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Biomaterials Science
Reviews

Title

Amphiphilic Polymer therapeutics: An alternative platform in the fight against antibiotic resistant bacteria

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Abstract

As we are on the cusp of the "Post-antibiotic" era due to rapid spread of drug resistant bacteria, there is an urgent need for new antimicrobials that are not susceptible to bacterial resistance mechanisms. In this review, we will discuss recent development of "polymer therapeutics" with antimicrobial activity. Learning from host-defence peptides, we propose the biomimetic design of synthetic polymers to target bacterial cell membranes which act by compromising the membrane integrity. The discussion is extended to the future challenges and opportunities of antimicrobial polymers for clinical applications.

1. Introduction

The emergence of antibiotic resistance in bacteria has been one of the most pressing issues in facing clinical practitioners in hospital and community settings, significantly reducing the number of treatment options available for bacterial infections.[1-3] An United Nations (UN) report on drug resistance by the Interagency Coordination Group (IADG) in 2019, reported to the secretary-general of the UN that at least 700,000 deaths a year are already caused by drug-resistant diseases in the world, including 230,000 deaths from multidrug-resistant tuberculosis.[4] The UN also warns the number of deaths due to drug-resistant diseases could be increased to 10 million globally per year by 2050 if no action is taken to address this problem. Similar guidance and warnings have been issued from the World Health Organization (WHO) and the United States Centers for Disease Control (CDC).[5, 6] We are on the cusp of the "Post-antibiotic" era due to rapid spreading of resistant and, in some cases, multi-drug resistant bacteria that cannot be eliminated by any clinically approved antibiotics. Bacteria may develop resistance mechanism(s) when they are exposed to sub-lethal concentrations of antibiotics, through lateral gene transfer with other bacteria, or through mutation of alternative resistance or detoxification pathways.[7-9] Many researchers have been making tremendous efforts to identify molecular targets which are effective in inhibiting bacterial growth but do not contribute to resistance development.[7, 10, 11] However, the development of new antibiotics has been hampered by several factors including multiplicity in the modes of resistance development, poor patient compliance with dosing regimens, and lack of research focus in the pharmaceutical industry due to questionable financial return on investment.[12] In addition to traditional microbiology, medicine, and pharmacology, interdisciplinary approaches incorporating material science are emerging as viable alternatives in the fight against antimicrobial resistance.

In this minireview, we will first introduce the antibiotic resistance and tolerance mechanisms, which are related to a large range and dimension of complexity from a single cell to multicellular aggregates, namely biofilms. The discussion will be extended to the development of polymer therapeutics to address the antibiotic resistance challenge. Learning from host-defence antimicrobial peptides in the innate immune system, we envision the design of synthetic polymers which selectively target bacterial cell membranes and act by compromising the membrane integrity, regardless planktonic or in biofilm, and will, ideally, be less susceptible to resistance development (**Fig. 1**). The general purpose of this review is not a comprehensive review of the literature, but instead discussing the

potential contribution of polymer therapeutics for prevention territorial expansion of evil drug-resistant bacteria as well as their challenges and opportunities in clinical applications.

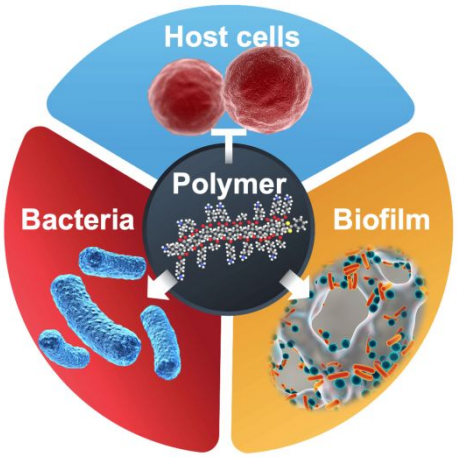

Figure 1. Antimicrobial polymer therapeutics effective against planktonic bacteria and bacterial biofilms with drug-resistance.

