



Stimuli-responsive nanocarriers for bacterial biofilm treatment

Meng Ding, Wei Zhao, Ling-Jie Song*^{ORCID}, Shi-Fang Luan*^{ORCID}

Received: 30 April 2021 / Revised: 17 May 2021 / Accepted: 19 May 2021 / Published online: 4 August 2021
© Youke Publishing Co., Ltd. 2021

Abstract Bacterial biofilm infections have been threatening the human's life and health globally for a long time because they typically cause chronic and persistent infections. Traditional antibiotic therapies can hardly eradicate biofilms in many cases, as biofilms always form a robust fortress for pathogens inside, inhibiting the penetration of drugs. To address the issues, many novel drug carriers emerged as promising strategies for biofilm treatment. Among them, stimuli-responsive nanocarriers have attracted much attentions for their intriguing physicochemical properties, such as tunable size, shape and surface chemistry, especially smart drug release characteristic. Based on the microenvironmental difference between biofilm infection sites and normal tissue, many stimuli, such as bacterial

products accumulating in biofilms (enzymes, glutathione, etc.), lower pH and higher H₂O₂ levels, have been employed and proved in favor of “on-demand” drug release for biofilm elimination. Additionally, external stimuli including light, heat, microwave and magnetic fields are also able to control the drug releasing behavior artificially. In this review, we summarized recent advances in stimuli-responsive nanocarriers for combating biofilm infections, and mainly, focusing on the different stimuli that trigger the drug release.

Keywords Nanocarriers; Stimuli responsive; Bactericidal; Biofilm eradication; Drug release

Meng Ding and Wei Zhao have contributed equally to this work.

M. Ding, W. Zhao, L.-J. Song*, S.-F. Luan*
State Key Laboratory of Polymer Physics and Chemistry,
Changchun Institute of Applied Chemistry, Chinese of
Academy, Changchun 130022, China
e-mail: songlj@ciac.ac.cn

S.-F. Luan
e-mail: sfluan@ciac.ac.cn

M. Ding
College of Applied Chemistry and Engineering, University of
Science and Technology of China, Hefei 230026, China

W. Zhao
School of Chemistry and Chemical Engineering, Yantai
University, Yantai 264005, China

S.-F. Luan
National Engineering Laboratory of Medical
Implantable Devices, Key Laboratory for Medical
Implantable Devices of Shandong Province, WEGO Holding
Company Limited, Weihai 264210, China

1 Introduction

Biofilms, the microbial aggregations enclosed in slippery and sticky extracellular matrix, represent the most common growth mode of bacteria in nature [1]. Generally, biofilms are formed as follows: bacteria adhere to suitable biotic or abiotic surfaces first and then secrete extracellular polymer substances (EPS, containing polysaccharides, proteins and DNA, etc.) to wrap themselves, forming a heterogeneous three-dimensional biofilm structure [2]. The formation of biofilms established the protection for bacteria against antibiotics and host immune attack. Consequently, bacteria inside biofilms are 10–1000-fold more resistant to antibiotics than their planktonic counterparts, and these pathogens cause about 80% infections clinically, including notorious cystic fibrosis, native valve endocarditis, otitis media, periodontitis, implant relevant infections, etc. [3–5]. The traditional therapies for biofilm infections have focused on indwelling medical devices, using physical–



mechanical approaches to disrupt biofilms or surface-coating/eluting substrates releasing antibiotics to prevent bacteria adhesion [6]. However, most therapies have entailed conventional antibiotics for high concentration and long incubation, which would lead to long and expensive treatment and would easily increase the risk of adverse outcomes, such as high toxicity and drug resistance [6–8]. Thus, it is of great necessity to develop innovative therapeutics for biofilm infections.

Nanomaterials arise as promising tools to combat biofilm infections in recent years, which can act as therapeutics themselves or as drug delivery systems carrying various antibiotics or bactericidal agents to biofilms [9–12]. The flexible tunability in their size, shape, surface hydrophobicity/hydrophilicity and charge facilitates the penetration into biofilms and circulation safely in bloodstream. For example, the nanoscale size corresponds to the dimensions of water-filled channels in biofilm structures, and the negatively charged extracellular matrix has high affinity with positively charged materials. Also, the high surface-to-volume ratios of nanomaterials benefit modification of specific functional groups for practical applications, such as targeting to infection sites and releasing drugs upon stimulations [9, 13]. Choosing right nanocarriers with proper interior characteristics enables the loading of desired drugs and thus removes the barriers in their physiological applications [14]. Moreover, the smart responsiveness, namely the stimuli responsiveness, endows nanocarriers with the ability to release drugs on demand, which enhances the accumulation of bactericidal agents in biofilms sites, lowers the toxicity toward surrounding tissue and thus improves the drug efficiency [15, 16]. Because of the rapid advances in nanotechnology, it is not difficult to construct stimuli-responsive nanocarriers for effectively combating biofilms infections.

It has been fully validated that the microenvironment of biofilms is markedly different from the normal tissue around the infection sites [17]. First, the extracellular and intracellular substances produced by bacteria and accumulated in the biofilms are able to act as triggers of responsive nanocarriers. For example, specific enzymes such as lipase, hyaluronidase (HAS), glutamyl endonuclease are suitable to activate the responses due to their enzymolysis [15, 16, 18]. According to the different substrate characteristics of enzyme degradation, nanocarriers with corresponding structures can be built to satisfy the need of practical applications. Besides, glutathione (GSH), the most abundant thiols in many bacterial cells (ranges from 0.1 to 10.0 mmol·L⁻¹), can also stimulate the conversion of nanocarriers to activated states or [19]. The additional advantage of using GSH as stimulus is that the consumption of this protective substance can increase the sensitivity of bacteria to other damages, thus enhancing the

bactericidal effects. Second, other substances with high concentration or easy to be converted into stimuli are also popular in designing responsive nanocarriers. H₂O₂ in biofilm is also abundant, which is well known as a host antibacterial chemical, but recently proved to come from bacteria as well [20, 21]. This metabolic byproduct of aerobic bacteria will be decomposed into water and oxygen under specific nanocarriers' catalysis, to improve the efficiency of photodynamic therapy (PDT) [22]. Moreover, it is usually hypoxic in the interior of biofilms because outer layers consumed oxygen preferentially, which compels the inner layer cells to ferment and produce acidic metabolic products [16]. The acidic metabolic products from all bacteria would make the microenvironmental pH lower than 7.4, both inside and around biofilms [23, 24]. Various pH-responsive drug delivery nanosystems can be established based on this characteristic, such as charge switchable and acid degradable nanocarriers. Finally, many external stimuli provide the strategy to construct responsive drug delivery nanosystems, such as light, heat, microwaves and magnetic field [25]. These artificially controllable stimuli offer a drastically different perspective to design nanocarriers.

In this review, we summarized recent advances in stimuli-responsive drug nanocarriers used for biofilm treatment and focused on the construction of responsive nanocarriers and their responses to various triggers. There are tremendous numbers of relative works in this field, and only a few representative ones were introduced here. The dual- or multi-responsive nanocarriers are very prevailing, so some nanocarriers can be classified into two or more categories, but they would be introduced once in this review to avoid repetition.

2 Nanocarriers responsive to bacterial products

2.1 Enzyme-responsive nanocarriers

2.1.1 HAS-responsive nanocarriers

Bacterial HAS are classes of enzymes that degrade predominantly hyaluronic acid (HA) and also capable to degrade chondroitin and chondroitin sulfates [26]. HAS is mainly produced by gram-positive bacteria to initiate their infections on host skin or mucosal surfaces [27]. For gram-negative bacteria, HAS is often periplasmic rather than extracellular, so they are less likely to function in pathogenesis [27]. In the design of HAS-responsive nanocarriers, HA is often used as capping agent to guard the drugs inside.

Using HA to coat graphene-mesoporous silica nanosheet (GS), Qu et al. constructed a targeted “on-demand” drug

delivery nanosystem to treat bacterial infections [28]. Ascorbic acid (AA), a non-toxic prodrug of H_2O_2 , and HA-dopamine conjugates (HA-DA) were encapsulated in GS, followed by modification of the GS surface by peroxidase-like ferromagnetic nanoparticles (MNPs, Fe_3O_4) loading vancomycin (Van) (Fig. 1a). The nanocarriers AA@GS@HA-MNPs were capable to target bacteria and accumulate at infection sites, where the HA-DA was decomposed by bacterial HAS and led the AA out. Under catalysis of MNPs, AA generated hydroxyl radical ($\cdot\text{OH}$) to damage biofilm components and bacteria structures without inducing drug resistance. Meanwhile, the graphene in GS had excellent photothermal properties, also contributing to the elimination of biofilms.

Later, Liu et al. [29] constructed a similar HAS-responsive nanosystem, using mesoporous Ru nanoparticles (Ru NPs) to load AA and act as photothermal agents and then coated NPs with HA. The ciprofloxacin-loading MoS_2 with peroxide-like ability was further immobilized on the surface, which would catalyze AA to $\cdot\text{OH}$ after drug release triggered by HAS. The constituted AA@Ru@HA- MoS_2 for chemo-photothermal therapy showed remarkable bactericidal properties. Compared with AA@GS@HA-MNPs, AA@Ru@HA- MoS_2 exhibited broader antibacterial activity toward multi-drug-resistant bacterial infections.

HAS responsiveness can also be integrated with other responsiveness to obtain dual- or multi-responsive nanocarrier. For example, Xing et al. used MNPs as cores and coated them with multilayer films containing antibiotic gentamicin (Gen), tannic acid and silver nanoparticles and finally capped with HA as responsive shells [30]. Under the

acidic microenvironment of biofilm with overexpressed HAS, Gen and Ag^+ were rapidly released from the nanocarriers MNPs@Ag@HA, exhibiting broad-spectrum antibacterial capability. Intriguingly, this work employed magnetism rather than catalytic properties of MNP to guide the MNPs@Ag@HA penetration within the biofilm upon magnetic field navigation, which greatly facilitated the biofilm elimination (Fig. 1b).

2.1.2 Lipase-responsive nanocarriers

Lipases are enzymes able to catalyze the hydrolysis and synthesis of esters, whose major substrates are carboxyl ester bonds in acylglycerols. They are widely distributed throughout bacteria, and some pathogenic bacteria secrete lipases during colonization in hosts, such as *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) [31, 32]. As a result, the extracellular lipases are often used as triggers of nanocarriers containing carboxyl ester bonds.

