica Acta*. 2001;1514(2):191–205. doi:10.1016/S0005-2736(01)00369-8

96. Salvati A, Åberg C, Dos Santos T, et al. Experimental and theoretical comparison of intracellular import of polymeric nanoparticles and small molecules: toward models of uptake kinetics. *Nanomedicine*. 2011;7(6):818–826. doi:10.1016/j.nano.2011.03.005
97. Torchilin VP. Multifunctional, stimuli-sensitive nanoparticulate systems for drug delivery. *Nat Rev Drug Discov*. 2014;13(11):813–827. doi:10.1038/nrd4333
98. Carafa M, Marianecci C, Rinaldi F, Santucci E, Tampucci S, Monti D. Span® and Tween® neutral and pH-sensitive vesicles: characterization and *in vitro* skin permeation. *J Liposome Res*. 2009;19(4):332–340. doi:10.3109/08982100903014994
99. Nowroozi F, Almasi A, Javidi J, Haeri A, Dadashzadeh S. Effect of surfactant type, cholesterol content and various downsizing methods on the particle size of niosomes. *Iran J Pharm Res*. 2018;17(2):1.
100. Taymouri S, Varshosaz J. Effect of different types of surfactants on the physical properties and stability of carvedilol nano-niosomes. *Adv Biomed Res*. 2016;5(1). doi:10.4103/2277-9175.178781
101. Kumar GP, Rajeshwarrao P. Nonionic surfactant vesicular systems for effective drug delivery—an overview. *Acta Pharmaceutica Sinica B*. 2011;1(4):208–219. doi:10.1016/j.apsb.2011.09.002
102. Nematollahi MH, Pardakhty A, Torkzadeh-Mahanai M, Mehrabani M, Asadikaram G. Changes in physical and chemical properties of niosome membrane induced by cholesterol: a promising approach for niosome bilayer intervention. *RSC Adv*. 2017;7(78):49463–49472. doi:10.1039/C7RA07834J
103. Basiri L, Rajabzadeh G, Bostan A. Physicochemical properties and release behavior of Span 60/Tween 60 niosomes as vehicle for α-Tocopherol delivery. *LWT*. 2017;84:471–478. doi:10.1016/j.lwt.2017.06.009
104. Gharbavi M, Amani J, Kheiri-Manjili H, Danafar H, Sharafi A. Niosome: a promising nanocarrier for natural drug delivery through blood-brain barrier. *Adv Pharmacol Sci*. 2018;2018:1–15. doi:10.1155/2018/6847971
105. Haddadian A, Robattorki FF, Dibah H, et al. Niosomes-loaded selenium nanoparticles as a new approach for enhanced antibacterial, anti-biofilm, and anticancer activities. *Sci Rep*. 2022;12(1):21938. doi:10.1038/s41598-022-26400-x
106. Mirzaie A, Peirovi N, Akbarzadeh I, et al. Preparation and optimization of ciprofloxacin encapsulated niosomes: a new approach for enhanced antibacterial activity, biofilm inhibition and reduced antibiotic resistance in ciprofloxacin-resistant methicillin-resistance *Staphylococcus aureus*. *Bioorg Chem*. 2020;103:104231. doi:10.1016/j.bioorg.2020.104231
107. Palanisamy P, Crossia WF, Prakash D, Antonyraj APM. Nanoformulation of stavudine-loaded niosomes: enhancing drug delivery, entrapment efficiency, and controlled release for improved antiretroviral therapy. *J Orthopaed Rep*. 2025;100677. doi:10.1016/j.jorep.2025.100677
108. Shadloo A, Peyvandi K, Shojaeian A, Shariat S. How the designed processing parameters affect the liquid mixture density and viscosity of the tretinoin-loaded niosomes at different temperatures? *Heliyon*. 2024;10(18):e37925. doi:10.1016/j.heliyon.2024.e37925
109. Wagdi MA, Salama A, El-Liethy MA, Shalaby ES. Comparative study of niosomes and spanlastics as a promising approach for enhancing benzalkonium chloride topical wound healing: in-vitro and in-vivo studies. *J Drug Delivery Sci Technol*. 2023;84:104456. doi:10.1016/j.jddst.2023.104456
110. Allen TM, Cullis PR. Liposomal drug delivery systems: from concept to clinical applications. *Adv Drug Delivery Rev*. 2013;65(1):36–48. doi:10.1016/j.addr.2012.09.037
111. Bertrand N, Leroux JC. The journey of a drug-carrier in the body: an anatomo-physiological perspective. *J Control Release*. 2012;161(2):152–163. doi:10.1016/j.jconrel.2011.09.098
