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REVIEW

Uncovering potential targets for MRSA infection treatment: a nanomedicine perspective

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ABSTRACT

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a formidable global health challenge, responsible for severe infections with unacceptably high mortality rates. Conventional antibiotics, though essential, face growing limitations due to resistance, poor penetration into biofilms, and inability to eliminate intracellular reservoirs. These shortcomings underscore the urgent need to explore MRSA-specific targets, including toxin secretion, quorum sensing, biofilm formation and efflux pumps in the design of intelligent antibiotic delivery systems. Nanocarriers provide an ideal platform to address these gaps by enhancing drug stability, penetration, and site-specific delivery, while enabling the co-administration of antibiotics with anti-virulence agents at otherwise inaccessible infection sites.

Areas covered: This review discusses emerging MRSA therapeutic targets, cell wall/membrane synthesis, quorum sensing, biofilms, virulence factors, and efflux pumps, and how nanocarrier-based systems have been engineered to exploit them. Advances from 2015–2025 are analyzed, highlighting nano-enabled strategies that enhance antibiotic efficacy, neutralize toxins, disrupt biofilms, and achieve high drug accumulation at infection foci.

Expert opinion: Targeting MRSA's virulence pathways through nanocarrier systems offers a paradigm shift beyond traditional antibiotics. The next decade will require not only optimization and mechanistic validation but also innovative material design, scalable manufacturing, and integration into clinical practice to realize the promise of nanocarrier-enabled anti-MRSA therapies.

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1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a major global health threat, maintaining its classification as a high-priority pathogen on the recently published World Health Organization (WHO) Bacterial Priority Pathogens List (2024) [1]. Ranked among the leading causes of infection in both healthcare and community settings by the Global Burden of Disease study, MRSA is responsible for a substantial proportion of deaths linked to antimicrobial resistance (AMR), particularly in high-income countries, where nearly half of AMR-related deaths are attributed to *Staphylococcus aureus* and *Escherichia coli* [2]. The clinical and economic burdens of MRSA are significant; for instance, MRSA infection could be responsible for fatal sepsis, pneumonia, and higher rates of myocardial infarction and heart failure in patients with bacteremia [3]. In addition, it accounts for 56.8% of nosocomial bacteremia infections with a 30-day mortality rate of 19.1%, while MRSA-associated endocarditis carries a reported mortality rate ranging from 17% to 50% [4]. These figures highlight the urgent need for global coordination and sustained research efforts to mitigate the impact of MRSA and develop effective strategies to combat AMR.

Current treatment strategies for MRSA infections rely heavily on a limited arsenal of antibiotics, including vancomycin, linezolid, daptomycin, and newer agents such as ceftaroline and delafloxacin [5]. These therapies predominantly target essential bacterial functions such as cell wall synthesis, protein translation, and membrane integrity [6]. However, increasing resistance, mediated by mechanisms like cell wall thickening, ribosomal mutations, and efflux pumps, has significantly compromised their efficacy [7]. Moreover, conventional dosage forms often fail to achieve therapeutic concentrations at the infection site, especially in intracellular reservoirs or within protective biofilm communities [8]. MRSA's capacity to form biofilms and engage in quorum sensing (QS) not only enhances its survival and virulence but also limits antibiotic penetration and immune system recognition [9]. These factors collectively hinder treatment success and promote chronicity and recurrence. Importantly, most current therapies do not directly target biofilm formation, QS, or other recently identified virulence-associated pathways, leaving critical aspects of MRSA pathophysiology unaddressed. This underscores the pressing need to exploit these non-traditional but highly relevant targets to design more innovative and effective therapeutic strategies.

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Article highlights

- Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a major global health threat with substantial clinical and economic impact.
- Conventional antibiotics are limited by resistance, poor biofilm penetration, and inability to eradicate intracellular reservoirs.
- Targeting MRSA-specific pathways, such as toxin secretion, quorum sensing, biofilm formation, and efflux pumps, presents novel therapeutic opportunities.
- Nanocarrier-based systems enable precise targeting of these pathways while co-delivering antibiotics and anti-virulence agents to otherwise inaccessible sites.
- Current evidence shows that such nanosystems enhance drug accumulation, reduce biofilm burden, and mitigate toxin-mediated host damage, leading to improved outcomes in experimental MRSA models.

In recent years, nanomedicine has emerged as a transformative frontier in combating multidrug-resistant pathogens, notably MRSA [10]. Nanocarrier-based systems offer distinct physicochemical and functional advantages that address the limitations of conventional antibiotic formulations, including enhanced drug solubility, stability, protection from enzymatic degradation, and controlled or sustained release [11]. These platforms also improve pharmacokinetics and biodistribution, enabling passive or active targeting through surface modifications such as ligands that recognize bacterial epitopes or infected tissue markers, thereby enhancing therapeutic precision while minimizing systemic toxicity [11,12]. Crucially, nanocarriers can penetrate dense biofilm matrices and access intracellular reservoirs, key protective niches where MRSA evades both immune clearance and antimicrobial activity [13]. Beyond improving antibiotic efficacy, nanocarriers can be strategically designed to deliver anti-virulence agents targeting QS, biofilm architecture, and toxins, targets largely inaccessible by conventional therapies [14–17]. Their ability to co-deliver multiple agents with synergistic or complementary actions further augments their therapeutic utility [18]. Collectively, these features position nanomedicine as an ideal platform for both enhancing conventional antimicrobial strategies and exploiting precise, underutilized targets central to MRSA pathogenesis and persistence.

This review presents a focused analysis of emerging molecular and cellular targets for MRSA treatment and how nanocarrier technologies can be strategically employed to exploit these pathways. It highlights recent advances in nano-enabled approaches targeting the MRSA cell wall, QS, biofilm formation, virulence factors, and efflux pumps, and discusses the associated challenges and future directions for clinical translation. To the best of our knowledge, this is the first review to systematically synthesize studies utilizing nanocarriers to target uniquely identified therapeutic pathways in MRSA infections. The literature search encompassed experimental studies published between 2017 and 2025, sourced from Web of Science, PubMed, Scopus, and Google Scholar, using search terms including 'MRSA + Target + Nano + drug delivery system + cell wall + biofilm + quorum sensing + efflux pump + virulence factors.' This review offers a timely and novel contribution to the field, supporting the development of more targeted, effective, and sustainable nanomedicine-based therapies for MRSA.

2. Potential therapeutic targets in MRSA

Despite the availability of multiple antimicrobial agents against *Staphylococcus aureus*, the effective treatment of MRSA remains a major clinical challenge due to the pathogen's rapid evolution of resistance mechanisms and its ability to evade host immune responses [7]. Current therapeutic strategies predominantly target essential bacterial processes such as cell wall biosynthesis (e.g. vancomycin and β-lactams active against penicillin-binding protein 2a (PBP2a)), protein synthesis (e.g. linezolid), and membrane integrity (e.g. daptomycin) [6]. However, rising treatment failures, biofilm-associated persistence, and recurrent infections highlight the limitations of these traditional bactericidal approaches.

Emerging evidence has uncovered several alternative therapeutic targets that go beyond classical pathways, offering novel strategies to combat MRSA (Figure 1). These include QS systems that mediate bacterial communication and virulence regulation; biofilm formation, which contributes to chronicity and antibiotic tolerance; virulence factors such as toxins and surface proteins involved in immune evasion; and efflux pumps that actively expel antibiotics and contribute to multidrug resistance [19]. Exploiting these non-traditional targets holds potential for attenuating pathogenicity, disrupting resistance networks, and resensitizing MRSA to existing antibiotics. This section outlines the theoretical underpinnings of these emerging targets and presents the rationale for nanomedicine-enabled strategies in MRSA treatment.

2.1. Cell wall and cell membrane

The bacterial cell wall, primarily composed of peptidoglycan, is essential for maintaining shape, structural integrity, and resistance to osmotic stress. In MRSA, resistance to β-lactams is largely mediated by PBP2a, encoded by *mecA*, which reduces drug binding [20]. Despite this, cell wall biosynthesis remains an indispensable and druggable target. Key components for intervention include cytoplasmic enzymes MurA-MurF, responsible for early peptidoglycan precursor synthesis, and Fem proteins, which assemble the pentaglycine bridge critical for cell wall stability [21]. Wall teichoic acids (WTAs) are another attractive target, as they contribute to cell division, adhesion, and virulence, and are essential for proper peptidoglycan crosslinking. Similarly, lipid II, a membrane-anchored peptidoglycan precursor, offers a crucial node for disrupting cell wall synthesis. Beyond biosynthetic targets, the cell membrane itself represents a vulnerability; agents that perturb membrane integrity can induce rapid bacterial death through loss of membrane potential and leakage of cytoplasmic contents [6]. Collectively, the cell wall and membrane constitute structurally and functionally essential components of MRSA, making them central targets for therapeutic intervention.

2.2. Quorum sensing and biofilm formation

QS in MRSA is a cell-density-dependent regulatory system that orchestrates the expression of virulence factors, including toxins, adhesins, and enzymes critical for colonization and immune evasion. The accessory gene regulator (*agr*) system is the primary QS pathway, controlling the timing and

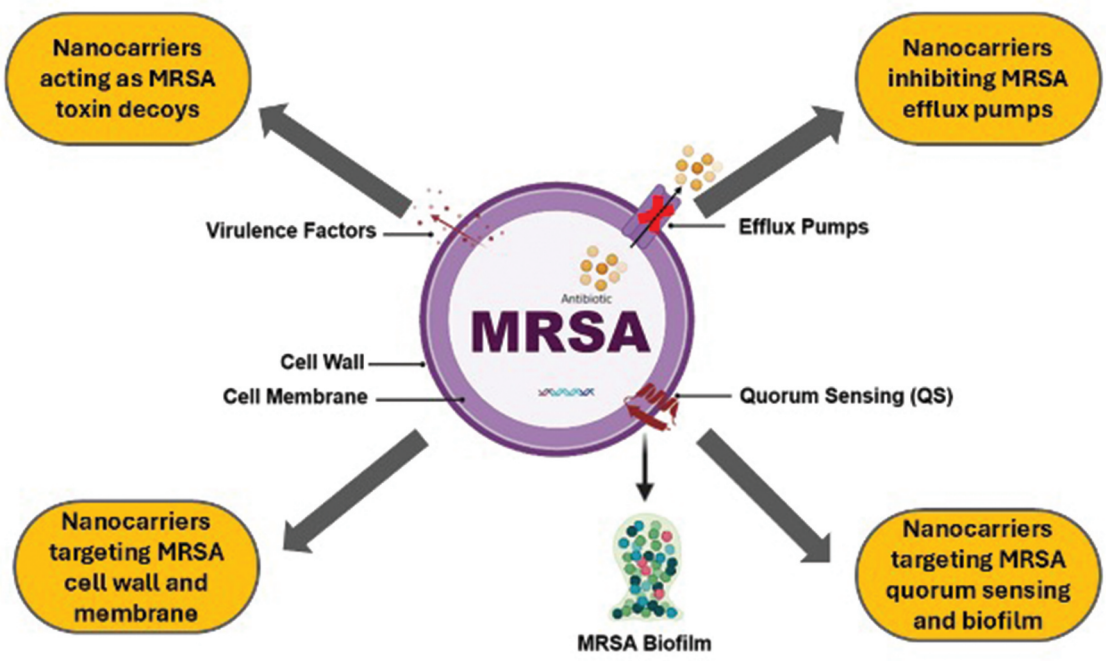

Figure 1. Emerging therapeutic targets in MRSA and novel targeting strategies employing nanocarrier-based systems.

magnitude of multiple virulence determinants. Genes such as *fnbA*, *fnbB*, *icaA-D*, and clumping factor genes drive intercellular adhesion and biofilm formation, structured bacterial communities encased in an extracellular polymeric substance (EPS) matrix [22]. This architecture shields MRSA from host immune defenses, impedes antibiotic penetration, and facilitates persistence and recurrence. The combined effects of QS-regulated virulence and biofilm fortification contribute to tissue damage, chronic inflammation, and impaired bacterial clearance, underlining their direct impact on host pathology [23].