The combination of pH and lipase responsiveness is very prevalent in this field. For example, Shi et al. reported mixed-shell-polymeric-micelles (MSPM) composed of hydrophilic poly(ethylene glycol) (PEG) and lipase-degradable, pH-responsive poly(β -amino ester) (PAE) [33]. After entering acidic pathological sites, the PAE block was protonated and switched into positive, and thus enhanced the accumulation of nanocarriers, as well as the penetration of Triclosan, a hydrophobic antibiotic loaded in PAE cores. Once the micelles reached the internal of biofilm, the lipase secreted by bacteria decomposed the PAE cores and released the antibiotics loaded. This method for

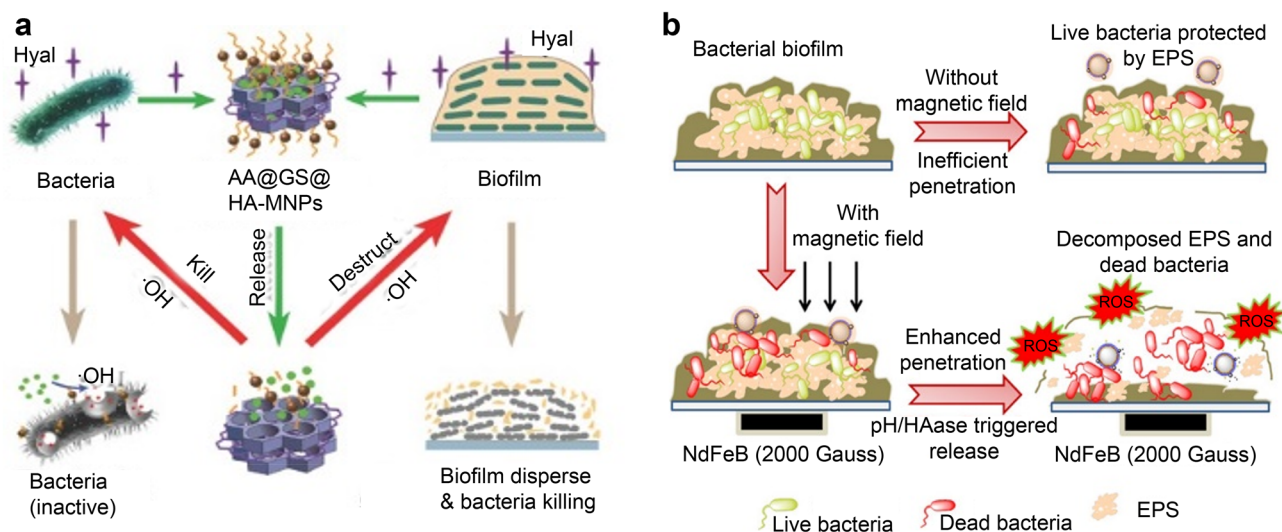


Fig. 1 a Schematic illustration of AA@GS@HA-MNPs and its response to enzymes. Reproduced with permission from Ref. [26]. Copyright 2016, Wiley. b MNPs@Ag@HA penetrate into biofilms and release drugs. Reproduced with permission from Ref. [28]. Copyright 2018, American Chemical Society

drug delivery and release provided novel ideas for improving commercial hydrophobic antibiotics therapies (Fig. 2a). Later, they further applied this strategy in the treatment of oral biofilms, using PEG-PAE with ester-linked Triclosan to build up self-assembled micelles [34]. Similarly, the micelles turned positive after entering the acidic microenvironment of biofilm, and the Triclosan was released under the catalyzation of lipase (Fig. 2b). The antibiofilm efficiency was fully demonstrated in a multi-drug-resistant *S. aureus* infection mice model and multi-species oral biofilms collected from orthodontic patients. The comprehensive research showed a promising prospective of this stimuli-responsive nanocarriers for clinical application.

Similar idea was embodied in the work of Li et al., who designed size/surface charge adaptive micelles for drug delivery [35]. Antibiotic azithromycin was conjugated with block copolymers composed of PEG and poly (aspartamide) (PAsp), which self-assembled into micelles with positively charged surfaces. Subsequently, *cis*-aconityl-D-tyrosine (CA-Tyr) was anchored on the surface via electrostatic interaction, ultimately endowing nanocarriers DOEAz@Tyr with negative charges. In the acidic biofilms, the *cis*-aconityl linker was broken down and D-Tyr was released to disperse biofilm matrix first, leaving the positive charged micelles. The micelles further shrunk into smaller size to penetrate into biofilm better, where the azithromycin was released through lipase degradation. The antibiofilm efficiency was demonstrated both *in vitro* and *in vivo* using a catheter implantation model. More significantly, the pharmacokinetics of free drugs and micelles in rats were investigated, and the results indicated that DOEAz@Tyr had longer blood circulation time than free azithromycin, due to the enhanced accumulation at infection sites.

Cationic polymers with antibacterial property have also been proved promising for combating pathogenic bacteria, which could be further combined with enzyme-responsive polymers for preparation of many intriguing polymer nanomaterials [36, 37]. By using poly (ϵ -caprolactone (PCL) and quaternary as corresponding enzyme degradable shell and bactericidal core, a series of reverse polymer micelles (RMs) were synthesized by Xu et al. [38]. The PCL blocks in RMs were efficiently hydrolyzed in the presence of bacterial lipases, leading to the release of quaternary biocidal agents (QBAs). To exploit their application, the RMs were impregnated into commercial gelatin sponge (GS) (Fig. 3). Owing to the difference in bacterial outer membrane, the RM-coated GS showed better antibacterial properties toward gram-positive *S. aureus* and *Bacillus subtilis* than gram-negative *P. aeruginosa*.

2.1.3 Other enzyme-responsive nanocarriers

Except the common HAS and lipases, other bacteria-secreted certain enzymes can also act as stimuli.

Gelatinases are extracellular proteases produced by bacteria, which are able to hydrolyze or decompose gelatin and are often associated with pathogenesis [39]. According to this, gelatinase-sensitive gelatin nanoparticles (GNPs) encapsulating chloramphenicol (CAM) were loaded into the matrix of microneedles patches, which was designed to treat *Vibrio vulnificus* biofilms [40]. During use process, the microneedles initially penetrated EPS and disrupt biofilm physically, and then the GNPs were degraded by gelatinase to leak CAM out (Fig. 4a). With this interesting microscopic structure to deliver antibiotic, the therapeutic performance of chloramphenicol was notably improved, and the off-target toxicity was reduced at the same time.

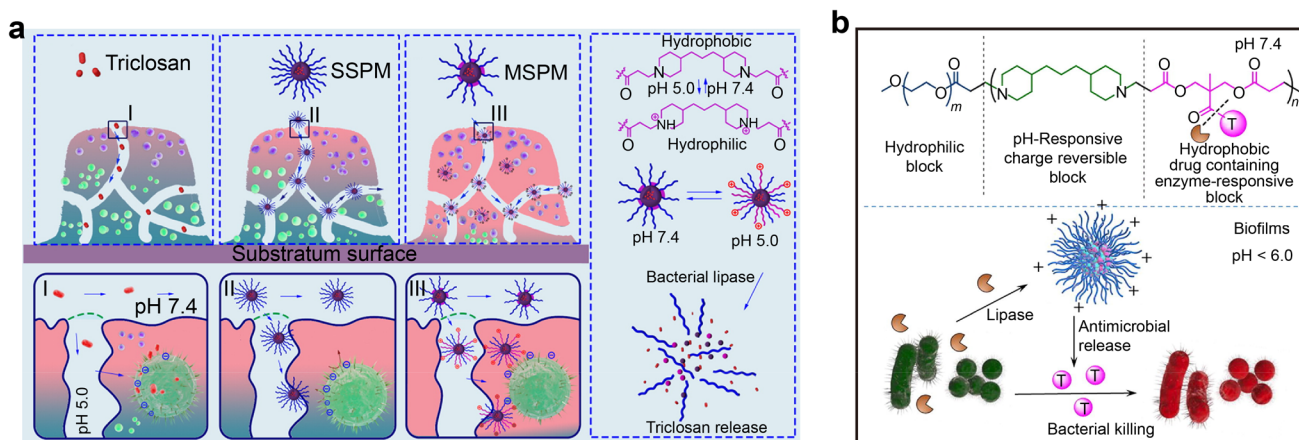


Fig. 2 **a** Conversion of triclosan-loaded MSPM in biofilm microenvironment. Reproduced with permission from Ref. [33]. Copyright 2016, American Chemical Society. **b** Self-assemble micelles for oral biofilm treatment. Reproduced with permission from Ref. [34]. Copyright 2018, Elsevier

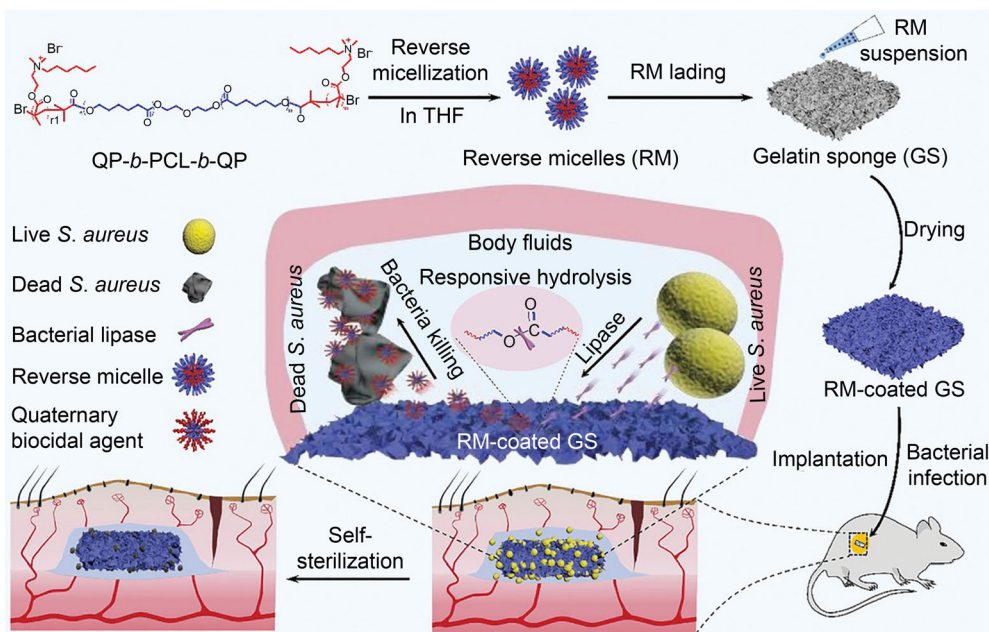


Fig. 3 Construction and self-sterilization of RMs-coated GS in infection site. Reproduced with permission from Ref. [38]. Copyright 2021, Wiley

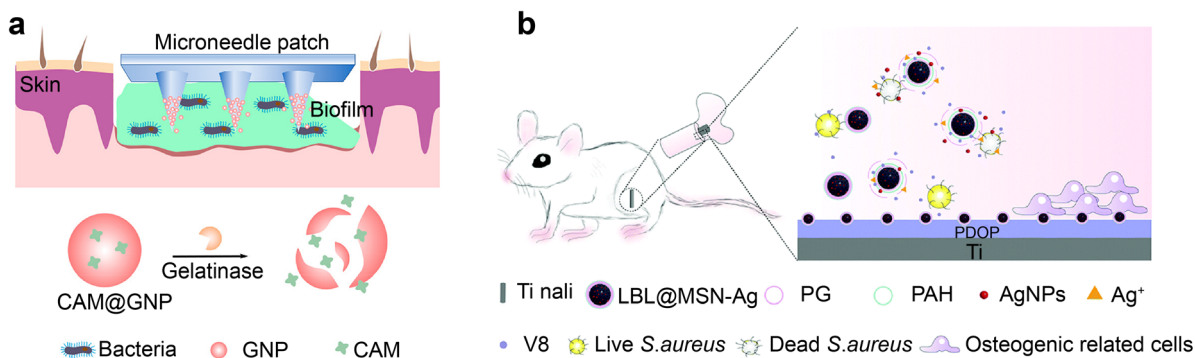


Fig. 4 **a** Microneedles penetrate biofilms and release antibiotics in response to gelatinase. Reproduced with permission from Ref. [40]. Copyright 2019, American Chemical Society. **b** V8 enzyme induced release of Ag NPs and consequent death of *S. aureus*. Reproduced with permission from Ref. [41]. Copyright 2019, Royal Society of Chemistry

This microneedle patch-assisted antibiofilm strategy shows great potential in treating skin superficial infections.

