Recent development of nanomedicine for the treatment of bacterial biofilm infections

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Abstract
Bacterial biofilm related infections are ever growing issues for global medical community. Traditional antibiotic therapy is usually ineffective for treating them because the bacteria inside biofilms have evolved with multiple mechanisms to evade antibiotic challenge. Hence, effective therapeutic strategy with novel antibiofilm mode of action is highly desired. In this context, nanomedicine has drawn great attentions and has been proven promising to prevent and eliminate bacterial biofilms. In this review, we focus on the recent advance of nanotechnology-based strategies and nanoagents for combating bacterial biofilm infections. First, typical antibiofilm nanotechnologies utilized different chemical, physical, and biological properties of nanomaterials are discussed. Second, smart nanoagents that can responsive to biofilm microenvironment, including pH, H2O2, and enzymes, are shown. Third, some promising antibiofilm approaches, such as theranostics, biofilm structure destruction, and quorum sensing inhibition, are also demonstrated. Finally, we conclude the current antibiofilm nanotechnologies and discuss the challenges and future directions in this field.

KEYWORDS
antibiofilm, bacterial biofilms, EPS, nanomedicine, responsive
1 INTRODUCTION

In nature, bacteria usually exist as the form of biofilms. During the formation of biofilms, bacteria adhere to the inert or biological surfaces, and enclose themselves within the self-produced extracellular polymeric substances (EPS), including exopolysaccharides, proteins, extracellular DNA (eDNA), and so on.1,2 Owing to the protection of EPS, bacteria inside biofilms not only have the ability to withstand the host innate immune system, but also greatly improve their resistance to antibiotics, compared with their planktonic forms.3,4 Previous studies have indicated that about 60-70% of bacterial infections are associated with the formation of biofilms, which usually causes recalcitrant chronic infections, such as cystic fibrosis pneumonia, endocarditis osteomyelitis, implant-related infections, chronic wounds, dental caries, periodontitis, chronic otitis media, musculoskeletal infections, and so on.5–8 Besides the threat to human health, biofilm infections also cause heavy financial burden to the society. For example, >80 billion dollars are annually spent to fulfill the healthcare cost for treating oral biofilm related diseases in the United States.9

As an important survival strategy, bacterial biofilms have several underlying mechanisms to meet the challenge of environmental stress, which make them hard to be completely eradicated. First, the EPS of biofilms not only provide physical barrier to inhibit the penetration of antibiotics, but also inactivate the antibiotics by enzymatic decomposition or adsorption, which help the microbes in biofilms to gain antibiotic resistance up to 1000 times to the corresponding planktonic bacteria.10,11 Besides, the spatially organized EPS provide a strong fortress for bacteria inside and protect them against the attack of immune cells.12,13 Second, the heterogeneous physiological states inside biofilms can induce the bacteria to take different metabolic pathways compared with their planktonic forms, which enable the bacteria inside to escape from metabolic interference by antibiotics and cause the regrowth of biofilms after treatment.1,13–16 Therefore, due to the complex physical, chemical, and biological properties of biofilms, significant challenges have been posed for the traditional therapeutics to defeat biofilm-associated infections.12,14,17 It is crucial to develop innovative antibiofilm technology for combating bacterial biofilms.

Nanomedicine has shown great promise for the diagnosis and therapy of various diseases.18,19 Due to their small size, high specific surface area, unique physical properties, high chemical activity, and flexibility in material engineering, nanomaterials have been extensively exploited for the control of bacterial biofilm infections.20–22 In view of this, nanomaterial-based antibiofilm strategy has received great attention over the past decade. Typically, drug delivery nanocarriers have been designed to control the release of antimicrobials into the biofilm-infected tissues to enhance the availability and decrease the adverse side-effects of antibiotics.23 Besides, photothermal nanomaterials could locally generate heat under light irradiation and thermally ablate bacteria for photothermal therapy (PTT) of biofilm infections.24–26 In addition, some photosensitive nanoagents could produce toxic reactive oxygen species (ROS) and irreversibly damage the cell components of bacteria for photodynamic therapy (PDT) of biofilm infections.27,28 Furthermore, some nanomaterials with enzyme-like catalytic activity could also kill bacteria and disrupt the matrix of biofilm through the catalytic generation of ROS.29–32 These antibiofilm nanotechnologies, as above mentioned, have several advantages, such as high efficiency, low possibility for resistance, high controllability, noninvasiveness, and short time. Based on the antibiofilm nanotechnology, more and more intelligent nanoagents with multiple functionalities have been ingeniously designed for targeting the microenvironment and extracellular matrix of biofilms, aimed not only for the kill of the bacteria in biofilms.6,29,33,34 Therefore, the antibiofilm nanotechnology has become unprecedentedly prosperous for developing new strategies and nanoagents to treat bacterial biofilm infections.

In this review, we will summarize recent efforts in the development of nanomedicine for the treatment of bacterial biofilm infections (Scheme 1). Typical antibiofilm technologies by utilizing the intrinsic properties of...
TABLE 1 Typical antibiofilm approaches based on nanotechnology

| Approach    | Mechanism of action                                                                 | Advantage                                                                                                         | Shortage                                                                 |
|-------------|--------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|
| DDS         | Utilize nanomaterials as unique vehicles to load and deliver the drugs to bacterial biofilms | Improve the penetration and accumulation of drugs in biofilms, enhance the therapeutic efficacy and reduce the side-effect | Limited therapeutic efficacy to kill the bacteria with high antibiotic resistance |
| PTT         | Use nanomaterials with light-heat conversion property to ablate bacteria under laser irradiation | Efficient for various bacterial biofilms, high controllability of the therapeutic parameters, no common drug-resistance problems | Lateral damage of healthy tissues, not feasible for the biofilm infections deeply in the body |
| PDT         | ROS generation by nano-photosensitizers and oxidative damage of vital cellular components of bacteria | General treatment for various bacterial biofilms, high controllability, easy to operate, difficult for bacteria to develop resistance | Limited penetration of tissues due to the relative short light wavelength, possible phototoxicity to normal skin tissues |
| Catalytic therapy | Using nanocatalysts to generate ROS for killing bacteria and destroying the biofilm structure | Damage the EPS of biofilms and weaken the resistance of bacteria in biofilms to environmental stress | Low therapeutic efficacy without the addition of H$_2$O$_2$, lack of targeting ability to bacterial biofilms |

nanomaterials are first shown in brief. Then, the importance of biofilm microenvironment for the development of intelligent stimuli-responsive nanoagents is discussed. Additionally, promising strategies about the integration of diagnostic function into the therapeutics, targeting the chemical structure and biological signaling of biofilms are also mentioned. Lastly, we conclude the current developed antibiofilm nanotechnologies and the challenges remained in this field. The aim of this review is to give a relatively comprehensive demonstration of the promising nanomedicine for the treatment of bacterial biofilm infections with the emphasis of newly proposed and promising therapeutic strategies.