Because QS and biofilms are central to pathogenicity but not essential for survival, they represent promising therapeutic targets aimed at attenuating virulence and persistence rather than directly killing the pathogen. Understanding these regulatory systems provides critical insight into non-bactericidal strategies for controlling MRSA infections.

2.3. Virulence factors

Virulence factors are central to MRSA pathogenicity, driving tissue damage, immune evasion, and persistent colonization. Key determinants include secreted toxins such as α-hemolysin, Panton-Valentine leukocidin (PVL), and *Staphylococcal* enterotoxins, as well as cell-surface proteins like protein A and multiple adhesins [24]. These molecules orchestrate diverse biological effects: toxins disrupt host cell membranes and trigger uncontrolled inflammation, while adhesins and protein A enable bacterial adherence, immune modulation, and evasion of opsonophagocytic killing. Collectively, these functions allow MRSA to establish deep-seated infections, resist host defenses, and disseminate within tissues [25].

Importantly, virulence factors are not essential for bacterial survival, but they are indispensable for disease progression. This makes them appealing therapeutic targets, as anti-virulence strategies can disarm the pathogen, reduce host damage, and complement conventional antimicrobial approaches without exerting direct bactericidal pressure, reducing the likelihood of resistance development.

2.4. Efflux pumps

Efflux pumps are membrane transport proteins that actively expel diverse antimicrobial agents, including fluoroquinolones, tetracyclines, macrolides, and biocides, from the cytoplasm to the extracellular environment. This reduces intracellular drug concentrations to sub-therapeutic levels, allowing survival, treatment failure, and the development of multidrug resistance [26]. Key MRSA efflux systems include *NorA*, *NorB*, *NorC*, *MepA*, and *Tet38*, largely belonging to the major facilitator superfamily (MFS) and the multidrug and toxic compound extrusion (MATE) families. Their expression is often upregulated in response to antibiotic pressure or mutations, providing strong adaptive advantages [27]. Efflux activity not only protects against antibiotics but also promotes resistance evolution by maintaining prolonged sub-inhibitory drug exposure.

Given their broad substrate specificity and critical role in antimicrobial resistance, efflux pumps represent exciting emerging molecular targets for restoring antibiotics efficacy. A clear understanding of these systems is essential for the rational design of therapeutic strategies aimed at overcoming efflux-mediated resistance mechanisms.

3. Nanocarriers-targeting potential therapeutic pathways in MRSA

3.1. Nanocarriers targeting MRSA cell wall and cell membrane

Recent studies have explored nanocarriers designed to target MRSA cell wall and membrane or interfere with their structural integrity. Table 1 summarizes these studies, detailing the nanocarrier type, targeted cell envelope component, key characterizations and main findings. These studies are further organized into three mechanistic categories: (1) teichoic acid-targeting nanocarriers, (2) peptidoglycan-targeting nanocarriers, and (3) nanocarriers targeting cell membrane integrity.

3.1.1. Nanocarriers targeting teichoic acid

Several studies demonstrate nanomaterial-mediated targeting of teichoic acids to disrupt MRSA cell wall. Wang et al. [28] reported that graphene oxide exhibits 27-times adsorption affinity for teichoic acid via π-π interaction, leading to increased activity of autolysin atlA and consequent peptidoglycan hydrolysis and cell death, as shown in Figure 2. Similarly, Romero-Urbina et al. [29] found out that silver nanoparticles (AgNPs) bind to the polyanionic teichoic acid via electrostatic interactions, inducing destabilization of the peptidoglycan layer and subsequent pore formation, cytoplasmic leakage, and cell lysis. Moreover, Sadeghi et al. [30] showed that gold nanoparticles (AuNPs) conjugated with berberine electrostatically interact with teichoic acids, promoting membrane damage and deeper penetration of berberine into the bacterial cell wall and membrane. Moreover, Huang et al. [31] designed Zn^{2+} -gallic acid nanoflowers (ZGNFs) with selective bacterial adhesion mediated by strong interaction between quinone residues on the surface of ZGNF and amino groups on teichoic acids. The targeted binding to teichoic acids enhanced Zn^{2+} accumulation at the cell wall, which in turn perturbed metal-ion homeostasis and caused cell wall damage.

These studies collectively demonstrate the therapeutic potential of teichoic acid-targeting nanocarriers against MRSA. Future development could enhance efficacy by loading β-lactams or glycopeptides into this system to simultaneously achieve teichoic acid targeting and antibiotic-mediated killing of MRSA.

3.1.2. Nanocarriers targeting peptidoglycan layer

Peptidoglycan, a structural component of MRSA's cell wall [32], is a promising therapeutic target. Recent studies demonstrate that nanocarriers designed to either structurally mimic peptidoglycan or inhibit its biosynthetic enzymes exhibit anti-MRSA activity. This subsection reviews innovative approaches targeting the MRSA peptidoglycan layer through nanomaterial-based strategies.

Yang et al. [33] developed aminosaccharide-functionalized AuNPs to mimic N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) in peptidoglycan layer. Microscopy analysis revealed that D-glucosamine (GluN) conjugate (Au-GluN) damaged & collapsed cell wall by disrupting the connection between C-1 and C-4 of hydroxyls of NAG and NAM. This approach highlights the utility of structural mimicry in design of cell wall-targeted nanocarriers. Similarly, Kalita et al. [34] designed a dual-action lysozyme-capped Au nanoclusters with ampicillin to target MRSA. The lysozyme hydrolyzed β (1 → 4) glycosidic bonds in the peptidoglycan, while enhancing ampicillin accumulation, simultaneously compromising membrane integrity and cell wall biosynthesis. This approach highlights the potential of enzymatic-antibiotic synergy in peptidoglycan-targeted anti-MRSA therapies.

Targeting peptidoglycan and inducing membrane depolarization, Guo et al. [35] developed a phototheranostic nanoplatform, Van-OA@PPy, which combines vancomycin (Van), oleic acid (OA) and polypyrrole (PPy). They exploited vancomycin's high affinity for the D-Ala-D-Ala in MRSA peptidoglycan precursors, enabling specific adhesion to the bacterial cell wall, and the polypyrrole's efficient photothermal conversion under near-infrared (NIR) irradiation. This dual-function system achieved selective MRSA targeting through vancomycin-peptidoglycan interactions while inducing membrane/cell wall disruption via NIR-triggered hyperthermia, as evidenced by marked membrane depolarization and ultrastructural damage. We believe that harnessing a cell wall-active antibiotic as both a targeting ligand and a payload within a responsive nanocarrier offers a promising strategy for targeting MRSA.

3.1.3. Nanocarriers targeting cell wall and membrane integrity

These nanocarriers disrupt bacterial membranes and wall integrity through electrostatic interaction, physical injury, membrane depolarization and oxidative stress.

Leveraging electrostatic interactions, Liu et al. [36] developed quaternary ammonium chitosan-functionalized mesoporous silica nanoparticles (MSN-NH_2-CFP@HACC) that exploit electrostatic interactions to target MRSA membranes, enhancing intracellular cefoperazone delivery while disrupting membrane integrity and suppressing β-lactamase activity. The *in vitro* and *in vivo* results demonstrated effective bacterial capture, inhibition of β-lactamase activity, and significant clearance of intracellular MRSA. Similarly, Qiu et al. [37] developed cationic poly(lactic-co-glycolic acid) (PLGA) nanoparticles that adhered electrostatically to MRSA, inhibiting biofilm formation, degrading mature biofilms, and inducing membrane damage. Both studies demonstrate the utility of charge-driven nanocarriers for MRSA targeting and eradication.

Oxidative stress is another potent strategy for MRSA cell wall disruption, as demonstrated by several nanomaterial systems. For instance, Hamida et al. [38] and Ali et al. [39] reported that AgNPs induce structural damage through ROS-mediated and physical mechanisms, respectively, causing wall disintegration and membrane rupture. Similarly, Ye et al. [40] and Zhang et al. [41] fabricated copper-containing ferrite ($Cu@Fe_3O_4$) nanoparticles and conjugated polypeptides-AuNPs (Au@PNPs), respectively, which generated oxidative stress leading to membrane shrinkage, wall deformation, and cytoplasmic leakage. These studies collectively establish

Table 1. Summary of nanocarriers targeting MRSA cell wall/membrane components.

Nanocarrier type	Target	Key evaluations and characterization	Targeted disease/model	Main findings	Ref.
Graphene oxide (GO) NPs	Teichoic acid	• Adsorption experiment on GO interaction with teichoic acid and peptidoglycan • SEM and TEM analysis of bacterial cells morphology after GO exposure • Cell wall degradation fluorescence study	MRSA infection/*in vitro*	• GO had 27X adsorption to teichoic acid than peptidoglycan via π-π interaction. • 99% mortality in Gram-positive vs 25% in Gram-negative • Addition of exogenous teichoic acid reduced anti-MRSA activity by 4–5-fold	[28]
Silver nanoparticles (AgNPs)	Teichoic acid	• Particle size: 5 nm- 33 nm • Electron microscopy analysis of bacterial cells morphology after AgNPs exposure	MRSA infection/*in vitro*	• Imaging shows AgNPs densely around teichoic acid • Thinning and permeabilization of the cell wall (40 nm in untreated vs 32 nm in treated MRSA cells) • AgNPs and cell wall interaction caused membrane disruption, peptidoglycan damage and cell leakage	[29]
Gold nanoparticles (AuNPs) conjugated with berberine (BER)	Teichoic acid	• Particle size: 49.38 nm • ROS and live/dead staining assays	MRSA-infected wounds/*in vivo*	• Binding to teichoic acid and cell membrane damage • AuNPs-BER had lower MIC against MRSA (109.5 μg/ml) compared to free BER (165 μg/ml). • AuNPs-BER and free BER had MRSA survival rate of 2.7% and 26%, respectively.	[30]
Zn2±gallic acid nanoflowers (ZGNFs)	Teichoic acid	• High resolution TEM (HR-TEM) imaging • Confocal laser scanning microscopy	MRSA-induced keratitis/*in vivo*	• ZGNFs induced aggregation of MRSA bacteria unlike in Gram – bacteria. • ZGNFs didn't interact with mammalian cells. • TEM images showed intact peptidoglycan structure. • TEM showed amorphous teichoic acid morphology with indistinct border	[31]
Aminosaccharide-functionalized gold nanoparticles (AuNPs)	Peptidoglycan	• Particle size: approximately 7 nm • Live/dead staining assays • TEM and SEM imaging	MRSA infection/*in vitro*	• AuNPs and D-glucosamine (GluN) exhibited synergistic antibacterial activity. • Increased permeability of Au_GluN treated MRSA • Au_GluN damaged & collapsed cell wall.	[33]
Lysozyme-gold nanoclusters-ampicillin (AUNC-L-Amp)	Peptidoglycan	• Bacteria morphological characterization • Membrane permeability assay of MRSA persister	MRSA-infected diabetic wounds/*in vivo*	• 50–89% fold increase in antibacterial activity of AUNC-L-Amp compared to Free-Amp • AUNC-L-Amp reverted the MRSA resistance toward ampicillin • AUNC-L-Amp inhibited MRSA persister cells	[34]
Phototheranostic nanoparticles (Van-OA@PPy + NIR)	Peptidoglycan + Membrane integrity	• Bacterial membrane potential monitoring • Bacteria morphological characterization	MRSA-induced subcutaneous abscess/*in vivo*	• Van-OA@PPy + NIR had lowest red/green fluorescence ratio indicating significant membrane damage via photothermal effect. • Van-OA@PPy + NIR caused sunken/disrupted cell membranes	[35]
Chitosan-modified mesoporous silica NPs (MSN-NH2-CFP@HACC)	Membrane integrity	• Bacteria morphological characterization • Bacterial targeting and capture assay • β-Lactamase activity assay • Live/dead bacterial fluorescent staining	MRSA-induced bacteraemia/*in vivo*	• Significant bacteria aggregation over time as observed in SEM • MSN-NH2@HACC reduced β-lactamase activity by 50%.	[36]
Cationic poly (D, L-lactide-co-glycolide) NPs (CNPs)	Membrane integrity	• Bacteria morphological characterization • Measurement of interaction force by atomic force microscopy • Biofilm eradication assay	MRSA infection/*in vitro*	• CNPs adsorbed on MRSA cells • MRSA cells had scattered cell mass • CNPs inhibition of biofilm formation was concentration dependent.	[37]
Silver nanoparticles (D-SNPs)	Membrane integrity	TEM imaging	MRSA infection/*in vitro*	• TEM micrographs showed intact untreated MRSA cell wall. • D-SNPs caused MRSA shape deformation, damage to peptidoglycan layers, disruption of the cell wall, pore formation and cell lysis.	[38]
Silver nanoparticles (AP-AgNPs)	Membrane integrity	• SEM and TEM imaging • Membrane permeability & integrity tests • Biofilm inhibition assay	MRSA-induced superficial skin infection/*in vivo*	• AP-AgNPs caused MRSA membrane permeability and significant cell damage with content leakage. • 80% clearance of mature MRSA biofilms.	[39]

(*Continued*)

Table 1. (Continued).