Glutamyl endonuclease (V8) is an extracellular virulence factor of *S. aureus*, which can break down amid linkages. Based on this, Cai et al. established V8 enzyme-responsive nanoplatform using poly-L-glutamic acid (PG) as degradable motif [41]. The mesoporous silica loading Ag (Ag@MSN) was coated with PG and polyallylamine hydrochloride (PAH) through layer-by-layer assembly and deposited on the surface of Ti substrate, which is often used as orthopedic implants materials. Once the biofilm formed on the Ti implant, decomposition of PG led to the release of MSN-Ag, which could further continuously generate silver ions to kill bacteria (Fig. 4b). The highlight of this work is that PG has the potential for accelerating tissue

regeneration, consistent with the practical application of the material. This ingenious design utilizes the two important functions of one component to precisely match the application scenario rather than simply combines two kinds of components with different functions together, obviously reducing the complexity of synthesis process.

Nowadays, bacterial drug resistance has become a serious problem all over the world. One of the major mechanisms of drug resistance is that bacteria produce enzymes to destroy conventional antibiotics. For example, β -lactamases are enzymes usually produced by bacteria to resist β -lactam antibiotics, such as penicillin and cephalosporin. β -lactamase from gram-negative bacteria can hydrolyze the amide bond in four-membered β -lactam [42]. Another similar enzyme related to bacterial resistance is

penicillin G amidase (PGA), which catalyzes the hydrolysis of penicillin as its name implied [43]. As a consequence, selective drug release can be achieved using nanocarriers specifically degradable by these enzymes. Liu et al. developed the bacterial strain-selective nanocarriers for drug delivery to kill resistant bacteria [44], which was based on the amphiphilic diblock copolymers with enzyme-responsive property. Then, they self-assembled into micelles and loaded the antimicrobial agents into either hydrophobic bilayers or aqueous interiors. The antimicrobial agents were released by bacterial enzyme-cleavable self-immolative side linkages, rather than the degradation of the main chain. This design could avoid wide variation of micelles in degradation kinetics and release properties caused by the diversity of enzymes. The micelles were proved to be capable of carrying not only multiple antibiotics, but also large molecules such as proteins. Under the catalysis of enzymes, the terminal groups on the side chains of PEG-b-PP and PEG-b-PC were uncapped and spontaneously rearranged, resulting in morphological transition of micelles to release cargos. The degradation process of nanocarriers was investigated in detail in this work, revealing more mechanisms about enzyme-cleavage nanocarriers and providing valuable information for following research works.

By summarizing recent researches about enzyme-responsive nanocarriers, it can be concluded that most nanocarriers achieved responsive drug release through enzyme-degradable motifs, and many of them chose the commonly bacteria-secreted enzymes as stimuli, namely HAS and lipases, to realize broad-spectrum antibacterial purpose. However, the existing challenge is how to prove that the nanocarriers were decomposed by enzymes inside biofilms as they were supposed to be, rather by enzymes outside biofilms, because the surrounding normal tissue also had opportunities to trigger drug release by host enzymes with similar functions. Besides, with the discovery of the importance of probiotics, more attentions have been paid on selectively killing of pathogenic bacteria over probiotics, and therefore more intelligent and more targeted nanocarriers need to be constructed by rational choice of enzymes.

2.2 Other factors-responsive nanocarriers

GSH, a tripeptide present in many prokaryotic cells, is a water-soluble thiol performing important functions in bacteria, for example, protecting bacteria against environmental stresses, such as osmotic shock, toxins, acidity and oxidative stress [45, 46]. The concentration of GSH ranges from 0.1 to 10.0 mmol·L⁻¹ in bacteria, so it can apparently be a stimulus for drug release [19]. Ji et al. developed a GSH-responsive antibiofilm nanosystem with gas and PDT

synergistic therapy capacities [47]. α -cyclodextrin (α -CD) conjugated nitric oxide (NO) prodrug (α -CD-NO) and chlorin e6 (Ce6) prodrug (α -CD-Ce6) were integrated with pH-sensitive poly (ethylene glycol) (PEG) block polypeptide copolymers (PEG-(KLAKLAK)₂-DA) through host-guest interaction, forming a supramolecular nanocarrier α -CD-Ce6-NO-DA. The negative charged nanocarriers could switch into positive charged ones under acidic microenvironment of biofilms, followed by the NO release under the stimulus of GSH in biofilms. NO can either destroy bacteria and biofilm structures by forming reactive nitrogen species (RNS) or induce biofilm dispersion by quorum signaling (Fig. 5). In addition, NO consumed GSH to increase the sensitivity of bacteria to oxidative damages of PDT and simultaneously generated RNS in the reaction with reactive oxygen species (ROS) from PDT. Owing to the combined therapy efficacy, the nanocarrier showed significant antibiofilm performances in vivo.

Some interesting nanocarriers were constructed based on chelation between bacterial products and the materials. Calcium phosphate (CaP) is reported to interact with cell walls of *S. aureus*, which may be caused by chelation of phosphate with the major endogenous metals in the cell walls of gram-positive bacteria. Therefore, CaP can be used as a nanocarrier modifier for targeting and responding to bacteria. Besides, liposomal carriers, which are frequently used in nanomedicine, release cargos once adhere on and fuse with peptidoglycan in gram-positive bacteria cell walls, greatly promoting the intracellular accumulation of drugs because release sites were very close to cytoplasm. Combining these two components together, 1,2-dioleoyl-sn-glycero-3-phosphate (DOPA) liposomes coated with CaP had the ability to target and adhere to *S. aureus* biofilms and release drugs near bacterial membranes [48]. The dispersion of the loaded drugs in liposomes and surrounding media was investigated using fluorescent drugs, acridine orange (AO) or 5,10,15,20-Tetrakis (1-methyl-4-pyridinio) porphyrin (TMP). The results demonstrated that the interactions between liposomes and bacteria could break down the CaP shells, thus causing the drugs release. It is therefore more accurate to say that the nanocarriers respond to the structural components of bacteria, rather than what they secreted. Although the antibacterial activity of nanocarriers was not investigated and authors did not develop particular application, this work expanded existing research scope of responsive nanocarriers.

Metal-organic frameworks (MOFs) are hybrid materials composed of metal ions or clusters and organic ligands, which are widely used in industrial and biomedicine fields, including catalysis, gas storage, nanomachine, biosensing and drug delivery [49]. MOFs with iron clusters are likely to degrade in the presence of bacterial siderophores, which scavenge iron extracellularly for bacteria, so iron-based

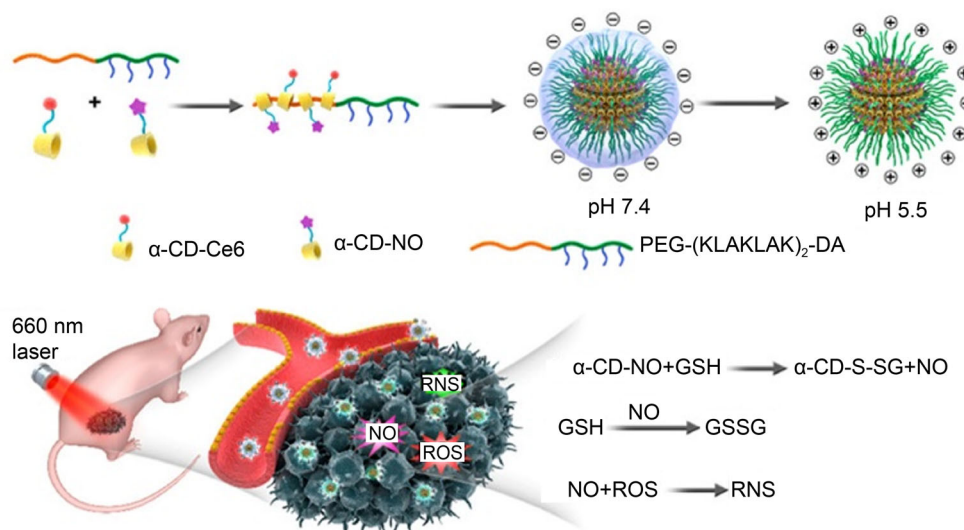


Fig. 5 Supramolecular nanocarrier α -CD-Ce6-NO-DA response to low pH and GSH and cascade reactions. Reproduced with permission from Ref. [47]. Copyright 2020, American Chemical Society

MOFs have potential to become responsive nanocarriers as well [50, 51]. De Vos et al. compared the antibiofilm performances of MIL-88B(Fe) and its hydrophobic variant MIL-88B(Fe)PA, both loading antibacterial agent 5-(4-Chlorophenyl)-N-(2-isobutyl)-2-aminoimidazole (IMI) [52]. The results showed that MIL-88B(Fe) was stable in water and partially degradable in the presence of external iron chelators. The abilities to inhibit biofilm growth of both MOFs were examined. Upon the chelation by bacteria, MIL-88B(Fe) showed better antibiofilm inhibition than the hydrophobic MIL-88B(Fe)PA, while the latter was only effective with additional chelating agent. This was mainly because that MIL-88(Fe)PA had higher affinity with IMI and was more difficult to release IMI. Although these two MOF materials did not show significant antibiofilm performances, this work provided a new perspective about responsive nanocarriers and reminded researchers that higher drug loading capability is not equal to better performance.

3 Nanocarriers responsive to environmental factors

3.1 pH-responsive nanocarriers

Similar to tumors, the microenvironment of biofilm is also acidic due to the anaerobic fermentation caused by hypoxia in the interior of biofilms [23]. This characteristic motivates the development of lots of pH-responsive nanocarriers for biofilm treatments. Based on their mechanism, the responsive nanoparticles can be mainly classified into two categories: (1) protonable nanoparticles that undergo

changes of physicochemical properties when surrounding pH varies; (2) acid degradable nanoparticles containing acid sensitive linkages in the structures. It is noteworthy that pH responsiveness often combines with other stimuli responsiveness and only a few of representative works are introduced here.

In both cancer and infections therapies, acid-induced protonation and charge reversal polymers are very common materials in the construction of pH-responsive nanocarriers. By properly designing, pH responsiveness can be achieved by surface modification of pre-prepared nanocarriers loading drugs or micelles formed from pH-sensitive polymers. The negative charge of nanoparticles before response is conducive to their circulation in the body fluid and reduces the nonspecific adhesion to proteins. When stimulated by the acidic environment of biofilm, the surfaces of the nanoparticles become positively charged, which facilitates their accumulation and penetration. This intelligent response improves the biocompatibility and accumulation at pathological sites of nanoparticles.