112. Gunn JS, Bakaletz LO, Wozniak DJ. What's on the outside matters: the role of the extracellular polymeric substance of gram-negative biofilms in evading host immunity and as a target for therapeutic intervention. *J Biol Chem*. 2016;291(24):12538–12546. doi:10.1074/jbc.R115.707547
113. Hussaini IM, Oyewole OA, Sulaiman MA, Dabban AI, Sulaiman AN, Tarek R. Microbial anti-biofilms: types and mechanism of action. *Res Microbiol*. 2024;175(3):104111. doi:10.1016/j.resmic.2023.104111
114. Kalia VC. Quorum sensing inhibitors: an overview. *Biotechnol Adv*. 2013;31(2):224–245. doi:10.1016/j.biotechadv.2012.10.004
115. Krzyżek P. Challenges and Limitations of Anti-quorum Sensing Therapies. *Front Microbiol*. 2019;10:2473. doi:10.3389/fmicb.2019.02473
116. Almatroudi A. Biofilm resilience: molecular mechanisms driving antibiotic resistance in clinical contexts. *Biology*. 2025;14(2):165. doi:10.3390/biology14020165
117. Dieltjens L, Appermans K, Lissens M, et al. Inhibiting bacterial cooperation is an evolutionarily robust anti-biofilm strategy. *Nat Commun*. 2020;11(1):107. doi:10.1038/s41467-019-13660-x
118. Hetta HF, Ramadan YN, Rashed ZI, et al. Quorum sensing inhibitors: an alternative strategy to win the battle against Multidrug-Resistant (MDR) bacteria. *Molecules*. 2024;29(15):3466. doi:10.3390/molecules29153466
119. Rodríguez-Urretavizcaya B, Vilaplana L, Marco MP. Strategies for quorum sensing inhibition as a tool for controlling Pseudomonas aeruginosa infections. *Int J Antimicrob Agents*. 2024;64(5):107323. doi:10.1016/j.ijantimicag.2024.107323
120. Tetz GV, Artemenko NK, Tetz VV. Effect of DNase and antibiotics on biofilm characteristics. *Antimicrob Agents Chemother*. 2009;53(3):1204–1209. doi:10.1128/AAC.00471-08
121. Ashkezari S, Abtahi MS, Sattari Z, et al. Antibiotic and inorganic nanoparticles co-loaded into carboxymethyl chitosan-functionalized niosome: synergistic enhanced antibacterial and anti-biofilm activities. *J Drug Delivery Sci Technol*. 2023;83:104386. doi:10.1016/j.jddst.2023.104386
122. Targhi AA, Moammeri A, Jamshidifar E, et al. Synergistic effect of curcumin-Cu and curcumin-Ag nanoparticle loaded niosome: enhanced antibacterial and anti-biofilm activities. *Bioorg Chem*. 2021;115:105116. doi:10.1016/j.bioorg.2021.105116
123. Karygianni L, Ren Z, Koo H, Thurnheer T. Biofilm matrixome: extracellular components in structured microbial communities. *Trends Microbiol*. 2020;28(8):668–681. doi:10.1016/j.tim.2020.03.016
124. Koo H, Xiao J, Klein MI, Jeon JG. Exopolysaccharides produced by *Streptococcus mutans* glucosyltransferases modulate the establishment of microcolonies within multispecies biofilms. *J Bacteriol*. 2010;192(12):3024–3032. doi:10.1128/JB.01649-09
125. Stewart PS, Franklin MJ. Physiological heterogeneity in biofilms. *Nat Rev Microbiol*. 2008;6(3):199–210. doi:10.1038/nrmicro1838
126. Giuli MV, Hanieh PN, Forte J, et al. pH-sensitive niosomes for ATRA delivery: a promising approach to inhibit Pin1 in high-grade serous ovarian cancer. *Int J Pharm*. 2024;649:123672. doi:10.1016/j.ijpharm.2023.123672
127. Rinaldi F, Hanieh P, Chan L, et al. Chitosan glutamate-coated niosomes: a proposal for nose-to-brain delivery. *Pharmaceutics*. 2018;10(2):38. doi:10.3390/pharmaceutics10020038
128. Wang Z, Wang X, Xu W, et al. Translational challenges and prospective solutions in the implementation of biomimetic delivery systems. *Pharmaceutics*. 2023;15(11):2623. doi:10.3390/pharmaceutics15112623

129. Tan RYH, Lee CS, Pichika MR, Cheng SF, Lam KY. pH responsive polyurethane for the advancement of biomedical and drug delivery. *Polymers*. 2022;14(9):1672. doi:10.3390/polym14091672