Nanocarrier type	Target	Key evaluations and characterization	Targeted disease/model	Main findings	Ref.
Copper-containing ferrite nanoparticles (Cu@Fe NPs)	Membrane integrity	• TEM and confocal imaging • Membrane permeability test • Biofilm inhibition assay	MRSA-induced non-lethal systemic and localized infections/*in vivo*	• Cu@Fe NPs caused MRSA bacteria adhesion and membrane permeability content leakage. • Excellent anti-MRSA activity with MIC of 1 µg/ml	[40]
Polypeptide-gold nanoparticles (Au@P NPs)	Membrane integrity	• Membrane depolarization assay • Intracellular ROS assay • SEM and TEM imaging	MRSA infection/*in vitro*	• Mild-membrane depolarization • Au@P NPs disrupted MRSA membrane via ROS production	[41]
Daptomycin-loaded liposomes	Membrane integrity	• Flow cytometry measurement of interaction of MRSA with liposomes	MRSA-induced pneumonia/*in vivo*	• Enhanced binding and specificity toward MRSA bacterial cells	[42]

Abbreviations: GO, graphene oxide; NPs, nanoparticles; AgNPs, silver nanoparticles; AuNPs, gold nanoparticles; Zn^{2+}, zinc ions; ZGNFs, Zn^{2+} -gallic acid nanoflowers; BER, berberine; ROS, reactive oxygen species; MIC, minimum inhibitory concentration; TEM, transmission electron microscopy; SEM, scanning electron microscopy; HR-TEM, high-resolution transmission electron microscopy; AFM, atomic force microscopy; NIR, near-infrared irradiation; PPy, polypyrrole; OA, oleic acid; MSN, mesoporous silica nanoparticles; HACC, hydroxypropyltrimethyl ammonium chitosan chloride; CFP, cefoperazone; CNPs, cationic poly(D,L-lactide-co-glycolide) nanoparticles; D-SNPs, dendrimer-stabilized silver nanoparticles; AP-AgNPs, antimicrobial peptide – functionalized silver nanoparticles.

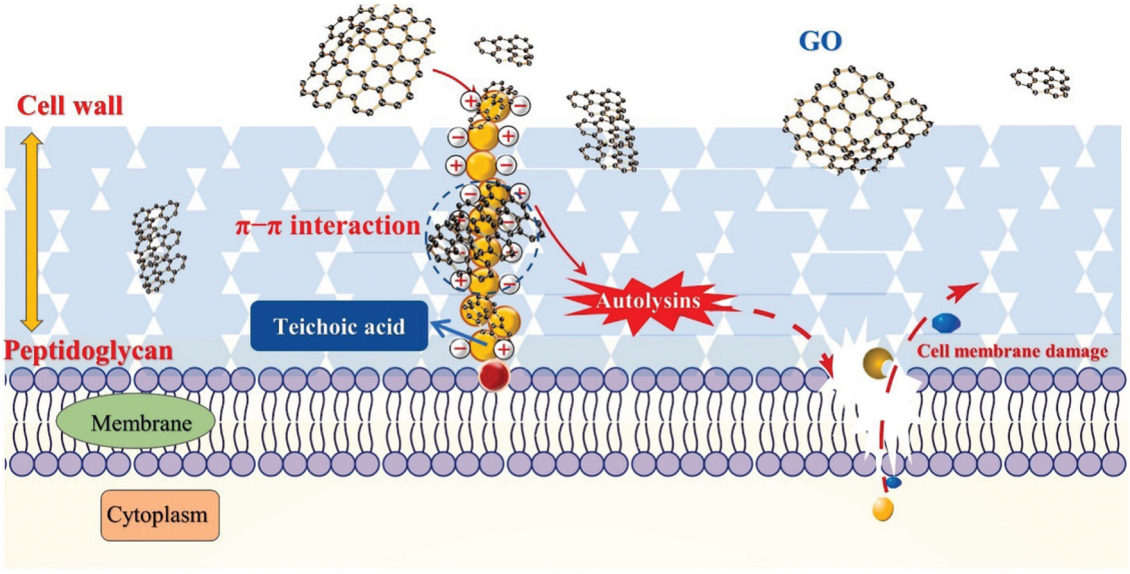

Figure 2. Interaction of graphene oxide (GO) with MRSA cell wall components. GO preferentially adsorbs to teichoic acids via π–π interactions, enhancing autolysin activity and promoting peptidoglycan hydrolysis, which ultimately leads to cell wall degradation. Adopted with permission from [28].

Abbreviations: GO, graphene oxide; MRSA, methicillin-resistant *Staphylococcus aureus*.

oxidative nanomaterials as effective agents against MRSA through combined physical and redox-mediated damage.

Membrane-binding and depolarization is also a potential strategy for MRSA cell wall disruption. Jiang et al. [42] exploited daptomycin's inherent membrane-binding and depolarization activity to fabricate a liposomal daptomycin delivery system with dual function as an antibiotic and a targeting ligand against MRSA pneumonia. The partial conjugation of daptomycin onto the liposome surface enhanced binding to and disruption of MRSA membrane, while encapsulation of additional daptomycin led to increased lung accumulation, superior bacterial clearance, and improved survival in a mouse model compared to conventional PEGylated liposomal daptomycin.

In summary, teichoic acids, peptidoglycan, and membrane disruption represent viable targets for anti-MRSA nanotherapeutics. Current evidence supports the development of nanocarriers capable of teichoic acid disruption, peptidoglycan synthesis inhibition, physical membrane destabilization, and synergistic antibiotic delivery. Further innovation should focus on engineering multifunctional nanocarriers that simultaneously target teichoic acids, inhibit peptidoglycan synthesis, destabilize the cell wall/membrane and deliver antibiotics to fully combat MRSA resistant mechanisms.

3.2. Nanocarriers targeting quorum sensing and biofilm

Various nanocarriers, including metallic, mesoporous silica, lipid-based, polymeric, and hybrid nano-constructs as well as hydrogels, have been investigated for their ability to disrupt QS and eradicate MRSA-associated biofilms. Table 2 summarizes these reported nanosystems, detailing carrier types, encapsulated agents, QS-interference mechanisms, and corresponding biofilm eradication efficiencies. While metallic and

Table 2. Summary of the reported nanocarriers designed to target QS and biofilms associated with MRSA infections.

Type of NPs	Biofilm inhibition mechanism	Drug loaded	Biofilm inhibition	Targeted disease/model	Ref.
Metallic NPs	• Targeting *agrA*, *sea*, and *seb* QS-quenching-induced genes	AgNPs	• NA. Only gene expression that leads to QS quenching was investigated	MRSA and MSSA infection/*in vitro*	[44]
Hybrid metallic NPs	• Inhibit *icaA* and *icaD* genes • Improved penetration through EPS.	AgNP s- oxacillin	• Complete eradication of biofilm	MRSA infection/*in vitro*	[45]
Hybrid metallic NPs	• Downregulation of *agrA*, *agrB*, *RANI111*, and *hla* genes	AgNPs	• Complete eradication of biofilm at 4 ug/ml	*Staphylococcus aureus* infection/*in vitro*	[46]
Hybrid metal NPs	• Upregulation of *agrA* and *icaR* genes • Downregulation of *crtM*, *sigB*, *sarA*, and *fnbA* genes	Ag-Metformine-NPs	• The system showed significant ability to inhibit biofilm formation, reaching 86% for METF-NPS and Ag-METF-NPs • No mature biofilm activity	*Staphylococcus aureus* and MRSA infection/*in vivo*	[47]
Hybrid metal NPs (loaded in liposome)	• Inhibition of EPS and production of pyocyanin • Increased ROS production	Lipopeptide and CuNPs	• The nanocarrier eradicated 82% of mature biofilm	MRSA and *Pseudomonas aeruginosa* biofilm/*in vivo*	[50]
Hybrid metallic NPs	• Improved biofilm penetration • Increased ROS production	• Copper oxide	• Significant reduction in mature biofilm reaching 83% under light	MRSA-induced abscess/*in vivo*	[51]
Metallic NPs	• Exhibition of OXD-like and GSH-Px-like enzyme activities. Promotion of ROS generation and induction of MRSA cuproptosis-like death	Cu-gallic acid-vancomycin (CuGA-VAN)	• Significant elimination of MRSA biofilm in a concentration-dependent manner	MRSA-induced wounds/*in vivo*	[52]
Hybrid metallic NPs	• Enhanced bacterial cuproptosis-like death • Increased production of ROS	Cu_2O-BSO	• 80% eradication of mature biofilm and inhibition of biofilm formation.	MRSA-induced pneumonia/*in vivo*	[48]
Metallic NPs (Mesoporous copper sulfide nanocage).	• Downregulation of all QS-quenching-induced genes • Increased the release of ROS through cuproptosis	Luteolin, mitoquinone	• Significant eradication of mature biofilm	Pneumonia/*in vivo*	[53]
Hybrid metal nanocomposite	• Enhanced penetration through the EPS • Downregulation of *ica* gene	Peptide LL37	• 81% biofilm formation inhibition	Bacterial infection/*in vitro*	[54]
Hybrid metallic NPs	• Decreased *agrA* expression	Zn NPs	• No biofilm study	MRSA and *Pseudomonas aeruginosa* infections/*in vitro*	[55]
Hybrid metallic NPs	• Exhibition of POD-like, GSH-Px-like, and CAT-like activities • Increased production of ROS • Disruption of MRSA metabolism	ZnO-CuS nanoflower	• Significant thinning and reduction in biofilm mass	Biofilm infection model of implants in mice/*in vivo*	[56]
Metallic NPs (Mesoporous silica NPs)	• Downregulation of all virulence genes, including *agr*, *clfA*, *pvl*, *tst*, *hla*, and *icaA*	Berberine	• 99.97% biofilm eradication	MRSA biofilm/*in vivo*	[57]
Metallic NPs (Graphene oxide (GO))	• Attenuation of *agr* level	Berberine and PBP2a aptamer for targeting agr	• 92.8% biofilm inhibition	MRSA biofilm/*in vitro*	[58]
Hybrid metal NPs	• Downregulation of all QS-quenching-related genes including *agr*, *spa*, *hla*, *icaA*, *icaD*, *clfa*, and *clfb* • Increased ROS production	Au NPs	• Eradication of 90% of mature biofilm • Significant reduction in the formation of biofilm, especially under NIR light.	MRSA-induced skin infection/*in vivo*	[43]
Hybrid metal NPs	• Downregulation of *icaA* and *cap8O* related to QS-quenching • Inhibition of EPS formation	CSIL for photothermal therapy (NIR photosensitizer) Indocyanine and lysostaphin (enzyme targeting MRSA cell wall)	• Significant eradication of biofilm, reaching 99.7% by improving penetration and inhibition of QS	Diabetic MRSA-infected wound/*in vivo*	[59]

(*Continued*)

Table 2. (Continued).