For example, Shi et al. designed hydrophilic mixed shell polymeric micelles (MSPMs) composed of copolymer poly(ethylene glycol)-block-poly(ϵ -caprolactone) (PEG-b-PCL) and poly(ϵ -caprolactone)-block-poly(β -amino ester) (PCL-b-PAE). MSPMs were proved to rapidly and completely changed their charges from negative to positive when pH dropped from neutral to slightly acidic [53]. Using this property, photosensitizer protoporphyrin IX (PpIX) was specifically delivered to the interior of biofilms. The results of in vitro and in vivo experiments showed that the surface charge adaptive nanocarriers were superior in the permeation and subsequent functioning of loaded drugs, as compared with the single shell polymeric micelles

without pH responsiveness. It is worth mentioning that Van was used as a contrast to illustrate the effectiveness of the constructed nanocarriers in eradicating vancomycin-resistant *Staphylococcal* biofilms, further highlighting the advantages of nanomaterials over clinical available therapies. Another outstanding point is the preliminary study on the circulation and metabolism of drug-carrying micelles in murine model, which made a small but tangible contribution to the transformation of nanomaterials to clinical application.

The use of protonable polymers can not only be used for constructing charge reversal nanocarriers, but also for preparing nanocarriers that can be disassemble in acidic environment due to charge changes. For example, Ling et al. reported pH-responsive nanoantibiotics (rAgNAs) via self-assembly of acid-sensitive poly(ethylene glycol)-poly(aminopropyl imidazole-aspartate)-polyalanine (PEG-PSB-PALA) and silver nanoclusters [54]. The negative charges endowed rAgNAs with high colloidal stability, which is beneficial for its long circulation in the body and enhanced accumulation at the biofilm infection sites. In acidic microenvironment, the protonated imidazole groups of PEG-PSB-PALA increased the electrostatic repulsion between polymers, resulting in the nanocarriers disassembly with the release of silver nanoclusters (Fig. 6a). Moreover, in acidic medium, leaching of silver ions from nanobiotics was fast enough to achieve local bactericidal concentration. The biofilm eliminating functions of nanoparticles and free Van were also compared in this work in vitro and in vivo. The methicillin-resistant *S. aureus* (MRSA) was used as a model bacterium to highlight the superiority of nanoparticles over Van.

Nanocarriers that release drugs through decomposition in acidic environments generally have acid-

unstable chemical bonds, for example, β -thiopropionate bonds. In the work of Zhang et al. [55], quaternary ammonium salt (QAS) containing polymers self-assembled into nanoparticles and cross-linked, introducing large amount of β -thiopropionate bonds. Thereafter, Ag NPs were encapsulated into pre-prepared nanoparticles to form micelles Ag@QAS@CM. The micelles could penetrate into the biofilm via electrostatic interaction between positively charged surface and negatively charged bacteria, followed by the release of Ag NPs and QAS fragments as bactericidal ingredients (Fig. 6b). The authors also used a commercial antibiotic for comparison, polyvinyl pyrrolidone protected AgNPs (P-AgNPs), and the results showed that Ag@QAS@CM had lower hemolysis rate and cytotoxicity than P-Ag NPs, but higher biofilm elimination efficiency than P-Ag NPs. Additionally, authors also proposed the existing problem in the future application of Ag NPs based on experimental phenomena, that is, the uncontrollable aggregation of Ag NPs.

In addition to structurally variable polymers, metal-organic materials can also be used as pH-responsive nanocarriers. Zeolitic imidazolate framework-8 (ZIF-8), an acid-labile MOF, has recently been widely explored as pH-responsive nanocarriers for therapy of cancers and bacterial infections, because of its simple synthesis and complete decomposition in acidic mediums [56, 57]. Besides, it can easily load a variety of drugs, including hydrophilic or hydrophobic small molecules, proteins, DNA and other biological macromolecules [58]. For instance, pH-responsive ZIF-8 nanocarriers for endophthalmitis were constructed with incorporated polyacrylic acid (PAA), ZIF-8-PAA [59], which further loaded ammonium methylbenzene blue (MB) as photosensitizer, and were decorated with Ag NPs and Van/NH₂-PEG on the surface (Fig. 7a). The

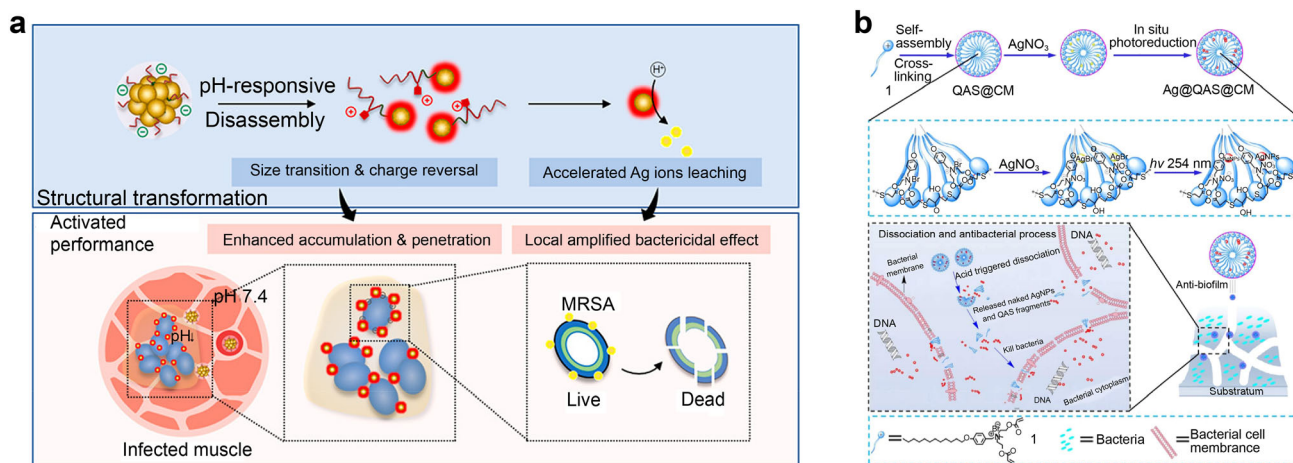


Fig. 6 **a** pH-responsive nanoantibiotics rAgNAs disassemble and release silver clusters under low pH. Reproduced with permission from Ref. [54]. Copyright 2019, American Chemical Society. **b** Preparation and antibiofilm mechanism of Ag@QAS@CM. Reproduced with permission from Ref. [55]. Copyright 2020, Elsevier

complex nanocarriers were demonstrated to degrade in the acidic biofilms as hypothesized and showed efficient anti-biofilm abilities in endophthalmitis animal model. The combination of powerful antimicrobial agents is similar to the cocktail of therapies used for HIV, which had excellent results unsurprisingly. However, the convoluted synthesis process and the tangled unknown interactions between each component may interfere with the translation into clinical application.

Another pH-responsive ZIF-8-based nanocarrier (Van@ZIF-8@PDA) was fabricated using similar strategy, loading Van as antibiotic and polydopamine (PDA) as photothermal agent [60]. The PDA was coated on the surface to improve the stability, dispersion and biocompatibility of ZIF-8 nanoparticles and the Van was encapsulated inside ZIF-8 in situ simply and rapidly (Fig. 7b). The nanocarriers possessed a dual responsiveness toward acidic environment and NIR-induced hyperthermia. Under both triggers, Van@ZIF-8@PDA showed effective anti-biofilm ability in vitro and in vivo. Although ZIF-8 has many advantages, such as the ability to carry multiple drugs, modifiable sites on surface and the facile synthesis, it also has inherent disadvantage of easy degradation in phosphate buffer saline media and other common mediums in biological experiments, which raises questions about whether it is stable in vivo to realize multiple functions as it functions in vitro [61, 62]. Up to now, not enough research has been done to shed light on this question.

3.2 ROS-responsive nanocarriers

In addition to its well-known acidic properties, biofilm infection sites also have higher ROS level than surrounding tissue, mainly because mammalian phagocytes kill invading microorganisms by producing ROS, including superoxide, hydrogen peroxide, singlet oxygen, etc. [20].

Besides, recent researches demonstrated that bacteria also produce H_2O_2 to inhibit inflammasomes for their colonization in hosts [21]. These ROS can undertake various functions in stimuli-responsive nanocarriers. For example, Qu et al. constructed a nanoplatfrom containing the porphyrin-based MOF dots and MnO_2 [22]. MnO_2 component could be degraded by H^+ and H_2O_2 in biofilm and catalyze the production of oxygen, alleviating hypoxia and facilitating PDT effect. Meanwhile, H_2O_2 served as trigger, as well as the auxiliary of PDT.

Additionally, H_2O_2 can also contribute to the release of drugs, as reported by Gong et al. [63]. They developed a dual-responsive nanoparticle to treat multi-drug-resistant (MDR) bacterial infections, using dextran as hydrophilic shell and poly(β -amino ester)-guanidine-phenylboronic acid (PBAE-G-B) polymer as hydrophobic core to encapsulate hydrophobic drug, rifampicin (Fig. 8). The dextran possessed strong affinity with lectins expressed in bacteria and macrophage, making nanoparticles accumulated at infection sites. Subsequently, the phenylboronic ester could be decomposed into 4-hydroxybenzyl alcohol and boric acid/ester due to the oxidation of B-C bond by ROS, and the hydrophilic PBAE-G-B polymer converted into hydrophilic PBAE-G polymer under low pH, leading to the fast drug release. The NPs showed significant biofilm and intracellular bacteria elimination performance, as well as excellent therapeutic effects in *P. aeruginosa* pneumonia and peritonitis caused by MRSA.

4 Nanocarriers responsive to external stimuli

In addition to the substances produced inside or around biofilm infection sites, various exogenous energy can also trigger the drug release from rational designed nanocarriers. In these circumstances, the responsive behavior of

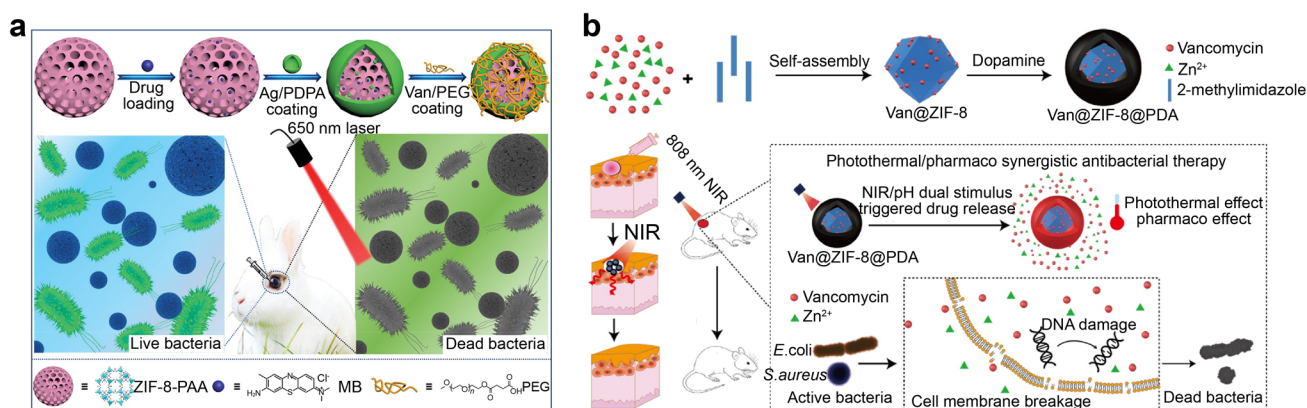


Fig. 7 a ZIF-8 based ZPMAVP applied for endophthalmitis treatment. Reproduced with permission from Ref. [59]. Copyright 2019, Wiley. b Dual-responsive ZIF-8 nanocarriers for synergistic photothermal/pharmacological therapy. Reproduced with permission from Ref. [60]. Copyright 2021, Elsevier

