Supramolecular Hydrogels for Antimicrobial Therapy

Benhui Hu[a], Cally Owh[b], Chee Pei Lin[b], Wan Ru Leow[a], Xuan Liu[c], Yun-Long Wu[c], Peizhi Guo[d], Xian Jun Loh*[b] and Xiaodong Chen*[a]

Key learning points

(1) Supramolecular hydrogels can be conferred inherent antimicrobial properties when assembled by antimicrobial gelators such as peptides and positively charged amphiphiles.

(2) Encapsulating antimicrobial agents of either antibiotics or antimicrobial metal nanoparticles into hydrogel networks can also achieve antimicrobial functions.

(3) Integration of more functions further improves the performance and expands the arsenal of antimicrobial hydrogels.

(4) Perspectives of antimicrobial therapy: coupling with progressive efforts in artificial intelligence and biomedical engineering.

The emergence of drug-resistant microbes has become a threat to global health, and the microbial infections severely limit the use of healthcare materials. To achieve efficient antimicrobial therapy, supramolecular hydrogels demonstrate unprecedented advantages in medical application due to the tunable and reversible nature of supramolecular interactions and the capability of hydrogel to incorporate various therapeutic agents. Herein, the antimicrobial hydrogels are categorized according to their inherent antimicrobial property or based on their role in encapsulating antimicrobial materials. Moreover, strategies to further enhance the antimicrobial efficacy are highlighted, such as the incorporation of antifouling activity or the enabling of response towards physiological cues. We envision that supramolecular hydrogels, in combination with modern medical technology and devices, will contribute to the development of efficient and safe systems for antimicrobial therapy.

1. Introduction

Bacterial infections currently pose a serious global challenge that both threatens public health and causes heavy economic burdens.[1] The presence of pathogenic bacteria in susceptible interfaces or wounds can lead to infections, which causes a drastic increase in toxins that may significantly extend or disturb the healing process. Moreover, serious bacterial infections can result in the surgical failure of medical implant and devices, which increases the risk of sepsis and even death. This problem is further exacerbated by the rise in the global antimicrobial resistance crisis, where antimicrobial agents traditionally used in limiting, preventing, or eliminating pathogenic microbial growth have been rendered ineffective.[2]

The advent of antimicrobial resistance can be attributed to several reasons. First of all, although bacteria are unicellular microorganisms with relatively complex structures, their reproduction can occur rapidly through binary fission.[3] This rapid generation time, along with the massive quantities of microbes present in small volumes, allow for an increased chance of local population variation, resulting in continuous growth and evolution and increased adaptability against antimicrobial agents.[2] In addition, the survival cost of maintaining this evolutionary resistance has proven to be low, as antimicrobial resistant microbes rarely lose their resistance even in the absence of the threatening agent.[4] Finally, traditional antibiotic treatments are associated with over-prescription and improper use, culminating in the progressive rise of antibiotic resistance of various pathogens.

The consequences of antimicrobial resistance are dire. It has been noted that infection by an antibiotic-resistant strain of bacteria has the capacity to double the length of hospitalization, the mortality rate, and possibly the morbidity in comparison to a drug-susceptible strain.[4] Thus, in addressing the global problem of antibiotic resistance, the development of alternative antimicrobials and their corresponding formulations have become an urgent need.

Among the candidates for alternative antimicrobials, supramolecular biomaterials based on organized intermolecular self-assembly are receiving increasing attention in many branches of medicine. These hydrogels are a class of macromolecular antimicrobial agents with proven efficacies in combating multi-drug resistant infections. This ability stems from the inherent mechanism through which these agents act on the microbes. While traditional antibiotics act on intracellular targets without destroying the bacterial morphology, macromolecular agents function through disrupting the microbial membrane, and, as a result, reduces the chances of the microbe developing resistance. Furthermore, they exhibit excellent biocompatibility, programmable antimicrobial capability and adjustable mechanical strength.[5]

The existence of abundant gelators for the formation of diverse hydrogels, in conjunction with the development of antimicrobial research, promotes the elegant design and fabrication of antimicrobial supramolecular hydrogels. Such hydrogels can be endowed with antimicrobial functions through their self-assembled components or the incorporation of other agents. The integration of additional properties into these supramolecular hydrogels could enable them to serve as a versatile platform to meet more specific requirements in antimicrobial therapy. In view of this, the review will first introduce three types of hydrogels with inherent antimicrobial properties. The review will next proceed to discuss hydrogels that encapsulate antimicrobial agents such as antibiotics or nanoparticles. Afterwards, the review will focus on the strategies to integrate different properties to further improve the antimicrobial performance of supramolecular hydrogels. Overall, the review aims to link the fundamental understanding of hydrogels and their functionalization to the rational design of supramolecular systems for antimicrobial therapy.

2. An overview of supramolecular hydrogels

The main idea that differentiates supramolecular systems from those of traditional chemistry is the focus on reversible weaker and non-covalent intermolecular interactions. Compared to many conventional chemically crosslinked hydrogels, which may exhibit brittle, opaque and lack of self-healing properties, such systems offer attractive advantages as they allow for different physical properties, simpler synthesis, multi-functionality, as well as increased efficacies. Furthermore, they are capable of forming self-assemblies into complex structures through various means, including hydrogen bonding, ionic and associative interactions, host-guest complexation, metal-ligand complexation, π-π stacking, electrostatic interactions and even from low molecular weight constituents.[6-9] Based on the formation mechanism, supramolecular materials could also be classified into those formed through the one-dimensional assembly of molecular stacking motifs or through the crosslinking of polymeric precursors via molecular recognition motifs (Fig. 1a).[10]

Of particular interest are supramolecular materials assembled by two or more molecular entities via non-covalent binding interactions, which greatly enrich the formation of new types of structures at various levels. As supramolecular assemblies often cooperatively integrate two or more of the above-mentioned self-assembly interactions, the assemblies can be endowed with unique synergistic properties rather than the mere combination of each component's individual properties. Thereafter, understanding the complex interplay within the organized supramolecular assembly can help to decipher the natural supramolecular self-assembly and disassembly, and promote the in-deep fundamental research in biomedical applications.[11]

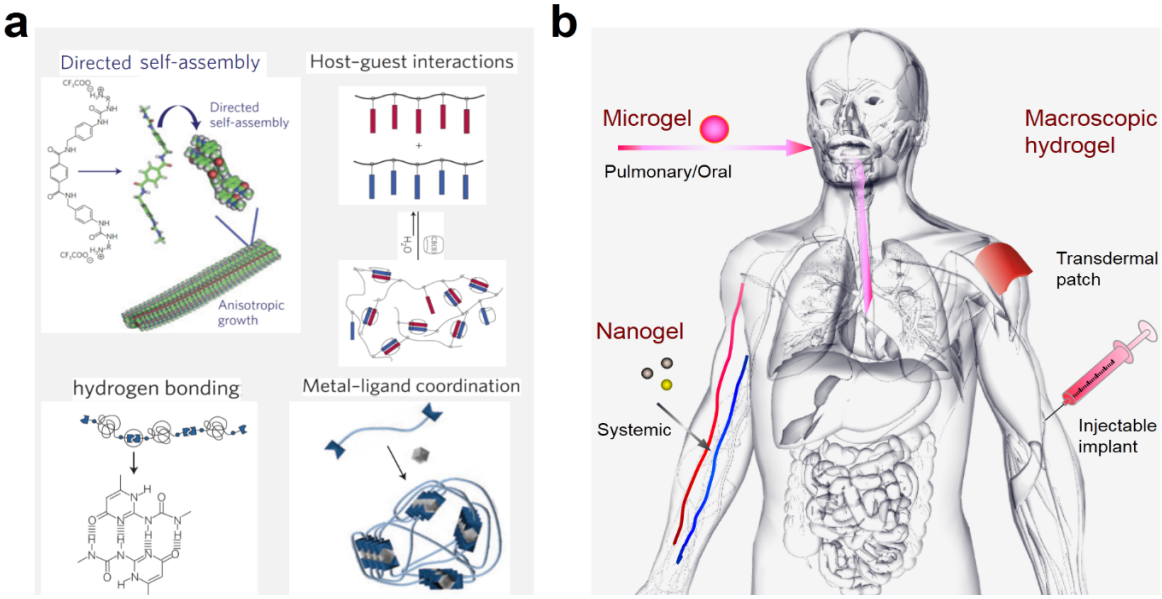

Fig. 1 Supramolecular materials assembled through directed molecular stacking or molecular recognition motifs for biomedical applications. (a) Illustration of one-dimensional assembly via stacking of molecular containing urea groups and aromatic groups (upper left), and examples formed by the crosslinking of polymeric precursors *via* molecular recognition motifs including crosslinking by host–guest interactions (upper right), chain extension through terminal hydrogen-bonding moieties (lower left) and oligomers based on coordination of metals with ligands (lower right). Figure reproduced from ref. 10 with the permission of Nature Publishing Group. (b) Macroscopic hydrogels can be suitable for transepithelial delivery and implantable dressings, and *in situ*-gelling hydrogels are injectable. In addition, microgels are suitable for pulmonary and oral delivery while nanogels are usually used for systemic administration. Reproduced with permission of BioDigital, Inc.