2. Drug resistance/tolerance in bacteria

Development of drug resistance – a complex problem. Drug resistance is the ability of cells to survive at higher concentrations of drugs than the typical lethal dose. When bacterial cells are insensitive to therapeutic agents, there are two main categories that can cause this phenomenon: resistance and tolerance.[13] The narrowly defined "resistance" is characterized by genetic mutations in the bacterium,[14, 15] which induces alteration of the drug target, enhanced drug efflux systems, or cell-mediated drug inactivation. On the other hand, "tolerance" is defined as decreased responsiveness to drugs caused by cellular adaptation as a result of exposure to them.[16, 17] These bacterial resistance and tolerance mechanisms involve a large range and dimension of complexity. In a single cell, specific functional dysregulations are caused by mutations, transcriptional, and translational alterations which change cellular biological processes such as cell growth, metabolism, differentiation, and division. Additionally, bacteria that are exposed to one antibiotic are also capable of developing resistance mechanisms against multiple other antibiotics, resulting in cross resistance.[18] An additional layer of complexity arises from the heterogeneity of responses between cells in population (**Fig. 2**). In some cases, a small subpopulation in a clonal bacterial population, known as persisters, survive after exposure to a high concentration of antibiotics that cause the majority of bacteria in that infection to be eradicated.[19] Multicellular assemblies (i.e. biofilms) can also impact bacterial survival by limiting antibiotic access to the bacterial cells. This is further complicated by environmental factors physically

surrounding a biofilm which can also influence drug effectiveness. These influences are hierarchically associated with one another, and multiple and collective defects combine to result in treatment failure.

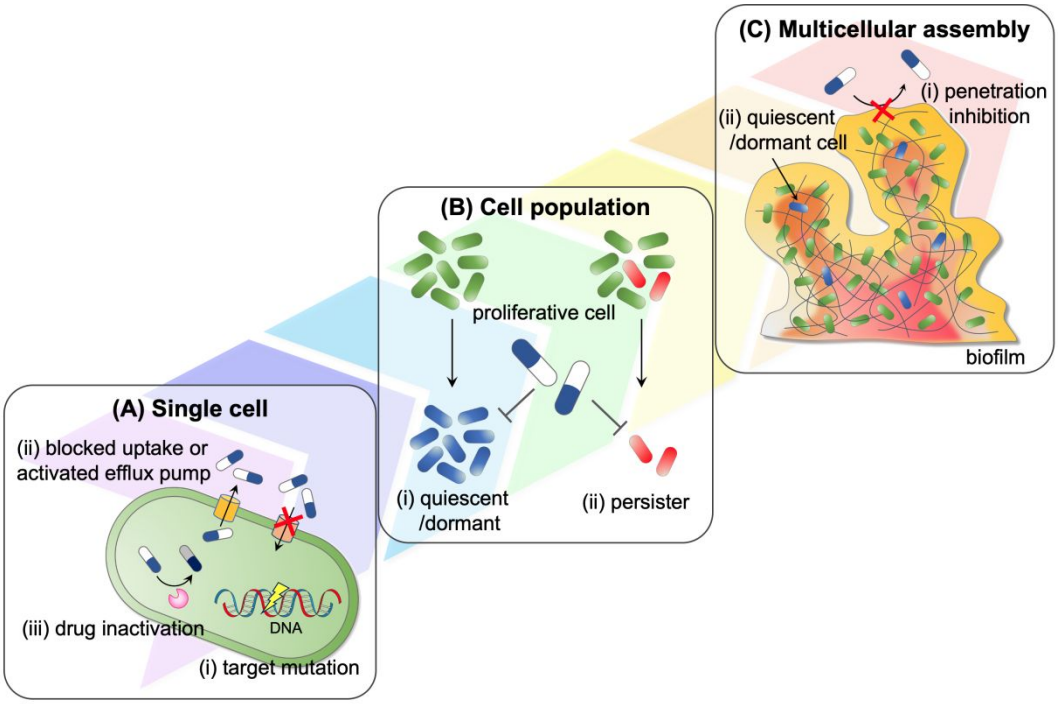

Figure 2. Drug resistance mechanisms in bacteria. (A) In a bacterial cell, there are several mechanisms to gain resistance owing to gene/protein alterations; (i) mutation of drug target protein gene to alter the structure of an active or regulatory site, (ii) reduction of drug concentration by decreasing uptake and enhancing efflux, and (iii) inactivation of drugs by modification or degradation enzymes. (B) In a clonal bacterial cell population, there are several types of sub-populations with different susceptibility to drug treatment; (i) dormant or quiescent cells are minimally susceptible to antibiotics which target cellular metabolic processes, and (ii) persisters, a subpopulation of a clonal bacterial population, which survive despite exposure to a lethal concentration of drugs. (C) Bacterial cells aggregate to build multicellular assemblies, called biofilms, which protect embedded bacteria by crosslinked polymer network matrix to reduce penetration of drugs (i). Also, bacteria in biofilms often stay in quiescent or dormant mode (ii).