Type of NPs	Biofilm inhibition mechanism	Drug loaded	Biofilm inhibition	Targeted disease/model	Ref.
Hybrid Metallic NPs (MOFs)	• Inhibition of PIA and downregulation of *ica* gene • Downregulation of fibronectin-binding protein • Increased production of ROS	(1) CaO2	• 70% biofilm eradication by ROS and under microwave	Osteomyelitis/*in vivo*	[60]
Hybrid lipid polymer NPs	• Improved biofilm penetration by mimicking the arginine-rich fragments in viral protein transduction domains	Levofloxacin	• Eradication of more than 81% of mature biofilm	Wound healing/*in vivo*	[66]
Polymeric NPs	• Positive charge-induced biofilm penetration and destruction	Rutin	• Inhibition of 22.5–37.5% of biofilm formation at ½ MIC	*Staphylococcus aureus* infection/*in vitro*	[67]
Niosome	• Downregulation of *icaR* gene • Improved biofilm penetration	Vancomycin	• 2–8-fold reduction in biofilm formation compared to bare vancomycin in all tested strains	MRSA biofilm eradication/ *in vitro*	[68]
Polymeric NPs	• Improved biofilm penetration	Composed of F-127 surfactant, tannic acid (TA), and biguanide-based polymetformin	• 1.8 $\log_{10}$ reduction in bacterial CFUs following treatment with the nanosystem compared to VCM *in vivo* • Mature biofilm eradication and biofilm formation revealed the significant superiority of the system	MRSA-induced excisional wound/*in vivo*	[69]
Polymeric NPs	• Improved biofilm penetration	Linezolid	• The nanocarrier results in 80% mature biofilm eradication compared to free drug, which showed 35% eradication at 2x concentration of the nanocarrier.	MRSA infection/*in vitro*	[70]
Polymeric NPs	Downregulation of *agrA*, *agrB*, and *agrC* genes	Histidine Kinase inhibitor peptide	• Inhibition of 60% of biofilm formation in MRSA and 72% in MSSA	MRSA infection/*in vitro*	[71]
Nano emulsion	• Downregulation of *crtM*, *sigB*, *sarA*, and *fnbA* genes	NA	• Inhibition of 77.88–94.52% of biofilm formation	*Staphylococcus aureus* and *Pseudomonas aeruginosa* infection/*in vivo*	[72]
polymeric NPs	• Downregulation of *agrB*, *agrC*, *agrD*, and *agrA* and *hld* genes • Promotion of ROS production	Glabrol, streptomycin	• NA	Intracellular bacteria/*in vivo*	[73]
Hydrogel	• Inhibition of *agrB*, *agrC*, *agrD*, and *agrA* genes expression • inhibition of coagulase (*coa*), *Staphylococcal* protein A (*spa*), Panton-Valentine Leukocidin (*pvl*), α-toxin (*hla*), and serine protease (*ssp*).	Hyper-branched poly-L-lysine (HBPL) as QS quencher	• Inhibition of 90% of biofilm formation • Destruction of 90% of mature biofilm	Wound healing and infection/*in vivo*	[75]
Hydrogel	• Downregulation of *agrA*, *agrC* and *hlg* genes. • interference with bacterial metabolism	Pravastatin (induces NO production)	• NA	Diabetic wound healing/ *in vivo*	[76]

Abbreviations: NPs, nanoparticles; QS, quorum sensing; MRSA, *Methicillin-resistant Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; EPS, extracellular polymeric substances; ROS, reactive oxygen species; AgNPs, silver nanoparticles; CuNPs, copper nanoparticles; Zn NPs, zinc nanoparticles; Au NPs, gold nanoparticles; GO, graphene oxide; MOFs, metal – organic frameworks; PIA, polysaccharide intercellular adhesin; NIR, near-infrared; POD-like, peroxidase-like activity; GSH-Px-like, glutathione peroxidase–-like activity; CAT-like, catalase-like activity; MIC, minimum inhibitory concentration; NA, not assessed; VCM, vancomycin; EPS, extracellular polymeric substance; PBP2a, penicillin-binding protein 2a.

hybrid metallic nanoparticles dominate current research, polymeric, lipid-based, hydrogel, and hybrid systems are also emerging as promising alternatives. These trends underscore opportunities to diversify nanocarrier platforms to improve MRSA targeting and combat bacterial resistance.

3.2.1. Metallic and hybrid metallic nanoparticles

Metal nanoparticles have shown significant potential in eradicating bacterial infections and associated biofilms by damaging cell integrity, generating reactive oxygen species (ROS), and inhibiting DNA replication [43]. Various metals have been used to construct metallic and hybrid metallic nanoparticles, including Ag, Cu, Zn, graphene oxide (GO), silica (Si), and Au. Notably, four studies have utilized AgNPs, either alone, in combination with oxacillin to enhance antibacterial activity, with metformin to improve QS inhibition, or encapsulated within chitosan nanoparticles to increase biofilm penetration [44–47]. All demonstrated significant QS inhibition with complete eradication of mature biofilms, except the study by Baei et al., which did not assess biofilm eradication [44].

In contrast to Ag, Cu is a trace element known for its good biocompatibility and potent antibacterial activity, primarily through the induction of cuproptosis-like death in bacteria [48,49]. Several Cu-based hybrid nanoparticles have been designed to inhibit QS and combat biofilms. For example, Kannan et al. co-loaded CuNPs with lipopeptides, which inhibited EPS production and virulence factors in MRSA, while enhancing ROS generation for biofilm eradication [50]. To address CuNPs' cytotoxicity toward normal cells, Chen et al. encapsulated ultra-small CuNPs in pH-responsive liposomes, enabling targeted release at infection sites and minimizing off-target effects [51]. Interestingly, Hu and colleagues reported chemically stable Cu-gallic acid-vancomycin (CuGA-VAN) nanoneedles that exhibited dual oxidase-like (OXD-like) and glutathione peroxidase – like (GSH-Px-like) enzyme activities, promoting ROS generation and inducing MRSA cuproptosis-like death. This strategy resulted in rapid disruption of MRSA biofilms and concurrently enhanced wound repair [52]. More recently, Hu et al. amplified cuproptosis by combining copper oxide with buthionine sulfoximine, a GSH synthesis inhibitor, which depleted GSH levels and improved QS inhibition compared with earlier approaches [48]. Similarly, Zhang et al. developed a biomimetic Cu-S nanocage system co-loaded with luteolin and mitoquinone as QS quenchers, coated with a macrophage cell wall for selective targeting of bacteria. This design not only improved cuproptosis activity but also offered protection to normal cells through selective targeting [53].

ZnO nanoparticles have also demonstrated notable antibacterial effects. Rashki et al. incorporated ZnO into a nanocomposite formulation to deliver an antimicrobial peptide, achieving significant biofilm inhibition, up to 81%, QS suppression, and enhanced biofilm penetration attributed to the positive charge of chitosan [54]. In comparison, Alidoust et al. employed rutin-coated ZnO nanoparticles to enhance bioavailability, resulting in potent QS inhibition against both MRSA and *Pseudomonas aeruginosa* [55].

Beyond monometallic platforms, bimetallic nanozymes have been developed to enhance redox-mediated QS disruption in resilient biofilms. Sun et al. developed ZnO-CuS bimetallic nanozyme nanoflowers exhibiting peroxidase-, catalase-, and glutathione peroxidase – like activities, which promoted sustained ROS generation and intracellular GSH depletion in MRSA. As illustrated in Figure 3, this redox imbalance disrupted bacterial metabolic pathways and biofilm integrity, thereby enhancing QS inhibition and reinforcing cuproptosis-like antibacterial effects while maintaining favorable biosafety [56].

Various other metallic and inorganic nanomaterials, such as GO and Si, have been employed as drug carriers through adsorption mechanisms. For instance, GO was used for the co-delivery of DNA aptamers and berberine, while Si nanoparticles were employed for berberine delivery alone. Both nanosystems demonstrated strong anti-biofilm efficacy and complete biofilm eradication capabilities [57,58].

Given ongoing concerns over the safety and cytotoxicity of metal nanoparticles, recent studies have integrated external stimuli, such as phototherapy, NIR irradiation, and microwave irradiation, to boost antibacterial efficacy, lower nanoparticle dosage, and improve biocompatibility. Ye et al. developed a hybrid metal nanoparticle via gelation of epigallocatechin gallate (EGCG) with AuNPs. Under NIR irradiation, this system exhibited substantial biofilm prevention and eradication capabilities [43]. Similarly, Deng et al. engineered Si-based nanomotor co-loaded with indocyanine green (ICG) and lysostaphin. This nanomotor demonstrated near-complete MRSA biofilm eradication both *in vitro* and *in vivo*, while effectively suppressing virulence gene expression [59]. Recently, Cheng et al. introduced a manganese-doped porphyrin metal-organic framework loaded with calcium peroxide. Upon microwave activation, this platform generated ROS, leading to approximately 70% biofilm eradication via inhibition of the two-component signaling system associated with QS [60].

Taken together, metal and hybrid metal nanoparticles exhibit considerable potential in QS inhibition and biofilm eradication. Therefore, we believe that incorporating stimuli-responsive and biomimetic design strategies alongside these nanoparticles is expected to further enhance targeted delivery, improve therapeutic outcomes, and minimize off-target toxicity.

3.2.2. Polymeric, lipid, and hybrid lipid-polymer nanocarriers

Owing to their excellent biocompatibility, high drug-loading capacity, and versatility in encapsulating both hydrophobic and hydrophilic drugs with controlled release profiles, polymeric nanoparticles, lipid-based carriers, and hybrid polymer-lipid systems have been widely employed to combat MRSA biofilms [61–65]. These nanocarriers primarily function by enhancing penetration through the EPS. For example, Li et al. developed polymeric nanoparticles from polymetformin and tannic acid, Esnaashari et al. encapsulated rutin within chitosan nanoparticles, Hemmati et al. formulated vancomycin-loaded niosomes, and Cai et al. designed a virus-inspired hybrid polymer – lipid nanoparticle coated with hyaluronic acid [66–69]. All these systems achieved effective EPS penetration, resulting in significant biofilm eradication.