Physically speaking, supramolecular hydrogels are three-dimensional (3D) crosslinked networks that contain large volumes of water, thus serving as soft and compliant hydrated materials. Hydrogels can be modulated in size and architecture, with the feature scale spanning from sub-nanometers to centimeters. The multiscale nature facilitates the modular design of the hydrogels and enables their biomedical application for various tissues in human body (Fig. 1b); for example, microgels and nanogels possess larger surface areas for bioconjugation (linking other functional biomolecule *via* stable covalent bonding), and are thus able to penetrate tissue barrier. In particular, microgels smaller than 5 μm are suitable for oral and pulmonary delivery, while nanogels of sizes between 10 to 100 nm are usually used for systemic administration, due to their ability to exit blood vessels and extravasate into tissues. Moreover, macroscopic hydrogels have diverse surface properties such as exposed functional groups and porous structures, which enable the reactivity of hydrogels towards the components of cell wall/membrane of bacteria; for example, the presence of an antibacterial group in a hydrogel can entrap bacteria and subsequently lead to its death. Other properties such as *in situ* gelling could enable hydrogels to be injected into targeted sites, where their mechanical structures would then be locally reformed. Such

hydrogels can be used as transdermal patches or implantable dressings.[12]

3. Potential applications of antimicrobial supramolecular hydrogels

Traditionally, supramolecular hydrogels have been envisioned to play major roles in multiple biomedical applications. Much focus has been placed on their potential in aiding wound-healing, as their high-water content provides a moist environment that enhances the cellular immunological response during the healing process. However, this property of hydrogels comes with a disadvantage: the presence of a highly hydrated environment is also attractive to pathogenic microbes and promotes the risk of microbial infection. As infection at the wound side could lead to dire consequences such as tissue morbidity or sepsis, the bestowment of antimicrobial properties to hydrogels is thus a primary concern leading to the development of materials for wound dressings and fillers.[13]

Conventional hydrogels are also currently employed in a host of medical applications that could benefit from antimicrobial activity, which could help in biomedical implants that face issues with infection at the implant-tissue interface. Apart from this, many medical devices such as catheters are plagued with issues of introducing nosocomial infections at the device insertion sites, that could be reduced through the release of bactericidal agents. Medical devices commonly used daily, such as contact lenses, are also prone to microbial attachment or biofilm formation. The mitigation of this could aid in reducing the risk of infection brought about by an item that has widespread and frequent use. In addition, these materials have been thought to have potential in topical treatments of microbial infections, such as in skin infections or acne treatment, possibly anchoring a place in the personal care market.

4. Inherent antimicrobial supramolecular hydrogels

Supramolecular hydrogels can be conferred antimicrobial properties when assembled from gelators with inherent antimicrobial effects. By molecular self-assembly in an aqueous environment, agents such as drugs or peptides can be transformed into the building blocks of such hydrogels without affecting their efficiencies. This is further supported by the natural tendency of amphiphilic or hydrophobic therapeutic agents, such as clinical drugs, to aggregate in water. With this, self-delivery of the agents in the form of hydrogels can be accomplished, and the concerns associated with loading capacities, as well as biocompatibility, biodegradability and bioresorbability of the carrier materials can be eliminated.

Herein, a summary is provided on the types of assembled supramolecular hydrogels that possess favorable physical and antimicrobial properties, such as hydrogels composed of antimicrobial peptides, hydrogels based on positively charged amphiphiles, and hydrogels formed by stereocomplexation.

4.1 Supramolecular hydrogels based on antimicrobial peptides

Antimicrobial peptides (AMPs) are evolutionally conserved in all classes of life and play an important role in innate immune system by conferring antimicrobial activities to physical components such as blood, leukocytes, and lymphatic tissue. The immunomodulating property of AMPs is based on their anti-inflammatory and immunostimulatory activities, enabling them to manipulate immune system to suppress the adverse health effects such as infections with precise regulation. AMPs is thus considered as a promising alternative to antibiotics. As AMPs mainly target the bacterial membranes or other generalized targets instead of specific proteins as most antibiotics do, they possess an edge over conventional antibiotics in tackling resistance strains due to the increased difficulty in developing resistance against them. The general antimicrobial mechanism of AMPs occurs through the interaction between positively charged AMPs and negatively charged lipid head groups of the outer membrane surface, leading to disruption or perturbation of membrane-associated events such as biosynthesis of cell wall and causes cytokinesis. Moreover, the AMPs are also able to translocate across the membrane to affect cytoplasmic targets. However, the different structures of the peptides were also found to be associated with some differences in the types of killing mechanism. For example, peptides composed of only leucines and lysines (LK peptides) have been designed based on the minimal requirement of an amphipathic structure. Properly modulating the composition (*i.e.* L/K molar ratio), charge periodicity and concentration of the peptides can obtain ideal secondary amphipathic structures and thus induce rapid bacterial cell death. The antibacterial action is composed of several key steps: partitioning and accumulation on the bacterial membrane, structural changes of the LK peptides, membrane permeabilization and depolarization. The sudden membrane depolarization could prohibit target bacteria from performing appropriate countermeasures and thus result in a rapid bacteria death. For those bacteria without cell

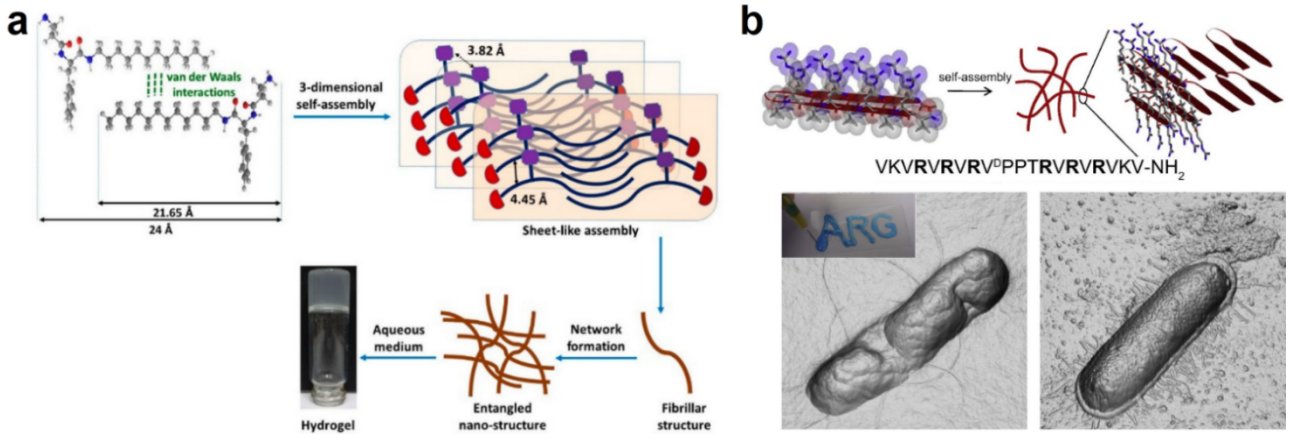

Fig. 2 Examples of AMPs-based supramolecular hydrogels. (a) Schematic illustration of the antibacterial hydrogel formation. Figure reproduced from ref. 17 with the permission of American Chemical Society. (b) Amphiphilic structure of arginine rich peptide designed to self-assemble into β-sheet rich fibrils (top), as well as the three-dimensional orthogonal projection images (derived from atomic force microscope (AFM) height data) of individual E. coli (normally around 2 μm in length) on the β-sheet rich hydrogel surface (left) and poly-L-Lysine control surface (right) after incubation at 37 °C for 2 hours (bottom), insert showing the injectable hydrogel delivered from a syringe. Figure reproduced from ref. 18 with the permission of Elsevier.

wall, the depolarization-induced massive liquid entry into the cytoplasm can also lead to fast cell burst. Inhibiting intracellular proteins is also proposed as the killing mechanism in some cases, reflecting many variables and possibilities in antibacterial action.[14]