Resistance/tolerance mechanisms. In a single bacterial cell, there are several strategies and routes to resistance by altering genes and/or proteins; active site mutation in drug target enzymes,[20] reduction of drug concentration by impaired/reduced uptake or activated efflux pump,[21, 22] drug inactivation by enzymes,[23] and DNA damage repair[24, 25]. As cellular response to drug treatment, a commonly observed phenomenon is a slow-down in general cellular metabolic processes, which leads to a dormant or quiescent stage of the cell cycle.[13, 26] Most traditional small-

molecule antibiotics target bacterial vital activities such as DNA synthesis, RNA synthesis, cell wall synthesis, or protein synthesis which are directly tied to cellular metabolism, growth, and cell division.[7, 27] In other words, the targets of many antibiotics are only present/active in actively growing bacteria. In response to this mode of antimicrobial action, some bacterial cells escape the drugs' attack by slowing metabolism, entering a dormant and/or quiescent cell state. Beyond the treatment-induced adaptation, a subpopulation of bacterial cells, persisters, may coincidentally be in a slow growing or dormant state and thus are intrinsically more tolerant to antibiotics compared to other subpopulations which are actively dividing.[19] These persister cells are a phenotypic variation of bacteria, not a genetic variant or mutation.[28, 29] Generally, bacterial cells can adhere to abiotic (implants) or tissue surfaces and assemble into biofilms in the host body.[30] Once adhered to the surface, these bacteria produce polysaccharides, lipids, proteins, and extracellular DNA as extracellular matrices to build the biofilm matrix. These components provide crosslinked polymer network, which forms a physical barrier preventing penetration of antibiotic molecules to bacteria deeply embedded in the biofilm.[31, 32] Thus, the actual concentration of the antibiotic that can reach bacterial cells in a biofilm is much lower than that in solution. Exposure of bacterial cells to low or sublethal antibiotic concentrations select for resistant bacteria, which contributes to resistance development. In addition, the bacteria in biofilms are often quiescent or dormant and thus they are inherently tolerant to many antibiotics as discussed above. Overall, long-term exposure to antibiotics causes the genetic or phenotypic changes coupled with physical and chemical environmental benefits of biofilm formation combine to benefit bacterial survival, resulting in the limitation of drug efficacy.

3. Polymer therapeutics to overcome drug resistance and tolerance in bacteria

Cell membrane as drug target. Since the discovery of penicillin by Dr. Alexander Fleming in 1928, many clinicians and researchers have devoted significant effort to develop antibiotic therapeutics to treat bacterial infections. However, as discussed above, while we administer these drugs for treatment, many bacterial species acquire resistant phenotypes which limit the number of effective treatments available to clinicians. The traditional small molecule drugs designed to target specific cellular components may not be able to escape from resistance mechanisms, therefore we have to attack alternative targets in bacteria. Here, we propose a non-traditional antibiotic design via a biomimetic approach to create antimicrobials which act by targeting bacterial cell membranes. The lipid bilayer structure of cell membranes in bacteria are not directly associated with the majority of resistance mechanisms and hence they are a promising candidate as a target site in bacteria. Though novel, the cell membranes are not an ideal drug target because the lipid bilayer structures are not specific to bacteria; all cells, from bacteria through complex higher organisms, have lipid bilayer cell membranes. In addition, the cell membranes do not have "defined" binding sites that enables the lock-and-key design of membrane-active therapeutic molecules compared to the traditional enzyme-inhibitor models. As an approach to address these challenges, we here propose to mimic

the biophysical traits and physiochemical architecture of naturally occurring antimicrobial peptides using synthetic polymers which show affinity for the bulk properties of the bacterial membrane compared to host membranes.

