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Recent nanotechnology-based strategies for interfering with the life cycle of bacterial biofilms

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Biofilm formation plays an important role in the resistance development in bacteria to conventional antibiotics. Different properties of the bacterial strains within biofilms compared with their planktonic states and the protective effect of extracellular polymeric substances contribute to the insusceptibility of bacterial cells to conventional antimicrobials. Although great effort has been devoted to developing novel antibiotics or synthetic antibacterial compounds, their efficiency is overshadowed by the growth of drug resistance. Developments in nanotechnology have brought various feasible strategies to combat biofilms by interfering with the biofilm life cycle. In this review, recent nanotechnology-based strategies for interfering with the biofilm life cycle according to the requirements of different stages are summarized. Additionally, the importance of strategies that modulate the bacterial biofilm microenvironment is also illustrated with specific examples. Lastly, we discussed the remaining challenges and future perspectives on nanotechnology-based strategies for the treatment of bacterial infection.

1 Introduction

Persistent bacterial infection has become a life-threatening challenge worldwide. Particularly, the emergence of drug-resistant pathogens prompts the over-consumption of antibiotics, which in turn exacerbates the rapid development of drug resistance.[1] In light of the current dilemma, great effort has been devoted to discovering new antibiotics or synthesizing novel antimicrobial drugs,[2,3] but their efficiency may not be high enough to keep up with the rise of drug resistance.[4] Some bacteria with intrinsic resistance are naturally insensitive to antimicrobial agents. Interestingly, drug resistance can also be acquired from other bacterial strains through specific biochemical routes, including gene mutation and gene transfer.

According to extensive research in the past few decades, biofilm formation is considered a vital pathway for the revolutionary development of resistance.[5] Biofilm formation is a mechanism for bacteria to protect them against the host immune system and hazardous environmental challenges.[6] The properties of the bacteria inside the biofilm are distinct from their planktonic counterparts, especially the susceptibility of pathogenic bacteria to conventional antibiotics.[7] Bacterial cells within the biofilm usually stay in slow growth states which enable the tolerance to the antimicrobial drugs that target physiological processes occurring during growth.[8] Moreover, the extracellular polymeric substance (EPS), as well as the biofilm microenvironment, plays vital roles in hindering the susceptibility of bacteria to antibiotics.[9] There is a diffusion-reaction inhibition effect exerted by EPS molecules during the penetration of antimicrobials in the biofilm, which limits the diffusion extent in the biofilm and quenches the activity of antimicrobial drugs. Additionally, attributed to the local metabolic activity and host immune response, there is an inherent acidic microenvironment (pH values of 4.5–6.5)[10–14] prevailing in a biofilm, leading to the disabling of antibacterial agents.[6] Unfortunately, numerous persistent infectious diseases are related to biofilm formation, such as caries, fibrosis pneumonia, and endocarditis.[15,16] Apart from infectious diseases, the urgent need to defeat biofilms also exists in various fields, including biomedical implants and food packaging.[17] Consequently, antibiofilm strategies, either preventing biofilm formation or eradicating them, have been a topic of great interest over the past few decades.

A typical life cycle of planktonic bacteria to biofilms is comprised of four stages, including the planktonic bacteria adhesion on either biological or inert surfaces, bacterial growth and EPS secretion, mature biofilm formation, and detachment of planktonic bacteria from the mature biofilm.[18] The irreversible attachment initiates various changes in the attached bacteria at the genetic level, boosting the biosynthesis of the matrix and tolerance to conventional antibiotics. Then, bacterial cells grow and aggregate with EPSs into a more complex three-dimensional structure. With the formation of mature biofilms, EPS, as a natural physical barrier, can not

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only protect the resident bacteria as described above, but also separate the biofilm cells from nutrients, thus leading to the emergence of subpopulations to adapt to the ever-changing microenvironment accompanied by cell crowding. Besides, bacterial cells can be detached from the biofilm and return to the planktonic mode which can initiate a new cycle for biofilm formation after their colonization on a new surface. In light of the biofilm formation life cycle and the pathways endowing biofilm bacteria with drug resistance, strategies that interfere with the biofilm life cycle, including inhibiting initial bacterial adhesion, inhibiting the biofilm formation, eradicating mature biofilms, and killing planktonic bacterial cells, have been widely explored. However, these strategies cannot be carried out with barely conventional antibiotics due to their limited biofilm penetration, insufficient bactericidal efficacy, poor targeting ability, developed drug resistance of bacteria, and barely any effect on the EPS.

Recent advances in nanotechnology have furnished promising alternative tools to combat biofilms and great effort has been devoted to leveraging nanomaterials for interfering with the biofilm life cycle. At the nanoscale, materials exhibit special and unique features. Moreover, diverse functionalities can be imparted for enhanced drug delivery, such as prolonged systemic circulation, improved drug solubility, enlarged drug loading capacity, targeted drug delivery and controlled drug release. Many approaches using nanotechnology-based drug delivery systems, including liposomes,[19–22] polymeric nano-vehicles,[23,24] and mesoporous silica nanoparticles,[25–28] have been verified and found to be feasible for combating drug-resistant bacterial strains.[29] Apart from being utilized as drug carriers, some nanomaterials with intrinsic antimicrobial effects, such as noble metal (*e.g.* gold,[30] silver[31]) nanomaterials, metallic oxide (*e.g.* iron oxide,[32] vanadium pentoxide[33]) nanomaterials, and carbon nanomaterials,[34–36] have also been developed as antibiotic-free antimicrobial agents that kill pathogens through various pathways (*e.g.* toxic metal ion leaching, generation of reactive oxygen species (ROS), and physical interactions) simultaneously.[4]

In this review, the current nanotechnology-based strategies for interfering with the biofilm life cycle, which includes inhibiting initial bacterial adhesion, promoting antimicrobial penetration into biofilms, and *in situ* activation of antimicrobial activities, as well as modulating the infection microenvironment, are summarized according to the requirements of different stages (Fig. 1). Finally, perspectives on the further development and challenges of nanotechnology-based antibiofilm strategies are discussed.

2 Engineered surfaces for adhesion inhibition

In the biofilm life cycle, the adhesion of planktonic bacteria plays a pivotal role in initiating biofilm formation.[18] Therefore, modified surfaces that prevent the non-specific attachment of planktonic bacterial cells and sterilize the adherent bacteria through a contact-killing effect or by releasing antibacterial agents, are highly desirable to avoid subsequent biofilm formation.[37,38] The properties of these surfaces are summarized in Table 1. In particular, biomimicry surfaces that are inspired by plants or animals are also highly feasible for preventing microbial adhesion.[39,40] The special nanostructured textures in nature (*e.g.* the nanopillar geometry of insect wings, nanogroove geometry of shark skin) play important roles in endowing an antimicrobial effect.[41]

2.1 Physical antiadhesion surfaces

To develop surfaces interdicting adhesion, surface topographical features, including charge, roughness, and wettability, have to be taken into consideration.[4]

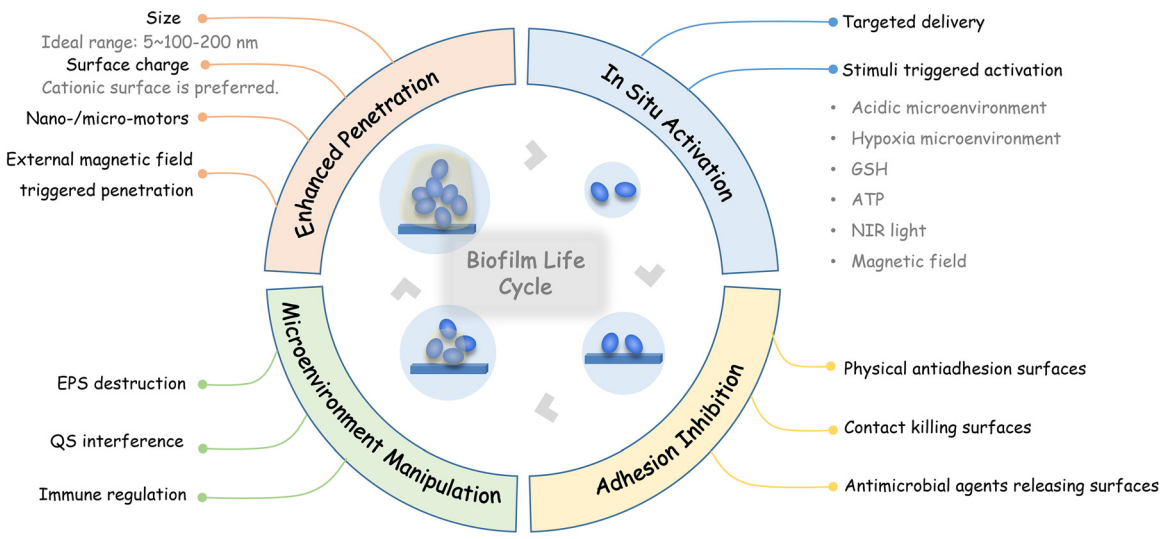

Fig. 1 Recent nanotechnology-based strategies for interfering with the biofilm life cycle.