Generally, AMPs are molecules containing around 15-50 amino acids. Although varied in composition of amino acid, secondary structure, biological properties and functions, AMPs are mostly cationic and amphipathic molecules. However, the naturally occurring AMPs pose several drawbacks, hindering their entrance into the market. Firstly, the relatively large size of the AMPs incurs high costs during large-scale production. Furthermore, they are susceptible to the environment pH and the presence of proteases. Finally, the salts and divalent cations present in the serum can also reduce their efficacy.[15]

Inspired by natural AMPs, synthetic amphiphilic peptides composed of cationic and hydrophobic residues have been prepared and applied as the molecular building blocks to assemble supramolecular hydrogels that exhibit inherent antimicrobial activity. Besides providing size tunability, the synthetic AMPs can also be modified to improve stability and achieve better targeting/killing efficiency. For instance, the alkyl chain length of the N-terminally located amino acid residues was found to be the key to thermal stability. Amphiphilic peptide-based hydrogels can mainly be form via various noncovalent interactions through molecular self-assembly. The efficacy of such antimicrobial hydrogels is dependent on various factors such as the length of the side chain, the charge density, the accessibility and the amphiphilicity.[16]

Recently, a series of peptides with a long fatty acyl chain covalently bonded to the C-terminus and a free amine group at the N-terminus have been synthesized. In an aqueous solution, these molecules formed hydrogels a nanofibrillar network structure. π–π stacking and β-sheet-like structure were involved in this supramolecular hydrogel. Interestingly, only hydrogels with longer alkyl chain length exhibited antibacterial activity against both Gram-positive bacteria (*B. subtilis* and *S. aureus*) and Gram-negative bacteria (*E. coli*) (Fig. 2a). A possible explanation could be that the peptides with longer alkyl chain length have more affinity toward bacterial membranes and hence, more interaction opportunities to cause membrane disruption. Besides, the hydrogels only induced a small amount of hemolysis at minimum inhibitory concentration (MIC) for *B. subtilis* and *E. coli*. They also exhibited proteolytic resistance to the enzymes proteinase K and chymotrypsin which suggested that they would be less prone to enzymatic degradation.[17]

In another study, an arginine rich peptide, which was folded into an amphiphilic β-hairpin conformation in buffer solution, soon self-assembled into a β-sheet rich fibrillar network (Fig. 2b, upper), forming a hydrogel. The hydrogel exhibited distinct antibacterial efficiency toward *E. coli* and *S. aureus via* disrupting the cell membrane (Fig. 2b, lower). Moreover, the hydrogel demonstrated shear-thinning behavior, which rendered it a potential injectable material (Fig. 2b, insert).[18]

Apart from deriving antimicrobial activity purely based on its constituents, it was suggested in another study that the antimicrobial activity of the hydrogels could more closely tied to their macromolecular properties, such as their supramolecular nanostructures and their rheological properties, instead of the efficacies of the individual peptides. The group tested three antimicrobial peptide sequences, $K_3W(QL)_6K_2$, $WK_2(QL)_6K$, and $K_2W(QL)_6K_2$, listed in order of decreasing bactericidal activity based on the results obtained from the MIC test of the peptide solution. However, incubating *S. aureus* pathogen with the formed hydrogels on agar plates showed that the hydrogel made up of $K_3W(QL)_6K_2$ had the worst bactericidal effect among the three peptides tested, which contradicted the results obtained from the solution-based MIC test. In the MIC test, the bacteria growth was inhibited with as little as 20 μM of the peptide ($K_3W(QL)_6K_2$) which was the lowest amount required out of the three peptides. With the aid of confocal microscope, the researchers found that the bacteria slowed down upon interaction with the hydrogels. They suggested that the stronger antimicrobial activity was likely accountable to the surface chemistry of the supramolecular hydrogel nanofibers and their bulk rheological properties. As the antimicrobial actions occurred entirely

on the hydrogel matrix surface, the surface positive charge density and the porous networks could trap the bacteria and reduce their movements. In these hydrogels, $K_2W(QL)_6K_2$ has the highest storage modulus, providing strongest suctioning force against the restrained bacteria. In contrast, $K_3W(QL)_6K_2$ has the lowest storage modulus and thus its antimicrobial activity is minimal.[19]

4.2 Supramolecular hydrogels based on positively charged amphiphiles

The success of AMPs in combating microbial activity, along with their aforementioned disadvantages, have spawned various different materials that attempt to capture their desirable properties while minimizing the bad. In particular, it was noted that the antimicrobial potency of AMPs was conferred upon to them by their physicochemical property of amphiphilic topology as opposed to their specific amino acid sequences. There was thus an interest in developing cationic amphiphiles that could imitate this topology.[20]

Positively charged amphiphiles are molecules with one or more positively charged head group(s) and lipophilic tail(s). These cationic amphiphiles with cationic and hydrophobic moieties are endowed with intrinsic broad-spectrum antimicrobial activity and serve in a variety of antimicrobial applications. As a mimic of natural AMPs, cationic amphiphiles function in a similar way, interacting with the negatively charged bacterial cell membrane and resulting in subsequent insertion. Hydrophobic counterparts then facilitate penetration and disruption of microbial membrane, resulting in the efflux of cellular contents and cell death. As the repair of the bacterial cell membrane is physiologically demanding, it is thought that this would reduce the chances of resistance development, making cationic amphiphiles attractive for antimicrobial drug development. Furthermore, cationic amphiphiles possess enhanced microbicidal activity as well as lower toxicity compared to small amphiphilic molecules, suggesting them to be more suitable for biomedical applications.[21]

The essential role of the amphiphilic topology is generally accepted for the insertion and disruption of the membrane. It was suspected that the membrane-disruption ability of cationic amphiphiles is related to molecular weight, cationic charges and hydrophobicity. The membrane-disruption capability would be enhanced with the increase of molecular weight until a critical molecular weight.[22] Interestingly, antimicrobial activity first increases and then decreases with the increase of hydrophobic alkyl chain length. It's probably because that the polymer chain could collapse or irreversibly aggregate with too many hydrophobic groups, thus reducing the antimicrobial potency. Hence, it's important to maintain the balance between hydrophilic and hydrophobic groups of antimicrobial cationic amphiphiles.

The self-assembly of cationic amphiphiles into a supramolecular structure occurs most easily in water or buffer solution via noncovalent interactions including hydrogen bonding, π–π stacking interaction, and van der Waals forces. A study on the synthesis of six dipeptide-based amphiphiles with varying head group architectures was also reported.[23] These amphiphiles also demonstrated excellent antimicrobial activities and water gelation abilities at room temperature. However, the most significant impact of these was thought to be their biocompatibility with various mammalian cell lines, which could potentially show microbial cell-specific toxicity.

Materials possessing both cationic and amphiphilic characteristics have been extensively utilized in antibacterial applications. Recently, a pyridinium-based amphiphile was synthesized and self-assembled into a hydrogel via hydrogen bonding and hydrophobic interaction. The antimicrobial activity of this hydrogel was derived from the pyridinium scaffold and the long alkyl chain. The positively charged amphiphiles could be adsorbed on the negatively charged microbial cell membrane by electrostatic interaction, followed by the self-promoted penetration of the hydrophobic chain into the cell membrane, leading to the release of the cytoplasmic constituents and bacterial death. Importantly, the pyridinium based amphiphile was non-toxic to mammalian cells, making it suitable for further clinical application.[24]

4.3 Supramolecular hydrogels formed by stereocomplexation

The hydrogen bond is one of the driving forces behind the formation of supramolecular assemblies. Insofar, it has been

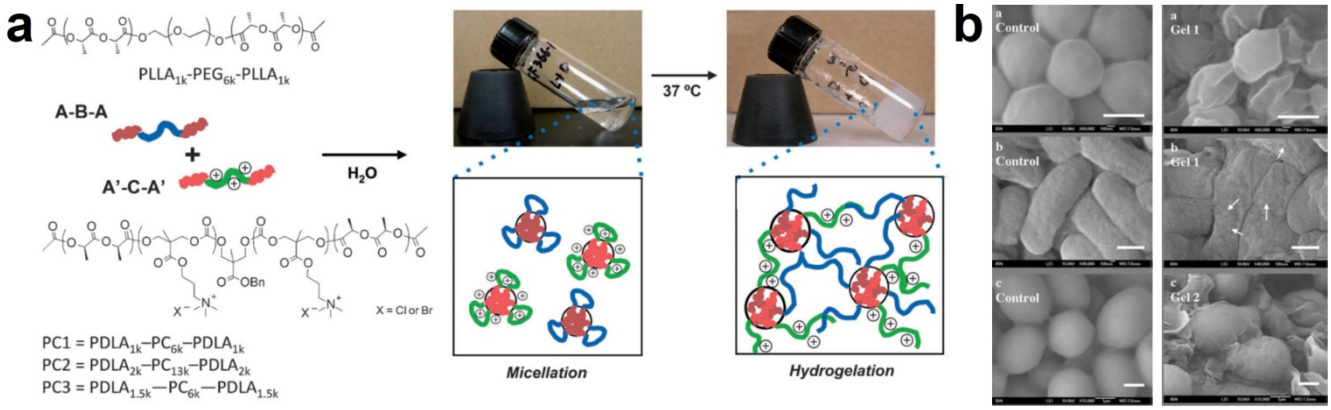

Fig. 3 Stereocomplexation strategy to form supramolecular hydrogel. (a) Formulation of the stereocomplexes comprising biodegradable polymer and a charged triblock polymer. (b) SEM images of the morphology of *S. aureus* (upper), *E. coli* (middle) and *Candida albicans* (bottom) before (control, left) and after (right) incubation with the gel formed from stereocomplexation. Scale bars: 500 nm. Figure reproduced from ref. 25 with the permission of Wiley-VCH.