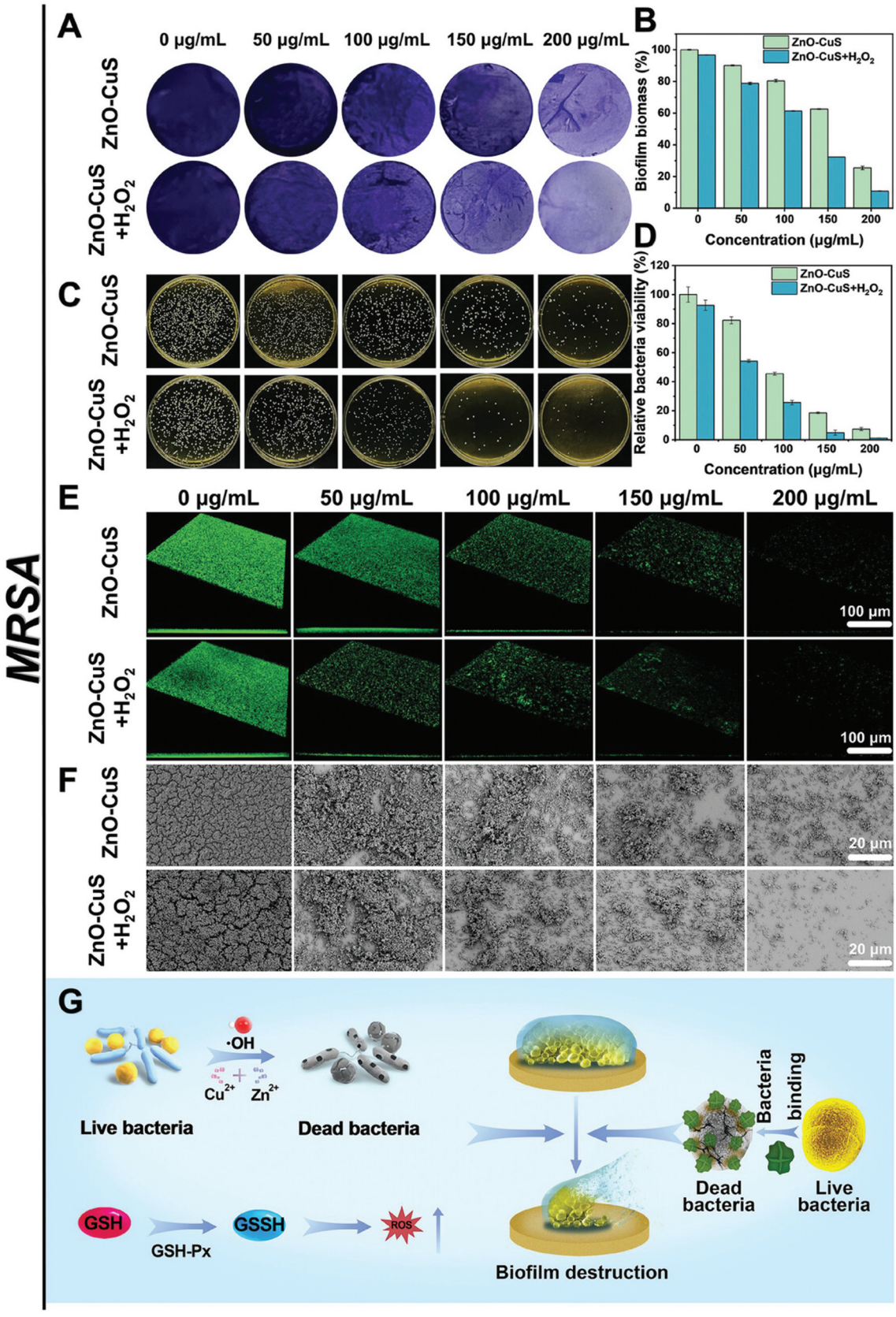

Figure 3. Biofilm-scavenging activity of ZnO-CuS nanoflowers against MRSA. (A) Crystal violet – stained images of MRSA biofilms following different treatments. (B) Quantification of MRSA biofilm biomass after the indicated treatments ($n = 3$). (C) Representative photographs of the spread plate method (SPM) of MRSA biofilms after different treatments. (D) Corresponding colony-forming unit (CFU) counts obtained from the SPM assay ($n = 3$). (E) Representative three-dimensional reconstructed images of MRSA biofilms stained with the NO1 probe following different treatments (scale bar = 100 μm). (F) Representative SEM images of MRSA biofilms after incubation under different treatment conditions (scale bar = 20 μm). (G) Proposed mechanism underlying the efficient in vitro antibiofilm activity of ZnO-CuS nanoflowers. Adapted with permission from [60].

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; CFU, colony-forming unit; SEM, scanning electron microscopy; SPM, spread plate method.

Furthermore, Li et al. engineered a pH-responsive poly(β-amino ester)-methoxy poly(ethylene glycol) nanocarrier, further coated with hyaluronic acid to confer enzyme responsiveness for targeted linezolid delivery. This system eradicated up to 80% of mature biofilms at the infection site [70].

A second strategy combines EPS penetration with QS inhibition, an approach effective for both biofilm prevention and eradication. For instance, Alenazi et al. employed Poly(amidoamine) (PAMAM) dendrimers to deliver a histidine kinase inhibitor peptide, Mosallam et al. developed a nanoemulsion-based prodrug derived from ascorbic acid, and Wang et al. co-loaded hyaluronic acid with a glabrol-streptomycin combination [71–73]. All these formulations significantly downregulated the *agr* system, a key regulator of MRSA biofilm formation.

The integration of QS inhibition with enhanced EPS penetration offers a synergistic and promising strategy for MRSA biofilm control. Future research aimed at identifying novel, biocompatible nanomaterials with intrinsic QS-inhibitory properties could open new avenues for combating AMR, particularly in MRSA infections.

3.2.3. Hydrogel

MRSA infections in chronic wounds impede the healing process due to persistent microbial colonization and biofilm formation [74]. In such cases, the dual approach of inhibiting biofilm development and eradicating resident bacteria can markedly accelerate wound healing, particularly in diabetic patients. To date, only two studies have reported hydrogels specifically designed to inhibit QS while simultaneously eradicating biofilms. Both utilized hyperbranched poly-L-lysine (HBPL) as the QS inhibitor. Lu et al. incorporated HBPL directly into the hydrogel matrix [75], whereas Tu et al. modified HBPL with manganese dioxide and crosslinked it with poly (PEGMA-co-GMA-co-AAm) (PPGA), subsequently loading the formulation with pravastatin sodium as a nitric oxide (NO) inducer [76]. Both hydrogel systems demonstrated strong *in vitro* and *in vivo* efficacy in QS inhibition and biofilm eradication.

To date, HBPL is the only material that has been incorporated into hydrogels for the effective targeting of biofilms and QS. Therefore, the development of novel materials with QS inhibitory properties and stimuli-responsive behavior holds significant promise for enhancing wound healing, particularly in vulnerable populations such as diabetic patients.

In summary, nanocarriers, including metallic, hybrid metallic, polymeric, lipid-based, hybrid lipid – polymer, and hydrogel systems, show strong potential to eradicate MRSA biofilms via QS inhibition, EPS penetration, and targeted antimicrobial delivery. Despite promising preclinical results, challenges remain in toxicity control, biofilm heterogeneity, and clinical translation. Future progress hinges on multifunctional, biocompatible designs with intrinsic QS-modulating activity, infection-responsive release, and proven efficacy in relevant MRSA infection models.

3.3. Nanocarriers acting as MRSA toxin decoys

MRSA produces a diverse range of toxins that play a central role in severe diseases such as sepsis and pneumonia. These toxins target host cells, including immune cells, platelets, and red blood cells (RBCs), causing immune evasion, hemolysis, and impaired platelet function [77]. This pathogenic role has inspired the development of nanocarriers designed to function as 'toxin decoys,' neutralizing toxins before they damage host tissues [78]. Table 3 summarizes studies on such nanosystems according to the coating strategy employed, including RBC-based, platelet-based, and other innovative approaches. Notably, most reported designs employ RBC membrane coatings, highlighting a gap in alternative strategies such as platelet membrane coatings.

3.3.1. RBC membrane-coated nanosystems

3.3.1.1. Conventional non-responsive nanosystems. RBC membrane-coated nanoparticles have emerged as promising MRSA toxin decoys, with PLGA nanoparticles representing the most widely investigated core material. Multiple studies have demonstrated their efficacy in neutralizing MRSA toxins and protecting host cells. Chen et al. developed biomimetic nanosponges comprising PLGA nanoparticles coated with human (hNS) [79] and mouse RBC membranes (RBC-NS) [77]. Both formulations significantly reduced toxin-induced cytotoxicity and hemolysis *in vitro* and protected mice from toxin-induced lethality. While the *in vivo* efficacy correlates directly with the neutralization capacity of the nanosponges, we believe that evaluation in MRSA infection models is warranted to better capture the complexity of MRSA-host interactions beyond toxin injection alone. Notably, the use of human cell-derived membranes in hNS may further enhance biocompatibility and reduce immunogenicity, supporting its potential for clinical application. Coburn et al. extended this concept to ocular infections, coating PLGA nanoparticles with rabbit or human RBC membranes. Pretreatment of bacterial supernatants with these nanosponges reduced hemolytic activity, and in combination with gatifloxacin, they lowered intraocular bacterial burden and preserved retinal function in a murine endophthalmitis model [80]. Similarly, Wu et al. incorporated the antibiotic tedizolid phosphate into RBC membrane-coated PLGA nanoparticles (RPTR-701Ns), which enhanced exotoxin adsorption, reduced RBC damage, accelerated wound healing, and achieved bacterial clearance in MRSA-infected wounds [81]. Overall, these studies demonstrate the potential of PLGA-based RBC nanosponges in neutralizing MRSA toxin, while simultaneously opening avenues to explore other polymeric cores for similar biomimetic designs.

Inorganic nanoparticles have also been adapted as RBC membrane-coated MRSA toxin decoys. Two studies have been reported, the first describing RBC membrane-coated Fe_3O_4 nanoparticles (RBC@Fe_3O_4) combining MRSA toxin capture with NIR photothermal antibacterial effects [82]. RBC@Fe_3O_4 completely inhibited toxin-induced hemolysis *in vitro* and reduced toxin-induced lethality, improving survival in a murine MRSA wound infection model. Notably, the system could be recycled without loss of bactericidal activity, offering an economic advantage. In the second study, Shi et al. developed biomimetic RBC membrane-enveloped molybdenum disulfide nanodots (EM@MoS_2) for implant-associated antibiofilm therapy, integrating hyperthermia/ROS generation, antivirulence activity, and immunomodulation. *In vitro* and *in vivo* evaluations demonstrated

Table 3. Summary of nanosystems reported as toxin decoys against MRSA toxins.

Nanosystem	Targeted disease/ model	Strategy	Key findings	Ref.
Human RBCs-coated PLGA NPs (hNS)	MRSA systemic infection/ *in vivo*	• Toxin neutralization: – Inhibits hemolysis – Prevents cytotoxicity	• Complete inhibition of MRSA toxin-induced hemolysis in a concentration-dependent manner. • hNS-absorbed toxins exhibited no cytotoxicity in human umbilical vein endothelial cells (HUVECs) and caused no lethality in mice, indicating effective toxin neutralization.	[79]
Mouse RBC membrane-coated PLGA NPs (RBC-NS)	MRSA systemic infection/ *in vivo*	• Toxin neutralization: – Inhibits hemolysis – Prevents cytotoxicity	• Significantly inhibited MRSA whole secreted proteins (wSP)-induced hemolysis and cytotoxicity. • Protected mice against wSP-induced lethality.	[77]
Rabbit and human RBC membrane-coated PLGA NPs	MRSA intraocular infections/ *in vivo*	• Toxin neutralization: – Inhibits hemolysis – Prevents retinal damage	• Reduced hemolytic activity of bacterial sterile culture supernatants *in vitro.* • Combination with gatifloxacin significantly reduced intraocular bacterial load and retinal function loss in a murine sterile endophthalmitis model compared to gatifloxacin alone or the nanosystem alone.	[80]
SD rats RBC membrane-coated Tedizolid Phosphate-loaded PLGA NPs	MRSA wound infection/ *in vivo*	• Immune escape • Toxin neutralization: – Inhibits hemolysis	• Reduced MRSA exotoxin – induced RBC damage by 17.13%. • Enhanced wound healing and bacterial elimination in MRSA-infected wounds in mice.	[81]
RBC membrane-coated Fe_3O_4 nanoparticles (RBC@Fe3O4))	MRSA systemic infection/ *in vivo*	• Toxin neutralization. • Photothermal therapy.	• Completely absorbed MRSA toxins and inhibited hemolytic activity *in vitro*. • Significantly reduced lethality and improved survival rates in in a murine MRSA wound infection model. • Superior treatment efficacy upon combination with NIR photothermal therapy, compared to single treatment.	[82]
RBC membrane-enveloped molybdenum disulfide nanodots (EM@MoS2)	MRSA biofilm on implants/ *in vivo*	• Toxin neutralization. • Laser irradiation-induced hyperthermia/ROS generation. • Immunomodulatory.	• Demonstrated significant synergistic antibiofilm activity and sustained immunomodulatory effects. • Achieved strong antibiofilm efficacy in both *in vitro* and *in vivo* evaluations.	[83]
Toxin-responsive RBC membrane nanobubble loaded with perfluorocarbon nanoemulsion and a photosensitizer IR780.	Subcutaneous MRSA infection/ *in vivo*	• Toxin-induced release of dissolved oxygen and the photosensitizer IR780. • Photodynamic therapy (PDT): NIR irradiation.	• Bound to and neutralized MRSA toxin, protecting healthy cells from cytotoxicity. • Toxin-induced pore formation enabled rapid therapeutic cargo release at infection sites. • In MRSA-infected mice, NIR-assisted treatment accelerated lesion healing, reduced bacterial load, and alleviated inflammation.	[84]
Exotoxins-responsive RBC membrane-coated gelatin nanoparticles loaded with vancomycin and chlorogenic acid (VC-SGNPs-R)	MRSA leg and skin infections/ *in vivo*	• Immune escape. • Toxin neutralization. • On-demand drug delivery.	• Demonstrated effective *in vivo* inflammation healing, wound repair, and MRSA eradication in leg and skin infection models. • Enabled on-demand drug release triggered by MRSA exotoxins in the infection microenvironment.	[85]
RBC membrane-coated redox-responsive vancomycin-loaded nanogel (RBC-nanogel)	Intracellular MRSA infection/ *in vitro*	• Toxin neutralization. • Redox-responsive for on-demand drug release.	• Neutralized MRSA-associated toxins extracellularly, enhancing bacterial uptake by macrophages. • Exhibited redox-responsive intracellular drug release, achieving superior bacterial growth inhibition compared to free antibiotics and non-responsive nanogels.	[86]
A thermosensitive Pluronic F127 Hydrogel embedded with RBC membrane-coated PLGA nanoparticles.	MRSA subcutaneous infection/ *in vivo*	• Toxin neutralization. • Thermo-responsive sol-gel transition.	• Rapid sol-gel transition near body temperature. • Sustained drug release with excellent biocompatibility and biodegradability. • Preserved *in vitro* and *in vivo* (mouse subcutaneous infection) toxin neutralization functionality.	[87]
Human platelet membrane-coated PLGA NPs (PNPs)	MRSA systemic infection/ *in vivo*	• Toxin neutralization. – Enhances platelet antimicrobial activity. – Supports the innate immune function. – Modulates the hyperinflammation of sepsis.	• Inhibited *S. aureus* toxin-induced platelet damage, preserving platelet activation and bactericidal function. • Prevented macrophage damage, maintaining oxidative burst, nitric oxide production, and bactericidal activity. • Reduced bacterial load in blood and improved survival in a mouse MRSA systemic infection model.	[89]