2 | NANOTECHNOLOGY FOR THE TREATMENT OF BIOFILMS

As an alternative to traditional antibiotic treatment, nanotechnology-based therapeutic strategy has shown great potential for combating bacterial biofilms and has drawn ever growing interest from various research fields. Nanomaterials with different chemical, physical, and biological properties have been exploited to prevent and treat bacterial biofilms by diverse mechanisms. Until now, many innovative antibiofilm nanomedicine approaches have been developed. For example, nanomaterials with large specific surface area and unique size effect have been used as drug delivery systems (DDS) to modulate the pharmacokinetics and efficacy of antibiotics; the photo-physical property of nanomaterials has been utilized for photothermal therapy (PTT) of biofilms by localized generation of hyperthermia; the photochemical property of nanomaterials to produce reactive oxygen species (ROS) has been adopted for photodynamic therapy (PDT) of biofilms; nanomaterials with high catalytic property have been exploited as enzyme-mimics to eradicate bacteria biofilms by generating reactive ROS (catalytic therapy). In this section, therapeutic nanotechnologies for combating bacterial biofilms are summarized, including DDS, PTT, PDT, and catalytic therapy, and the versatility of the nanoagents is discussed. Typical therapeutic approaches for bacterial biofilms based on nanotechnology are listed and compared in Table 1.

2.1 | Drug delivery systems

Currently, antibiotic therapy is the main clinical choice for the treatment of bacterial biofilm infections. However, due to the existence of unique niche in biofilms, bacteria have gained multiple weapons to resist antibiotics, such as limiting the penetration of antimicrobials, inactivation of drugs by extracellular enzymes or metabolic acids, adsorption antibiotics by biofilm components, metabolic change of the bacteria, and so on. The greatly enhanced resistance makes the bacteria in biofilms highly amenable to traditional treatment. The use of antibiotics with conventional dosage usually cannot kill all the bacteria in biofilms, which will result in relapsing of the infections after treatment. On the other hand, long-term use of antibiotics may further induce the issue of extreme resistance.

By using DDS, antimicrobial agents can be effectively delivered to the site of bacterial biofilm infections (Table 2). DDS not only enhance the solubility and biodistribution of antimicrobial agents, but also improve
TABLE 2

| Nanocarrier                  | Drug             | Application          | Reference |
|-----------------------------|------------------|----------------------|-----------|
| Liposomes                   | Triclosan        | Antibiofilm in vitro | 40        |
| PLGA NPs                    | Ciprofloxacin    | Antibiofilm in vitro | 46        |
| p(DMAEMA)-b-p(DMAEMA-co-BMA-co-PAA) NPs | Farnesol       | Carious lesion       | 95        |
| PCL-b-P(Lys-stat-Phe) / PEO-b-PCL NPs | Ciprofloxacin | Periodontitis        | 47        |
| AZM-DA NPs                  | Azithromycin     | Chronic lung infection | 121        |
| TPGS-PLGA NPs               | Azithromycin     | Lung infection       | 122        |
| MSNs                        | Ofloxacin/melittin | Implant-related infection | 48        |
| MOF-MSN NPs                 | Carbenicillin    | Subcutaneous abscess | 123        |
| MoS₂ NSs                    | Tetracycline     | Antibiofilm in vitro | 49        |
| Iron oxide NPs              | Tobramycin       | Antibiofilm in vitro | 50        |

Abbreviations: PLGA NPs, poly(lactic-co-glycolic acid) nanoparticles; AZM-DA NPs, azithromycin-conjugated amino-ended poly(amidoamine) dendrimer and 2,3-dimethyl maleic anhydride modified poly(ethylene glycol)-block-polylysine; TPGS, D-α-tocopheryl polyethylene glycol succinate; MSNs, supramolecular assemblies of heterogeneous mesoporous silica nanoparticles; MOF-MSN, metal-organic framework-coated mesoporous silica nanoparticles.

Their utilization and therapeutic efficacy. Currently, liposomes, polymers, and inorganic nanomaterials are commonly used as nanocarriers of DDS. Liposomes-based DDS have been exploited for the treatment of bacterial biofilms for a long time. In 2001, Robinson et al. used triclosan-loaded liposomes to treat oral biofilms. They demonstrated that the adsorption and efficiency of liposomal bactericide to bacterial biofilms are affected by bacterial species and strains. Alhajlan and co-workers found that liposomal formulations of clarithromycin reduced the minimum inhibitory concentration (MIC) of *Pseudomonas aeruginosa* (*P. aeruginosa*) from 256 μg/mL to 8 μg/mL, suggesting greatly improved antibacterial efficacy. Moreover, clarithromycin-loaded liposomes not only significantly reduced the bacterial growth in the biofilms by 3–4 log, but also showed lower cytotoxicity than the free clarithromycin.

Besides liposomes, polymer nanoparticles (NPs) have also been adopted as carriers for drug delivery. Among them, poly(lactic-co-glycolic acid) (PLGA) is a typical material used to construct DDS, due to its biocompatibility and biodegradability. Cheow et al. reported that PLGA-lipid hybrid NPs loaded with fluoroquinolone can inhibit the formation of *P. aeruginosa* biofilms. Although lipid coating did not improve the adsorbability of the NPs against the biofilms, the PLGA-lipid hybrid NPs still showed much improved inhibition of the biofilms. Taka-hashi and co-workers found that the antibiofilm activity of clarithromycin can be improved by using chitosan-functionalized PLGA as nanocarrier.

Baelo et al. prepared ciprofloxacin-loaded PLGA NPs functionalized with deoxyribonuclease (DNase) I, which can prevent the formation of *P. aeruginosa* biofilms and eradicate >99.9% of the established biofilms after repeated administration for 3 days. Xi et al. prepared dual-corona vesicles by using two block copolymers (PCL-b-P(Lys-stat-Phe) and PEO-b-PCL). In this design, PEO can provide stealthy effect by protein repelling and P(Lys-stat-Phe) has intrinsic antibacterial activity due to its positive charge (Figure 1A). These dual-corona vesicles loaded with ciprofloxacin hydrochloride (CIP) can eradicate both *E. coli* and *S. aureus* biofilms with 50% reduction of the normal dosage of CIP in vitro (Figure 1B), and can effectively disrupt the plaque biofilms in rat periodontitis model (Figure 1C).