demonstrated that the design of hydrogen bonding and electrostatic forces in supramolecular assemblies, affect their shape and structure, and can hence be used to adjust the stiffness of the hydrogels and their capabilities in cell signaling. This is because the cell wall and membrane of bacteria usually contain a large variety of oxygen-containing or nitrogen-containing compounds such as lipopolysaccharide, teichoic acid, and phosphatidyl glycerol phospholipid head groups, which are capable of forming multiple hydrogen bonds with antimicrobial agents.

Recently, a simple yet effective method using noncovalent interactions to produce charged hydrogels has been reported. Briefly, the gel is formed from stereocomplexation, a complexation of polymers with different tacticities or chiralities, of a polycarbonate triblock polymer and biodegradable poly(l-lactide)-b-poly(ethyleneglycol)-b-poly(l-lactide). The resultant stereocomplexes formed soluble micelles at room temperature and became supramolecular gels with ribbon-like and fiber-like structures upon heating to 37 °C (Fig. 3a).[25] Interestingly, the formed gel resulted in the significant enhancement of antimicrobial activity against both Gram-positive/Gram-negative bacteria and microbial biofilms. The antimicrobial mechanism was lysis of cell wall and membrane, as shown in the morphological changes of various bacteria after exposure to the gel (Fig. 3b).[25] Moreover, the gel remained nontoxic to mammalian cells and caused no significant hemolysis, rendering it ideal for topical and injectable applications. It is worth noting that the utilization of stereocomplexation strategy has enabled development of a variety of novel materials with unique functionalities and bioactivities.[26]

4.4 Supramolecular hydrogels formed from drug-based gelators

Despite the issues brought about by the overuse of conventional antibiotics, they remain significant players in the public health scene. Research efforts have thus also been directed into developing gelators from commercially-available drugs to improve their performance in various aspects. For example, it is thought that the self-assembly of such drug gelators may result in novel and potentially useful properties such as high local densities or multivalency, or even bioactive molecules possessing the ability to act in multiple roles.[27]

Inspired by this, researchers have made use of a broad-spectrum antibiotic, vancomycin, to design the small molecular antibiotic-modified gelator, vancomycin-pyrene. Vancomycin disrupts the metabolism of peptidoglycan (PG) and interferes the function of PG on exoskeleton of bacteria, whilst vancomycin-pyrene could form a supramolecular hydrogel at a low minimum gelation concentration of 0.36 wt% by virtue of the multiple hydrogen bonds and π-π stacking among the molecules. This was accompanied by the lack of reduction of potency, an undesirable effect commonly associated with antibiotic structural modifications. In fact, vancomycin-pyrene exhibited strong potencies nearly three orders of magnitude larger than its precursor, vancomycin, against vancomycin resistant enterococci. This was attributed to the self-assembly and aggregation could locally occur on the bacteria cell surface.[28]

5. Supramolecular hydrogels supporting antimicrobial agents

Apart from the direct formation of a supramolecular structure using inherently antimicrobial gelators, there exists another approach of utilizing these hydrogels as molecular composites in the delivery of encapsulated antimicrobial agents. Such a technique confers researchers with the ability to direct antimicrobial activity through both the polymeric carrier and the encapsulated agents if desired.[29] In addition, methodologies to incorporate metal nanoparticles with lower concentrations into the hydrogel networks have also been developed to achieve antimicrobial functions with reduced toxicity.

5.1 Supramolecular hydrogels incorporating antibiotics

A recurring theme in the efforts to combat multi-drug resistance in pathogenic microbes can be seen in the use of membrane-

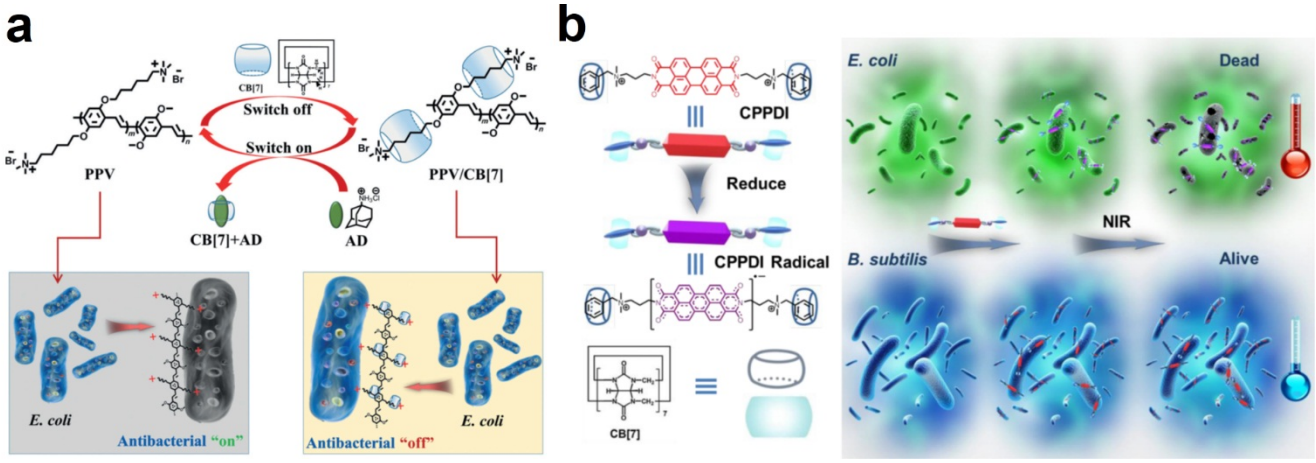

Fig. 4 Supramolecular complex performing antimicrobial activity on demand. (a) Supramolecular assembly and disassembly of poly(phenylene vinylene) regulated by amantadine for 'on-off' switch of its antimicrobial activity. Figure reproduced from ref. 31 with permission of Wiley-VCH. (b) Molecular structure of the supramolecular complex selectively triggered by bacteria with reductive ability. Figure reproduced from ref. 32 with permission of Wiley-VCH.

lytic elimination mechanisms and traditional antibiotics. However, recent studies have revealed that antibiotics, when used in conjunction with antimicrobial polymers, could achieve a synergistic effect, reducing drug resistance problems based on membrane-lytic mechanism of polymer, enhancing the permeability of bacteria membrane and facilitating the antibiotic penetration.[30] At the same time, the amount of polymer required for sufficient antimicrobial activity was reduced, allowing for the reduction of toxic side effects brought about by excessive carrier concentration. This was demonstrated through the integration of cationic polycarbonates with conventionally used antibiotics such as doxycycline, streptomycin or penicillin G, which was seen to effectively eliminate multidrug resistant bacteria like *P. aeruginosa*.

In a long-term point of view, effectively reducing bacterial resistance to antibiotics also requires avoiding the accumulation of active antibiotics in the environment. To address this issue, people recently developed a supramolecular antibiotics switch to perform antimicrobial activity on demand.[31] The reversible 'on-off' switch is controlled by the supramolecular assembly and disassembly process. As shown in Fig. 4a, the antimicrobial agent is composed of cationic poly(phenylene vinylene) (PPV) derivative with side chain of quaternary ammonium (QA) groups that could serve to penetrate the bacterial membrane and cause damage. The molecules cucurbit[7]uril (CB[7]) and amantadine are applied to switch-off or switch-on the antimicrobial function. Upon the addition of CB[7], the antimicrobial function could be switched off by virtue of the formation of a noncovalent complex between PPV and CB[7] with a cavity encapsulating QA groups. Further addition of amantadine could form the more stable CB[7]/amantadine complex and thus release the QA groups for the recovery of antimicrobial activity.