(*Continued*)

Table 3. (Continued).

Nanosystem	Targeted disease/ model	Strategy	Key findings	Ref.
Mouse Platelet Membrane-Derived Nanodiscs (PLT-NDs)	MRSA systemic infection/ *in vivo*	• Toxin neutralization	• Effectively neutralized purified toxins and complex wSP *in vitro* and *in vivo*. • Significantly improved survival rates in a murine MRSA model in a dose-dependent manner.	[90]
Monoclonal antibody-engineered cell membrane nanovesicles (ANVs)	MRSA myositis/ *in vivo*	• Toxin neutralization. • Sonodynamic therapy/ROS generation.	• Achieved complete sono-immunotherapeutic (SIT) eradication of MRSA myositis in mice. • ANVs-mediated SIT demonstrated full elimination of MRSA infection under image guidance.	[91]
Liposomal oxygen nanobubbles (NB) and fat extract (FE) embedded in a F-127 hydrogel (G(NB/FE))	MRSA wound infection/ *in vivo*	• Toxin neutralization. • Toxin-regulated on-demand phototherapy (TROP). • Supports growth factors release to accelerate wound healing.	• Preserved NB's ability to absorb MRSA toxins in situ, reducing toxin-induced cytotoxicity. • Hydrogel showed strong retention at MRSA skin infection site in mice. • Enabled TROP, enhancing MRSA clearance. • Maintained low inflammatory factor levels, promoting wound healing.	[92]

Abbreviations: MRSA, *Methicillin-resistant Staphylococcus aureus*; NPs, nanoparticles; PLGA, poly(lactic-co-glycolic acid); RBCs, red blood cells; hNS, human RBC membrane – coated nanosponge; NS, nanosponge; wSP, whole secreted proteins; HUVECs, human umbilical vein endothelial cells; NIR, near-infrared; PDT, photodynamic therapy; ROS, reactive oxygen species; MoS_2, molybdenum disulfide; IR780, near-infrared photosensitizer dye; VC-SGNPs-R, vancomycin and chlorogenic acid – loaded gelatin nanoparticles coated with RBC membrane; PLT-NDs, platelet membrane-derived nanodiscs; ANVs, antibody-engineered nanovesicles; SIT, sono-immunotherapy; NB, nanobubbles; FE, fat extract; F127, Pluronic F127 hydrogel.

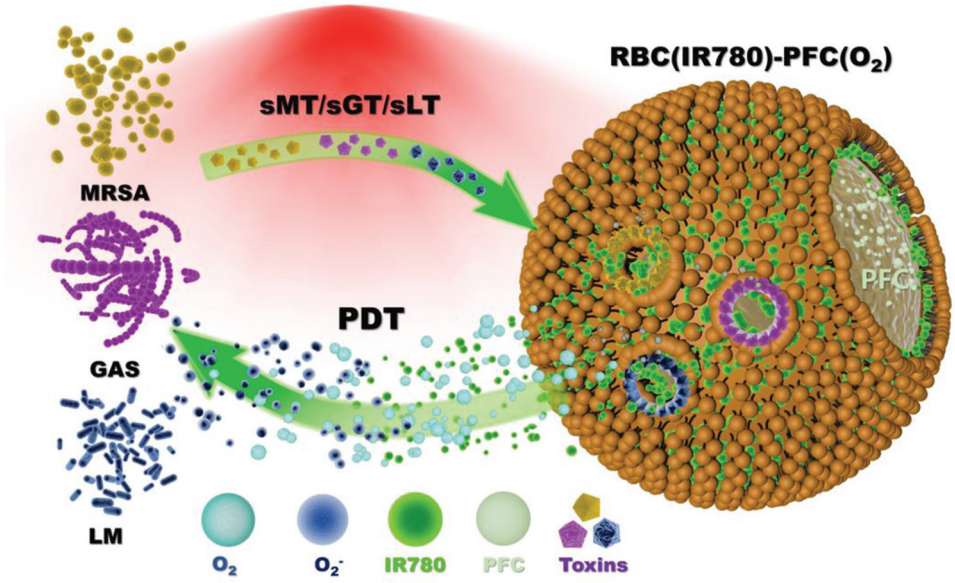

Figure 4. Biomimetic RBC-based nanobubbles for toxin-triggered, on-demand photodynamic antibacterial therapy. Toxin binding induces pore formation in the nanobubble membrane, resulting in the controlled release of the photosensitizer IR780 and oxygen, thereby enhancing NIR-activated antibacterial efficacy. Adopted with permission from [84].

Abbreviations: RBC, red blood cell; NIR, near-infrared.

synergistic antibiofilm effects. However, the source of the RBC membrane was not specified, which is critical for reproducibility and translation [83].

3.3.1.2. Stimuli-responsive nanosystems. Stimuli-responsive RBC membrane-coated nanosystems have emerged as advanced MRSA toxin neutralization, enabling on-demand payload release triggered by infection-specific cues. Two studies have leveraged MRSA pore-forming toxins (PFTs) to trigger targeted delivery [84,85]. As shown in Figure 4, Zhuge and coworkers [84] developed PFT-responsive nanobubbles derived from mouse RBC membranes for co-delivery of oxygen and the photosensitizer IR780. In a murine subcutaneous MRSA infection model, the system neutralized toxins, delivered cargo to infection sites, accelerated lesion recovery, reduced bacterial load, and alleviated inflammation, demonstrating a versatile gas-delivery and anti-infection platform. In the second study, Kamal et al. [85] engineered rat RBC membrane-coated gelatin nanoparticles (VC-SGNPs-R) responsive to bacterial exotoxins for co-delivery of chlorogenic acid and vancomycin. Triggered by MRSA exotoxins, the nanosystem achieved targeted drug release and exhibited strong anti-inflammatory, wound-healing, and antibacterial activity in murine leg and skin infection models. Of note, this work included comprehensive characterization and *in vitro* and *in vivo* evaluations of the nanosystem.

Beyond toxin-responsive systems, two other stimuli-responsive gel-based nanosystems have been explored as MRSA toxin decoys. Zhang et al. developed RBC membrane-coated, redox-responsive vancomycin-loaded nanogels crosslinked with disulfide bonds [86]. The RBC-nanogels neutralized MRSA toxins and rapidly released their payload in the reductive intracellular environment. However, the study did not include rheological characterization, an important quality control measure for gels. In the other study, Zuo and colleagues reported thermosensitive RBC membrane-coated PLGA nanoparticles embedded in a hydrogel (NS-pGel) for pore-forming toxin neutralization [87]. The system demonstrated rapid sol-gel transition near body temperature and effective toxin neutralization *in vitro* and *in vivo* in MRSA-induced local lesion and infection models.

Overall, RBC membrane-based MRSA toxin decoys represent a leading strategy for neutralizing bacterial toxins and combating MRSA infections, while highlighting opportunities for scale-up and clinical translation. Future work should target specific membrane proteins to improve specificity and feasibility and explore alternative cellular membranes with inherent toxin-binding potential to expand the range of effective decoys.

3.3.2. Platelet membrane-coated nanosystems

Platelets contribute to antibacterial defense by releasing antimicrobial peptides and sequestering toxins, functions that have inspired the development of platelet membrane – coated nanoparticles for MRSA toxin neutralization [88]. To date, two such systems have been reported [89,90]. In the first, Kim et al. engineered human platelet membrane-coated PLGA nanoparticles (PNPs) that neutralized MRSA toxins, thereby preserving platelet antimicrobial activity and immune function. In a murine MRSA sepsis model, PNPs significantly reduced bacterial blood counts and improved survival [89]. In the second, Sun et al. developed mouse platelet membrane-derived nanodiscs (PLT-NDs) capable of sequestering MRSA virulence factors, preventing target cell damage, and enhancing bacterial clearance. PLT-NDs neutralized both purified toxins and complex whole secreted proteins (wSP) of MRSA *in vitro* and *in vivo*,

Table 4. Nanocarrier-based targeting of efflux pumps against MRSA.

Nanocarrier type	Efflux pump inhibitor	Mechanism of action	Targeted disease/ model	Key findings	Ref
Metallic NPs	CuNPs and released Cu^{2+} ions	Inhibition of *NorA* activity	MRSA infection/ *In vitro*	• Inhibited MRSA *NorA* efflux pump at 0.5× MIC • Resensitized MRSA to ciprofloxacin by lowering its MIC	[93]
Metallic NPs	Thiolated chitosan	Direct binding of thiol groups to active sites of efflux pumps	MRSA infection/ *In vitro*	• Inhibited MRSA efflux pumps at 2.5 µg/ml	[94]
Metallic NPs	Cu-C_{60}-Cl NPs	Not specified	MRSA infection/ *In vitro*	• Cu-C_{60}-Cl directly inhibited the efflux pump • Cu-C_{60}-Cl showed superior efflux inhibition compared to Ag-C_{60}-Cl and C_{60}-Cl	[95]
Metallic NPs	Thiolated tocopheryl polyethylene glycol succinate	Suppression of *NorA* and *NorB* gene expression	Wound healing/ *in vivo*	• Significant efflux pump inhibition • *NorA* was downregulated by 21 ± 2.12%, and *NorB* by 35 ± 1.52%	[96]
Metallic NPs	Thiosemicarbazide	Inhibition of *NorB* efflux pump gene	MRSA infection/ *In vitro*	• Downregulated the expression of *NorB* by 49% • Co-delivery of Fe_3O_4@Glu-TSC and ciprofloxacin reduced *NorB* by 55%.	[97]
Metallic NPs	Thiosemicarbazide	Inhibition of transcription of *NorA*, *NorB*, *NorC* and *Tet38*	MRSA infection/ *In vitro*	• Co-treatment with ciprofloxacin at 0.5× MIC significantly downregulated *NorA* (5.4-fold), *NorB* (3.8-fold), *NorC* (2.1-fold), *Tet38* (3.4-fold)	[98]
Lipid-based NPs	D-α-tocopherol succinate	Inhibition of *NorA* and *NorB*	Systemic MRSA infection/ *in vivo*	• Inhibition of MRSA efflux pumps • TS showed binding energies of −63.45 kcal/mol (*NorA*) and −63.68 kcal/mol (*NorB*)	[99]
Lipid-based NPs	DSPC	Cationic liposome encapsulation acts as a delivery and efflux-bypassing system Downregulation of *NorB* and *MepA* gene expression	MRSA infection/ *In vitro*	• Efflux pumps *MepA* and *NorB* were upregulated under ciprofloxacin stress by 2.16- and 9.06-fold, respectively • CFL treatment drastically reduced *MepA* and *NorB* expression to 0.0048 and 0.0032, respectively	[100]
Lipid-based NPs	Eugenol-thiazole derivative	Inhibition of *NorA* and *MepA*	MRSA infection/ *In vitro*	• Enhanced antibiotic efficacy through reduction in MICs • Increased EtBr retention	[101]
Lipid-based NPs	Piperine	Not specified	MRSA infection/ *In vitro*	• Increased EtBr fluorescence and reduced dye extrusion • Enhanced gentamicin efficacy by raising intracellular concentration	[102]
Polymer-based NPs	Pyridinium amphiphile	Disrupts transmembrane potential, inhibiting *NorA* efflux pump	MRSA infection/ *In vitro*	• Inhibited NorA-mediated efflux • Restoration of MRSA susceptibility to ciprofloxacin	[103]
Polymer-based NPs	Chitosan	Positive charge-induced bacterial cell membrane destruction with subsequent disruption of the efflux pump system	MRSA infection/ *In vitro*	• Increased EtBr fluorescence • Enhanced antibacterial activity of ciprofloxacin	[104]
Nanocomposite NPs	Nitric oxide	Downregulation of *SepA*, *Tet38*, and *mecA* gene expression	MRSA-induced wounds/ *in vivo*	• Reduced *SepA* and *Tet38* expression by 2.4-fold and *mecA* by 3.3-fold • Attenuated MRSA drug resistance • Enhanced antibiotic sensitivity	[105]
Polymer-based NPs	Tocopherol succinate	Inhibition of *NorA* and *NorB*	Bacterial sepsis/ *in vivo*	• Efflux pump inhibition enhanced intracellular vancomycin accumulation and reduced MIC	[106]
Hybrid nanoparticles	Tannic acid	Inhibition of *NorA* by chelating metal ions and disrupts QS	MRSA infection/ *In vitro*	• Inhibition of *NorA* efflux pumps in MRSA • Enhanced ciprofloxacin efficacy	[107]