Besides the organic nanocarriers, inorganic nanomaterials with high surface to volume ratios, such as mesoporous nanoparticles and ultrathin nanosheets, have also shown promise for drug delivery. Nanomaterials with different compositions have been exploited as nanocarriers, including silica, metals, and metal compounds. For example, Yu and co-workers used mesoporous silica nanoparticles (MSN) to co-deliver antibiotics and antimicrobial peptide for the synergistic eradication of pathogenic biofilms (Figure 1D). Two kinds of MSNs, one conjugated with β-cyclodextrin and the other coated by adamantane with a magnetic core can self-assemble into superstructures by host-guest interactions, which can load both ofloxacin and melittin. The release of payloads in the MSNs can be triggered by the stimuli of bacteria and alternating magnetic field induced heating. Compared with free drugs, MSNs loaded with drugs exhibited much higher antibiofilm effect for killing the bacteria inside biofilms and reducing the biofilm biomass (Figure 1E,F), while their cytotoxicity to mammalian cells is neglectable. Owing to their intrinsic ultrahigh surface to mass ratio, MoS₂ nanosheets (NSs) were surface functionalized with biocompatible chitosan and used to deliver tetracycline. MoS₂ NSs-tetracycline nanocomposites showed synergetic antibacterial effect with reduced MIC values and enhanced uptake of antibiotics against both Gram-positive and Gram-negative drug resistant...
FIGURE 1  (A) Preparation of CIP-loaded dual corona vesicles and their application for the treatment of periodontitis. (B) Confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) images of bacterial biofilms after different treatments. (C) Photographs of the plaque model and SEM images of the biofilms on tooth after different treatments. Copyright 2019, American Chemical Society. (D) Supramolecular assemblies of heterogeneous MSNs to co-deliver antibiotics and the antimicrobial peptide for the eradication of biofilms. (E) In vitro antibiofilm activity and (F) in vivo treating implant-related infections of the MSNs. Copyright 2020, American Chemical Society
bacteria. In addition, MoS₂ NSs-tetracycline nanocomposites not only inhibited over 80% of the bacterial biofilm formation, but also efficiently damaged the structure of biofilms.

### 2.2 Photothermal therapy

Photothermal therapy (PTT) is a promising non-invasive therapeutic method by using photothermal agents to generate hyperthermia under laser irradiation. During the photothermal transformation, PTT agents can absorb the incident photons and relax non-radiatively, which can efficiently elevate the temperature of the surrounding environment and destroy the structure of cells. Previous studies demonstrated that the structure of bacterial cell walls can be destroyed at about 45-60°C and the integrity of bacterial biofilms can be disrupted at higher temperature. Hence, locally produced heat during PTT process can both kill bacteria inside biofilms and damage the bacterial biofilms, which is generally effective for various bacterial biofilms. Moreover, the parameters of PTT, such as irradiation location, power density, duration time, and so on, can be facilely controlled, which remarkably facilitates the practical use of PTT. Until now, various photothermal nanoagents have been developed for the treatment of bacterial biofilms by PTT (Table 3).

Pallavicini et al adopted gold nanostars (GNS) as photothermal agents for biofilm eradication under near infrared (NIR) light irradiation. GNS lowered the number of viable *Staphylococcus aureus* (*S. aureus*) in biofilms by two orders of magnitude under laser irradiation at low power density (808 nm, 0.09 W/cm²), compared with control group. Hsiao et al prepared N-(mercaptopropyl sulfonyl acid-substituted self-doped polyaniline)-chitosan (NMPA-CS) nanogels and utilized their photothermal property to treat methicillin-resistant *Staphylococcus aureus* (MRSA) subcutaneous abscesses of mice. Results demonstrated that NMPA-CS increased the temperature of the infected tissues to 55°C after 808 nm laser irradiation and kill 80% MRSA in vivo with obviously promoted recovery of the infected tissues.

Yuwen et al prepared MoS₂@polydopamine-Ag NSs (MPPINSs) for photothermal eradication of bacterial biofilms (Figure 2D). Compared with MoS₂ NSs without antibody functionalization, MPPINSs achieved about 4 log higher antibacterial efficiency both for in vitro *S. aureus* biofilms and in vivo focal bacterial infections after NIR laser irradiation (Figure 2E, F), suggesting the importance of shortening the distance between the nanoagents and the bacteria to improve the antibiofilm effect of PTT. Cao and co-workers constructed mesoporous silica supported silver-bismuth nanoparticles (Ag-Bi@SiO₂ NPs) as NIR light responsive photothermal nanoagents for the treatment of MRSA biofilm infections. Bi NPs in Ag-Bi@SiO₂ NPs can thermally disrupt the cell integrity of bacteria and enhance the release of silver ions to achieve excellent synergistic antibacterial effect. Mature MRSA biofilms were effectively obliterated with 69.5% reduction of the biofilm biomass by using Ag-Bi@SiO₂ NPs under NIR laser irradiation. In vivo results indicated that Ag-Bi@SiO₂ NPs killed 95.4% of MRSA in abscesses and significantly improved the recovery of the infected tissues.

Although PTT has been proven an effective strategy to treat bacterial biofilm infections, NIR-I light (650-950 nm)
is commonly used as the light source. In comparison, NIR-II light (1000-1700 nm) has higher skin-tolerance threshold, and deeper tissue penetration, which means better therapeutic efficacy of PTT. Therefore, PTT in NIR-II region is promising for efficient treatment of bacterial biofilm infections.

2.3  Photodynamic therapy

Photodynamic therapy (PDT) is an efficient method for the treatment of tumors and bacterial infections. During the PDT process, photosensitizers can absorb photons and transform from ground state (PS) to excited
state (PS*), which will further transfer the excited energy to O2 or other substrates to form highly toxic ROS, such as singlet oxygen. After that, the oxidative ROS react with various molecules in cells and damage their structures and functions, which can trigger cellular apoptosis or necrosis. (Figure 3A).69,71,72 PDT can kill various pathogens by oxidative damage of the vital biomolecules and membrane of bacteria (Table 4).70 Compared with antibiotic treatment, PDT is much difficult for bacteria to develop resistance, which can greatly improve the inactivation efficiency of drug-resistant bacteria.

Shen et al studied the antibacterial performance of g-C3N4 for the treatment of Staphyloccoccus epidermidis (S. epidermidis) biofilms.76 Under visible light irradiation, g-C3N4 can prevent the formation of biofilms and eradicate the mature biofilms. ROS quantification assays revealed that 1O2 rather than another ROS (O2•−, H2O2, ·OH, and holes) plays the most important role for the inhibition of biofilms.