Table 1 Summary of the properties of surfaces engineered for inhibiting bacterial adhesion

Surface type	Characteristics and antiadhesion mechanism	Advantages	Disadvantages
Physical antiadhesion surfaces	• Electrostatic repulsion between the negative surfaces and the negative bacterial membrane • Superhydrophobic surfaces capture stable air cushions • Superhydrophilic surfaces form a stable hydration layer	Inhibit the interaction of bacteria with the surfaces directly	Remain risks of bacterial attachment and initiation of biofilm formation
Contact-killing surfaces	Bacterial physical attachment on the surfaces: • Tethering positively charged molecules on surfaces to interfere with the integrity of the bacterial membrane • Decorating bactericidal peptides and nanoparticles on surfaces to kill pathogens directly • Manufacturing special nanostructured textures on surfaces to trap bacteria or rupture bacterial cells	Deactivate the adhered bacterial cells	Rely on the physical attachment of bacterial cells and kill bacteria in a limited range
Antimicrobial agents releasing surfaces	Decorating antibiotic-loaded nanocarriers, antimicrobial metal ions or ions-leaching metal nanoparticles on the surfaces	Deactivate both adhered bacteria cells and planktonic bacterial cells	Require rational design for controlled release to eliminate probable toxicity

Due to the inherent negative surface charge of bacterial cells, surfaces with negative charge are capable of suppressing bacterial adhesion through electrostatic repulsion. For example, Cheng Zhu *et al.* decorated negatively charged carbon nanodots on polymeric coatings, enabling a robust antiadhesive effect and effective inhibition of biofilm formation.[42] Similarly, graphene–silicone elastomer composite materials that were tailored with strong electronegativity could significantly expel both Gram-negative bacteria and Gram-positive bacteria through physical interaction.[43] On the other hand, although the positive surface usually cannot resist bacterial adhesion, it can interfere with the integrity of the bacterial membrane and lead to a contact killing effect,[44] which will be introduced in section 2.2.1.

Regarding roughness, bacterial adhesive abilities differ from micron-scale roughness and nanoscale roughness.[45] Although it has been well recognized that nanoscale roughness on surfaces generally provides better anti-adhesion properties, some studies verified that bacterial adhesion occurs more easily on rough surfaces in the range of nanoparticles.[28,46] For instance, Hao Song *et al.* constructed mesoporous silica nanoparticles with rough surfaces, which exhibited enhanced adhesion to the bacterial surface compared with mesoporous silica nanoparticles with smooth surfaces.[28]

Wettability is an important indicator of bacterial adhesion on a surface. It has been well recognized that the initial bacterial adhesion force on hydrophobic surfaces is higher than it on hydrophilic surfaces due to the hydrophobic interactions. On the other hand, there are some studies indicating that hydrogen bonding with hydrophilic surfaces can also help bacterial adhesion.[45] Consequently, surfaces with moderate wettability allow bacterial adhesion through hydrogen bonding or hydrophobic interactions,[47–49] which depends on not only the surface wettability but also the bacterial species.[50,51] However, superwettable surfaces can resist bacterial adhesion through the formation of natural barriers.[52] Superhydrophobic surfaces can capture stable air cushions, while superhydrophilic surfaces can form a stable hydration layer.[53,54] The surface of the lotus leaf is a typical natural superhydrophobic surface with self-cleaning and antifouling properties, which is attributed to its micro/nanostructure.[55] It is also worth noting that surface chemistry and surface roughness can be adjusted to influence the surface wettability.[55,56] For example, Gege Wang *et al.* endowed polylactic acid (PLA) surfaces with micron-/nanoscale roughness *via* an adjusted exfoliation process to form superhydrophobic surfaces.[57] The results turned out that the PLA surfaces with a higher roughness value (Ra = 302 nm) presented higher water contact angles, while pristine PLA surfaces were smoother (Ra = 7.155 nm) and exhibited hydrophilicity.

It has been reported that a conditioning film that is formed by the deposition of organic and inorganic molecules present in aqueous environments is a critical parameter for bacterial adhesion.[58] Components in the conditioning films with various properties, such as ionic strength, hydrophilicity and charge, can alter the physiochemical features of the surface.[59,60] Consequently, modifying the conditioning films can also influence bacterial adhesion. For example, Kim Doiron *et al.* utilized snow crab peptides that are natural organic matter in seawater to alter the nature of the conditioning film on mild steel, which consequently limits the biofilm formation and reduces the microbial-induced corrosion.[61]

2.2 Bactericidal surfaces

Although physical antiadhesion surfaces can largely repel bacteria, there remain risks of bacterial attachment and initiation of biofilm formation. To this end, strategies that use bactericidal surfaces have been proposed to inhibit biofilm formation. According to the bactericidal regime, these surfaces can be generally categorized as contact-killing surfaces and antimicrobial agent releasing surfaces. Additionally, the physi-

cal antiadhesive ability can also be integrated into bactericidal surfaces to maximize the inhibition of biofilm formation.[62,63]

2.2.1 Contact-killing surfaces. Different from the physical antiadhesion surfaces, contact-killing surfaces rely on the physical attachment with bacteria cells. Due to the inherent negative surface charge of bacterial cells,[64] contact-killing surfaces are usually fabricated by tethering positively charged molecules, such as quaternary ammonium compounds,[65–67] *N*-halamines,[68,69] zwitterions,[70,71] and guanidine-based compounds.[72,73] For instance, rechargeable biocidal poly(vinyl alcohol-*co*-ethylene) films incorporated with both *N*-halamine and zwitterionic moieties were capable of resulting in >99.9999% biocidal efficacy *via* contact killing.[74] Additionally, antimicrobial peptides (AMPs) can also be used to construct contact-killing surfaces.[75–78] For example, Miao Xu *et al.* prepared a hydrogel crosslinked with AMP *ε*-poly-L-lysine and catechol.[77] The as-fabricated AMP-containing hydrogel exhibited a contact-killing effect *in vitro* and showed a great wound disinfection effect *in vivo*.

Apart from tethering antibacterial groups/molecules, polymers and peptides, some nanoparticles with antimicrobial capability can also be grafted or loaded to the surface for contact killing. Additionally, the decoration of nanoparticles onto the surface may change the surface topographical features as well. Guanidine-based compounds with bacteriostatic properties can be utilized to fabricate antibacterial nanomaterials.[79] Hua Han *et al.* utilized guanidine-based compounds to construct nanogels by copolymerization.[73] The as-fabricated nanogels presented a strong contact-killing effect by destroying the cell membrane and causing bacterial lysis. Then the nanogels were grafted on the surface of cotton fibers, which converted the surface from hydrophilicity to hydrophobicity. Consequently, the cotton grafted with nanogels was able to repel most bacteria and further kill the immobilized bacteria.