In a follow-up study, a supramolecular complex through host-guest interactions was constructed.[32] It is interesting that the reductive ability from facultative anaerobic bacteria such as *E. coli* could turn this complex to supramolecular radical anions, which is active during photothermal therapy for killing bacteria. In contrast, the aerobic bacteria without sufficient reductive ability would not activate the antibiotic property of this supramolecular complex (Fig. 4b). Therefore, the different capacity to produce radical anions could be applied to selectively inhibit particular bacteria *via* photothermal therapy.

5.2 Supramolecular hydrogels containing nanoparticles

The bactericidal properties of silver against microorganisms has been known for centuries. In addition, gold and zinc oxide nanoparticles have also been proven to be endowed with antimicrobial activities. However, these nanoparticles have been proven to be inherently cytotoxic, and even potentially pose adverse effects on the immunological response, proliferation, protein synthesis, morphological structure and the migration of cells. Thus, individually administrating these metal nanoparticles may result in necrosis and apoptosis of mammalian cells.

Efforts to reduce the toxicity of metal nanoparticle have thus been made through either employing hydrogels as supports for loading nanoparticles or chemical reactions to form hydrogel-nanoparticles composite.[33] For example, volume-tunable hydrogels have been prepared to load Ag nanoparticles through swelling-shrinking cycles. The nanoparticles could diffuse into the gel network during swelling process and be encapsulated into the gel during shrinking step. Modulating the number of cycles would optimize the antimicrobial performance of the hydrogel.

In addition to metal nanoparticles, development of nanoparticle-stabilized liposomes has become an emerging platform for antimicrobial delivery.[34] The practical formulation of such nanodelivery system is highly desirable for clinical use. Supramolecular hydrogels have been used as the vehicles for integrating nanoparticle-stabilized liposomes, and such systems offer unique advantages such as sequential drug release and improved tissue localization. Recently, studies reported a formulation of hydrogel containing pH-responsive gold nanoparticle-stabilized liposomes for topical therapy regarding bacterial infection. The carboxyl-modified gold nanoparticles (AuC) stabilizing liposomes (AuC-liposomes) are incorporated in a polyacrylamide gel system with tunable release kinetics. The hydrogel maintains the structural integrity of AuC-liposomes and the released liposomes fuse with target bacteria in response to the infection-induced acidic environment (Fig. 5a).[35] A follow-up investigation further addressed the challenge from the associated high shear forces at the infection site. The nanoparticle-hydrogel (NP-gel) hybrid system mimicking mussel adhesion was developed by copolymerizing dopamine methacrylamide (catechol moiety producing mussel adhesion) into the gel network (Fig. 5b).[36] The superior adhesion and antimicrobial retention even under large shear stress enable this NP-gel system to work as an effective and reliable local therapeutic platform.

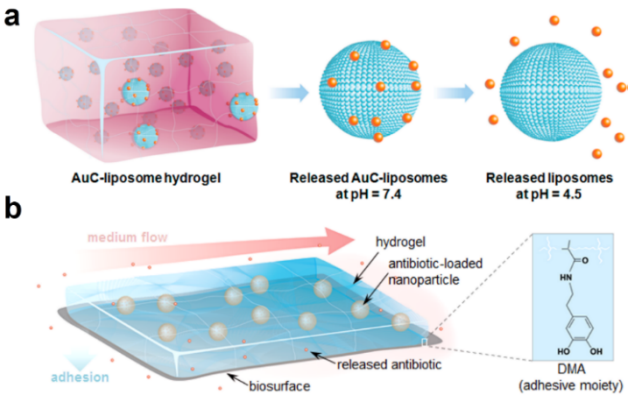

Fig. 5 Supramolecular hydrogel incorporating nanoparticles for therapy against microbial infections. (a) Schematic illustration of AuC-liposome hydrogel releasing AuC-liposomes in response to the pH of environment. Figure reproduced from ref. 35 with permission of American Chemical Society. (b) Illustration of the NP-gel system locally releasing antimicrobial agents under shear stress induced by medium flow. Figure reproduced from ref. 36 with permission of American Chemical Society.

6. Strategies of enhancing the antimicrobial efficacy of supramolecular hydrogels

Given that the membrane-targeted killing mechanism of antimicrobial supramolecular hydrogels has been deemed as the favorable path in combating antimicrobial resistance, the challenges and limitations remain for long-term use of these antimicrobial candidates due to either their cytotoxicity or continuously evolved drug resistance. Hence, researchers are developing new strategies for improving the performance and expanding the arsenal of antimicrobial hydrogels through the integration of more functions.

6.1 Supramolecular hydrogels formed by co-assembly

Antimicrobial activities of aforementioned supramolecular hydrogels were largely conferred by only one single gelator. However, supramolecular hydrogels formed by the co-assembly of two or more types of gelators or antibacterial compounds can drastically enlarge the scope of correlated applications. Based on this synergistic-killing strategy, a physically crosslinked hydrogel was prepared to perform the co-delivery of cationic antimicrobial poly-carbonates and traditional antimicrobial agent against bacteria growth and biofilm formation.[37] As shown in Fig. 6a, the synthesized hydrogel contains polycarbonate and poly(ethylene glycol) blocks, of which the mechanical property of the hydrogels can be maintained when incorporating positively-charged polycarbonates containing both alkyl/aromatic groups and vitamin E moieties. Furthermore, for the binary hydrogel, both the antimicrobial efficacy and biofilm eradication were enhanced when more hydrophobic positively-charged polycarbonates were incorporated. The authors also reported that synergistic antimicrobial effects are observed for the hydrogels which were composed of biocidal polycarbonates and antifungal fluconazole, which would contribute on designing novel antibacterial agents with attractive features, for example, reduction of drug concentrations and resistance, in practical therapy.

Another example is the preparation of a polyelectrolyte complex hydrogel incorporating chitosan as the cationic polyelectrolyte and γ-poly (glutamic acid) (γ-PGA) as the anionic polyelectrolyte, as shown in Fig. 6b.[38] The molar ratios of amine groups of chitosan to carboxylic acid groups of γ-PGA were variable and the simple ionic interaction resulted in the homogeneous polyelectrolyte complex hydrogel. Antibacterial tests on both Gram-positive and Gram-negative bacteria confirmed the satisfactory antibacterial ability. Furthermore, the positive charge and hydrophilicity of this hydrogel also enhanced the adsorption of serum proteins and promoted fibroblast cell attachment and proliferation, demonstrating improved biocompatibility.

Recently, 9-fluorenylmethoxycarbonyl (Fmoc)-based supramolecular hydrogels with inherent antibacterial activity and biocompatibility have been synthesized, of which the intermolecular interactions of Fmoc groups and hydrogen bonding of oligopeptides can easily promote the self-assembly of Fmoc-oligopeptides to form fibrous hydrogels. As the Fmoc-amino acids are normally used as precursor in synthesizing oligopeptides, it would be more cost-effective to directly use commercially Fmoc-amino acid as gelator for forming supramolecular hydrogel. To drive the gel formation, phenylalanine has been chosen as the candidate due to the enhanced intermolecular π-π stacking interactions from phenyl groups. Moreover, leucine has also been incorporated as it possesses intrinsic anti-inflammatory properties. Based on