To further improve the PDT efficacy for bacterial biofilm treatment, other therapeutic modalities have also been integrated into the nanoagents. Ma et al designed a
TABLE 4  PDT nanoagents for the treatment of bacterial biofilms

| Nanoagent         | Light irradiation | Application                  | Reference |
|-------------------|-------------------|------------------------------|-----------|
| g-C₃N₄           | White light, 4.4 mW/cm², 6 h | Anti-biofilm in vitro       | 127       |
| Ce6&CO@FADP       | 665 nm, 11 mW/cm², 8 min | Subcutaneous abscess        | 75        |
| α-CD-Ce6-NO-DA    | 660 nm, 0.2 W/cm², 1 min   | Subcutaneous infection       | 128       |
| MOF dots@MnO₂-HSA | Visible light, 300 W, 20 min | Subcutaneous abscess       | 104       |
| Ag/g-C₃N₄        | Visible light, 300 W, 3 h | Anti-biofilm in vitro       | 129       |
| CMC NPs-MB       | 650 nm, 0.2 W/cm², 5 min   | Subcutaneous abscess        | 111       |
| Ti-RP-IR780-RGDC  | 808 nm, 2 W/cm², 10 min    | Bone implant infection      | 74        |
| DNase-AuNCs      | 808 nm, 2 W/cm², 10 min    | Oral biofilms               | 76        |
| ZPMAVP           | 630 nm, 0.2 W/cm², 5 min   | Bacterial endophthalmitis    | 73        |

Abbreviations: g-C₃N₄, graphitic carbon nitride; Ce6&CO@FADP, chlorin e6 conjugated with CO@fluorinated amphiphilic dendritic peptide; α-CD-Ce6-NO-DA, α-cyclodextrin conjugated with nitric oxide prodrug, Ce6, and block peptide copolymer; MOF dots@MnO₂-HSA, porphyrin-based metal organic framework encapsulated by human serum albumin and manganese dioxide; CMC NPs-MB, methylbenzene blue loaded carboxymethyl chitosan nanoparticles; Ti-RP-IR780-RGDC, titanium bone implants coated by red phosphorus, IR780, and peptide; DNase-AuNCs, deoxyribonuclease functionalized gold nanoclusters; ZPMAVP, ZIF-8-PAA-MB@AgNPs@Van-PEG.

therapeutic nanoplatform (Ce6&CO@FADP) by combining PDT and CO delivery. This multifunctional nanoagent contains both photosensitizer Ce6 and CO source CORM-401. During PDT process, CO can be rapidly released with singlet oxygen, which presents remarkable synergistic antibiofilm performance. Chen et al prepared ZPMAVP as a PDT/chemotherapy dual-function nanoagent for the treatment of endophthalmitis (Figure 3B). Under light irradiation, methylbenzene blue (MB) acts as photosensitiser to produce ROS, while the silver nanoparticles (AgNPs) release toxic silver ions at the same time to synergistically enhance the inhibition of bacterial biofilms (Figure 3C, D). Since the convenience for light operation to eyes, this phototherapy has the promise for ophthalmic diseases. Tan et al prepared red phosphorus, IR780, and peptide (RGDC) functionalized titanium bone implants (Ti-RP-IR780-RGDC) for antibiofilm application (Figure 3E). This design integrates the ROS generation ability of red phosphorus and the photothermal effect of IR780, which provides a novel therapeutic method with the combination of PDT and PTT. This dual-mode therapy presents an effective and rapid approach for the treatment of implant-related biofilm infections (Figure 3F). Xie et al prepared DNase-decorated AuNCs (DNase-AuNCs) as multifunctional therapeutic nanoagents with both PDT/PTT and eDNA degradation capabilities. The introduction of DNase can facilitate the hydrolysis of the eDNA in biofilm matrix and the destruction of biofilm structure. Under NIR laser irradiation, DNase-AuNCs can disperse up to 80% of the biofilm and kill ~90% of the bacteria inside biofilms. Additionally, DNase-AuNCs can effectively eradicate the multidrug-resistant (MDR) S. aureus and MDR P. aeruginosa biofilms formed on the orthodontic devices, indicating their potential use for medical devices.

2.4 | Catalytic therapy

ROS are important weapons for the immune system against microorganisms, which can be generated by the NADPH oxidase in the innate immune cells. ROS can inactivate bacteria by irreversible damaging the proteins, DNA, and polysaccharides. Importantly, ROS can inhibit the formation of bacterial biofilms and even break down the structure of mature biofilms, which is not easy to achieve by other therapeutic methods.

With the development of nanomedicine, various catalytic nanomaterials have been employed for the treatment of bacterial biofilms by ROS generation (Table 5). Since the discovery of the peroxidase-like property of Fe₃O₄ NPs in 2007, many nanomaterials with different compositions and structures have been found with enzyme-like catalytic activity, which are also named nanozymes. For example, nanomaterials with oxidase (OXD)-like and peroxidase (POD)-like activities can catalyze the generation of H₂O₂ and •OH, respectively. Nanozymes can efficiently generate ROS under mild biological environments, which is similar to that of natural enzymes in immune cells. Thus, nanozymes have been extensively studied for catalytic therapy of bacterial infections (Figure 4A).

Sun et al reported that graphene quantum dots (GQDs) have POD-like activity and high antibacterial activity. GQDs can convert H₂O₂ into •OH to improve the antibacterial effect against both S. aureus and E. coli. In addition, GQDs can inhibit the formation of biofilms and destroy the formed biofilms in the presence of low concentration of H₂O₂. GQD-Band-Aids showed excellent antibacterial performance for mice wound infections. Wang et al prepared AuNPs-graphitic carbon nitride (Au/g-C₃N₄) nanohybrids for the treatment of bacterial biofilms and
**TABLE 5** Nanozymes for combating bacterial biofilms by catalytic therapy

| Nanozyme         | Enzyme mimicry | Application                  | Reference |
|------------------|----------------|------------------------------|-----------|
| GQDs             | POD            | Wound infection              | 89        |
| MSN-AuNPs        | OXD/POD        | Biofilm prevention and destruction | 130    |
| Au/g-C3N4 nanohybrids | POD           | Wound and lung infections    | 82        |
| Fe3O4 NPs        | POD            | Dental caries                | 91        |
| Ferumoxytol NPs  | POD            | Tooth decay                  | 92        |
| Fe3O4 NPs        | POD            | Tooth decay                  | 114       |
| Dex-NZM          | POD            | Oral biofilms                | 51        |
| nFeS             | POD            | Oral biofilms and wound infection | 32        |
| CeO2 nanorods    | Haloperoxidase | Biofouling prevention        | 131       |
| DMAE             | DNase          | Biofilm matrix cleavage      | 112       |
| MOF/Ce           | DNase/POD      | Subcutaneous abscess         | 23        |

Abbreviations: POD, peroxidase; OXD, oxidase; GQDs, graphene quantum dots; MSN-AuNPs, mesoporous silica nanoparticles coated by AuNPs; Dex-NZM, dextran-coated Fe3O4 NPs; nFeS, nano-iron sulfides; DMAE, DNase-mimetic artificial enzyme; MOF/Ce, cerium (IV) complexes functionalized metal organic frameworks.

wound disinfection (Figure 4B,C).82 Au/g-C3N4 nanozyme with peroxidase-activity showed efficient destruction of the existing antibiotic-resistant *S. aureus* biofilms in vitro at low concentration (20 mg/mL) with the presence of H2O2 at bio-safety levels (10 μM). Moreover, in vivo results further indicated that Au/g-C3N4 nanozyme could effectively treat MRSA wound infections and lung infections with low inflammation and accelerated recovery.