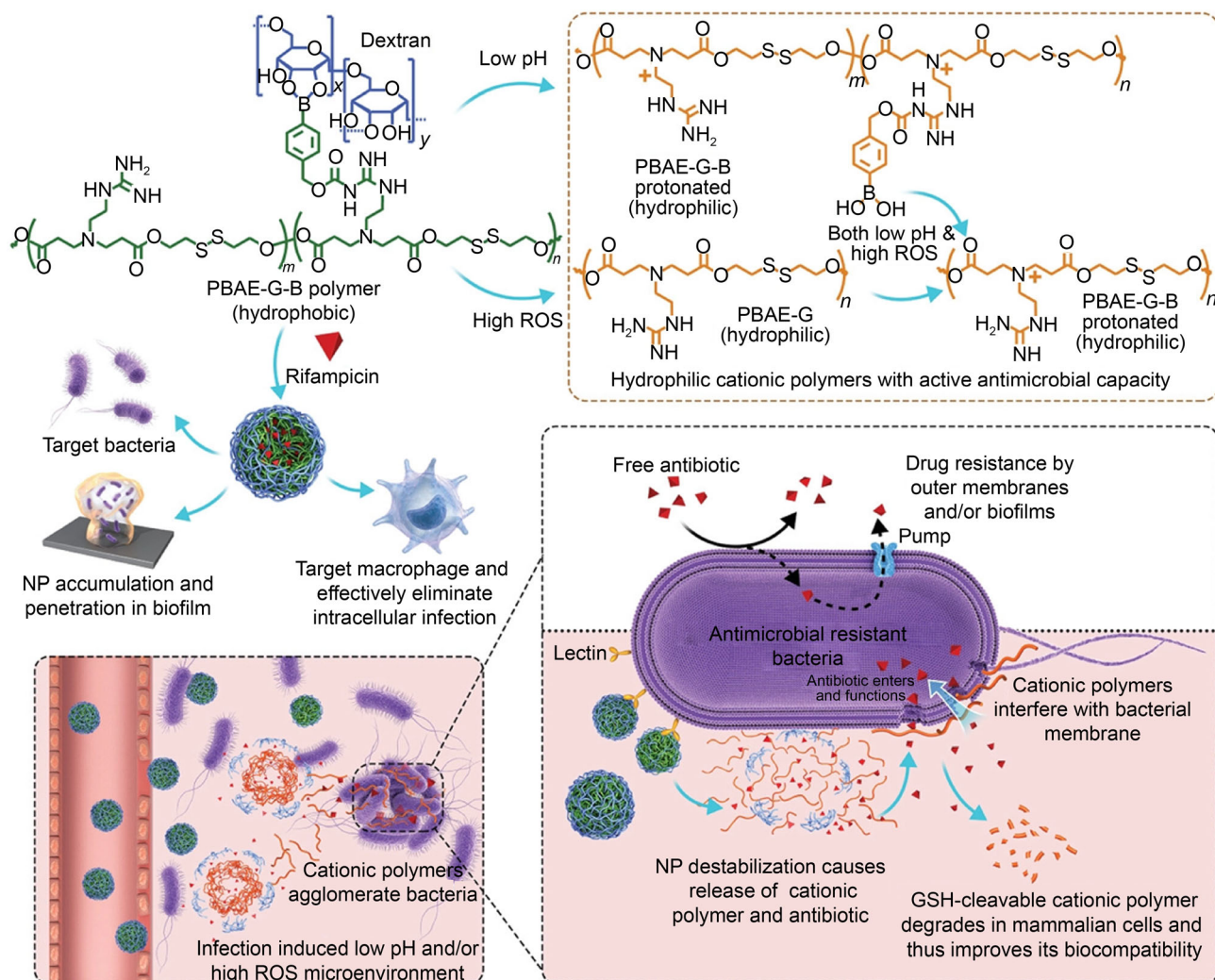


Fig. 8 Composition and antibacterial mechanism of dual-responsive nanocarrier loading rifampicin. Reproduced with permission from Ref. [63]. Copyright 2021, Wiley

nanocarriers is more controllable and precise because external stimuli are applied artificially.

4.1 Light or thermo-responsive nanocarriers

As a relatively inexpensive and convenient external stimulus, light is extensively used for controllable drug release from responsive nanocarriers with high spatial and temporal precision [64]. Particularly, light in near-infrared (NIR) region is suitable for treatment of biofilm infections as the longer wavelength has deeper tissue penetration and almost negligible harm to living organisms. Besides, NIR light can be transformed into heat by photothermal agents, thus can also act as a thermal trigger. This conversion is frequently used in light responsive materials because light is much easier to control than heat. In the work of Li et al., NIR-responsive liposomes loading antibiotics and NIR photothermal agent (cypate) were formed by theros-

sensitive phospholipid, distearoyl phosphatidylcholine (DSPC) and quaternized cholesterol [65]. The liposomes were stable at normal physiological temperature and could penetrate into biofilms via electrostatic interaction and deliver antibiotics to the inside (Fig. 9). Subsequently, the liposomes underwent the phase transition from gel to liquid due to the photothermal effect of cypate under the NIR laser irradiation, leading to the release of drug. Besides, the heat from cypate enhanced the permeability of bacterial cell membranes and further promoted the bactericidal activity of antibiotics. The experimental results verified that synergistic therapy eliminated pathogenic biofilms effectively both in vitro and in vivo.

In addition to cypate, carbon quantum dots (CQDs) can transform NIR optical energy into heat as well. Besides, CQDs have been proved to possess significant drug loading ability, due to their large specific surface areas and the relatively stable interaction with various molecules. Based

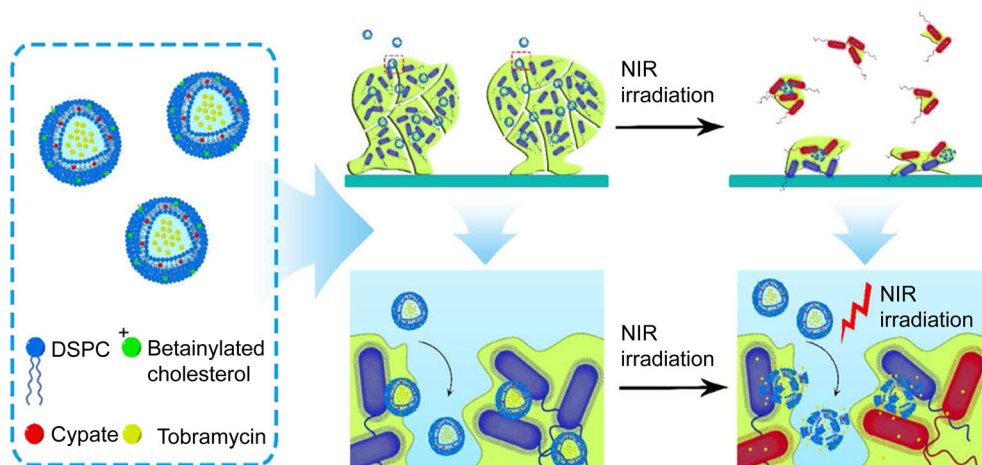


Fig. 9 Liposomes for killing bacteria in biofilm by release of antibiotic tobramycin under NIR irradiation. Reproduced with permission from Ref. [65]. Copyright 2018, American Chemical Society

on these useful properties, Wan et al. incorporated azithromycin and tobramycin into CQDs and encapsulated CQDs into poly lactic-co-glycolic acid (PLGA) [66]. The CQD-PLGA hybrid nanoparticles possessed high drug loading capacity and excellent photothermal performance. Temperature of hybrid nanoparticles could be raised to the glass transition temperature of PLGA under NIR laser irradiation for rapid drug release. However, the antibiotics were not released all at once, and the rest could be used for long-term therapeutics. Likewise, the increase in temperature facilitated the entry of drugs into bacteria. The antibiofilm ability of hybrid nanoparticles was demonstrated in vitro.

As mentioned above, NO has been widely regarded as an emerging antibacterial gas molecule. Up to now, many novel nanomaterials have been developed to deliver NO. For example, a multifunctional $\text{Fe}_3\text{O}_4@\text{PDA}@\text{PAMAM}@\text{NONOate}$ nanocomposite, containing magnetic Fe_3O_4 clusters, photothermal agent PDA, cationic poly(amidoamine) dendrimers PAMAM and NO donor N-diazeniumdiolate (NONOate), was reported by Xue et al. [67]. Under 808-nm laser irradiation, Fe_3O_4 clusters and PDA generated heat to raise local temperature, which caused NO release from NONOate. The release of NO could be triggered on demand through intermittent laser irradiation, but weak spontaneous NO leakage was still inevitable (Fig. 10a). With synergistic therapeutic effects of photothermal and NO, the nanocomposites showed effective biofilm eradication. Another novel feature of this work is that the strong electrostatic interaction between nanoparticles and bacteria was utilized to realize the highly efficient separation of bacteria under external magnetic fields, which was conducive to remove nanomaterials and bacterial debris from biological environment to reduce their potential toxicity.

As another NO producer, L-arginine (L-Arg) can produce NO upon ROS catalysis. Besides, ROS reacts with NO to generate highly harmful RNS, contributing to the bactericidal efficacy. It is predictable that the combination of PDT and NO therapy would result in enhanced biofilm elimination with mutual promotion. In the work of Cai et al., mesoporous polydopamine (MPDA) was modified with L-Arg and further loaded the photosensitizer indocyanine green (ICG) via π - π stacking [68]. Under NIR irradiation, the increased local temperature triggered the release of ICG from MPDA due to the disruption of thermo-sensitive noncovalent interaction between aromatic structures. ICG was then stimulated by NIR irradiation for ROS generation, destroying the biofilm components and making bacteria vulnerable to hyperthermia (Fig. 10b). Meanwhile, RNS was produced to enhance the therapeutic effect of PDT. Therefore, the bacteria within biofilms were synergistically and thoroughly killed by ROS, RNS and low-temperature ($< 45^\circ\text{C}$) photothermal therapy (PTT), which markedly reduced the potential thermal damage to surrounding tissues. This all-in-one phototherapeutic nanoplatform achieved multiple functions upon single NIR irradiation, presenting a safe, compact efficient strategy for biofilm treatment.