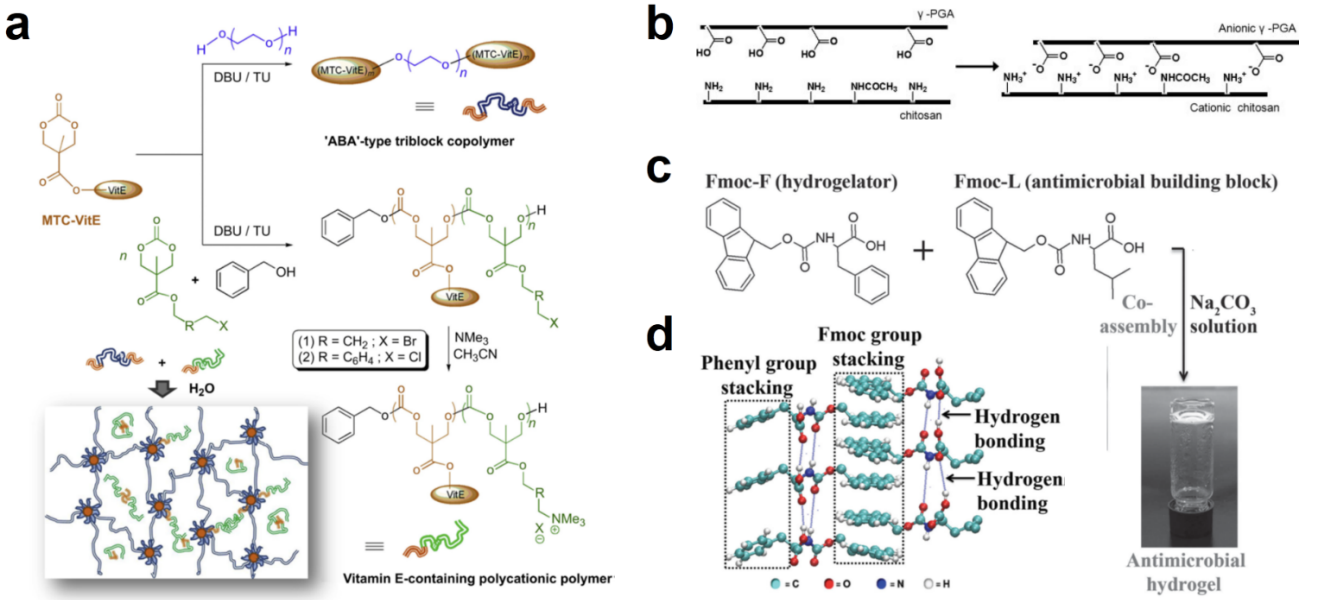

Fig. 6 Various Co-Assembled Hydrogels. (a) Synthesizing the ABA-type polycarbonate and poly (ethylene glycol) triblock copolymers containing vitamin E. Figure reproduced from ref. 37 with permission of Elsevier. (b) Schematic illustration of forming the ionic interactions between chitosan and γ-PGA. Figure reproduced from ref. 38 with permission of Elsevier. (c) Illustration of preparing the antimicrobial hydrogel by co-assembly of commercial Fmoc-F and Fmoc-L. (d) Proposed molecular arrangement of Fmoc-F and Fmoc-L in the co-assembled hydrogel, showing the intermolecular π-π stacking interactions and intermolecular hydrogen bonding drive the co-assembly. Figure reproduced from ref. 39 with permission of Wiley-VCH.

these two points, a strategy to co-assembly of Fmoc-phenylalanine (Fmoc-F) and Fmoc-leucine (Fmoc-L) has been developed (Fig. 6c).[39] The method is simple without complicated synthesis and purifications. The co-assembled elastic hydrogels are formed due to the multiple intermolecular non-covalent interactions among these two types of molecules, as shown in Fig. 6d.[39] Interestingly, this co-assembled (F+L) hydrogel shows preferential antimicrobial effect for gram-positive bacteria compared to that of Gram-negative bacteria, yet remaining biocompatible to normal mammalian cells. The selectivity should be attributed to the interactions between the hydrogel and the Gram-positive bacteria *e.g. S. aureus*. It is observed during their contact, the Fmoc-L can insert into *S. aureus* and release based on the hydrophobic interactions, in which the bacterial wall and membrane would be disrupted, resulting in the bacteria death. Overall, this supramolecular hydrogel demonstrates great potential in eliminating the problem raised from bacterial drug resistance and would be utilized as the coatings in clinical devices for treating skin and wound infections.

6.2 Hydrogels integrating both antimicrobial and antifouling properties

Generally speaking, the hydrophobic nature of supramolecular hydrogel results in a preferential absorption of protein on its surface. This surface fouling by protein would promote the attachment of bacteria and fungi, which may subsequently develop to form biofilms and enhance the risk of infections. In particular, implanted hydrogels could absorb the bodily fluids, resulting in the accumulation of either proteins or microorganisms and resulting in in severe health problems including circulation blocks, blood clots and inflammatory responses.

To address this concern, studies attempting to develop simultaneously antifouling and antimicrobial hydrogels have been reported. One approach is integrating both nonfouling and antimicrobial components into one supramolecular structure via copolymerization. An example representing this approach is a PEG-based hydrogel chemically incorporating polycarbonate containing QA groups. Organocatalytic ring opening polymerization was applied for synthesizing the block copolymer and addition of tetra-sulfhydryl PEG to the solution of star-shaped PEG conjugating aminated polycarbonate led to the final gelation. This PEG-based hydrogel could be *in situ* immobilized to the surface of rubber material (Fig. 7a), showing advanced antifouling and antimicrobial activity (Fig. 7b).[40] Tests on animals demonstrated that the hydrogel could effectively kill broad-spectrum pathogenic microbes, even including the multidrug-resistant bacteria, yet remain negligible side effects on host cells.

Another approach is integrating cationic active derivative with zwitterionic materials to encompass both antimicrobial and nonfouling attributes on a single surface. For example, derivatives of zwitterionic carboxybetaine with hydroxyl groups are in antifouling zwitterionic form at neutral or basic condition and convert to a cationic charged form under acidic condition (Fig. 7c).[41] The hydrogel surface could thus exhibit either active biocidal state or ultralow-fouling state, in response to external environments. The conversion overcomes the conventional limited antimicrobial capacity against individual or planktonic bacterial cell, as the controlled release of antimicrobial agents could work synergistically with the zwitterionic form in preventing bacterial colonization and inhibiting proliferation.

Although the aforementioned attempts could prevent multidrug-resistant infections and be used for biomedical coatings, the risk of rupture or damage of the hydrogel

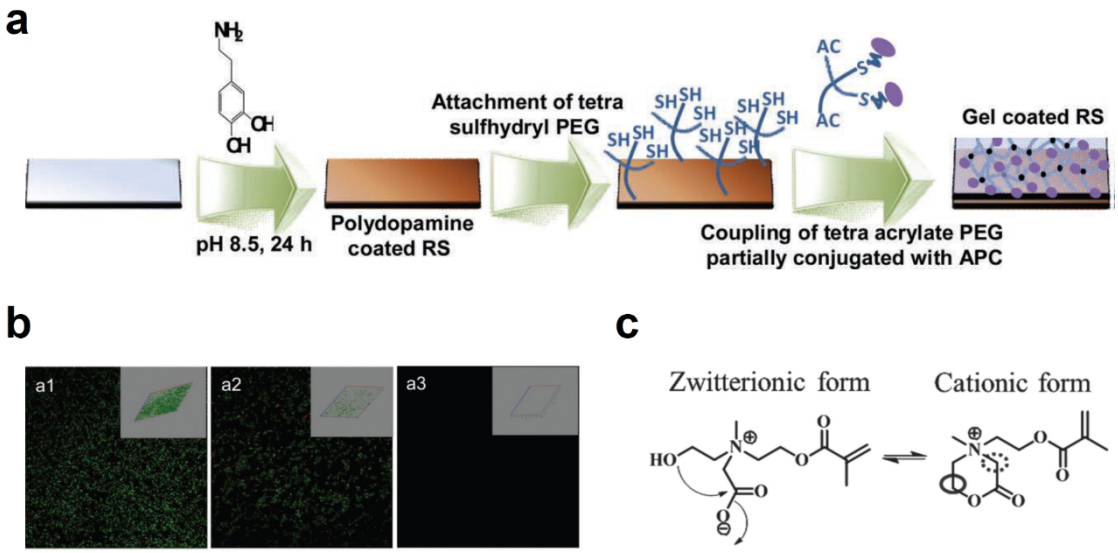

Fig. 7 Hydrogel coatings exhibiting antimicrobial and antifouling functions. (a) Schematic illustration of an antimicrobial hydrogel *in situ* immobilized onto the surface of a commonly used catheter materials. (b) Images showing attached bacteria after exposure to three different surfaces for 1 day: a1) Bare surface; a2) surface coated with PEG hydrogel; a3) surface coated with hydrogel with both antimicrobial and antifouling properties. Figure reproduced from ref. 40 with permission of Wiley-VCH. (c) poly(2-((2-hydroxyethyl) (2-(methacryloyloxy)ethyl)(methyl) ammonio) acetate) switching between zwitterionic form and cationic charged form. Figure reproduced from ref. 41 with permission of Wiley-VCH.

structure remains significant due to the repeated daily movements. To ensure a long-term usage, people recently developed a self-healable hydrogel network based on self-assembly of a triblock copolymer in ABA type.[42] The self-healing capability was constructed from the mussel inspired catechol-mediated hydrogen bonding and hydrophobic interactions, and the thermos-reversibility from its PEG-based blocks endows the hydrogel moldable and injectable. Integrating antimicrobial functions into self-healable supramolecular hydrogels could maintain its structural and functional integrity during bioengineering application, especially for implantation.

6.3 Stimuli-responsive hydrogels for antimicrobial therapy

The progression of hydrogels into the arena of stimuli-responsiveness can revolutionize the way the antimicrobial agents are delivered. These stimuli-responsive hydrogels can be strategized for different uses: (i) to achieve sustained release with minimum toxicity to the surrounding cells and tissues; (ii) to form responsive surfaces that possess both bactericidal ability and self-cleaning ability for long term usage.