3.1. Host-defense antimicrobial peptides (AMPs)

Learning from the natural immune system, host-defense antimicrobial peptides (AMPs) have been a part of immune systems in most organisms throughout evolution to protect from pathogens.[33-35] These molecules show potent inhibitory effects against a number of pathogens, such as bacteria, fungi, parasites, and viruses, as well as some drug-resistant bacteria. The conservation through evolution and efficacy against otherwise resistant organisms indicates the AMP mode of action is independent from that of typical antibiotics which trigger development of resistance mechanisms. AMPs are relatively short peptides which act by disrupting bacterial membranes and/or target the intracellular components such as DNA.[36-38] The prominent and essential trait of this peptide family is cationic amphiphilicity; the cationic side chains facilitate the binding of AMPs to anionic bacterial cell walls and cytoplasmic membranes while the hydrophobic side chains are inserted into the nonpolar core of the lipid membrane, disrupting the structural integrity of membranes and compromise the barrier function of membranes. Because the bacterial cell membranes are highly negatively charged as compared to those of human cells, AMPs preferably bind to bacteria over human cells, leading to cell selectivity.[36, 37, 39, 40] Indeed, many experimental studies have demonstrated the membranolytic mechanism of AMPs.[41-43] Some AMPs enter the cytoplasm and bind to DNA.[44, 45] Recently, Weisshaar and coworkers directly demonstrated that human AMP LL-37 enters into *E. coli* and alter the motility of DNA and proteins, which is essential to their biological function.[46] Similarly, a number of Trp-rich AMPs have been identified to interact with bacterial DNA without causing significant membrane damage.[47-49] Because many conventional antibiotics are bacteriostatic at low concentrations, bacteria can recover once the antibiotics are removed. However, AMPs are bactericidal, resulting in bacterial cell death and thus no ability to recover after the AMPs are cleared. Unfortunately, AMPs have not translated well into the clinic due to poor bioavailability and high cost of production.[38]

3.2 AMP-mimetic polymers

Harnessing these beneficial properties of AMPs is a promising avenue to create new antimicrobial agents to mitigate the resistance mechanism in bacteria. To that end, many studies have been devoted to developing antimicrobial polymers which mimic the functions and molecular signatures of AMPs. These polymers have cationic and hydrophobic groups which are essential to selectively target and disrupt bacterial cell membranes (**Fig. 3**). Many studies on the structure-activity relationships have been reported, and the optimization of monomer compositions and block sequences of cationic and hydrophobic monomers for potent activity have been extensively examined.[50, 51] In addition, nano- and micro-structures of polymers and particles have been also investigated for

their antimicrobial activity.[52] While many studies on antimicrobial polymers have been reported, the focus below is on the selected studies that particularly address drug resistance in bacteria (**Table 1**).

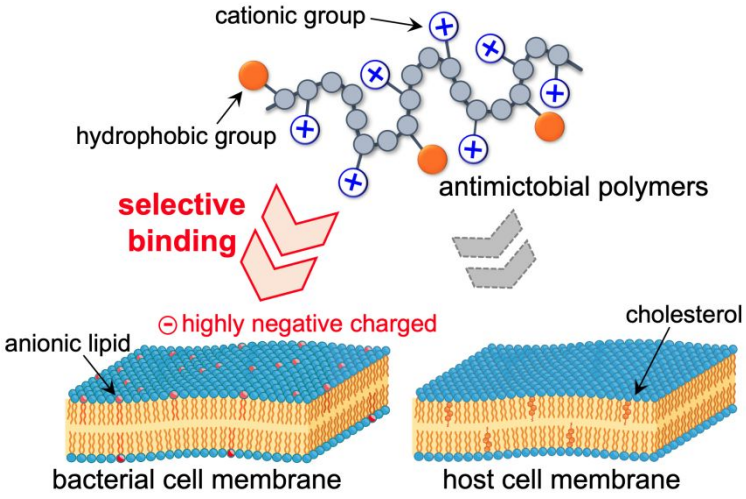

Figure 3. Design of antimicrobial polymers and their mechanism mimicking properties of the host-defense antimicrobial peptides (AMPs) to eliminate bacteria. The polymers own cationic charged groups and hydrophobic groups in a polymer chain which show global amphiphilicity. The polymers selectively bind to negatively charged bacterial cell membranes rather than host cell and disrupt it to cause cell death.