In some cases, UV light is also used to trigger the response of nanoparticles. For example, by anchoring azobenzene motif on two antibacterial amphiphilic tripeptides (Ala-Gly-Gly-OH and Gly-Gly-Ala-OH), two kinds of supramolecular peptide systems responsive to UV light were constructed [69]. The hydrophobic azobenzene changed into *cis*-form under UV irradiation, making the tripeptides more hydrophilic via the increased dipole moment. The photoisomerization led to the disassembly of supramolecular systems and thus released free peptides to combat bacteria and biofilms. Interestingly, after 2-h

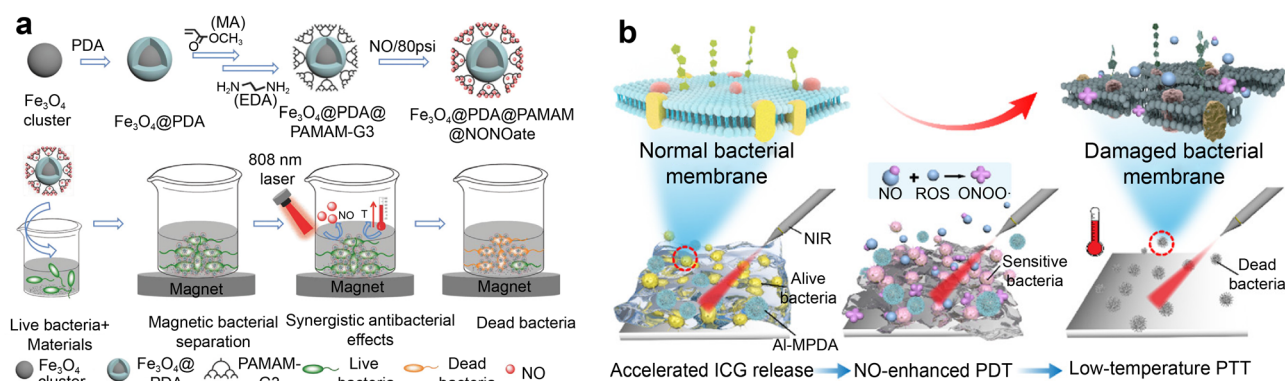


Fig. 10 a Synthetic route of $\text{Fe}_3\text{O}_4@PDA@PAMAM@NONOate$ and its bacteria capture process. Reproduced with permission from Ref. [67]. Copyright 2018, Wiley. b ICG released from MPDA and NO generated for synergistic antibacterial therapy. Reproduced with permission from Ref. [68]. Copyright 2020, American Chemical Society

visible light irradiation, the *cis*-form azobenzene returned into its *trans*-form, and the structures of supramolecular systems were also recovered. The supramolecular assemblies formed by tripeptides and β -cyclodextrin were in dynamic equilibrium between assembly and disassembly, which would be destroyed by adamantane through host-guest interaction, namely the competitive replacement of β -cyclodextrin. The supramolecular assemblies showed antibacterial and antibiofilm abilities *in vitro*.

Additionally, UV light can also act as the first stimuli to trigger a series of reactions. 2-nitrobenzaldehyde (*o*-NBA), a pH-jump reagent, would generate acid *in situ* under the irradiation of UV light, converting light stimulus into pH stimulus. According to this, Han et al. encapsulated *o*-NBA and antibiotic rifampicin into ZIF-8, successfully turning the acid-decomposed ZIF-8 into light-triggered nanocarriers for rifampicin release [70]. The nanocarriers showed good bactericidal activity and biofilm eradication performance. This ingenious conception exploited more application of pH-responsive nanocarriers, broadening the mind of researchers significantly. Although the abovementioned works provided inspiration on developing new light-responsive nanocarriers, the usage of UV light is problematic for some practical applications, due to its shortcomings, e.g., limited penetration depth in tissue and undesired damage to human bodies. Of course, the potential medical applications of these strategies would be more extensive, if the UV light can be replaced by light with longer wavelengths.

4.2 Magnetic field and microwave-responsive nanocarriers

Formidable magnetic fields are able to penetrate into the whole human body, and according to this property, magnetic fields are widely used in modern medicine, for example, magnetic resonance imaging (MRI), which is

universally used in the diagnosis of various diseases. Therefore, magnetic nanoparticles have the potential to target specific biofilm infection sites and further permeate protective biofilm matrix aided by the energy from external magnetic fields. Besides, they have been proved to cause local hyperthermia and/or mechanical forces, which have the potential to disrupt polymer materials and lead to drug release.

Zink et al. constructed a drug-delivery supramolecular nanocarrier H-MEL + G-OFL that could respond to alternative magnetic fields (AMF), heat and bacteria [71]. In the presence of *P. aeruginosa*, heating and AMF, the nanocarriers released most of antimicrobial peptide melittin and antibiotic ofloxacin rapidly, while in the absence of these stimuli, only negligible leakage of cargos was observed during 7-days incubation, which indicated the stability of nanocarriers (Fig. 11). The nanopatform showed high efficiency of biofilm elimination both *in vitro* and *in vivo* using an implantation model. This work offered a novel idea for construction of responsive nanocarriers for co-delivery of drugs with different distinct physical and chemical properties. Regrettably, the synthesis process was complicated, which may obstacle its clinical application.

Superparamagnetic iron oxide nanoparticles (SPIONs) have been proven to have antibacterial capability and regarded as a potential magnetically localizable therapy agent. The smaller the SPIONs are, the easier they can penetrate into biofilms, but the magnetic field positioning is also weakened. Additionally, the inherent hydrophobic SPIONs are difficult to disperse homogeneously in aqueous physiological media, greatly limiting the clinical application. To address these issues, Webster et al. encapsulated the hydrophobic SPIONs and hydrophilic methicillin into the cavity of polymersomes (iron oxide encapsulating polymersomes, IOP) [72]. In the treatment of methicillin-resistant *Staphylococcus epidermidis* biofilms, IOP with methicillin exhibited obviously enhanced penetration depth

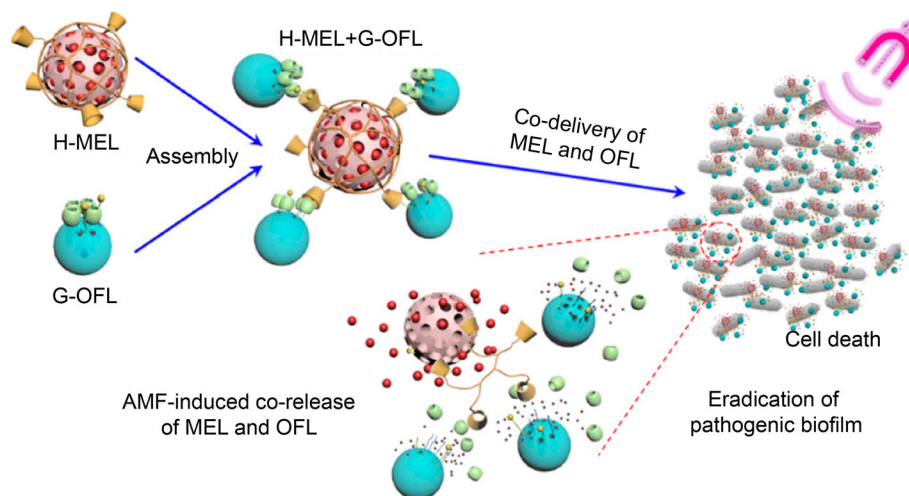


Fig. 11 Preparation of H-MEL + G-OFL and its drug release in response to alternative magnetic fields. Reproduced with permission from Ref. [71]. Copyright 2020, American Chemical Society

with the help of external magnetic field, thus giving rise to efficient biofilm elimination, as compared to free SPIONs and methicillin. This work improved existing magnetically localizable therapy for biofilms and weakened the resistance of biofilm to antibiotics via increasing drug penetration. However, the drawback was that the process of SPIONs and methicillin release from the nanocarriers was not further explored.

Additionally, the magnetic loss of iron oxide NPs is large enough to enable them to produce huge magnetic calor when excited by microwave (MV) via converting the MV energy to heat, which has been widely used for microwave caloric therapy (MCT). Combining the magnetic targeting and MCT, Wu et al. constructed a dual-targeting and MV-responsive nanocarriers for precise and synergistic therapy for combating MRSA-induced osteomyelitis (Fig. 12) [73]. Briefly, mesoporous iron oxide NPs were modified with dielectric carbon nanotubes (CNTs) with good MV absorption ability to obtain neural-network-like $\text{Fe}_3\text{O}_4/\text{CNT}$. Antibiotic gentamicin (Gent) was then encapsulated into nanocomposites $\text{Fe}_3\text{O}_4/\text{CNT}$ for synergistic chemotherapy, and further integrated with 1-tetradecanol, a phase change material to control Gent release. The synthesized $\text{Fe}_3\text{O}_4/\text{CNT}/\text{Gent}$ had selective and effective bacteria-capturing ability due to the strong interaction between nanocomposites and the amino groups on the bacteria surfaces. Upon external MV stimulation, $\text{Fe}_3\text{O}_4/\text{CNT}/\text{Gent}$ increased local temperature to kill bacteria and induced the melting of 1-tetradecanol to release Gent, which greatly assisted the elimination of MRSA infection in deep tibial plateau efficiently. Moreover, the application of external magnetic field also concentrated pathogenic bacteria at the infection sites, largely avoiding the spreading of MRSA via blood and the consequent

sepsis, as well as organ infections. This work utilized MV as a novel responsive trigger and demonstrated attractive results for treating deep tissue infections, showing inimitable superiorities and provided an encouraging alternative to visible light or NIR light-based phototherapy in clinical settings.

In another example, Wu et al. further developed a Prussian blue (PB) MOF as a MV-responsive material, aiming at rapid therapy of osteomyelitis [74]. PB is a representative mesoporous MOF with excellent abilities to convert MV energy into heat and release iron ions. Because of the insertion of Na^+ , the release of Fe(II) and Fe(III) ions from PB was accelerated and the spin state of iron ions became irreversible. This resulted in the enhanced permeability of bacterial membranes to iron ions, which further activated the Fenton reaction to induce the bacterial death via the generation of highly harmful $\cdot\text{OH}$. The bactericidal mechanism of PB MOF was thoroughly investigated and illustrated. It is not surprising that synergy of MV, hyperthermia and oxidative damages showed remarkable antibacterial capability, offering a fast, noninvasive therapeutic method for deep tissue infections.

In general, external magnetic fields or microwave with strong penetration abilities have great potential to replace light in the treatment of deep tissue infections. For external magnetic field stimulus, it may guide the nanocarriers to precise location and concentrate them in the pathological sites, especially in vivo. However, due to its weak bactericidal capacity, synergistic therapies, such as chemotherapy, PDT or PTT, are often needed. In addition, another prominent feature of magneto-responsive nanocarrier is that they can capture and separate bacteria, reducing the adverse effects of bacterial debris and the risk of bacterial spread. For external microwave stimulus, MCT is often

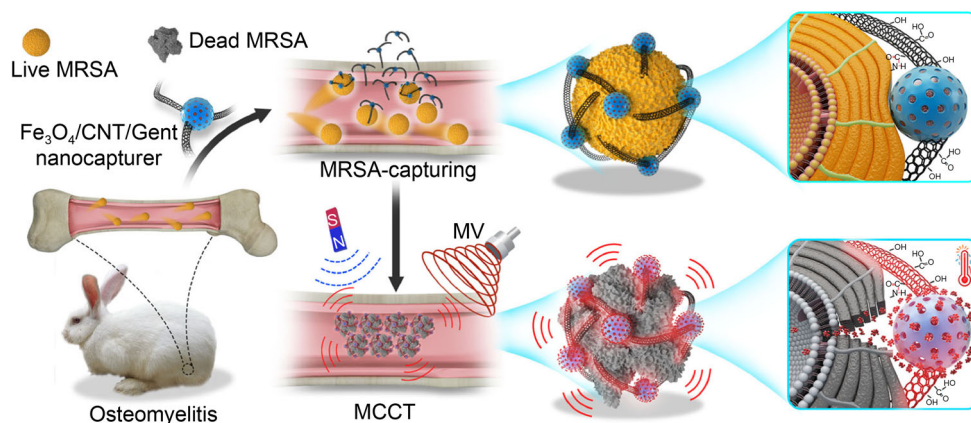


Fig. 12 Bacterial capture and antibacterial mechanism of $\text{Fe}_3\text{O}_4/\text{CNT}/\text{Gent}$ under microwave and external magnetic field. Reproduced with permission from Ref. [73]. Copyright 2020, Springer Nature

used to kill bacteria by heat, during which progress the potential side effects can also be minimized by synergy therapies. With rational design, these two stimuli can be united harmoniously for optimized biofilm treatments.