Inspired by the special nanostructured textures in nature, surfaces with specific nanopatterns may trap bacteria or rupture bacterial cells and thus can be developed for contact-killing surfaces. Among them, nanopillars are a nanopattern that has been widely investigated. For instance, Martyna Michalska *et al.* fabricated a nanopillar geometry inspired by insect wings on black silicon.[80] The as-fabricated biomimicry surfaces presented tunable antibacterial effects. The longer (up to 7 μm) and exceedingly sharp pillars presented a strong bactericidal effect on various bacterial species, while the shorter (<2 μm) and blunt pillars appeared effective for specific bacterial species. They illustrated that the strong adhesion effect between bacterial cells and nanopillars, which was dependent on the topography of the nanopillar array and the properties of bacterial cells, was the main mechanism for the bactericidal effect of as-fabricated nanopillar patterned surfaces.[80] Similarly, Joshua Jenkins *et al.* also explored the morphological effects of mimetic titanium nanopillars on bacteria.[81] They found that nanopillars with topography that mimic the surface of dragonfly wings could deform and penetrate the bacterial envelope rather than rupturing or lysing bacterial cells. Interestingly, such mimetic titanium nanopillars performed physiological effects on bacteria, including inhibiting cell division and increasing intracellular oxidative stress.[81] Besides, integrating bactericidal compounds onto the nanopillar array can obtain a synergistic antibacterial effect as evidenced by Goro Choi *et al.*, who fabricated a polymeric nanopillar array covered by an ionic polymer layer containing quaternary ammonium compounds.[82]

2.2.2 Antimicrobial agent releasing surfaces. Antimicrobial agent releasing surfaces can not only inhibit the adhered bacteria but also influence the planktonic bacterial cells. Rita M. Mendes *et al.* compared the antimicrobial activity of sophorolipid-releasing surfaces with the contact-killing strategy using sophorolipid-tethered surfaces.[83] The results turned out that the releasing strategy achieved a better inhibitory effect on biofilm formation, which might be attributed to the bactericidal effect in a wider range of microenvironments by releasing antimicrobials rather than the local bactericidal effect that only kills the adhered cells on the surfaces.

Some antimicrobial metal ions (*e.g.*, Cu^{2+} [84] and Ag^{+} [85]) and ion-leaching metal nanoparticles (*e.g.*, silver nanoparticles (AgNPs),[86] metal–organic framework (MOF) nanoparticles[87]) are easily decorated on biomaterial surfaces and thus usually utilized to fabricate antimicrobial agent releasing surfaces. AgNPs have been well-recognized and widely adopted as bactericidal nanomaterials. Ag^{+} leaching from the surface of AgNPs plays a major antimicrobial role. Therefore, AgNPs can be deemed as a carrier of Ag^{+}. Hossein Yazdani-Ahmadabadi *et al.* used AgNP assemblies to form silver coatings that provided a sustained release of Ag^{+} ions.[86] Consequently, the fabricated silver coatings exerted a long-term anti-adhesion effect (>30 days) and a long-term antibacterial effect *in vitro* on various bacterial species for up to 28 days. MOFs consisting of crystalline porous coordination polymers have emerged as antibacterial materials in recent years. Similarly, MOFs can sustainably release the stored metal ions and thus achieve a long-term antibacterial effect.[88] Xiaoxue Yao *et al.* prepared omniphobic porous hydrogels loaded with zinc imidazolate framework 8 (ZIF–8) nanoparticles as wound dressings.[38] Anchoring of ZIF–8 nanoparticles endowed hydrogels with a reentrant architecture and increased roughness, which was beneficial for eliminating the adhesion of pathogens. Bactericidal Zn^{2+} could be gradually released from ZIF-8 nanoparticles. With long-term antiadhesion and antibacterial effects, the ZIF-8 nanoparticle loaded hydrogel successfully accelerated wound closure *in vivo*.

For antimicrobial agent releasing surfaces, antibacterial agents are incorporated and usually released in a controlled manner.[38] Moreover, to improve the antibacterial efficacy and reduce the potential toxicity, stimuli-responsive moieties are usually introduced to allow stimuli-triggered drug release for enhanced specificity to pathogens.[10,89] At the site of infection, the acidic microenvironment as well as different types of molecules secreted by the bacteria can be used as triggers.[90] For instance, a pH-responsive grafted bilayer constructed by Hyun-Su Lee *et al.*[10] could responsively release loaded antibiotics in

the acidic microenvironment to perform a significantly enhanced antibacterial activity.

3 Tailored nanomaterials for enhanced biofilm penetration

After the formation of biofilm architectures, the EPS acts as a natural fortress to protect the bacterial cells within the biofilm. The susceptibility of conventional antibiotics and the toxic metal ions to the enwrapped bacteria might be diminished by chelation or enzymatic degradation during the diffusion process. Thus the bacterial strains within the biofilm can survive the challenge of antimicrobials with sublethal concentrations, which can further promote resistance to the exposed antimicrobials. Therefore, it is a desirable strategy to combat biofilms by improving the penetration capacity and retaining the sterilization activity in the meanwhile. Utilizing nanocarriers to deliver conventional antibiotics or toxic metal ions is an alternative to maintain their disinfecting activity. Additionally, it has been noted that the physicochemical properties of nanomaterials, such as size, surface charge, and surface ligands, can alter their penetration extent in biofilms.

3.1 Size

Regarding size, the ideal diameter for nanomaterials to treat biofilms has been arrived at as the range between 5 and 100–200 nm and no larger than 500 nm.[6] Specifically, compared to large nanoparticles of limited biofilm penetration capability, small nanoparticles with a size of <20 nm have been reported to penetrate biofilms to the deep layers.[23,91,92] For instance, Xiaokai Chen *et al.* synthesized epoxy group-functionalized organosilica nanodots (OSiNDs) with a high photoluminescence quantum yield.[93] Due to the ultrasmall size (≈6.4 nm), OSiNDs were able to penetrate biofilms and thus could be used as a universal platform for imaging or therapy. Considering that small-sized nanoparticles can be readily cleared from the body and larger nanoparticles have prolonged circulation time and are liable to accumulate at the infection site due to the host immune response, size transition triggered by the external or internal stimuli might favor both accumulation and penetration.[94–96] Our group fabricated pH-responsive silver nanoassemblies composed of ultrasmall AgNPs with a diameter of about 4 nm and pH-sensitive polymeric ligands.[97] The as-fabricated pH-responsive silver nanoassemblies could undergo structural transformation in the acidic microenvironment in the biofilm and disassembled into dispersed AgNPs. The resulting size transition from ~150 nm to ~8 nm enabled enhanced penetration and further bactericidal effect activation.[97]

3.2 Surface charge

Surface charge is also a crucial factor affecting the penetration capacity.[23,91,92,98] Yong Liu *et al.* found that the micellar nanocarriers with surface-adaptive charge reversal properties could perform enhanced penetration while those micellar nanocarriers with a negative charge could only penetrate to a limited extent.[23] Xiaoning Li *et al.* compared the penetration capacity of quantum dots (QDs) with different surface modifications.[98] Their results demonstrated that cationic QDs could readily penetrate fully into biofilms while neutral or anionic QDs could hardly penetrate or accumulate into biofilms. Therefore, nanomaterials with a positive charge tend to have better penetration capacity.[99,100] Although cationic nanocarriers are preferred for penetration, their surfaces easily absorb proteins and form a corona, which may cause an enlarged size and hinder their penetration to biofilm. Based on the fact that poly(ethylene oxide) (PEO) with a negative charge can resist protein adhesion, Yuejing Xi *et al.* utilized PEO to construct nanocarriers to avoid the blocking of EPS in the biofilms.[101] With the further incorporation of positively charged polymer and loading of antibiotics, the as-fabricated nanoformulation could reduce dental plaque as verified in a rat periodontitis model. Depending on the different requirements in blood circulation and biofilms, biofilm microenvironment-responsive charge-reversal strategies are also often used to improve the penetration and accumulation of antimicrobials in bacterial biofilms while reducing their effects on normal tissues.[102–104] For example, AgNPs functionalized with carboxyl betaine groups presented an acidic-responsive charge reversal from negative to positive, which benefited their antibacterial effects deep in the biofilm and reduced their possible toxicity to healthy tissues.[105]