Gao et al demonstrated the utilization of Fe3O4 NPs with POD-like activity for the destruction of the EPS in biofilms.90 Typical components of EPS, such as proteins, nucleic acids, and polysaccharides, can be oxidatively cleaved by H2O2 with the catalytic Fe3O4 NPs. Besides the bacteria killing ability, Fe3O4 NPs can also prevent and destroy the bacterial biofilms. Due to their biocompatibility and versatility, Fe3O4 NPs were further applied to treat oral biofilms.51,91,92 Gao and co-workers showed that Fe3O4 NPs (0.5 mg/mL) can reduce the number of live *Streptococcus mutans* (*S. mutans*) in biofilms by more than 5 log and degrade the biofilm matrix with 1% H2O2 in 5 min, indicating the effectiveness and rapidness of catalytic therapy.91 The onset and severity of dental caries in vivo can also be significantly reduced by Fe3O4 NPs after short time treatment (1 min). Later, Liu et al revealed that ferumoxytol NPs with pH-dependent catalytic activity can disrupt *S. mutans* biofilms and prevent tooth decay.92 The generation of •OH from H2O2 by ferumoxytol NPs can be facilitated in acidic oral biofilm microenvironment, which causes bacterial death by damaging cell membranes and EPS. Results of the experiments using different biofilm models proved that ferumoxytol NPs can efficiently prevent and treat biofilm-induced oral diseases. Since ferumoxytol has been approved by the FDA of the United States to treat iron deficiency, its clinical translation for the treatment of dental caries is promising.

Though Fe3O4 NPs are effective antibiofilm nanoagents, the lack of targeting ability of Fe3O4 NPs still largely limits their use. Naha et al prepared dextran-coated Fe3O4 NPs (Dex-NZM) that can selectively target biofilms for the treatment of tooth decay (Figure 4D,E).51 As a polysaccharide derived from microorganisms, dextran can be incorporated into the biofilm matrix by *S. mutans*-derived glucosyltransferases. Thus, the selectivity of Fe3O4 NPs for biofilm binding is improved by dextran coating, while their binding for gingival cells is reduced. Dex-NZM not only can efficiently target *S. mutans* biofilms and degrade the EPS via catalytic activation of H2O2 under pathological acidic conditions, but also can effectively treat the oral biofilm-associated infections without obviously adverse effects on the surrounding host tissues and oral microbiota diversity.

Besides, many other nanozymes with different components and multiple functions have also been designed and studied for combating bacterial biofilms. Liu et al demonstrated that MOF/Ce-based nanoagents with dual enzyme-like properties can both disrupt biofilms and kill the bacteria inside.23 The cerium (IV) complexes with DNase mimic activity can hydrolyze eDNA and disrupt the established biofilms, while the MOF with peroxidase-like activity can kill bacteria with the combination of H2O2. In vivo antibiofilm study further exhibited that MOF/Ce can kill bacteria and promote wound healing against bacteria-induced subcutaneous abscess. Xu et al prepared nanosized iron sulfides (nFeS) by the transformation of natural organosulfur compounds to inorganic polysulfides. nFeS have excellent antibacterial efficiency compared to the organosulfur, which originates from the POD-like catalytic
property of nFeS and the enhanced release of bactericidal hydrogen polysulfanes. In vivo results showed that the *S. mutans* biofilms on human teeth and *P. aeruginosa* infected wounds can be effectively treated by nFeS.

### 3 MICROENVIRONMENT RESPONSIVE ANTIBIOFILM NANOAGENTS

During the formation of biofilms, bacteria can generate and develop unique microenvironment, which provides opportunity to design effective therapeutic nanoagents with biofilm-targeting and stimuli-responsive capabilities. First, EPS act as the barrier between the bacteria and surrounding environments, which not only limits the oxygen to enter the deep layer of biofilms, but also hinders the diffusion of the acidic metabolites of bacteria. Moreover, the immune cells usually gather around the bacterial biofilms, consume the oxygen, and release ROS, such as H$_2$O$_2$. In addition, many types of enzymes can be produced by the bacteria in biofilms, which are important for the biofilm formation and dispersal. Hence, the biofilm microenvironment...
composed of distinct chemical and biological characteristics, including pH, hypoxia, H$_2$O$_2$, enzymes, and so on, which can be used as internal stimuli for targeted and triggered therapy. Various therapeutic nanoagents with different responsive types have been designed and studied for the treatment of bacterial biofilms and the typical examples are summarized as follows (Table 6).

3.1 pH-responsive therapeutic nanoagents

Since the diffusion of acidic metabolites produced by bacteria in biofilms is usually hindered by the EPS matrix, the pH in biofilm microenvironment is usually lower than 7, and can be even lower in certain situations. This distinct pH facilitates the chemical or physical change of nanoagents, which can be utilized to specifically target the bacterial biofilms and trigger the release of payloads. pH-responsive therapeutic nanoagents with different functions, such as drug delivery, PTT, and PDT, have been developed.

Various pH-sensitive drug delivery nanocarriers with acid-responsive decomposition or reversible protonation ability have been designed for acid-triggered delivery of drugs. Among them, charge switchable polymer ligands, such as poly(β-amino ester), have shown great promise. Liu et al prepared mixed-shell-polymeric micelles (MSPMs) modified with hydrophilic PEG and poly(β-amino ester) (Figure 5A). MSPMs have negative surface charges in physiological conditions (neutral) and switch to positive charges in acidic conditions (acidic), which allows MSPMs to effectively penetrate and accumulate in S. aureus biofilms owing to the negative charge of bacterial surface (Figure 5B). Once attached to the bacteria surface, the micelles release the drugs in biofilms, which significantly improves the killing effect of the bacteria inside biofilms.

Hu et al designed zwitterion-modified gold nanoparticles (AuNP-N-C) by using both weak electrolytic 11-mercaptoundecanoic acid (HS-C$_{10}$-COOH) and strong electrolytic (10-mercaptodecyl)trimethylammoniumbromide (HS-C$_{10}$-N$_4$) as ligands. AuNP-N-C with mixed charges showed quickly transition from negative charges in neutral conditions (pH 7.4) to positive charges in acidic conditions (pH 5.5), which enable AuNPs dispersed well in healthy tissues and aggregated in MRSA infected tissues. The aggregation of AuNPs significantly enhanced the light absorption in NIR region due to plasmon resonance coupling effect, which subsequently enhanced the therapeutic efficacy of MRSA biofilms by PTT and reduced the side effects at the same time.