Abbreviations: MRSA, Methicillin-resistant *Staphylococcus aureus*; NPs, nanoparticles; CuNPs, copper nanoparticles; Cu^{2+}, copper ions; MIC, minimum inhibitory concentration; EtBr, ethidium bromide; QS, quorum sensing; MFS, major facilitator superfamily; MATE, multidrug and toxic compound extrusion; DSPC, 1,2-distearoyl-sn-glycerol-3-phosphocholine.

improving survival in infected mice [90]. Notably, the use of human-derived platelet membranes, as in Kim's study, may confer superior translational potential due to enhanced biocompatibility.

3.3.3. Miscellaneous

Beyond cell membrane coatings, alternative strategies for MRSA toxin neutralization have emerged [91,92]. Pang et al. engineered HEK 293T cell-derived nanovesicles (ANVs) displaying toxin-absorbing monoclonal antibodies for enhanced α-toxin capture. Loaded with a sonosensitizer, these ANVs enabled ultrasound-triggered ROS generation, achieving accelerated bacterial clearance. Guided by real-time magnetic resonance imaging (MRI) and photoacoustic imaging, the system achieved complete sono-immunotherapeutic eradication of MRSA myositis in mice, representing a 'one-arrow, two-hawks' approach that bypasses antibiotic limitations and mitigates resistance risk

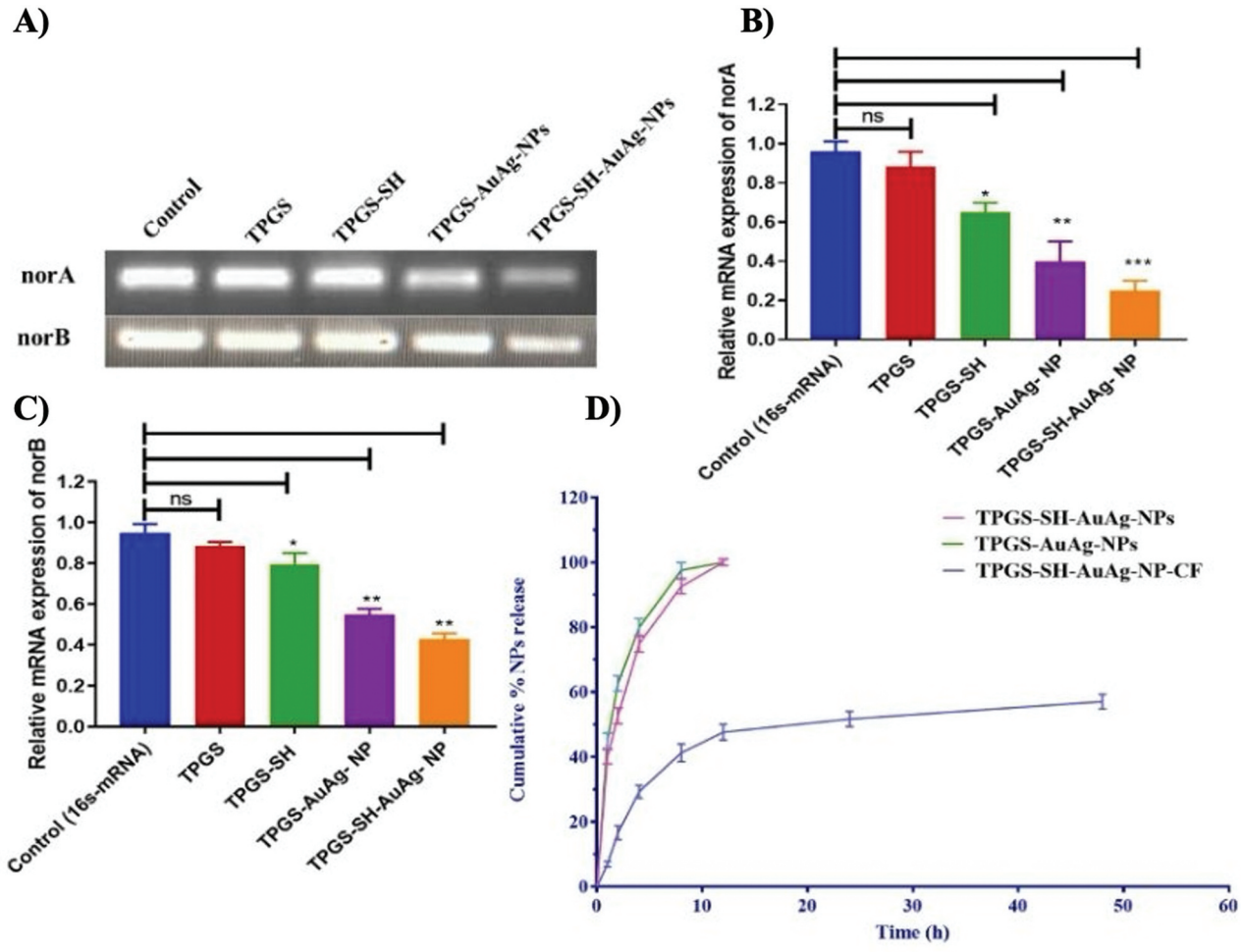

Figure 5. (A) Agarose gel electrophoresis analysis of *norA* and *norB* gene expression following different treatments. (B) Quantification of relative *norA* mRNA expression. (C) Quantification of relative *norB* mRNA expression (*ns*, non-significant). (D) *In vitro* drug release profiles of TPGS-Au-Ag-NP and thiolated TPGS-Au-Ag-NP formulations. Adopted with permission from [96].

Abbreviations: *norA*, *norB*, multidrug efflux pump genes; TPGS, D-α-tocopheryl polyethylene glycol succinate; NP, nanoparticle; mRNA, messenger ribonucleic acid.

[91]. Similarly, Wang et al. developed a dual-function platform combining phototherapeutic liposomal oxygen nanobubbles with fat extract (FE) embedded in an F-127 hydrogel (G(NB/FE)) [92]. This system employed a toxin-regulated on-demand phototherapy (TROP) strategy to both neutralize MRSA toxins and release FE-derived growth factors, promoting angiogenesis and collagen deposition for wound healing. In a murine skin infection model of MRSA, G(NB/FE) enhanced bacterial clearance, mitigated toxin-induced cytotoxicity, and accelerated tissue repair. Of note, they reported comprehensive *in vitro* and *in vivo* evaluations, supporting reproducibility and potential scalability. Collectively, these non-membrane-based MRSA toxin decoys show strong therapeutic promise, offering multifunctional platforms that unite toxin sequestration with targeted therapy and underscoring opportunities for further optimization and clinical translation.

Overall, MRSA toxins represent a unique therapeutic target, and nanocarrier-based decoys offer a powerful strategy to neutralize these virulence factors and mitigate infection. Future research should focus on identifying key toxin-binding components, refining nanodecoy specificity, exploring alternative cellular or synthetic membranes, and rigorously validating efficacy in complex, clinically relevant MRSA infection models.

3.4. Nanocarriers inhibiting MRSA efflux pumps

Nanocarrier-mediated strategies have shown considerable potential in overcoming efflux pump-driven resistance in MRSA. These systems act through multiple mechanisms, including direct inhibition of pump activity, downregulation of efflux pump gene expression, disruption of the proton motive force required for pump function, and alteration of membrane integrity to impair efflux. A wide range of nanocarrier platforms, metallic, lipid-based, polymeric, and hybrid or composite, have been investigated for these purposes. Table 4 summarizes various nanocarriers targeting MRSA efflux pumps, their mechanisms of action, and key findings.

3.4.1. Metallic nanocarriers

Various metallic nanocarriers, including Cu, Ag, Au, Fe, and Zn, have shown inhibition of the MRSA efflux pump. Similar to

their application and success in targeting QS, Christena et al. previously investigated the efflux pump inhibitory potential of CuNPs against MRSA [93]. Their study revealed that CuNPs inhibited the MRSA efflux pump at a sub-inhibitory concentration (0.5 ×minimum inhibitory concentration, MIC). The study suggested that both CuNPs and the ions released from the CuNPs, as confirmed by EDTA chelation experiments, inhibited the activity of *NorA*. However, it remains unclear whether the inhibitory action resulted from direct binding to the efflux pump or indirectly from the loss of the proton motive force. Importantly, CuNPs resensitized MRSA to antibiotics by reducing the MIC of ciprofloxacin. We believe that the ability of CuNPs to inhibit efflux pump activity at relatively low concentrations offers a significant safety advantage, as these doses are likely to be nontoxic. We therefore support its use as a bacterial adjuvant rather than a primary antibacterial agent, due to toxicity issues.

Another study designed thiolated chitosan-coated cobalt-doped ZnO (Co-ZnO NPs) [94]. The nanocarrier effectively inhibited efflux pumps in MRSA at a concentration of 2.5 µg/ml. The mechanism was attributed to the thiol groups binding to active sites of efflux pumps, thereby blocking drug extrusion from bacterial cells. Similarly, Ibrahim et al. synthesized Ag- and Cu-coordinated chlorofullerene nanoparticles (Ag-C_{60}-Cl and Cu-C_{60}-Cl) and evaluated their effect on MRSA efflux pump activity [95]. Cu-C_{60}-Cl demonstrated superior MRSA efflux pumps inhibitory activity compared to Ag-C_{60}-Cl and bare C_{60}-Cl. However, further studies are still needed to elucidate their mechanism of action.

Singh et al. fabricated a redox-sensitive tocopheryl polyethylene glycol succinate-coated bimetallic Au-Ag-NPs (TPGS-SH-Au-Ag-NP) [96]. Ethidium bromide (EtBr) accumulation assay and flow cytometry revealed TPGS-SH-Au-Ag-NPs to have a significant efflux pump inhibition in MRSA. Moreover, TPGS-SH-Au-Ag-NP treatment with noticeably reduced mRNA expression of the *NorA* and *NorB* efflux pump genes in MRSA compared to other treatments (Figure 5(A)). Gene expression analysis showed strong downregulation of *NorA* (21 ± 2.12%) and *NorB* (35 ± 1.52%) compared to controls (Figure 5(B,C)), indicating suppression of key efflux systems. These results offer an attractive therapeutic option that could increase and sustain intracellular drug concentrations for longer durations, as shown in Figure 5(D), thereby reducing the likelihood of MRSA resistance.