Table 1. Antimicrobial polymers addressing drug resistant bacteria.

Polymers/Macromolecules	Drug resistant bacteria[a]	Reference
Single polymer chains		
Cationic/amphiphilic poly(maleic anhydride)s	MRSA, VRE, MDR *A. baumannii*	66, 67
Cationic/amphiphilic guanidine hydrochloride polymers	96 clinical isolated MDR bacteria	71
Cationic/amphiphilic polymethacrylates	MRSA, VRSA	73
Cationic/amphiphilic polymethacrylates + antibiotics	MDR *P. aeruginosa*	96
Cationic polyionenes	MRSA, MDR *E. coli*, *A. baumannii*, *K. pneumonia*	74, 75
Cationic/amphiphilic synthetic random peptide polymers	Clinical isolated MDR *S. aureus*, *S. haemolyticus*, *P. aeruginosa*, *E. coli*, *K. Pneumoniae*, *A. baumannii*	76, 77
Nanoparticles/micelles/macromolecules		
Cationic chitosan-graft-polylysine nanoparticle	MRSA	68, 69
Cationic di-block copoly(beta-peptide)s	MDR MRSA (planktonic and biofilm)	79

Mixed cationic/biodegradable triblock polymer micelle (PLLA-PEG-PLLA & PDLA-CPC-PDLA) and its hydrogels	MRSA (planktonic and biofilm), VRE	83

[a)] MRSA, methicillin-resistant *S. aureus*; VRSA, vancomycin-resistant *S. aureus*; VRE, vancomycin-resistant *Enterococci*; MDR, multi-drug resistant

3.3 Selectivity to bacteria over human cells

AMPs and AMP-mimetic polymers rely on the inherent differences between bacteria and host cells in both lipid compositions and physicochemical properties of cell membranes cells to allow selective targeting of bacterial cells over host cells. A hallmark of bacterial membranes is the highly anionic surface charge which arises from anionic lipids and anionic groups in the polysaccharide coat around the cells.[53, 54] Alternatively, mammalian cell membranes have a generally net neutral surface,[55] while the cell-surface glycosaminoglycans (GAGs) are negatively charged[56]. This is in stark contrast to the mammalian membrane which is primarily composed of zwitterionic phosphatidylcholine lipids and cholesterol.[55, 57] Mammalian cells do contain small quantities (~12 mol%) of anionic lipids such as phosphatidylinositol and phosphatidylserine, these lipids are localized to the inner leaflet of the cell membrane and thus are not exposed to the extracellular environment.[58, 59] While specific lipid and polysaccharide compositions vary from species to species in bacteria, phospholipids such as phosphatidylglycerol and cardiolipin and the polysaccharide-linked lipids such as lipopolysaccharide (LPS) and lipoteichoic acid (LTA) impart the anionic character to the bacterial surface. The strong net negative charge of the bacterial surface as compared to the human cell surface allows for electrostatic forces to drive the binding of AMPs and polymers to the bacteria over human cells.[60-62] This net charge difference is at the core of how AMP-mimetic polymers selectively target bacterial cells in the complex host environment.

3.4 Antimicrobial polymers to mitigate drug resistance in bacteria

Many studies reported that the activity of AMP and AMP-mimetic polymers is inherently broad spectrum including drug resistant bacteria (**Table 1**).[52, 63-71] Nosocomial (hospital-acquired) bacterial pathogens with multi-drug resistance have been highlighted by "ESKAPE", an acronym standing for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*..[8, 72] AMP-mimetic polymers have shown potent activity against these ESKAPE pathogens. For example, methacrylate random copolymers inhibited growth of clinical isolates of methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA).[73] Another recent example is that polyionenes with rigid amide bonds showed potent *in vitro* activity against clinically isolated multi-drug resistant ESKAPE pathogens.[74] The same polymer was used to treat drug resistant *K. pneumoniae* lung infections in a mouse model, showing that antimicrobial mechanism of AMP-mimetic polymers could address drug-resistant bacterial infections *in vivo*.[75] Additionally, a short synthetic peptide polymer killed clinically isolated multi-drug resistant bacteria in multiple