6.3.1 Sustained release of antimicrobial agents

(1) pH responsive carriers

The over dosage of antimicrobial agents can be detrimental to mammalian cells. The ideal system would be similar to a battery works. The antimicrobial agents loaded in the hydrogel systems can be released on demand in manner akin to the charge flow in a battery upon activation. Not only would the lifetime of the antimicrobial agents be prolonged, it would also minimize the potential adverse effects on the mammalian cells and host tissues. Such a controlled release can be achievable with stimuli-responsive hydrogels. An example of such a system was made up of poly(acrylic acid) and p(2-(dimethylamino)ethyl methacrylate). As hydrogen bonds were responsible for holding the gel together, the gel was pH sensitive. The acidic environment brought about by the presence of the bacteria was emulated in the study. The release of vancomycin and levofloxacin was shown to be very responsive to pH change with almost complete release of antibiotics after 7 hours.[43]

(2) Mechano-responsive carriers

Antibiotics are also often used in greater dosages than actually required to account for the dilution in the blood stream or gut. This over-dosage carries a risk as it could exert adverse effects on the cells and tissues. To overcome this problem, hydrogels exhibiting mechano-responsive properties were developed. In a study, a hydrogel synthesized from peptides was demonstrated to form a viscous solution after being shaken and it regained its gel form after being left to stand without disturbance for 6 hours.[44] Another gel exhibited the same properties but achieved shorter gelation time of tens of minutes.[17] The thixotropic property appealed to the localized delivery of antimicrobial agents as it can be injected along with the gel into the intended location. This could also aid in the reduction of medical costs due to the reduced amount of the antimicrobial agents required to kill the bacteria.

6.3.2 Responsive surface with contact-killing performance

A sustainable antibiofouling property of medical devices is critical, especially for debridement and re-epithelization of the wound. Surface modification to enable the killing of bacteria upon contact is an effective method to prevent bacterial growth and even eradicate formed biofilm. Switchable capabilities could be endowed on the device surface to release the bacteria after killing, maintaining the cleanliness of the surface and extending the lifetime of the device. To achieve this, temperature-responsive polymer-based coatings have thus been developed, whereupon their stimuli-responsiveness allows for the switching from a cell-repellent surface a to cell-adherent one. Specifically, a smart coating capable of reversibly switching between bacteria-repellent and bactericidal function has been prepared via modification of the composition of a thermoresponsive oligo(ethylene glycol) methacrylate-based copolymer.[45] This coating contains antimicrobial peptides and could kill bacteria at room temperature. When the temperature raises to physiological condition, the surface turns hydrophobic through the collapse of the polymer chains and buries the antimicrobial peptides beneath, reducing the peptides-induced risk of hemolytic activity, yet preserving the antifouling property. A variety of the loaded agents have also been incorporated into this polymer-based coating by layer-by-layer technique, realizing a bacteria-triggered release of antimicrobial agents.[46] The smart coatings are self-defensive as they only release antimicrobial payload to kill bacteria when and where the bacteria approach the surface, and the correlate triggers are either the bacteria-induced pH variations or enzymes secreted by formed biofilms. As shown in Fig. 8a, the pH-induced release of antimicrobial agent collapsed the surface coating to a denser and smoother film. The released agents could efficiently kill the bacteria both on the surface and in the surrounding medium.[47]

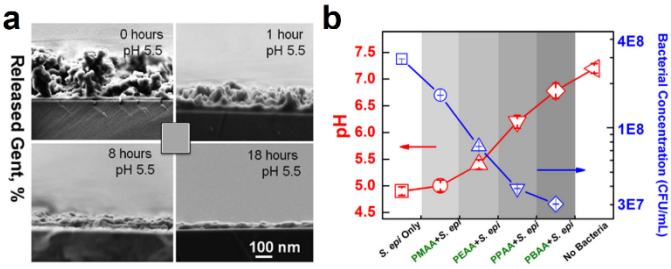

Fig. 8 Some typical examples of stimuli-responsive hydrogel-based system for antimicrobial treatment. (a) SEM images showing the releasing of antimicrobial agents from a film at various times. Figure reproduced from ref. 47 with permission of American Chemical Society. (b) The resultant concentration and pH of the medium after 24h culture of *Staphylococcus epidermidis* in solution containing various PaAA acids. Figure reproduced from ref. 48 with permission of Elsevier.

Further exploiting of this strategy enables the development of hydrogel coatings where the surface itself could become antimicrobial in response to the growth of bacteria. The biologically active coatings are manufactured from a family of polyanionic poly (2-alkylacrylic acids) (PaAAs), in which the hydrophobicity is dependent on the length of the included alkyl side chain. Under normal physiological environment (pH around 7.4), the coating remains hydrophilic and non-toxic to osteoblasts. However, in acidified condition caused by bacteria growth, the coating becomes

selectively toxic to staphylococcal bacteria. Moreover, the pH decrease would be concomitantly less due to smaller quantity of bacteria (Fig. 8b).[48] The antimicrobial mechanism is proposed as the insertion of the hydrophobic segments into the bacterial walls to cause damage and death. It is worth noting that the hydrogel coatings are not preloaded with any antimicrobial agents and thus do not need the antimicrobial supply. Unlike the continuously eluting coatings, this preload-free coating also diminishes the risk of the selection pressure from antibiotic-resistant bacteria.

7. Conclusions and perspectives

In coming years, advancement of antimicrobial supramolecular hydrogels will leverage new principles and methods in supramolecular chemistry to prepare more supramolecular structures with higher precision. One vision for the future of this field would attempt to mimic the innate immune system in pathogen recognition and inflammatory signaling to defense against diseases. Although supramolecular hydrogels integrating stimuli-responsive properties can achieve on-demand release of antimicrobial agents with a high level of control, realizing release of various components at controllable rates remains a challenge. Obtaining this multiple release could provide more solutions to perform 'cocktail therapy' against bacterial infections and significantly reduce the drug resistance of microorganisms. The potential of this combination therapy is exemplified from the fact of sequential signaling of various growth factors during natural tissue repair and regeneration. Ideally, the sequential release system would be endowed with multiple responsive capabilities, of which the response to a particular stimulus is predictable and precise. To build such intelligent systems will probably utilizing more cutting-edging technologies such as machine learning and even deep learning.

Alive materials is another category besides intelligent systems that would be efficient for antimicrobial therapy. The strategy involves using a particular bacterium or a combination of various bacteria to eliminate the harmful pathogens or balance the microbiota. Very recently, a 3D-printing technology has been developed to embed bacteria within complex biocompatible materials, forming 'living materials' capable of generating bacterial cellulose and degrading pollutants[49]. Applying this technology with supramolecular hydrogels may open the field of rationally designing bacteriotherapy to cure infections and other forms of persistent dysbiosis.

Progress in flexible bioelectronics and devices highlight more opportunities and extend the integration capability of supramolecular hydrogels for antimicrobial therapy.[50] Attempts to implant bioresorbable sensors and thermal therapy devices have been reported to achieve non-antibiotic bacteriocides for surgical site infections. The design and principles in these devices could be integrated with supramolecular hydrogels to create next-generation bioelectronics with safe therapeutic functions for long-term use. The hybridized devices could actively respond to the in-vivo environment and carry out therapeutic activities accordingly.

In summary, the development of supramolecular assembly and exploring of molecular recognition or intermolecular communications between antimicrobial agents and bacteria cell wall/membrane provide in-deep fundamental insights for designing supramolecular hydrogels with efficient and broad-spectrum antimicrobial activities. Continued fundamental advancements in molecular engineering and expanding arsenal of materials will further broaden and deepen the strategies applicable for antimicrobial hydrogels. Coupled with progressive efforts in biology and artificial intelligence, we expect that the expanding study of supramolecular hydrogels will enhance therapeutic impact and improve human healthcare.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors thank the support by the National Research Foundation, Prime Minister's Office, Singapore, under the NRF Investigatorship (NRF-NRFI2017-07).