3.3 Others

Apart from altering the physicochemical properties of nanomaterials, enhanced penetration can also be achieved by exerting special physical and mechanical features of nanomaterials. Magnesium-based micromotors developed by Liangfang Zhang's group presented efficient motor propulsion under acidic conditions and were of great potential for antibiotic delivery with enhanced penetration to the bacterial biofilm.[106] Similarly, Tingting Cui *et al.* fabricated a self-propelled nanoswimmer for biofilm penetration using mesoporous silica asymmetrically functionalized with gold nanoparticles (AuNPs).[107] Upon exposure to near-infrared (NIR) light irradiation, the photothermal conversion of AuNPs triggered the thermophoretic motion of the nanoswimmers and resulted in a rapid penetration into the biofilm. Moreover, magnetic materials with the assistance of an external magnetic field can reach the deep side of biofilms.[108,109] Naside Gozde Durmus *et al.* prepared silver-conjugated superparamagnetic iron oxide nanoparticles for the eradication of antibiotic-resistant biofilms.[110] In the presence of an external magnetic field, the antibacterial effect of silver was further enhanced, which might be attributed to the improved penetration of the nanoparticles within the biofilm. Besides, as verified by Kecheng Quan *et al.*, the distribution of magnetic nanoparticles in biofilms was dependent on the exposure time of the external magnetic field.[111] They identified that there was an optimal exposure time for magnetic nanoparticles to distribute homogeneously in biofilms

rather than accumulating in their top or bottom layer. Apart from magnetic force-triggered penetration, the photochemical internalization effect has also been demonstrated beneficial for biofilm permeability.[112]

Some studies also observed that some biomaterials presented inherent biofilm penetration ability. For example, galactose can bind to LecA in the EPS and thus can be incorporated with bactericidal agents to promote their penetration.[113,114] Yiwen Zhu synthesized a series of tunable block ratio diblock copolymers PαGal$_{50}$-*b*-PGRB$_n$ that could self-assemble into micelles spontaneously (Fig. 2).[114] Galactose moieties in the polymers were used to target *Pseudomonas aeruginosa* (*P. aeruginosa*) biofilm due to the carbohydrate–protein interaction between galactose and LecA in the EPS. The penetration and dispersion of micelles in the biofilm enabled their effective photodynamic bactericidal activities inside the biofilm.[114]

4 Stimuli-responsive nanomaterials for *in situ* activation

To treat superficial bacterial infections such as diabetic wounds, a local antimicrobial delivery strategy is usually adopted to create high localized concentrations for biofilm prevention.[6,39] However, for deep-seated infectious diseases, unspecific distribution of antimicrobial agents upon systemic administration may not only cause severe side effects but also be invalid to treat the biofilm-related infection. An infection site-targeted delivery system is an alternative to decrease the unspecific distribution. Sazid Hussain *et al.*[25] used a cyclic 9-amino-acid peptide CARGGLKSC, a *Staphylococcus aureus* (*S. aureus*) targeting peptide screened *via* phage display in the *S. aureus*-induced pneumonia mice model, to modify vancomycin-loaded porous silicon nanoparticles. The as-constructed

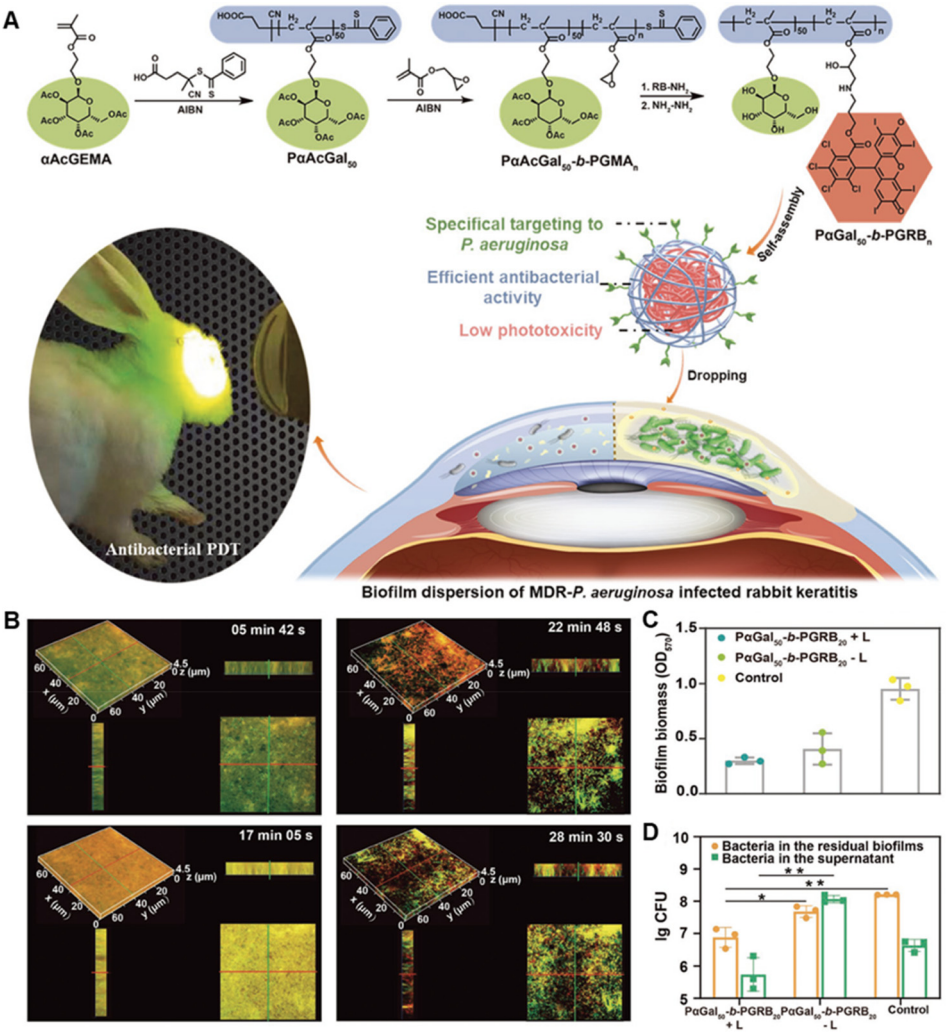

Fig. 2 (A) Schematic illustration of the synthesis of biofilm-targeted copolymers PαGal$_{50}$-*b*-PGRB$_n$ and their antibacterial PDT against *P. aeruginosa* biofilm. (B) The penetration of PαGal$_{50}$-*b*-PGRB$_{20}$ in the mature *P. aeruginosa* biofilm. (C) Biofilm eradication effect of PαGal$_{50}$-*b*-PGRB$_{20}$. (D) Bactericidal effect of PαGal$_{50}$-*b*-PGRB$_{20}$ on the biofilm bacteria. Adapted with permission from ref. 114, copyright 2022, Wiley.

antibiotic delivery system presented specific accumulation in the *S. aureus*-infected tissues and minimized systemic side effects with a reduced dose. Additionally, the targeted systems exhibited better therapeutic effects on *S. aureus*-induced pneumonia than the untargeted delivery systems or free vancomycin with equivalent doses. Moreover, due to the possible diffusion inhibition effect of the EPS in the biofilm, a higher concentration of antimicrobials is required for the bactericidal purpose.[115] Therefore, it is highly desirable to develop stimuli-responsive nanoformulations with *in situ* activation features to improve antibiofilm efficacy and eliminate systemic toxicity.[116]