Horev et al prepared therapeutic nanoagents using the diblock copolymer p(DMAEMA)-b-p(DMAEMA-co-BMA-co-PAA), which can self-assemble into nanoparticles with size about 21 nm in water. As a hydrophobic antibacterial agent, farnesol was loaded inside the hydrophobic core of p(DMAEMA-co-BMA-co-PAA) in the polymer NPs. In acidic biofilm microenvironment, DMAEMA and PAA could be protonated, which results in the decomposition of the polymer NPs and triggers the release of farnesol. Besides, this polymer NPs could bind to the EPS with high affinity owing to the cationic coronas, which enhances the permeation of the farnesol into the interior of bacterial biofilms (Figure 5C). Compared with free farnesol, the farnesol-loaded polymer NPs showed fourfold antibacterial efficiency and over twofold enhancement in biofilm removal from saliva-coated hydroxyapatite surfaces for the treatment of S. mutans biofilms (Figure 5D,E).

3.2 Enzyme-responsive therapeutic nanoagents

Bacterial biofilm matrix contains numbers of extracellular enzymes, which may help bacteria to gain nutrients, assist...
Thus, the enzymes in biofilm microenvironment are ideal targets for the design of stimuli-responsive therapeutic nanoagents. Hyaluronidase (Hyal) is widely overexpressed by diverse kinds of bacteria, which has been utilized as a bacteria-relevant stimulus to trigger the release of antimicrobials. Thus, HA is a reasonable choice to construct Hyal-responsive nanoagents. Ji et al prepared HA coated sandwich-like graphene-mesoporous silica nanosheets (GS), and then loaded ferromagnetic nanoparticles (MNPs) as catalyst and ascorbic acid (AA) as prodrug to prepare AA@GS@HA-MNPs. In bacterial biofilms, HA can be degraded by Hyal and release the dispersal of biofilms, or serve as virulence factors.

FIGURE 5  (A) Antimicrobials encapsulated in MSPM NPs with stealthy effect and improved penetration in bacterial biofilms. (B) The CLSM images of MSPM NPs incubated with S. aureus biofilms at different pH conditions. Copyright 2016, American Chemical Society. (C) Scheme of the pH-responsive polymer NPs loaded with farnesol for the prevention and treatment of bacterial biofilms. (D) The number of colony forming units and (E) biofilm removal efficiency after different treatments. Copyright 2015, American Chemical Society.
the encapsulated AA. During the oxidation of AA by MNPs, detrimental •OH forms, effectively decomposes the biofilm structure, and inactivates the embedded bacteria (Figure 6A). Owing to the photothermal effect of graphene, AA@GS@HA-MNPs could achieve chemophotothermal synergistic antibacterial activity against both Gram-positive (S. aureus) and Gram-negative (E. coli) bacteria under laser irradiation. For S. aureus biofilms, AA@GS@HA-MNPs could markedly destroy the structure of biofilm and cause the reduction of biofilm biomass by >80% (Figure 6B,C). In vivo experiments showed that the contamination of biofilm on the surface of implanted catheter has been eliminated after treatment by AA@GS@HA-MNPs. The histological analysis also indicated that no live bacteria and signs of infection remained in the infected tissues after treatment.

Liu et al constructed enzyme-responsive multifunctional therapeutic nanoagent (AA@Ru@HA-MoS2) by functionalized AA-loaded mesoporous Ru NPs with HA, MoS2, and ciprofloxacin (CIP) (Figure 6D).102 In biofilms, HA can be decomposed by the Hyal secreted from bacteria with the release of AA, which is further catalytically oxidized by MoS2 to generate bactericidal •OH. Meanwhile, Ru NPs can also be used as photothermal agents for synergistic chemo-photothermal therapy. The treatment by AA@Ru@HA-MoS2 with NIR laser irradiation showed high bacteria inactivation efficiency and better wound healing for both Gram-positive (S. aureus) and Gram-negative (P. aeruginosa) bacteria infected wounds from mice (Figure 6E-G).

3.3 | H2O2-responsive therapeutic nanoagents

Biofilm infections usually accompany the infiltration of polymorphonuclear leukocytes (PMNs) around the pathogenic tissues, which induces the production of high level H2O2.94,103 Additionally, the EPS in biofilms also cause hypoxic conditions, which may restrict the efficacy of oxygen-dependent therapies, including antibiotics and PDT. The high level of H2O2 inside biofilm-infected tissues could be used as an oxygen source to relieve hypoxia.

Deng et al used Hf(IV)-based porphyrin to prepare metal organic framework (pMOF) dots, then encapsulated them by human serum albumin (HSA) and MnO2 as multicomponent nanoplatforms (MMNPs) to treat bacterial biofilms (Figure 7A).104 MMNPs can gradually degrade in acidic biofilm microenvironment, owing to the reaction of MnO2 with H2O2 and H+, and release O2 to facilitate the formation of 1O2 during PDT (Figure 7B). Treatment by MMNPs combined with both H2O2 and light showed high antibacterial efficiency for both Gram-negative E. coli (99%) and Gram-positive S. aureus (90%) (Figure 7C-E). For in vitro S. aureus biofilms, the pMOF dots exhibited high penetration due to the MMNPs degradation in acidic microenvironment with the presence of H2O2 (Figure 7F,G). The crystal violet staining assay indicated that biofilms treated by MMNPs with H2O2 and light showed almost completely disrupted structure (Figure 7H,I). For animal experiments, the infected mice treated by MMNPs with light showed outstanding bacteria inactivation efficiency and improved tissues healing (Figure 7J-L).

4 | PROMISING ANTIBIOFILM STRATEGY

The biological, chemical, and physical underpinning of the biofilm formation is complicated, which has posed great challenges during the development of effective antibiofilm strategy.1,14,15 Along with the advances in the understanding of the biofilms, more potential therapeutic targets have been realized for the prevention and control of bacterial biofilms.23,81,105 On the other hand, nanomaterials have great flexibility in controlling the composition, morphology, surface, and size, which makes them versatile platforms to integrate with other functional components. Therefore, the convergence of the advanced biology and nanotechnology has sparked the development of innovative antibiofilm strategy. For example, diagnostic modality can be introduced to the therapeutic nanoagents to form theranostic nanoagents; specific enzymes can be integrated into the therapeutic nanoagents to overcome the EPS barrier in biofilms; quorum sensing inhibitors can be loaded in nanoagents to reduce the virulence of biofilms.