References

1. D. L. Heymann, *Cell*, 2006, **124**, 671-675.
2. C. A. Michael, D. Dominey-Howes and M. Labbate, *Front. Public Health*, 2014, **2**, 145.
3. E. R. Angert, *Nat. Rev. Microbiol.*, 2005, **3**, 214-224.
4. S. B. Levy and B. Marshall, *Nat. Med.*, 2004, **10**, S122-129.
5. A. S. Hoffman, *Adv. Drug Delivery Rev.*, 2012, **64**, 18-23.
6. E. Ye, P. L. Chee, A. Prasad, X. Fang, C. Owh, V. J. J. Yeo and X. J. Loh, *Materials Today*, 2014, **17**, 194-202.
7. E. A. Appel, F. Biedermann, U. Rauwald, S. T. Jones, J. M. Zayed and O. A. Scherman, *J. Am. Chem. Soc.*, 2010, **132**, 14251-14260.
8. Y. Q. Li, B. W. Zhu, Y. G. Li, W. R. Leow, R. Goh, B. Ma, E. Fong, M. Tang and X. D. Chen, *Angew. Chem., Int. Ed.*, 2014, **53**, 5837-5841.
9. M. Burnworth, L. M. Tang, J. R. Kumpfer, A. J. Duncan, F. L. Beyer, G. L. Fiore, S. J. Rowan and C. Weder, *Nature*, 2011, **472**, 334-337.
10. M. J. Webber, E. A. Appel, E. W. Meijer and R. Langer, *Nat. Mater.*, 2016, **15**, 13-26.
11. B. H. Hu, W. R. Leow, P. Q. Cai, Y. Q. Li, Y. L. Wu and X. D. Chen, *ACS Nano*, 2017, **11**, 12302-12310.
12. J. Y. Li and D. J. Mooney, *Nat. Rev. Mater.*, 2016, **1**, 16071.
13. W. W. Xu, Q. A. Song, J. F. Xu, M. J. Serpe and X. Zhang, *ACS Appl. Mater. Interfaces*, 2017, **9**, 11368-11372.
14. L. Beven, S. Castano, J. Dufourcq, A. Wieslander and H. Wroblewski, *Eur. J. Biochem.*, 2003, **270**, 2207-2217.
15. A. Giuliani and A. C. Rinaldi, *Cell. Mol. Life Sci.*, 2011, **68**, 2255-2266.
16. T. K. Nguyen, S. J. Lam, K. K. K. Ho, N. Kumar, G. G. Qiao, S. Egan, C. Boyer and E. H. H. Wong, *ACS Infect. Dis.*, 2017, **3**, 237-248.
17. N. Nandi, K. Gayen, S. Ghosh, D. Bhunia, S. Kirkham, S. K. Sen, S. Ghosh, I. W. Hamley and A. Banerjee, *Biomacromolecules*, 2017, **18**, 3621-3629.
18. A. S. Veiga, C. Sinthuvanich, D. Gaspar, H. G. Franquelim, M. Castanho and J. P. Schneider, *Biomaterials*, 2012, **33**, 8907-8916.
19. L. Jiang, D. Xu, T. J. Sellati and H. Dong, *Nanoscale*, 2015, **7**, 19160-19169.
20. B. Findlay, G. G. Zhanel and F. Schweizer, *Antimicrob. Agents Chemother.*, 2010, **54**, 4049-4058.
21. C. R. A. Maria and D. M. C. L. Dias, *Int. J. Mol. Sci*, 2013, **14**, 9906-9946.
22. M. F. Ilker, H. Schule and E. B. Coughlin, *Macromolecules*, 2004, **37**, 694-700.
23. R. N. Mitra, A. Shome, P. Paul and P. K. Das, *Org. Biomol. Chem.*, 2009, **7**, 94-102.
24. S. Brahmachari, S. Debnath, S. Dutta and P. K. Das, *Beilstein J. Org. Chem.*, 2010, **6**, 859-868.
25. Y. Li, K. Fukushima, D. J. Coady, A. C. Engler, S. Q. Liu, Y. Huang, J. S. Cho, Y. Guo, L. S. Miller, J. P. K. Tan, P. L. R. Ee, W. M. Fan, Y. Y. Yang and J. L. Hedrick, *Angew. Chem., Int. Ed.*, 2013, **52**, 674-678.
26. Z. B. Li, B. H. Tan, T. T. Lin and C. B. He, *Prog. Polym. Sci.*, 2016, **62**, 22-72.
27. F. Zhao, M. L. Ma and B. Xu, *Chem. Soc. Rev.*, 2009, **38**, 883-891.
28. B. G. Xing, C. W. Yu, K. H. Chow, P. L. Ho, D. G. Fu and B. Xu, *J. Am. Chem. Soc.*, 2002, **124**, 14846-14847.
29. V. W. Ng, J. M. Chan, H. Sardon, R. J. Ono, J. M. Garcia, Y. Y. Yang and J. L. Hedrick, *Adv. Drug Delivery Rev.*, 2014, **78**, 46-62.
30. V. W. L. Ng, X. Y. Ke, A. L. Z. Lee, J. L. Hedrick and Y. Y. Yang, *Adv. Mater.*, 2013, **25**, 6730-6736.

31. H. T. Bai, H. X. Yuan, C. Y. Nie, B. Wang, F. T. Lv, L. B. Liu and S. Wang, *Angew. Chem., Int. Ed.*, 2015, **54**, 13208-13213.
32. Y. C. Yang, P. He, Y. X. Wang, H. T. Bai, S. Wang, J. F. Xu and X. Zhang, *Angew. Chem., Int. Ed.*, 2017, **56**, 16239-16242.
33. P. Thoniyot, M. J. Tan, A. A. Karim, D. J. Young and X. J. Loh, *Adv. Sci.*, 2015, **2**, 1400010.
34. D. Pornpattananangkul, L. Zhang, S. Olson, S. Aryal, M. Obonyo, K. Vecchio, C. M. Huang and L. F. Zhang, *J. Am. Chem. Soc.*, 2011, **133**, 4132-4139.
35. W. W. Gao, D. Vecchio, J. M. Li, J. Y. Zhu, Q. Z. Zhang, V. Fu, J. Y. Li, S. Thamphiwatana, D. N. Lu and L. F. Zhang, *ACS Nano*, 2014, **8**, 2900-2907.
36. Y. Zhang, J. H. Zhang, M. G. Chen, H. Gong, S. Tharnphiwatana, L. Eckmann, W. W. Gao and L. F. Zhang, *ACS Appl. Mater. Interfaces*, 2016, **8**, 18367-18374.
37. A. L. Z. Lee, V. W. L. Ng, W. X. Wang, J. L. Hedrick and Y. Y. Yang, *Biomaterials*, 2013, **34**, 10278-10286.
38. C. T. Tsao, C. H. Chang, Y. Y. Lin, M. F. Wu, J. L. Wang, J. L. Han and K. H. Hsieh, *Carbohydr. Res.*, 2010, **345**, 1774-1780.
39. I. Irwansyah, Y. Q. Li, W. X. Shi, D. P. Qi, W. R. Leow, M. B. Y. Tang, S. Z. Li and X. D. Chen, *Adv. Mater.*, 2015, **27**, 648-654.
40. S. Q. Liu, C. Yang, Y. Huang, X. Ding, Y. Li, W. M. Fan, J. L. Hedrick and Y. Y. Yang, *Adv. Mater.*, 2012, **24**, 6484-6489.
41. B. Cao, Q. Tang, L. L. Li, J. Humble, H. Y. Wu, L. Y. Liu and G. Cheng, *Adv. Healthcare Mater.*, 2013, **2**, 1096-1102.
42. L. Li, B. Yan, J. Q. Yang, W. J. Huang, L. Y. Chen and H. B. Zeng, *ACS Appl. Mater. Interfaces*, 2017, **9**, 9221-9225.
43. Z. Lu, J. Zhang, Z. Yu, Q. Liu, K. Liu, M. Li and D. Wang, *New J. Chem.*, 2017, **41**, 432-436.
44. A. Baral, S. Roy, S. Ghosh, D. Hermida-Merino, I. W. Hamley and A. Banerjee, *Langmuir*, 2016, **32**, 1836-1845.
45. X. Laloyaux, E. Fautre, T. Blin, V. Purohit, J. Leprince, T. Jouenne, A. M. Jonas and K. Glinel, *Adv. Mater.*, 2010, **22**, 5024-5028.
46. S. Pavlukhina, Y. M. Lu, A. Patimetha, M. Libera and S. Sukhishvili, *Biomacromolecules*, 2010, **11**, 3448-3456.
47. I. Zhuk, F. Jariwala, A. B. Attygalle, Y. Wu, M. R. Libera and S. A. Sukhishvili, *ACS Nano*, 2014, **8**, 7733-7745.
48. Y. M. Lu, Y. Wu, J. Liang, M. R. Libera and S. A. Sukhishvili, *Biomaterials*, 2015, **45**, 64-71.
49. M. Schaffner, P. A. Ruhs, F. Coulter, S. Kilcher and A. R. Studart, *Sci. Adv.*, 2017, **3**, eaao6804.
50. Y. Q. Liu, K. He, G. Chen, W. R. Leow and X. D. Chen, *Chem. Rev.*, 2017, **117**, 12893-12941.