130. Hajinezhad MR, Fathi-karkan S, Roostaee M, et al. Engineering pH-responsive niosomes with ergosterol and CHEMS for controlled carfilzomib release: insights from *in vitro* and *in vivo* studies. *Drug Dev Ind Pharm*. 2025;51(9):1122–1137. doi:10.1080/03639045.2025.2530161
131. Meoli CM, Riccardi D, Baldino L. Optimization of chitosan-coated niosomes encapsulating doxycycline hyclate for pH-responsive drug release. *Ind Eng Chem Res*. 2025. doi:10.1021/acs.iecr.4c04456
132. Hu Q, Katti PS, Gu Z. Enzyme-responsive nanomaterials for controlled drug delivery. *Nanoscale*. 2014;6(21):12273–12286. doi:10.1039/C4NR04249B
133. Jeong GJ, Rather MA, Khan F, Tabassum N, Mandal M, Kim YM. pH-responsive polymeric nanomaterials for the treatment of oral biofilm infections. *Colloids Surf B*. 2024;234:113727. doi:10.1016/j.colsurfb.2023.113727
134. Al-Madboly LA, Aboulmagd A, El-Salam MA, Kushkevych I, El-Morsi RM. Microbial enzymes as powerful natural anti-biofilm candidates. *Microb Cell Fact*. 2024;23(1):343. doi:10.1186/s12934-024-02610-y
135. Nielsen SM, Johnsen KK, Hansen LBS, et al. Large-scale screening identifies enzyme combinations that remove in situ grown oral biofilm. *Biofilm*. 2024;8:100229. doi:10.1016/j.bioflm.2024.100229
136. Billings N, Birjiniuk A, Samad TS, Doyle PS, Ribbeck K. Material properties of biofilms—a review of methods for understanding permeability and mechanics. *Rep Prog Phys*. 2015;78(3):036601. doi:10.1088/0034-4885/78/3/036601
137. Stewart PS. Mechanisms of antibiotic resistance in bacterial biofilms. *Int J Med Microbiol*. 2002;292(2):107–113. doi:10.1078/1438-4221-00196
138. Ramakrishnan R, Singh AK, Singh S, Chakravortty D, Das D. Enzymatic dispersion of biofilms: an emerging biocatalytic avenue to combat biofilm-mediated microbial infections. *J Biol Chem*. 2022;298(9):102352. doi:10.1016/j.jbc.2022.102352
139. Ding M, Zhao W, Song L, Luan S. Stimuli-responsive nanocarriers for bacterial biofilm treatment. *Rare Met*. 2022;41(2):482–498. doi:10.1007/s12598-021-01802-4
140. Thorn CR, Howell PL, Wozniak DJ, Prestidge CA, Thomas N. Enhancing the therapeutic use of biofilm-dispersing enzymes with smart drug delivery systems. *Adv Drug Delivery Rev*. 2021;179:113916. doi:10.1016/j.addr.2021.113916
141. Lahiri D, Nag M, Dey A, et al. Immobilized enzymes as potent antibiofilm agent. *Biotechnol Progr*. 2022;38(5):e3281. doi:10.1002/btpr.3281
142. Wei Y, Lv J, Zhu S, Wang S, Su J, Xu C. Enzyme-responsive liposomes for controlled drug release. *Drug Discovery Today*. 2024;29(7):104014. doi:10.1016/j.drudis.2024.104014
143. De La Rica R, Aili D, Stevens MM. Enzyme-responsive nanoparticles for drug release and diagnostics. *Adv Drug Delivery Rev*. 2012;64(11):967–978. doi:10.1016/j.addr.2012.01.002
144. Badran MM, Alsubaie A, Salem Bekhit MM, et al. Bioadhesive hybrid system of niosomes and pH sensitive in situ gel for itraconazole ocular delivery: dual approach for efficient treatment of fungal infections. *Saudi Pharm J*. 2024;32(12):102208. doi:10.1016/j.jsps.2024.102208
145. Shrestha S, Wang B, Dutta PK. Commercial silver-based dressings: in vitro and clinical studies in treatment of chronic and burn wounds. *Antibiotics*. 2024;13(9):910. doi:10.3390/antibiotics13090910
146. Maitz J, Merlino J, Rizzo S, McKew G, Maitz P. Burn wound infections microbiome and novel approaches using therapeutic microorganisms in burn wound infection control. *Adv Drug Delivery Rev*. 2023;196:114769. doi:10.1016/j.addr.2023.114769
147. Buchholz N, Budia A, Cruz JDL, et al. Urinary stent development and evaluation models: in vitro, ex vivo and in vivo—A European Network of Multidisciplinary Research to Improve Urinary Stents (ENIUS) initiative. *Polymers*. 2022;14(9):1641. doi:10.3390/polym14091641