4.1 | Thera nostic nanoagents for bacterial biofilms

Theranostics combines both diagnostic and therapeutic modalities in one entity, which could provide the pathological information to guide the treatment at the same time. Compared with single-mode agents for diagnosis or treatment, theranostic agents could overcome undesirable differences in biodistribution and selectivity.106–108 Therefore, theranostic agents are attractive for effective diagnosis and therapy of bacterial biofilm infections at one time.

Ding et al used Au@Ag NPs for simultaneous imaging and treatment of bacterial biofilms.109 Au@Ag NPs have strong two-photon photoluminescence (2PPL) property at aggregated condition. In S. aureus biofilms, positively charged Au@Ag NPs aggregated on the negatively charged bacterial surface, with significantly enhanced 2PPL, which
FIGURE 6  (A) Preparation of AA@GS@HA-MNPs and their application for the treatment of bacterial biofilms. (B) Therapeutic efficacy of AA@GS@HA-MNPs for *S. aureus* biofilms. (C) Crystal violet staining and live (green)/dead (red) staining images of *S. aureus* biofilms after different treatments. Copyright 2016, Wiley-VCH. (D) Preparation of AA@Ru@HA-MoS₂ and their use for combating bacterial biofilms. (E) Photographs of bacterial culture from the skin of bacteria infected mice after treatment. (F) Photographs of bacteria infected wounds and (G) the wound size of mice after different treatments. Copyright 2019, American Chemical Society
was successfully used for optical imaging of *S. aureus* biofilms. After aggregated in *S. aureus* biofilms, Au@Ag NPs also displayed excellent photo-induced antibacterial activity under laser irradiation and effectively eradicated the biofilms in vitro.

Xiu et al designed MnO$_2$-based theranostic nanoagents for simultaneous detection and therapy of biofilm infections in vivo.$^{110}$ In this work, MnO$_2$ NSs were modified with bovine serum albumin (BSA), PEG, and Ce6 to form MnO$_2$-BSA/PEG-Ce6 nanosheets (MBP-Ce6 NSs, Figure 8A). In acidic biofilm microenvironment, MBP-Ce6 NSs could decompose and release Mn$^{2+}$ with Ce6, following activated fluorescence (FL) and magnetic resonance (MR) signals for FL/MR dual-mode imaging of infected tissues (Figure 8B-E). Meanwhile, the overexpressed H$_2$O$_2$ in biofilm infected tissues could be catalyzed by MnO$_2$ to generate O$_2$. The biofilm-infected tissues show obvious hypoxia relief after the treatment by MBP-Ce6 NSs (Figure 8F). The in vivo bacteria inactivation efficiency is about 2.5 log (99.7%) and the recovery of infected tissues is significantly promoted after the treatment by MBP-Ce6 NSs with laser irradiation (Figure 8G).

Wang et al synthesized carboxymethyl chitosan nanoparticles (CMC) combined with MB for simultaneous imaging and eradicating of bacterial biofilms.$^{111}$ In acidic biofilm microenvironment, pH-responsive CMC-MB NPs can release MB for in situ fluorescent imaging of *S. aureus* biofilms (Figure 8H). After irradiated by a 650 nm laser (202 mW/cm$^2$, 5 min), the CMC-MB NPs showed efficient biofilm eradication capability and notable wound healing of subcutaneous *S. aureus* infection in rabbit model (Figure 8I).

### 4.2 EPS destruction for combating bacterial biofilms

Previous study revealed that EPS not only provide shelter for bacteria, but also act as adhesion sites for the bacterial colonization, which has been recognized as an important factor for the recurrence of the stubborn biofilm-related infections.$^{5,33}$ Hence, destruction of the EPS is important for completely eradicate bacterial biofilms, which not only facilitates the penetration of antibiotics, but also prevents the attachment of planktonic bacteria to form new biofilms.$^1$ Recently, innovative nanomedicine adopted this strategy has been used to enhance the treatment of biofilms by physical or chemical routes.
FIGURE 8  (A) Preparation and theranostic application of MBP-Ce6 NSs. (B) Fluorescent images and (C) fluorescent intensity of biofilm infected mice after treatment with MBP-Ce6 NSs. (D) MR images and (E) MR signal intensity of the infected tissues after treatment. (F) Immunofluorescence analysis and (G) photographs of the infected tissues after treatment.110 Copyright 2020, American Association for the Advancement of Science. (H) CLSM images of *S. aureus* stained without or with CMC-MB NPs. (I) Photographs and hematoxylin-eosin (H&E) staining sections of the infected tissues after treatment.111 Copyright 2019, American Chemical Society

Enzyme-mimic nanomaterials could destroy the EPS structure through chemical ways by broken down the chemical bonds of the main components, such as exopolysaccharides, proteins, and nucleic acids. Chen et al designed DNase-mimetic artificial enzymes (DMAE) for eliminating biofilms.112 DMAE were fabricated by physically confining Au NPs on the surface of Fe₃O₄/SiO₂ NPs and followed by the assembly of Ce(IV)-nitrilotriacetic acid (NTA) complex on the exposed surface of Au NPs to form cooperative catalytic centers (Figure 9A). DMAE
have nuclease-mimic property, which can accelerate the hydrolysis of eDNA in biofilm matrix and cause the disintegrating of biofilms. After the DMAE treatment, mature $S. \textit{aureus}$ biofilms showed distinctly reduction of biofilm biomass during long time (120 h), while the natural enzyme (DNase I) only showed 24 h inhibition of biofilm formation (Figure 9B,C).

Besides the chemical route to remove EPS, Teirlinck et al proved that the laser induced vapour nanobubbles (VNBs) formed around Au NPs can locally disturb the biofilm matrix and facilitate the penetration of antibiotics. The bacterial biofilms were first treated with Au NPs and irradiated by high-intensity and ultra-short laser pulse (1.69 J/cm$^2$, 7 ns). After laser energy absorbed by the Au NPs, their temperature can simultaneously increase by several hundred degrees and heat the surrounding water to evaporate. Subsequently, the water VNBs implode to puff the biofilm matrix and expand the space between sessile bacteria, which substantially increases the penetration of antibiotics. As demonstrated, the combination of VNBs and tobramycin treatment showed greatly enhanced bacterial inactivation efficiency by about
80 times for *Burkholderia multivorans* (*B. multivorans*) biofilms, 20 times for *P. aeruginos*a biofilms, and 25 times for *S. aureus* biofilms, compared with sole tobramycin treatment.