strains both *in vitro* and *in vivo*.[76, 77] Antimicrobial methacrylate copolymers did not induce the resistance development in *E. coli*, and the *E. coli* exposed to the polymers did not exhibit or develop any cross-resistance to conventional antibiotics.[78] While traditional antibiotics target proliferating bacteria, antimicrobial polymers act by disrupting bacterial cell membranes which are not dependent on metabolic activity or the cell division process. Therefore, the polymers were expected to kill slowly proliferating or dormant bacteria. Indeed, the methacrylate copolymers reduced the number of viable *S. aureus* both in the exponential and stationary growth phases, indicating that the membrane-active mode of the polymers is effective in killing low proliferating bacteria.[78] Recently, also, enantiomeric glycosylated cationic block co-beta-peptides have been shown to be active against nutrient-starved persister MRSA.[79]

Traditionally, membrane disruption is the consensus hypothesis for the antimicrobial mechanism of these amphiphilic polymers. However, it should be highlighted that Yang and Hedrick recently demonstrated that the cationic guanidinium-functionalized polycarbonates exerted antimicrobial effects by entering into bacterial cell and causing precipitation with cellular components.[80] Another example of intercellular activity of polymers is that polyhexamethylene biguanide (PHMB) polymer internalize in the cells and bind to DNA, causing cell division arrest and chromosome condensation.[81] Locock and coworkers recently demonstrated using super-resolution fluorescence imaging that methacrylate copolymers exerted their antimicrobial effects by mechanisms involving membrane permeation as well as cellular uptake, interaction with intracellular targets and possible complexation with bacterial DNA.[82] Again, these results are in parallel to many of the findings for AMPs, thus it is not surprising that a polymer that faithfully mimics the AMP physiochemical properties will also mimic the function and mechanism.

In addition to planktonic bacteria, antimicrobial polymers have also been tested for eradication of biofilms. For example, multi-drug resistant *S. aureus* biofilm colonized on plate was disrupted and eliminated by enantiomeric block co-beta-peptide.[79] Disruption of MRSA biofilm was also reported by injectable and biodegradable hydrogels which are constructed by non-covalent crosslinking of antimicrobial polymer micelles.[83] Further, it was recently demonstrated that cariogenic dental *S. mutans* biofilm could be removed under hydrodynamic cyclic stress for 30 sec using an antimicrobial polymer solution (**Fig. 4**).[84]

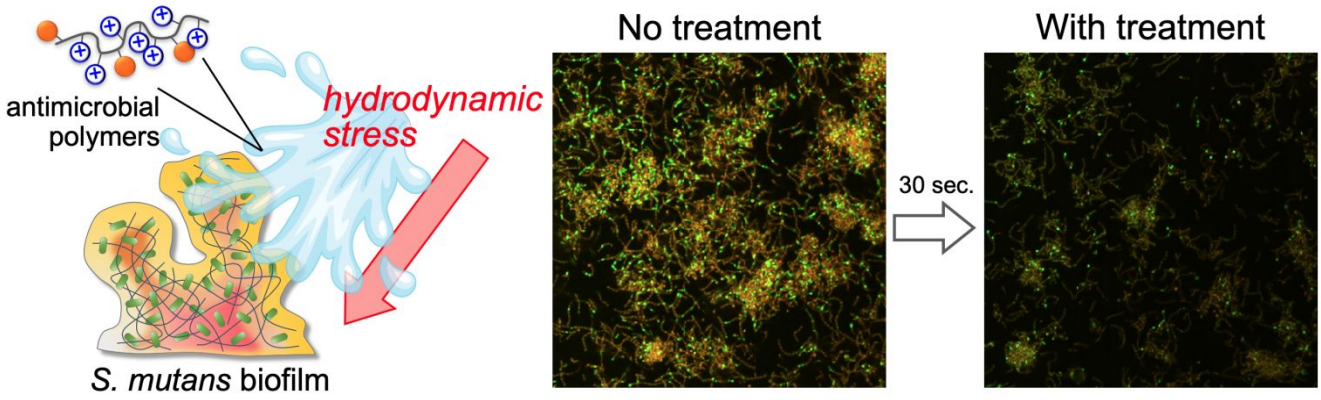

Figure 4. Anti-biofilm activity of antimicrobial polymers. The cariogenic bacterium *S. mutans* biofilm was removed by hydrodynamic cyclic stress for 30 sec using antimicrobial polymer solution. Reprinted with permission from ref 84. Copyright 2017 American Chemical Society.