148. Corrado B, Cammarano A, Dello Iacono S, et al. A comprehensive review of progress in preventing urinary infections associated with the use of urinary catheters: a dual analysis of publications and patents. *Infect Dis Rep*. 2025;17(3):64. doi:10.3390/idr17030064
149. Høiby N, Bjarnsholt T, Moser C, et al. ESCMID* guideline for the diagnosis and treatment of biofilm infections 2014. *Clin Microbiol Infect*. 2015;21:S1–25. doi:10.1016/j.cmi.2014.10.024
150. Riccardi D, Baldino L, Reverchon E. Liposomes, transfersomes and niosomes: production methods and their applications in the vaccinal field. *J Transl Med*. 2024;22(1):339. doi:10.1186/s12967-024-05160-4
151. Rathi DNG, Norman A, Mohd Esa N, Che Abdullah CA. Niosomes as versatile nanocarriers in diabetes research. *Int J Mater Res*. 2025;116(8):613–634. doi:10.1515/ijmr-2024-0270
152. Blanco-Cabra N, Alcàcer-Almansa J, Admella J, Arévalo-Jaimes BV, Torrents E. Nanomedicine against biofilm infections: a roadmap of challenges and limitations. *WIREs Nanomed Nanobiotechnol*. 2024;16(1):e1944. doi:10.1002/wnan.1944
153. Lan J, Zou J, Xin H, et al. Nanomedicines as disruptors or inhibitors of biofilms: opportunities in addressing antimicrobial resistance. *J Control Release*. 2025;381:113589. doi:10.1016/j.jconrel.2025.113589
154. Lee NK, Chang JW. Manufacturing Cell and Gene Therapies: challenges in Clinical Translation. *Ann Lab Med*. 2024;44(4):314–323. doi:10.3343/alm.2023.0382
155. Mayrink NNV, Alcoforado L, Chioro A, et al. Translational research in health technologies: a scoping review. *Front Digit Health*. 2022;4:957367. doi:10.3389/fdgth.2022.957367
156. Ferraz MP. Advanced nanotechnological approaches for biofilm prevention and control. *Appl Sci*. 2024;14(18):8137. doi:10.3390/app14188137
157. Farah H, Kadhim-Abosaoda M, Mohaisen-Mousa H, et al. Nanomedicine strategies against biofilm-associated infections: advances, challenges, and translational barriers. *MicrobiologyOpen*. 2026;15(1):e70210. doi:10.1002/mbo3.70210
158. Hemmati J, Chegini Z, Arabestani MR. Niosomal-based drug delivery platforms: a promising therapeutic approach to fight *Staphylococcus aureus* drug resistance. *J Nanomater*. 2023;2023:1–18. doi:10.1155/2023/5298565
159. Xie Y, Liu H, Teng Z, Ma J, Liu G. Nanomaterial-enabled anti-biofilm strategies: new opportunities for treatment of bacterial infections. *Nanoscale*. 2025;17(10):5605–5628. doi:10.1039/D4NR04774E
160. Adeniji OO, Nontongana N, Okoh JC, Okoh AI. The potential of antibiotics and nanomaterial combinations as therapeutic strategies in the management of multidrug-resistant infections: a review. *IJMS*. 2022;23(23):15038. doi:10.3390/ijms232315038
161. Mohanta YK, Chakrabartty I, Mishra AK, et al. Nanotechnology in combating biofilm: a smart and promising therapeutic strategy. *Front Microbiol*. 2023;13:1028086. doi:10.3389/fmicb.2022.1028086

162. Zafari M, Adibi M, Chiani M, et al. Effects of cefazolin-containing niosome nanoparticles against methicillin-resistant *Staphylococcus aureus* biofilm formed on chronic wounds. *Biomed Mater*. 2021;16(3):035001. doi:10.1088/1748-605X/abc7f2
163. Osman N, Mohammed M, Omolo CA, et al. Hyaluronidase inhibitor surface-modified niosomes: an efficient strategy for antibacterial and anti-biofilm drug delivery. *Materialia*. 2023;30:101826. doi:10.1016/j.mtla.2023.101826
164. Shamkani F, Barzi SM, Badmasti F, et al. Enhanced anti-biofilm activity of the minocycline-and-gallium-nitrate using niosome wrapping against *Acinetobacter baumannii* in C57/BL6 mouse pneumonia model. *Int Immunopharmacol*. 2023;115:109551. doi:10.1016/j.intimp.2022.109551