4.1 Acidic microenvironment

Due to the bacterial metabolic activity, there is an inherent acidic microenvironment in the biofilm. Hence, the acidic microenvironment of a biofilm is distinguished from the normal physiological conditions, which can be utilized to develop pH-responsive drug delivery systems for *in situ* activation.[117] Benjamin Horev *et al.* developed polymeric nanocarriers to deliver the antibacterial drug farnesol to oral biofilms.[24] In the acidic microenvironment, the nanocarrier underwent core destabilization, which induced the release of farnesol *in situ* and facilitated its therapeutic effect. Some antibacterial peptides can also be assembled and fabricated into pH-responsive nanoparticles. The acidic microenvironment may cause their structural transformation and thus enable a biofilm-specific activity.[95,118] Similar strategies are also adopted for the photosensitizer delivery for selective antibacterial photodynamic therapy (PDT). For example, Lei Xia *et al.* constructed pH-responsive supramolecular-based nanoparticles through the host–guest recognition of carboxylatopillar[5]arene (CP5) and a quaternary ammonium group functionalized tetrafluorophenyl porphyrin (TFPP-QA).[119] Under acidic conditions, the supramolecule-based nanoparticles dissociated and the exposed positive TFPP-QA could disrupt the bacterial membrane. Moreover, upon light irradiation, the anchoring of TFPP-QA on the bacterial surface benefited the PDT.

However, the acidic microenvironment might quench the activity of some conventional antibiotics. In contrast, some metal ion-leaching-based antimicrobial agents, such as AgNPs and MOF, can be promoted in the acidic conditions and thus enable the *in situ* amplification of the bactericidal effect.[97,105,120] Typically, due to the structural characteristics of MOFs, antibiotics can be loaded and released along with acid-induced degradation.[121] Consequently, leaching metal ions and antibiotics can synergistically combat pathogens. Moreover, due to the ion-leaching efficiency being related to the specific surface area, smaller nanoparticles with a higher specific surface area generally exhibited accelerated ion leaching. Therefore, combined with the pH-responsive size-transition strategy, the antimicrobial effect based on metal ion-leaching can be further amplified.[97]

4.2 Other endogenous stimuli

A biofilm is a heterologous structure with steep gradients of not only pH values but also nutrients, oxygen, electron acceptors and donors, and redox conditions.[8,122] These characteristics can also be utilized as endogenous stimuli for *in situ* activation in the biofilm microenvironment (Table 2).

Due to the fast growth of bacteria and rapid oxygen consumption, bacteria at a depth in biofilms experience hypoxic conditions.[123] Based on overexpression of azoreductase by bacteria in the hypoxic biofilm microenvironment, Juan-Juan Li *et al.* synthesized lactose-modified azocalix[4]arene (LacAC4A) and fabricated supramolecular nanoformulation using LacAC4A for hypoxia-responsive antibiotic delivery.[124]

Besides, overexpression of glutathione (GSH) was also observed in the bacterial biofilm microenvironment.[125] Dengfeng Hu *et al.* conjugated GSH-sensitive α-cyclodextrin to

Table 2 Representative nanoformulations that are activated *in situ* by endogenous stimuli

Endogenous stimuli	Nanoformulations	Outcomes	Ref.
pH	Nanoassembly of chitosan–polyethylene glycol–peptide conjugate	Enhanced penetration to the biofilm. Expose the α-helical peptide at the bacterial cell membrane.	95
pH	Nanoassembly composed of AgNPs and imidazole-containing polymer	Enhanced penetration to the biofilm. Accelerated Ag^+ leaching in the biofilm.	97
pH and lipid	Peptide nanoparticles (pHly-1 NPs)	Coil-helix conformational transition for killing cariogenic bacteria.	118
pH	Supramolecular nanoformulation composed of CP5 and TFPP-QA	Release photosensitizer *in situ.*	119
pH	Au/Ag hybrid nanoparticles by coating gold nanorods with silver	Acid-triggered Ag^+ release and *in situ* photoacoustic imaging.	120
pH	ZIF-8 coated with polydopamine loading vancomycin	Release antimicrobials *in situ.*	121
Azoreductase (hypoxia)	Supramolecular nanoformulation self-assembled by lactose-modified LacAC4A	Hypoxia-responsive antibiotic delivery.	124
GSH and pH	Supramolecular nanoformulation composed of α-cyclodextrin conjugated prodrugs and pH-sensitive polypeptide copolymer	Enhanced penetration to the biofilm due to the pH-responsive charge reversal. GSH-responsive drug release. GSH consumption benefits the PDT.	102
ATP	IAA carrying ZIF-8 and HRP co-loaded in the polyacrylamide hydrogel microspheres	ATP-triggered release of IAA enabling the cascade amplification of antibacterial effect by the reaction of IAA and HRP.	127

nitric oxide (NO) and chlorin e6 (Ce6) respectively to obtain GSH-activated antibacterial prodrugs.[102] These prodrugs together with pH-sensitive polymers were then fabricated into supramolecular nanoformulations through host–guest recognition. The supramolecular nanoformulations underwent charge reversal in the acidic biofilm microenvironment and thus successfully penetrated the biofilm. Then these GSH-prodrugs were activated by the overexpressed GSH in the biofilm and released bactericidal NO and a photosensitizer. During the activation process, GSH consumption was beneficial for the Ce6-mediated PDT as well.[102]

Living bacteria can secret adenosine triphosphate (ATP) and thus the extracellular ATP is abundant in biofilms.[126] Based on the affinity of ATP with Zn^{2+}, Yuhao Weng *et al.* designed an ATP-activated platform for the co-delivery of indole-3-acetic acid (IAA) and horseradish peroxidase (HRP) to biofilms (Fig. 3).[127] IAA carrying ZIF-8 and HRP were co-loaded in polyacrylamide hydrogel microspheres. IAA was loaded to ZIF-8 that was self-assembled by Zn^{2+} and 2-methylimidazole to avoid premature interaction with HRP before arriving at the bacterial biofilm. Due to the stronger affinity between ATP and Zn^{2+} than it between Zn^{2+} and 2-methylimidazole, ZIF-8 dissociated in the biofilm and released IAA. ROS generated from the reaction of IAA and HRP damaged the bacterial membrane and triggered more ATP leakage, resulting in cascade amplification of the antibacterial effect.[127]

4.3 NIR light

Light with short wavelengths (ultraviolet or near-ultraviolet visible light) presents low penetration with high phototoxicity. As feasible non-invasive external stimuli, red light and NIR light with deep penetration capacity have been widely used in various biomedical applications, such as antitumor and infection treatment. Zhiqiang Shen *et al.* developed coumarin-based nitrogen oxide donors that could be activated by red-light irradiation through a photoredox catalysis reaction.[113] With a rational design, NIR light irradiation can also be utilized to trigger the release of antibacterial molecules in biofilms by increased temperature *via* the photothermal effect.[107,128,129] For instance, Xile Hu *et al.* fabricated glycosheets self-assembled by the galactose- and fucose-based ligands on a molybdenum disulfide thin-layer.[130] *In situ* thermal release of antibiotics triggered by NIR light together with white light-triggered ROS generation enabled the effective