Mechanical removal of the biofilm by water jets is a direct and simple method for the destruction of bacterial biofilms, which has been utilized in the clinical treatment of dental biofilms.⁴⁴ Although this method is effective, its application is limited to the biofilms in the open body cavity. Magnetic NPs (eg, Fe₃O₄ NPs) can be manipulated by the external magnetic fields with controlled movement, which can be used as robots to deliver/release drug cargos at desired place and destroy the structure of biofilms at the same time. Hwang et al designed catalytic antimicrobial robots (CARs) for precise, efficient, and controllable killing, degrading, and removing of biofilms.¹¹⁴ In this work, two kinds of CARs were constructed by using Fe₃O₄ NPs as both magnetic active materials and peroxidase-like catalyst (Figure 9D,E). For removing the biofilm on the accessible surface, the biohybrid type CARs were formed under magnetic field. When Fe₃O₄ NPs were incubated in *S. mutans* biofilms with H₂O₂, they can generate highly oxidative ·OH for bacteria killing and EPS degradation. Then, the biohybrid CARs were precisely manipulated using a magnetic field gradient to completely remove the biofilms, including dead bacteria and degraded EPS (Figure 9F,G). CARs of the other type were prepared by embedding Fe₃O₄ NPs into gels with specific shapes in 3D-printed model. The polymeric soft robots can be used to treat biofilms on biotic (teeth) and abiotic (catheter or implant) surfaces. 3D-modeled CARs with helicoid shape could effectively disrupt and remove the biofilms in the isthmus of tooth canal (Figure 9H,I).

### 4.3 Quorum sensing inhibition for bacterial biofilm control

Quorum sensing (QS) plays an important role in cell-to-cell communication between bacteria, which can control the formation and dispersal of biofilms.¹⁵⁻¹⁵ Also, QS influences the generation of antibiotic resistance and the activation of virulence pathways of bacteria inside biofilms.¹⁶⁻¹⁷ Therefore, the inhibition of QS has attracted great attention for the bacterial biofilm control.

For this purpose, quorum sensing inhibitors (QSI)-containing nanomaterials have been exploited to interfere the QS of bacterial biofilms. Sun et al loaded both the QSI (luteolin, L) and antibiotics (ampicillin, A) into graphitic hollow carbon nitride nanospheres (HCNS/A&L), which were further capped by HA to form HCNS/A&L@HA. In bacterial biofilms, HA can be decomposed by Hyal to release luteolin for QS inhibition and ampicillin for bacteria killing.¹¹⁸ After light irradiation, HCNS could generate ROS and further eliminate the residual bacteria for synergistic treatment of biofilm infections (Figure 10A). *S. aureus* in biofilms treated by HCNS/A&L@HA with light irradiation could be effectively inactivated and the biofilm structure can also be destructed (Figure 10B). In periprosthetic infected (PPI) mice model, those after HCNS/A&L@HA treatment showed obvious scab formation with complete healed tissues at implantation sites (Figure 10C).

Lehr et al designed multifunctional nanoagents by co-loading QSI and antibiotics (tobramycin, Tob) for the treatment of pulmonary *P. aeruginosa* infections.¹¹⁹ Self-assembled squalenyl hydrogen sulfate were used as nanocarrier to load Tob and the potent pqsR inverse agonistic QSI to form Tob&QSI-SqNPs (Figure 10D). Compared with the treatment by Tob alone, Tob&QSI-SqNPs showed improved penetration inside *P. aeruginosa* biofilms and significantly enhanced therapeutic efficacy in relevant biological barriers (mucin/human tracheal mucus, biofilm), with 16-fold lower Tob concentration for the complete eradication of *P. aeruginosa* biofilms (Figure 10E,F).

### 5 SUMMARY AND FUTURE OUTLOOK

During the long process of evolution, bacteria have gained capability to protect themselves by forming biofilms with biomacromolecules. After the formation of biofilms, bacteria not only can evade the hunting of immune cells, but also become more resistant to antibiotics, which make it a great challenge to treat the bacterial biofilms formed in human body. Even worse, the discovery of novel antibiotics has greatly slowed down during last decades, while the number of drug-resistant bacteria strains continually increases. In the “post-antibiotic era,” the threat of bacterial biofilm infections has motivated the development of novel antibiofilm strategy.

Due to the rapid progress of nanomedicine in past decade, nanotechnology-based strategies, such as drug delivery, PTT, PDT, catalytic therapy, and so on, have shown great promise for the treatment of biofilm infections. First, nanomaterials can encapsulate, deliver, and release the antimicrobial agents to the biofilms, which can significantly improve their accumulation in biofilms, increase their local concentration, and decrease their systematic toxicity. Second, the rich physical and chemical properties of nanomaterials have provided great opportunity for the development of novel therapeutic approaches with completely different mechanisms to combat bacterial biofilms compared with antibiotics.²³,⁸¹,¹²⁰ For example, liposomes containing antibiotics can deliver drugs to
biofilms and enhance their therapeutic efficacy; Au nanostructures can transform the light energy into localized hyperthermia for the physical killing of bacteria; photo-active nanomaterials can generate bactericidal ROS under light irradiation; nanozymes could catalytically inactivate bacteria by ROS. Hence, nanotechnology has provided great feasibility for the development of effective therapeutic strategies with unique mechanisms, which poses high difficulty for the bacteria to evolve resistance. Second, the versatility to engineer nanomaterials with different sizes, surface charges, compositions, and other properties, also have great practical significance for the development of multifunctional therapeutic nanoagents. For instance, the immune cells in human body can response to the invasion of pathogens, accumulate at the infected sites, and recognize and attack the bacteria. To mimic these processes, researchers have prepared various targeting nanoagents by utilizing the unique biofilm microenvironment with acidic pH, high H$_2$O$_2$ level, enzymes, and so on. In this context, intelligent
stimuli-responsive nanoagents have been exploited, which further enhanced the specificity and efficacy of the treatment. Third, as the most flexible and versatile strategy, nanomedicine can evolve with the state-of-art concepts from biology, medicine, chemistry, and material science, which can be expected to develop novel therapeutic strategy for the treatment of bacterial biofilm infections. For example, with the rapid progress of precise medicine in cancer, the success of theranostics has inspired us to develop nanoagents with both therapeutic and diagnostic modalities. Along with the comprehensive understanding of the biology for bacterial biofilms, EPS and QS have been regarded as curial factors contributing to the virulence and resistance of bacterial biofilms. Nanoagents with EPS destruction and QS inhibition functions may address these insurmountable obstacles for traditional medicine.

Despite great potential of the antibiotic nanomedicine, challenges still exist before its successful translation in clinic. Most currently studied antibiotic approaches remain tenuous, and few of them are available for clinical use. To achieve this aim, great efforts should be devoted to use clinically relevant models to evaluate the efficacy of the strategies, thoroughly study the biosafety of the nanoagents, comprehensively understand the nanomaterial-biofilm interactions, develop effective routes to control the quality of nanoagents during scalable production, and reduce the complexity and cost of the fabrication. And we can expect that greater achievement will be made through the close cooperation of scientists from diverse fields, including biology, chemistry, material science, and medicine.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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