Safarnejad et al. developed Fe_3O_4 magnetic nanoparticles functionalized by glutamic acid and conjugated with thiosemicarbazide (Fe_3O_4@Glu-TSC) to target the *NorB* efflux pump gene in MRSA [97]. The Fe_3O_4@Glu – TSC NPs downregulated the expression of *NorB* by 49%, compared to controls. When Fe_3O_4@Glu-TSC was combined with ciprofloxacin, *NorB* expression was reduced by 55%. A similar study designed ZnO nanoparticles conjugated with thiosemicarbazide and functionalized with glutamic acid (ZnO@Glu-TSC) to target efflux pump genes *NorA*, *NorB*, *NorC*, and *Tet38* in MRSA [98]. The study demonstrated that co-treatment with ZnO@Glu – TSC NPs and ciprofloxacin at half their MICs resulted in a significant downregulation of these efflux pump genes by 5.4-, 3.8-, 2.1-, and 3.4-fold, respectively, compared to ciprofloxacin alone. The proposed mechanism involved inhibition of transcription, possibly mediated by ROS or direct interactions with transcription factors, highlighting the potential of ZnO@Glu-TSC nanoparticles as efflux pump inhibitors.

3.4.2. Lipid-based nanocarriers

Lipid-based nanocarriers have been widely explored for their ability to inhibit efflux pumps and restore antibiotic activity against MRSA. Dlamini et al. studied ciprofloxacin-loaded nanostructured lipid carriers (CIP-NLCs) incorporating D-α-tocopherol succinate (TS), a *NorA* and *NorB* inhibitor [99]. EtBr accumulation assays confirmed significant efflux inhibition, and molecular simulations showed strong binding of TS to *NorA* and *NorB*, with free binding energies of −63.45 kcal/mol and −63.68 kcal/mol, respectively.

Another study evaluated ciprofloxacin liposome-encapsulated formulations (CFL) for their ability to suppress the expression of the *MepA* and *NorB* efflux pumps in MRSA [100]. Treatment with CFLs reduced NorB and MepA expression to 0.0032- and 0.0048-fold, respectively, compared to controls, whereas drug-free liposomes had minimal effects. This work highlights liposomal encapsulation as a promising means to both enhance drug efficacy and attenuate efflux-mediated resistance. Similarly, de Almeida et al. tested liposomal formulations incorporating eugenol-based thiazole derivatives against *NorA* and *MepA* in multidrug-resistant *Staphylococcus aureus* [101]. While the derivatives lacked direct antibacterial activity, they significantly potentiated antibiotics by inhibiting efflux pumps, as evidenced by reduced MIC values and increased EtBr retention.

Interestingly, Khameneh et al. further demonstrated the synergistic potential of lipid-based nanocarriers by formulating nanoliposomes co-loaded with piperine and gentamicin [102]. Piperine inhibited efflux pump activity, thereby increasing intracellular gentamicin concentrations and improving antibacterial efficacy. From our perspective, this work exemplifies how rational co-loading strategies can both block resistance mechanisms and enhance therapeutic performance.

Overall, lipid-based nanocarriers hold strong potential as future anti-MRSA therapeutics, offering versatile strategies to inhibit efflux pumps through co-delivery systems or genetic modulation.

3.4.3. Polymer-based nanocarriers

Two studies have reported the use of polymeric nanoparticles to inhibit MRSA efflux pump activity. Thiyagarajan et al. developed pyridinium amphiphile-loaded PLGA nanocarriers (C1-PNC) to reverse ciprofloxacin resistance [103]. C1-PNC disrupted the transmembrane potential essential for *NorA* function, leading to dose-dependent reductions in EtBr efflux and effective ciprofloxacin potentiation. Similarly, Ismail et al. designed chitosan-based pH-responsive, biomimetic nanoplexes loaded with ciprofloxacin (CS/SDC nanoplexes), which significantly inhibited efflux activity as demonstrated by EtBr assays [104]. The formulation also showed reduced MICs and faster bacterial killing compared to free ciprofloxacin, further confirming its ability to overcome efflux-mediated resistance.

3.4.4. Hybrid or composite nanostructures

Advanced nanocomposites, including lipid-polymer hybrid nanocarriers and multifunctional nanoparticles, have emerged as versatile platforms for targeting MRSA efflux pumps while simultaneously delivering antibiotics. Liang et al. developed Au nanostar/hollow polydopamine Janus nanostructure (GNS/HPDA JNPs) with NIR-controlled NO release to combat MRSA [105]. The nanosystem downregulated the expression of *MecA* and efflux pumps *SepA* and *Tet38* by 3.3- and 2.4-fold, respectively, attenuating drug resistance and enhancing antibiotic sensitivity. These results indicate that GNS/HPDA JNPs can effectively reverse efflux-mediated resistance, improving bacterial susceptibility to antibiotics.

Elhassan et al. developed two hybrid nanocarrier systems that combined antibiotic delivery with efflux pump inhibition [106,107]. The first system utilized a hyaluronic acid-lysine conjugate with tocopherol succinate and oleylamine to form a vancomycin-loaded hybrid nanosystem (VCM-HNLCs). TS functioned as a *NorA*/*NorB* inhibitor, increasing intracellular EtBr accumulation and enhancing vancomycin activity. The second system involved ciprofloxacin-loaded nanoparticles incorporating tannic acid, which inhibits *NorA* by chelating essential metal ions and disrupts QS pathways linked to efflux regulation. Both systems showed synergistic efflux pump inhibition and enhanced antibacterial efficacy, demonstrating the potential of hybrid nanostructures to simultaneously overcome resistance mechanisms and deliver antibiotics effectively.

Collectively, these studies underscore the promise of nanocarrier-based strategies to overcome MRSA efflux pump-mediated resistance. By enabling targeted delivery of efflux pump inhibitors and antibiotics, these systems have the potential to restore drug efficacy, enhance bacterial clearance, and offer a versatile platform for next-generation anti-MRSA therapies.

4. Conclusion

MRSA remains a formidable clinical challenge due to its multidrug resistance, virulence adaptability, and ability to evade host defenses. Conventional antibiotics often fail to address critical pathogenic mechanisms such as toxin secretion, QS, and biofilm fortification, resulting in persistent and recurrent infections. Nanocarrier-based systems offer a versatile and promising platform to overcome these barriers by enabling precise delivery of antibiotics and anti-virulence agents to otherwise inaccessible niches. By simultaneously targeting essential bacterial functions and virulence pathways, nano-enabled therapeutics have the potential to improve treatment outcomes and mitigate the emergence of further resistance.

Our analysis of the current literature highlights significant progress in nanomedicine-driven MRSA research. Notably, nanocarriers targeting biofilms and QS represent the most extensively studied approaches, comprising ~40% of the studies reviewed, with a clear evolution from conventional formulations to advanced hybrid and multifunctional designs. In addition, diverse nanocarrier systems have been engineered to disrupt the MRSA cell wall and membrane, neutralize toxins, and inhibit efflux pumps. Collectively, these strategies have enhanced drug accumulation at infection sites, reduced biofilm burden, and attenuated toxin-mediated hemolysis and host toxicity, leading to improved outcomes in experimental models of MRSA infection.

5. Expert opinion

Currently, the most recently published research focuses on nanocarriers targeting the MRSA cell wall and membrane, their biofilm and QS system, associated toxins, and MRSA efflux pumps. Such strategies have shown significant potential as emerging therapeutic avenues; they provide unique mechanisms for suppressing MRSA survival and dissemination, and in some cases, exert direct bactericidal effects. The proven ability of nanoplatforms targeting these virulence-associated pathways, in addition to essential bacterial processes, to increase the local concentration of antibiotics at infection foci could meaningfully reduce resistance development. Furthermore, several studies highlight the capacity of nanosystems to simultaneously engage multiple targets and processes, leading to synergistic outcomes that go beyond what conventional antibiotics can achieve.

The potential impact of these advances on real-world outcomes is substantial. If translated successfully, nanocarriers could reshape treatment guidelines by enabling targeted delivery of antibiotics and anti-virulence agents, particularly in settings where MRSA infections remain persistent and refractory to standard care. For example, integrating toxin-scavenging nanosystems into wound dressings or implant coatings could markedly reduce complications, hospital stays, and costs. Likewise, biofilm-penetrating nanocarriers may prolong the clinical lifespan of existing antibiotics, sparing the need for new drug classes and alleviating the burden on healthcare systems. However, translating these platforms into clinical use is not straightforward. Complex formulations, manufacturing scalability, reproducibility, regulatory uncertainty, and the need for rigorous safety evaluation remain major hurdles. In particular, limited data on immune dysregulation, chronic inflammation, nanoparticle persistence, and accumulation within immune organs, together with the absence of standardized long-term toxicity and immunotoxicity assessments, represent critical barriers to clinical translation. In addition, nanomedicine-based therapies require long-term pharmacokinetic and pharmacodynamic studies to establish dosing paradigms, cost-effectiveness, and compatibility with existing antimicrobial stewardship frameworks.

Looking ahead, a major focus should be on the rational design of nanocarriers and materials that target unique and underexplored pathways in MRSA biology. Beyond biofilms and toxin neutralization and efflux pumps, opportunities exist to develop 'next-generation nanobiotics' directed at essential enzymes such as MurF, which plays a critical role in peptidoglycan biosynthesis, or metabolic pathways such as fatty acid synthesis and teichoic acid assembly, and other intracellular targets, including β-lactamase, which remain largely untapped as therapeutic targets. Nanocarriers could be engineered to deliver inhibitors of these processes with enhanced stability and penetration. Similarly, nanoplatforms that disrupt regulatory systems such as two-component signaling

networks or that interfere with global virulence regulators could fundamentally alter MRSA pathogenesis. Novel material design, such as hybrid nanocarriers combining responsive polymers, peptides, and inorganic components, will be essential to achieve precision targeting and stimuli-responsive release in the infection microenvironment. Furthermore, future efforts should prioritize the development of biodegradable and immunocompatible nanomaterials, advanced surface engineering strategies to mitigate adverse immune responses, and more predictive preclinical models capable of accurately assessing long-term safety and immunological outcomes. Complementary technologies are also likely to accelerate progress. Artificial intelligence and machine learning can aid in the rational selection of nanomaterials and drug-target combinations, while CRISPR-based editing tools and phage-nanocarrier hybrids offer innovative ways to silence resistance genes or deliver payloads selectively to MRSA. Integration of advanced imaging, omics, and microfluidics platforms could further refine preclinical testing and accelerate translation.

The future of this field lies in combining antibiotic potentiation with targeted anti-virulence and resistance-suppressing strategies, moving beyond 'antibiotic-only' approaches toward versatile 'antibiotic-plus-nanotherapy' regimens. While other areas such as bacteriophage therapy and host-directed immunomodulators hold considerable promise, nanocarriers stand out for their modularity, adaptability, and potential for integration into existing treatment algorithms. Over the next five to ten years, nanocarriers are unlikely to replace antibiotics but will increasingly serve as indispensable adjuncts, particularly in chronic or device-associated MRSA infections where current options remain inadequate.

Looking five years ahead, it is reasonable to anticipate that simplified, modular nanocarrier platforms targeting biofilms, toxins, or unexplored enzymatic pathways will enter early-phase clinical trials, with initial applications in wound care and implant-related infections. Regulatory frameworks for nanomedicines will likely evolve to support scalable manufacturing and quality control, reducing barriers to adoption. At that point, clinicians may begin to rely on nanocarrier-based adjuncts not only as rescue therapies but as preventive and frontline tools for high-risk patients. The field is thus poised to transition from experimental promise to clinical reality, with nanocarrier-enabled strategies redefining the management of multidrug-resistant MRSA.

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