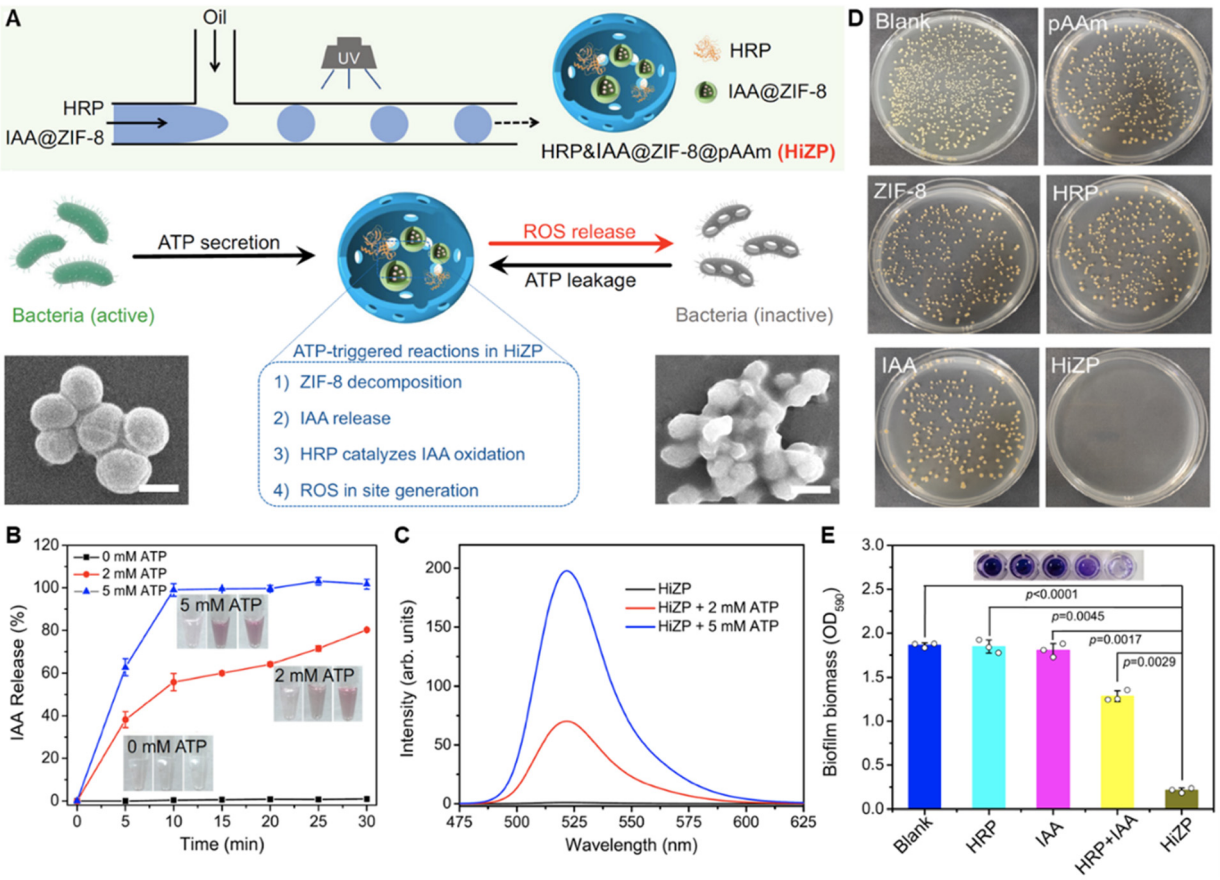

Fig. 3 (A) Schematic illustration of the construction and antibacterial mechanism of HiZP. (B) The ATP-responsive release profiles of IAA from IAA@ZIF-8. (C) ATP-responsive ROS generation of HiZP. (D) Antibacterial effect of HiZP on *S. aureus*. (E) Biofilm eradication effect of HiZP. Adapted with permission from ref. 127, copyright 2019, Springer.

therapy of multidrug-resistant bacterial infection on wounds. Besides, the photoacoustic effect can also be adopted for enhanced penetration into biofilms as well as controlled release *in situ*. As verified by Bing Cao *et al.*, NIR laser irradiation could trigger the gasification of perfluorohexane that was loaded in nanodroplets and resulted in photoacoustic cavitation and the release of loaded rifampicin in the biofilm.[131]

Apart from the NIR light-triggered antimicrobial release, nanomaterials with intrinsic NIR-activated properties are also utilized for *in situ* activation.[34,132] By incorporating photosensitizer molecules (*e.g.* IR780,[133] indocyanine green,[134] tetrafluorophenyl porphyrin,[119] Ce6,[112] Rose bengal[114]), PDT within the biofilm can occur upon NIR light application. Specifically, Gram-negative bacterial strains are more susceptible to PDT. However, PDT requires sufficient oxygen to generate destructive ROS while biofilm bacteria experience hypoxic conditions, especially for those deep in the biofilm. Insufficient oxygen may hinder the outcome of PDT. To this end, Xiaolin Sun *et al.* incorporated a MnO_2 nanolayer on the PDT nanocomposite to decompose the generated hydrogen peroxide (H_2O_2) during PDT and produce oxygen, resulting in promotion of ROS generation and improved PDT efficacy in the biofilm.[135] In addition, photothermal therapy (PTT) can also be adopted for *in situ* activation.[34,37,92,136,137] For example, Luoxiao Ran *et al.* utilized gold–silver alloy nanoparticles to carry gentamicin and wrapped them with polydopamine.[138] Upon NIR excitation, both gold–silver alloy nanoparticles and polydopamine performed photothermal effects, triggering the release of gentamicin and Ag^+ at the same time. Consequently, increased temperature (47 °C) *in situ* and released antibacterial molecules exhibited a synergistic antibiofilm effect. Specifically, some nanomaterials can simultaneously perform PDT and PTT for synergistic biofilm irradiation.[139,140]

As an external trigger, NIR light can be utilized together with both endogenous stimuli[103,104,141] and other external triggers[109] to achieve a more precise control and to promote the antibiofilm effect.

4.4 External magnetic field

Magnetic nanomaterials can be transported to the infection site upon the application of an external magnetic field. Besides, as evidenced by Aaron Elbourne *et al.*, gallium-based liquid metal droplets could transform their shape and equip themselves with sharp edges in response to a low-intensity rotating magnetic field.[142] Such magneto-induced shape transformation consequently resulted in damaged biofilm structure and ruptured bacterial cells. Among various magnetic nanoparticles, iron oxide nanoparticles (IONPs) have intrinsic peroxidase-like properties with the generation of bactericidal free radicals that can perform synergistic antibiofilm effects with magnetic field-triggered physical effects.[143,144]

Moreover, with an alternating magnetic field, local hyperthermia can be achieved by magnetic nanomaterials.[145] For instance, Jie Li *et al.* evaluated the antibiofilm effect of IONPs under different conditions and demonstrated that magnetic hyperthermia could affect the integrity of biofilm structure.[145] Similar to the photothermal effect-induced drug release, magnetic hyperthermia can trigger the release of antibacterial molecules in the biofilm as well. For instance, Jiaxing Wang *et al.* coated nitrosothiols on magnetic $CoFe_2O_4$@$MnFe_2O_4$ nanoparticles for synergistic biofilm eradication.[146] With an alternating magnetic field, magnetic hyperthermia damaged the dense structure of biofilms and enabled the penetration of nanoparticles. Moreover, thermosensitive nitrosothiols release antibacterial NO inside biofilms. The synergetic therapeutic effect was validated in implant-associated infection models.[146]

5 Nanocomposites for regulating infection microenvironment

Apart from the strategies targeting bacteria directly, manipulating the biofilm microenvironment, including destroying the EPS and inhibiting quorum sensing (QS), also represented a potential way to interfere with the life cycle of biofilms. Besides, invading pathogens can trigger the activation of innate immune reactions. Immune cells can combat bacterial infection through various pathways.[147] Therefore, regulating the immune microenvironment around the infection is a feasible approach to participate in antibiofilm.

5.1 EPS destruction

The EPS plays an essential role in maintaining the integrity of biofilms and protecting the enwrapped bacterial cells. Therapeutic strategies targeting the EPS have emerged as a topic of intense research attempts. Inhibiting the EPS has been verified as a robust antibiofilm strategy without the development of resistance.[148,149] Destroying the EPS in a mature biofilm can not only enable bacterial exposure but also promote the penetration and diffusion of antibacterial agents in the biofilm.