5 Summary and outlook

To date, nanomaterials have been widely developed and applied in biofilm infection treatments. Among them, stimuli-responsive nanocarriers have aroused mounting interests as they may solve the universal problems of conventional antibiotics or novel bactericidal agents, such as poor solubility or dispersity in water, difficulty in penetration into biofilms and low concentration in infection sites. Despite significant advances in this area, many challenges still remain for the development of stimuli-responsive nanocarriers. (1) The behaviors of most nanocarriers in physiological environments, especially in real infection sites, are not clear, mainly because the in-depth investigation is restricted by the lack of suitable instruments and characterization methods. Consequently, the effectiveness, safety and biocompatibility of many nanocarriers need to be demonstrated more deeply. (2) The responsive processes are mostly studied in the conditions with definite and tunable parameters *in vitro*, which is much different from the real and complex pathological conditions. Therefore, although the experiments *in vitro* and *in vivo* showed consistent efficient therapeutic results, the responsive processes *in vivo* need to be proved and illustrated substantially. (3) The intelligence and stability of stimuli-responsive nanocarriers should be further improved for targeting bacteria or biofilm infection microenvironments, with higher sensitivity and selectivity and lower drug leakages before the response. (4) The ingenious nanocarriers with multiple functions, such as diagnosing and imaging, are emerging as another rosy strategy for biofilm treatment,

which need full utilization of characteristics of bacteria and biofilms. (5) Last but not least, the construction of nanocarriers should be largely simplified as possible for clinical translation and application.

Overall, the future of stimuli-responsive nanocarriers is undoubtedly promising for their great variety in potential properties and functions and irreplaceable advantages over conventional therapies. Also, we believe that the collaborative efforts from academic research, physicians and industrial production will foster the real-life clinical infection applications of stimuli-responsive nanocarriers in the near future.

Acknowledgements This work was financially supported by the National Natural Science Foundation of China (Nos. 51873212 and 51803212), the Youth Innovation Promotion Association of Chinese Academy Science (No. 2017269), the Natural Science Foundation of Shandong Province (No. ZR2019MEM008) and Chinese Academy of Sciences-Wego Group Hightech Research & Development Program.

Declaration

Conflict of interest The authors declare that they have no conflict of interest.

References

- [1] Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. *Nat Rev Microbiol.* 2004;2(2):95.
- [2] Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S. Biofilms: an emergent form of bacterial life. *Nat Rev Microbiol.* 2016;14(9):563.
- [3] Wu H, Moser C, Wang HZ, Hoiby N, Song ZJ. Strategies for combating bacterial biofilm infections. *Int J Oral Sci.* 2015;7(1): 1.
- [4] Romling U, Balsalobre C. Biofilm infections, their resilience to therapy and innovative treatment strategies. *JAMA Intern Med.* 2012;272(6):541.

- [5] Jamal M, Ahmad W, Andleeb S, Jalil F, Imran M, Nawaz MA, Hussain T, Ali M, Rafiq M, Kamil MA. Bacterial biofilm and associated infections. *J Chin Med Assoc.* 2018;81(1):7.
- [6] Koo H, Allan RN, Howlin RP, Stoodley P, Hall-Stoodley L. Targeting microbial biofilms: current and prospective therapeutic strategies. *Nat Rev Microbiol.* 2017;15(12):740.
- [7] Levin-Reisman I, Ronin I, Gefen O, Braniss I, Shoresh N, Balaban NQ. Antibiotic tolerance facilitates the evolution of resistance. *Science.* 2017;355(6327):826.
- [8] Castaneda P, McLaren A, Tavaziva G, Overstreet D. Biofilm antimicrobial susceptibility increases with antimicrobial exposure time. *Clin Orthop Relat Res.* 2016;474(7):1659.
- [9] Makabenta JMV, Nabawy A, Li CH, Schmidt-Malan S, Patel R, Rotello VM. Nanomaterial-based therapeutics for antibiotic-resistant bacterial infections. *Nat Rev Microbiol.* 2021;19(1):23.
- [10] Liu Y, Shi L, Su L, Van Der Mei HC, Jutte PC, Ren Y, Busscher HJ. Nanotechnology-based antimicrobials and delivery systems for biofilm-infection control. *Chem Soc Rev.* 2019;48(2):428.
- [11] Wang Y, Jin Y, Chen W, Wang J, Chen H, Sun L, Li X, Ji J, Yu Q, Shen L, Wang B. Construction of nanomaterials with targeting phototherapy properties to inhibit resistant bacteria and biofilm infections. *Chem Eng J.* 2019;358:74.
- [12] Wang S, Gao Y, Jin Q, Ji J. Emerging antibacterial nanomedicine for enhanced antibiotic therapy. *Biomater Sci.* 2020;8(24):6825.
- [13] Wang LS, Gupta A, Rotello VM. Nanomaterials for the treatment of bacterial biofilms. *ACS Infect Dis.* 2016;2(1):3.
- [14] Mofazzal Jahromi MA, Sahandi Zangabad P, Moosavi Basri SM, Sahandi Zangabad K, Ghamarypour A, Aref AR, Karimi M, Hamblin MR. Nanomedicine and advanced technologies for burns: preventing infection and facilitating wound healing. *Adv Drug Deliv Rev.* 2018;123:33.
- [15] Alvarez-Lorenzo C, Garcia-Gonzalez CA, Bucio E, Concheiro A. Stimuli-responsive polymers for antimicrobial therapy: drug targeting, contact-killing surfaces and competitive release. *Expert Opin Drug Deliv.* 2016;13(8):1109.
- [16] Chen H, Jin Y, Wang J, Wang Y, Jiang W, Dai H, Pang S, Lei L, Ji J, Wang B. Design of smart targeted and responsive drug delivery systems with enhanced antibacterial properties. *Nanoscale.* 2018;10(45):20946.
- [17] Stewart PS, Franklin MJ. Physiological heterogeneity in biofilms. *Nat Rev Microbiol.* 2008;6(3):199.
- [18] Canaparo R, Foglietta F, Giuntini F, Della Pepa C, Dosio F, Serpe L. Recent developments in antibacterial therapy: focus on stimuli-responsive drug-delivery systems and therapeutic nanoparticles. *Molecules.* 2019;24(10):1991.
- [19] Masip L, Veeravalli K, Georgiou G. The many faces of glutathione in bacteria. *Antioxid Redox Signal.* 2006;8(5–6):753.
- [20] Nathan C, Cunningham-Bussell A. Beyond oxidative stress: an immunologist's guide to reactive oxygen species. *Nat Rev Immunol.* 2013;13(5):349.
- [21] Erttmann SF, Gekara NO. Hydrogen peroxide release by bacteria suppresses inflammasome-dependent innate immunity. *Nat Commun.* 2019;10(1):3493.
- [22] Deng Q, Sun P, Zhang L, Liu Z, Wang H, Ren J, Qu X. Porphyrin MOF dots-based, function-adaptive nanoplatforam for enhanced penetration and photodynamic eradication of bacterial biofilms. *Adv Funct Mater.* 2019;29(30):1903018.
- [23] Fulaz S, Hiebner D, Barros CHN, Devlin H, Vitale S, Quinn L, Casey E. Ratiometric imaging of the in situ pH distribution of biofilms by use of fluorescent mesoporous silica nanosensors. *ACS Appl Mater Interfaces.* 2019;11(36):32679.
- [24] Joshi VS, Sheet PS, Cullin N, Kreth J, Koley D. Real-time metabolic interactions between two bacterial species using a carbon-based pH microsensor as a scanning electrochemical microscopy probe. *Anal Chem.* 2017;89(20):11044.
- [25] Xiu W, Shan J, Yang K, Xiao H, Yuwen L, Wang L. Recent development of nanomedicine for the treatment of bacterial biofilm infections. *View.* 2020;2(1):20200065.
- [26] Archer NK, Mazaitis MJ, Costerton JW, Leid JG, Powers ME, Shirtliff ME. *Staphylococcus aureus* biofilms: properties, regulation, and roles in human disease. *Virulence.* 2011;2(5):445.
- [27] Hynes WL, Walton SL. Hyaluronidases of Gram-positive bacteria. *FEMS Microbiol Lett.* 2000;183(2):201.
- [28] Ji H, Dong K, Yan Z, Ding C, Chen Z, Ren J, Qu X. Bacterial hyaluronidase self-triggered prodrug release for chemo-photothermal synergistic treatment of bacterial infection. *Small.* 2016;12(45):6200.
- [29] Liu Y, Lin A, Liu J, Chen X, Zhu X, Gong Y, Yuan G, Chen L, Liu J. Enzyme-responsive mesoporous ruthenium for combined chemo-photothermal therapy of drug-resistant bacteria. *ACS Appl Mater Interfaces.* 2019;11(30):26590.
- [30] Wang X, Wu J, Li P, Wang L, Zhou J, Zhang G, Li X, Hu B, Xing X. Microenvironment-responsive magnetic nanocomposites based on silver nanoparticles/gentamicin for enhanced biofilm disruption by magnetic field. *ACS Appl Mater Interfaces.* 2018;10(41):34905.
- [31] Jaeger KE, Ransac S, Dijkstra BW, Colson C, van Heuvel M, Misser O. Bacterial lipases. *FEMS Microbiol Rev.* 1994;15(1):29.
- [32] Kiran S, Arshad Z, Nosheen S, Kamal S, Gulzar T, Majeed MS, Jannat M, Rafique MA. Microbial lipases: production and applications: a review. *J Biochem Biotechnol Biomater.* 2016;1(2):7.
- [33] Liu Y, Busscher HJ, Zhao B, Li Y, Zhang Z, Van Der Mei HC, Ren Y, Shi L. Surface-adaptive, antimicrobially loaded, micellar nanocarriers with enhanced penetration and killing efficiency in *Staphylococcal* biofilms. *ACS Nano.* 2016;10(4):4779.
- [34] Liu Y, Ren Y, Li Y, Su L, Zhang Y, Huang F, Liu J, Liu J, Van Kooten TG, An Y, Shi L, Van Der Mei HC, Busscher HJ. Nanocarriers with conjugated antimicrobials to eradicate pathogenic biofilms evaluated in murine in vivo and human ex vivo infection models. *Acta Biomater.* 2018;79:331.
- [35] Chen M, Wei J, Xie S, Tao X, Zhang Z, Ran P, Li X. Bacterial biofilm destruction by size/surface charge-adaptive micelles. *Nanoscale.* 2019;11(3):1410.
- [36] Ding X, Duan S, Ding X, Liu R, Xu FJ. Versatile antibacterial materials: an emerging arsenal for combatting bacterial pathogens. *Adv Funct Mater.* 2018;28(40):1802140.
- [37] Ding X, Wang A, Tong W, Xu FJ. Biodegradable antibacterial polymeric nanosystems: a new hope to cope with multidrug-resistant bacteria. *Small.* 2019;15(20):1900999.
- [38] Wang A, Duan S, Ding X, Zhao N, Hu Y, Ding X, Xu FJ. Bioswitchable antibacterial coatings enable self-sterilization of implantable healthcare dressings. *Adv Funct Mater.* 2021;31(18):2011165.
- [39] Grubb JD. *Methods in enzymology.* Edited by Colowick SP, Kaplan NO. New York: Academic Press Inc; 1994. 602.
- [40] Xu J, Danehy R, Cai H, Ao Z, Pu M, Nusawardhana A, Rowe-Magnus D, Guo F. Microneedle patch-mediated treatment of bacterial biofilms. *ACS Appl Mater Interfaces.* 2019;11(16):14640.
- [41] Ding Y, Hao Y, Yuan Z, Tao B, Chen M, Lin C, Liu P, Cai K. A dual-functional implant with an enzyme-responsive effect for bacterial infection therapy and tissue regeneration. *Biomater Sci.* 2020;8(7):1840.
- [42] Tooke CL, Hinchliffe P, Bragginton EC, Colenso CK, Hirvonen VHA, Takebayashi Y, Spencer J. Beta-Lactamases and beta-lactamase inhibitors in the 21st Century. *J Mol Biol.* 2019;431(18):3472.
- [43] Sevo M, Degrassi G, Skoko N, Venturi V, Ljubijankic G. Production of glycosylated thermostable *Providencia rettgeri*

- penicillin G amidase in *Pichia pastoris*. FEMS Yeast Res. 2002; 1(4):271.
- [44] Li Y, Liu G, Wang X, Hu J, Liu S. Enzyme-responsive polymeric vesicles for bacterial-strain-selective delivery of antimicrobial agents. Angew Chem Int Ed Engl. 2016;55(5):1760.
- [45] Chen NH, Counago RM, Djoko KY, Jennings MP, Apicella MA, Kobe B, Mcewan AG. A glutathione-dependent detoxification system is required for formaldehyde resistance and optimal survival of *Neisseria meningitidis* in biofilms. Antioxid Redox Signal. 2013;18(7):743.
- [46] Gales G, Penninckx M, Block JC, Leroy P. Role of glutathione metabolism status in the definition of some cellular parameters and oxidative stress tolerance of *Saccharomyces cerevisiae* cells growing as biofilms. FEMS Yeast Res. 2008;8(5):667.
- [47] Hu D, Deng Y, Jia F, Jin Q, Ji J. Surface charge switchable supramolecular nanocarriers for nitric oxide synergistic photodynamic eradication of biofilms. ACS Nano. 2020;14(1):347.
- [48] Rivero Berti I, Dell' Arciprete ML, Dittler ML, Minan A, Fernandez Lorenzo De Mele M, Gonzalez M. Delivery of fluorophores by calcium phosphate-coated nanoliposomes and interaction with *Staphylococcus aureus* biofilms. Colloids Surf B. 2016;142:214.
- [49] Zhu JY, Liang F, Yao YC, Ma WH, Yang B. Preparation and application of metal organic frameworks derivatives in electro-catalysis. Chin J Rare Met. 2019;43(2):186.
- [50] Wilson BR, Bogdan AR, Miyazawa M, Hashimoto K, Tsuji Y. Siderophores in iron metabolism: from mechanism to therapy potential. Trends Mol Med. 2016;22(12):1077.
- [51] Prabhakar PK. Bacterial siderophores and their potential applications: a review. Curr Mol Pharmacol. 2020;13(4):295.
- [52] Claes B, Boudewijns T, Muchez L, Hooyberghs G, Van Der Eycken EV, Vanderleyden J, Steenackers HP, De Vos DE. Smart metal-organic framework coatings: triggered antibiofilm compound release. ACS Appl Mater Interfaces. 2017;9(5):4440.
- [53] Liu Y, Van Der Mei HC, Zhao B, Zhai Y, Cheng T, Li Y, Zhang Z, Busscher HJ, Ren Y, Shi L. Eradication of multidrug-resistant *Staphylococcal* infections by light-activatable micellar nanocarriers in a murine model. Adv Funct Mater. 2017;27(44):1701974.
- [54] Wu J, Li F, Hu X, Lu J, Sun X, Gao J, Ling D. Responsive assembly of silver nanoclusters with a biofilm locally amplified bactericidal effect to enhance treatments against multi-drug-resistant bacterial infections. ACS Cent Sci. 2019;5(8):1366.
- [55] He DF, Yu YL, Liu FQ, Yao YC, Li PF, Chen JL, Ning N, Zhang SY. Quaternary ammonium salt-based cross-linked micelle templated synthesis of highly active silver nanocomposite for synergistic anti-biofilm application. Chem Eng J. 2020;382:122976.
- [56] He C, Liu D, Lin W. Nanomedicine applications of hybrid nanomaterials built from metal-ligand coordination bonds: nanoscale metal-organic frameworks and nanoscale coordination polymers. Chem Rev. 2015;115(19):11079.
- [57] Wang L, Zheng M, Xie Z. Nanoscale metal-organic frameworks for drug delivery: a conventional platform with new promise. J Mater Chem B. 2018;6(5):707.
- [58] Liang K, Ricco R, Doherty CM, Styles MJ, Bell S, Kirby N, Mudie S, Haylock D, Hill AJ, Doonan CJ, Falcaro P. Biomimetic mineralization of metal-organic frameworks as protective coatings for biomacromolecules. Nat Commun. 2015;6:7240.
- [59] Chen H, Yang J, Sun L, Zhang H, Guo Y, Qu J, Jiang W, Chen W, Ji J, Yang YW, Wang B. Synergistic chemotherapy and photodynamic therapy of endophthalmitis mediated by zeolitic imidazolate framework-based drug delivery systems. Small. 2019;15(47):e1903880.
- [60] Xiao Y, Xu M, Lv N, Cheng C, Huang P, Li J, Hu Y, Sun M. Dual stimuli-responsive metal-organic framework-based nanosystem for synergistic photothermal/pharmacological antibacterial therapy. Acta Biomater. 2021;122:291.
- [61] Velásquez-Hernández MdJ, Ricco R, Carraro F, Limpoco FT, Linares-Moreau M, Leitner E, Wiltche H, Rattenberger J, Schröttner H, Frühwirth P, Stadler EM, Gescheidt G, Amenitsch H, Doonan CJ, Falcaro P. Degradation of ZIF-8 in phosphate buffered saline media. CrystEngComm. 2019;21(31):4538.
- [62] Taheri M, Ashok D, Sen T, Enge TG, Verma NK, Tricoli A, Lowe A, Nisbet DR, Tsuzuki T. Stability of ZIF-8 nanopowders in bacterial culture media and its implication for antibacterial properties. Chem Eng J. 2021;413:127511.
- [63] Ye M, Zhao Y, Wang Y, Zhao M, Yodsanit N, Xie R, Andes D, Gong S. A dual-responsive antibiotic-loaded nanoparticle specifically binds pathogens and overcomes antimicrobial-resistant infections. Adv Mater. 2021;33(9):e2006772.
- [64] Ren Y, Liu H, Liu X, Zheng Y, Li Z, Li C, Yeung KWK, Zhu S, Liang Y, Cui Z, Wu S. Photoresponsive materials for antibacterial applications. Cell Rep Phys Sci. 2020;1(11):100245.
- [65] Zhao Y, Dai X, Wei X, Yu Y, Chen X, Zhang X, Li C. Near-infrared light-activated thermosensitive liposomes as efficient agents for photothermal and antibiotic synergistic therapy of bacterial biofilm. ACS Appl Mater Interfaces. 2018;10(17):14426.
- [66] Huang Z, Zhou T, Yuan Y, Natalie Klodzinska S, Zheng T, Sternberg C, Morck Nielsen H, Sun Y, Wan F. Synthesis of carbon quantum dot-poly lactic-co-glycolic acid hybrid nanoparticles for chemo-photothermal therapy against bacterial biofilms. J Colloid Interface Sci. 2020;577:66.
- [67] Yu SM, Li GW, Liu R, Ma D, Xue W. Dendritic Fe₃O₄@poly(dopamine)@PAMAM nanocomposite as controllable NO-releasing material: a synergistic photothermal and NO antibacterial study. Adv Funct Mater. 2018;28(20):1707440.
- [68] Yuan Z, Lin C, He Y, Tao B, Chen M, Zhang J, Liu P, Cai K. Near-infrared light-triggered nitric-oxide-enhanced photodynamic therapy and low-temperature photothermal therapy for biofilm elimination. ACS Nano. 2020;14(3):3546.
- [69] Chen L, Yang D, Feng J, Zhang M, Qian Q, Zhou Y. Switchable modulation of bacterial growth and biofilm formation based on supramolecular tripeptide amphiphiles. J Mater Chem B. 2019; 7(41):6420.
- [70] Song ZY, Wu Y, Cao Q, Wang HJ, Wang XR, Han HY. pH-responsive, light-triggered on-demand antibiotic release from functional metal-organic framework for bacterial infection combination therapy. Adv Funct Mater. 2018;28(23):1800011.
- [71] Yu Q, Deng T, Lin FC, Zhang B, Zink JJ. Supramolecular assemblies of heterogeneous mesoporous silica nanoparticles to co-deliver antimicrobial peptides and antibiotics for synergistic eradication of pathogenic biofilms. ACS Nano. 2020;14(5):5926.
- [72] Geilich BM, Gelfat I, Sridhar S, Van De Ven AL, Webster TJ. Superparamagnetic iron oxide-encapsulating polymersome nanocarriers for biofilm eradication. Biomaterials. 2017;119:78.
- [73] Qiao Y, Liu X, Li B, Han Y, Zheng Y, Yeung KWK, Li C, Cui Z, Liang Y, Li Z, Zhu S, Wang X, Wu S. Treatment of MRSA-infected osteomyelitis using bacterial capturing, magnetically targeted composites with microwave-assisted bacterial killing. Nat Commun. 2020;11(1):4446.
- [74] Wei S, Qiao Y, Wu Z, Liu X, Li Y, Cui Z, Li C, Zheng Y, Liang Y, Li Z, Zhu S, Wang H, Wang X, Che R, Wu S. Na⁺ inserted metal-organic framework for rapid therapy of bacteria-infected osteomyelitis through microwave strengthened Fenton reaction and thermal effects. Nano Today. 2021;37:101090.





Ling-Jie Song received her Ph.D. degree in Polymer Physics and Chemistry from Changchun Institute of Applied Chemistry, Chinese Academy Science (CIAC, CAS) in 2014. Afterward, she gained postdoctoral training at Chemical Department of Georgia Southern University with Professor Weihua Ming. Now, she is an associate professor at CIAC. Her research interest is the construction of bio-functional polymer surfaces, especially for

the smart antibacterial applications.



Shi-Fang Luan received his B.Sc. degree from Sichuan University in 2000 and obtained his Ph.D. degree in Polymer Physics and Chemistry from Changchun Institute of Applied Chemistry, Chinese Academy Science (CIAC, CAS) in 2006. After two-years post-doctoral research at Weigao Group, he returned to the State Key Laboratory of Polymer Physics and Chemistry of CIAC in 2009 and was promoted to full professor in 2016. His research interests

are mainly focused on the investigation of high-performance polymer material and surface as well as their bioapplication translation.