Some components exhibit an inherent EPS-disrupting effect and rhamnolipid is one of the typical examples. Rhamnolipid is a bacteria-secreted anionic surfactant that possesses negligible direct antibacterial effects but can impede bacterial adhesion and destroy the EPS in mature biofilms. Incorporating rhamnolipid into nanoparticles can eradicate biofilms effectively, especially for the *Helicobacter pylori* biofilm.[150,151] Some natural enzymes (*e.g.* dextranase or mutanase) can degrade the polysaccharides and thus cleave the biofilm matrix.[152] Inspired by the EPS degradation property of these natural enzymes, Anheng Wang *et al.* decorated protease alcalase on the surface of ciprofloxacin-loaded nanogels. Degraded EPS and enhanced penetration of ciprofloxacin in the depth of the biofilm could be achieved by the functionalized nanogels.[153]

It has been widely recognized that ROS benefits matrix degradation. Therefore, apart from the direct bactericidal effect, the EPS as well as the dense structure of biofilms can be destroyed simultaneously by PDT.[154] Most recently, taking lessons from natural enzymes,[155] some nanomaterials have

been found to mimic the activity of natural enzymes (oxidase, peroxidase, deoxyribonuclease), which mainly increase the oxidative stress by generating ROS within biofilms.[17,156–161] These artificial nanozymes, based on metal, metal oxide and carbon, have been explored for antibiofilm application.[143,162–166] Yanqiu Wang *et al.* proposed a strategy utilizing nanozymes with peroxidase activity with engineered bacteria generating H_2O_2 to degrade the biofilm EPS and eradicate the biofilm.[162] Using this strategy, significantly reduced bacteria number (5 log reduction), as well as biofilm matrix removal (85% reduction), could be achieved. In addition to the ROS-generating activities, more enzymatic activities of nanozymes have also been discovered and exploited. For example, DNase-mimicking activity is identified in cerium(IV) complexes, which can be utilized to hydrolyze extracellular DNA and thus disrupt established biofilms. Therefore, Zhengwei Liu *et al.* designed a series of MOF/Ce-based nanozymes to integrate the peroxidase-like activity of MOF and DNase-mimicking activity of cerium(IV) complexes for biofilm eradication (Fig. 4).[167] Interestingly, such a design could avoid the recurrence of biofilms as well.

Recently, a nanohole-boosted electron transport antibiofilm strategy rather than using simple enzymatic activities was proposed by Tonglei Shi *et al.*[168] Nanoholes with atomic vacancies in disulfide nanosheets served as electronic donors while bacterial biofilms served as electronic receptors. Boosted electron transport and redox reaction resulted in destruction of essential components in the EPS. Additionally, the proposed strategy also downregulated expression of biofilm formation-related genes and thus inhibited the EPS.[168]

5.2 QS Interference

QS is a process whereby bacteria communicate and present coordinated activities mediated by specific signaling molecules.[169,170] These molecules can sense the cell density and thus govern biofilm formation. Therefore, interfering with the QS is an effective microenvironment manipulating strategy for antibiofilm application.[62,171,172] QS inhibitors, such as acylase, lactonase, and curcumin, can degrade the extracellular QS signals and interfere with the biofilm life cycle, which requires targeted delivery to the infection site especially when there are already mature biofilms.[173,174] Aleksandra Ivanova *et al.* coated AgNPs with aminocellulose and acylase to obtain a nanoplatform for the delivery of antimicrobials and QS inhibitors deep into biofilms.[173] The efficient delivery of QS inhibitors could promote the therapeutic effect of antimicrobials with reduced minimum inhibitory concentration. Considering that natural haloperoxidase can also deactivate QS molecules and result in quorum quenching, Zijun Zhou developed cerium-based MOFs that were endowed with haloperoxidase-mimicking activity for an antibacterial effect and inhibiting the formation of antibiofilm.[175]

Apart from direct inhibition of the QS molecules, researchers also observed that modulating the biofilm microenvironment may regulate the QS. Dengfeng Hu *et al.* constructed perfluorohexane-loaded liposomes to deliver oxygen to the biofilm and tried to relieve the hypoxic conditions that were responsible for the antibiotic resistance.[176] The results turned out that such a strategy successfully retained the bacterial sensitivity to commercial antibiotics. Interestingly, the gene sequencing indicated that relieving biofilm hypoxia could restrain the QS as well, which requires further exploration.

5.3 Immune regulation

Macrophages and neutrophils are essential innate immune cells capable of defense against invading microbes. Moreover, both macrophages and neutrophils exhibited heterogeneous regulatory functions in response to various diseases and patho-

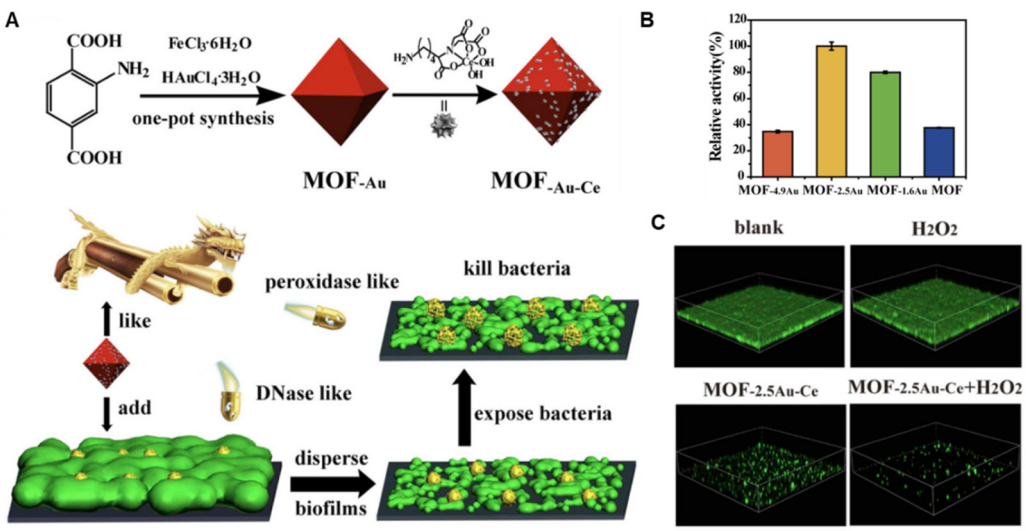

Fig. 4 (A) Schematic illustration of construction and antibiofilm mechanism of MOF_{-Au-Ce}. (B) Peroxidase-like activity of various MOFs. (C) Biofilm eradication effect of $MOF_{-2.5Au-Ce}$. Adapted with permission from ref. 167, copyright 2019, Elsevier.

logical courses. Therefore, these two cells are attractive targets and are usually paid more attention to when regulating the immune microenvironment for antibiofilm.

The polarization outcome of macrophages can be simply designated as pro-inflammatory (M1) and anti-inflammatory (M2) phenotypes. M1 macrophages can perform phagocytic and bactericidal effects while the formation of biofilms inhibits the M1 polarization. It has been reported that magnetic nanoparticles are capable of recruiting macrophages and influencing their polarization. Therefore, magnetic nanoparticles can be utilized not only for the direct antibacterial effect but also for macrophage-mediated immune therapy by promoting M1 polarization.[146] In contrast, M2 macrophages can accelerate tissue repair and benefit recovery after antibacterial management. As validated by Weijun Xiu *et al.*, after PDT-activated chemotherapy using hyaluronic acid-Ce6-metronidazole nanoparticles to treat bacterially infected wounds, an increased ratio of M2-like macrophages was observed in the microenvironment.[177] Consequently, PDT-activated chemotherapy-triggered M2 polarization promoted wound healing.

Similarly, the bactericidal function of neutrophils can also be suppressed. To eradicate biofilms and reactivate neutrophils for a synergistic effect, Wanbo Zhu *et al.* synthesized Fe_3O_4 nanoparticle dotted graphene oxide nanosheets and loaded them into a methacrylated hyaluronic acid microneedle patch.[178] Biofilm microenvironment and NIR triggered the disruption of biofilms and intracellular iron overload, which further resulted in a ferroptosis-like death. Interestingly, bac-

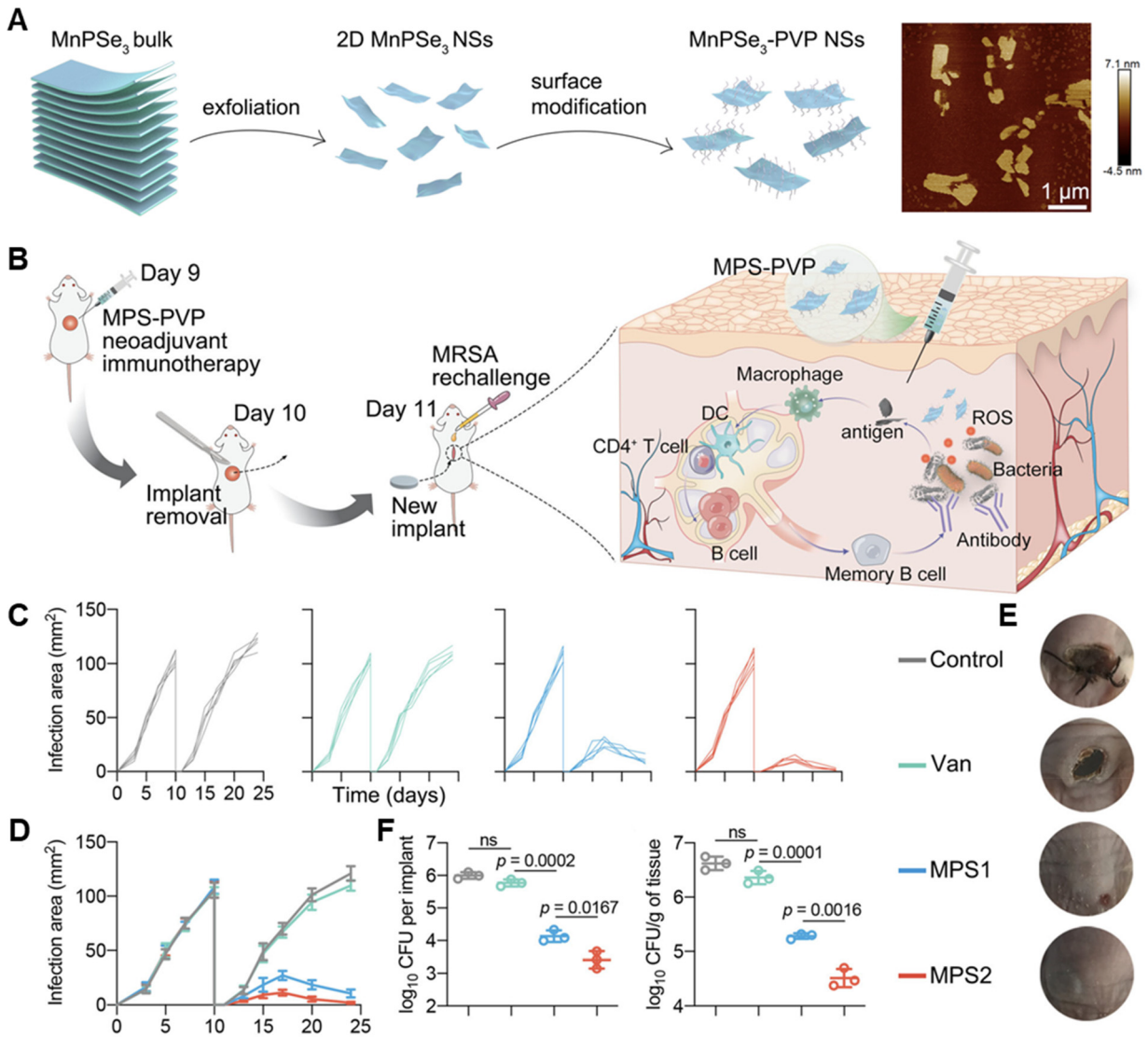

Fig. 5 (A) Schematic illustrations of the construction of MPS and its typical AFM images. (B) Schematic illustration of the experimental procedure of the presurgical neoadjuvant immunotherapy mediated by MPS-PVP. (C–E) Changes of individual infection area (C), average infection area (D) and representative pictures (E) of mice after treatments. (F) Statistical analysis of bacterial amounts in extracted implants and *peri*-implant tissues respectively. Adapted with permission from ref. 180, copyright 2022, Springer.

tericidal functions were reactivated in iron-nourished neutrophils and consequently eliminated the biofilm synergistically.

Apart from these nanocomposites integrating with antibacterial effect and immune regulation ability, some nanomaterials don't show intrinsic bactericidal functions but can significantly evoke the local innate immunity for immune antibacterial therapy.[179] For example, Chuang Yang *et al.* explored the potential of polyvinylpyrrolidone-modified manganese chalcogenophosphates nanosheets (MPS-PVP) in presurgical neoadjuvant immunotherapy to treat medical implant infections (Fig. 5).[180] MPS-PVP generated ROS that destructive the biofilm structure and led to the expression and exposure of bacterial-associated antigens. The following amplified priming of antigen-presenting cells and biofilm-infiltrating B cells enabled biofilm-specific humoral immune and memory responses. Therefore, immune-stimulatory MPS-PVP successfully mitigated residual infections and suppressed the infection recurrence after surgical removal of the infected implants.[180]

6 Conclusion and outlook

Immense progress in nanotechnology-based strategies for interfering with the biofilm life cycle has been achieved. According to the requirement of interfering with the biofilm life cycle in different stages, nanotechnology can be feasibly used to effectively inhibit initial bacterial adhesion, promote antimicrobial penetration to biofilms, boost antimicrobial activities *in situ*, and modulate the infection microenvironment.

However, there is still a long way to go for the antibiofilm exploration since a comprehensive understanding of the bacterial biofilm is still lacking.[170,181] First of all, the current understanding of the biofilm life cycle is limited and challenged by some new findings. And Karin Sauer *et al.* recently proposed a revised conceptual model of the biofilm life cycle, encompassing aggregation, growth and disaggregation independently of surfaces.[122] According to this model, strategies that combat pathogens directly rather than inhibiting bacterial adhesion are essential for infection management. Secondly, although the planktonic bacteria are considered to be more susceptible to antimicrobial agents and immune regulation, bacteria dissociated from the dispersal of biofilm are distinct from both biofilm bacteria and planktonic cells, which requires further exploration and development of more robust antibacterial strategies accordingly.[182]

In the current nanotechnology-based strategies for interfering with the biofilm life cycle, evoking immune function to antibacterial agents and antibiofilm is a rather promising future direction. The inherent interaction between nanomaterials and the immune system makes nanomaterials attractive in anti-infection immunotherapy. Moreover, the unique thermogenesis or ROS production of some nanomaterials in response to exogenous or external stimuli has been demonstrated capable of activating the immune system. If the autoimmune function can be fully utilized to treat bacterial infections, it can play a synergistic effect with antimicrobial materials. Consequently, the dosage of antimicrobial agents can be reduced, thereby reducing the exposure to antimicrobial agents and attenuating the progress of bacterial resistance to antimicrobial agents.

Despite the various antibacterial strategies, developing imaging strategies for the monitoring of bacterial infections deserves more attention. If the rapid diagnosis and identification of bacterial infection can be realized, the control of bacterial infection in the early stage can avoid the subsequent formation of bacterial biofilm. Moreover, imaging tools that are capable of indicating bacterial living mode can be used to guide the choice of strategies with rational design for interfering with the life cycle of bacterial biofilm. The diagnostic strategy based on nanotechnology has been demonstrated to be feasible in the theranostics of diverse diseases.[183–185] However, bacterial resistance to nanomaterials has been reported.[186–189] Therefore, close attention should be paid to the revolution of bacterial resistance to nanomaterials when using nanomaterials to treat or diagnose bacterial infection.

Author contributions

Jiahe Wu: conceptualization, visualization, writing – original draft, and writing – review & editing. Bo Zhang: writing – review & editing. Jianqing Gao: conceptualization, supervision, and writing – review & editing. Nengming Lin: supervision and writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

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