3.5 Challenges and opportunities in antimicrobial polymers

Resistance. AMPs are ancient weapons that have evolved over millions of years as a result of an escalating arms race with bacteria.[85] As AMPs have been attacking bacterial membranes for quite a long time, why haven't bacteria changed or evolved the lipid compositions or membrane structures to resist AMPs? It seems that bacteria cannot significantly change the lipid types or replace the components vulnerable to AMPs with alternative molecules, which we speculate may be too great of an evolutionary "step" to take through standard mutation and adaptation mechanisms. However, it has been reported that *S. aureus* actively reduces their susceptibility to AMPs by reducing the net negative charges of cell wall and membranes, resulting in reduced binding of AMPs to bacteria.[86] *E. coli* and *Salmonella* have also been reported to sense concentrations of AMPs using the PhoQP two component sensor system which is linked to activation of virulence.[87] In response, AMPs evolved to increase the net positive charge to overcome the reduced electrostatic binding to the bacterial surface.[86] Notably, the decreased susceptibility due to cell wall modification is moderate at best, especially when compared to antibiotic resistance phenotypes, and thus AMPs are currently still functionally active against many bacteria. This outcome, however, is a result of the long history of an ongoing arms race between AMPs and resistance mechanisms evolved in bacteria. This also suggests that bacteria may be able to eventually become resistant against antimicrobial polymers once we start widely using AMP-memetic antimicrobial polymers, posing a potential problem in clinical applications.[88] However, this seems to be an unlikely outcome due to the eons of exposure that has yielded only minimal resistance.

***In vivo* efficacy and selectivity.** Despite the net negative surface charge on bacteria is a significantly different from host cells which can be exploited, there are challenges to targeting bacteria in a mammalian host. The local microenvironments around bacterial infections can dramatically impact AMP-mimetic polymers to bind and exert

the antimicrobial activity. The rich density of charged molecules in the mammalian extracellular matrix and fluids provides a means of electrostatic screening of the charge-charge attractions between AMP-mimetic polymers and bacteria.[73, 89-91] These can arise from ionic species in the ECM such as small inorganic ions or proteins, as well as the proteoglycan components of the ECM such as glycosaminoglycans. Together, these effects work against the selectivity of AMP-mimetic polymers, providing non-productive sinks of binding instead of the intended bacterial target.

Synergistic usage with traditional antibiotics. Another strategy for addressing the antimicrobial resistance issue is to increase the intracellular antibiotic concentration using compromising the barrier function of bacterial cell membranes by membrane-active antimicrobials. The antimicrobial-induced membrane disruption can increase the permeability of target membranes and thus enhance cellular uptake of antibiotics, working against the action of efflux pumps. Many studies have been reported on the synergistic interactions between membrane active AMPs with antibiotics such as colistin with azithromycin,[92] daptomycin with β-lactams,[93, 94] and synthetic AMPs with conventional antibiotics,[95] just to name a few. Similarly, antimicrobial polymers are do not necessarily have to be used as mono-therapeutic; a recent report in this field is that synthetic antimicrobial polymers showed synergetic effects with doxycycline and colistin against drug resistance *P. aeruginosa*.[96] There are also *in vitro* studies that demonstrated enhanced activity of Polymyxin B with ionic liquids.[97] These studies demonstrated the possibility that antibiotics to which bacteria developed resistance can be revived again for treatment.

AMP-mimetic design and macromolecular antibiotics. To date, more than 5,000 sequences of AMPs have been reported and are stored in several databases.[98] Interestingly, the sequences and native conformations (α-helices, β-sheets, and globules) of these AMPs are very diverse and do not share common sequences, which reflects the fact that the activity of AMPs does not rely on specific ligand-receptor interactions which would preferentially favor certain 3-D folds. However, one common feature is the global cationic amphiphilicity in which cationic and hydrophobic domains on helical or globular structures are segregated to opposite sides, capable of disrupting bacterial cell membranes. Therefore, the segregated cationic amphiphilicity is nature's antibiotic design that has been evolutionarily optimized to enable antimicrobial functions of AMPs. In other words, this may implicate that specific peptide sequences are not necessarily required, but the key features to replicate are not only the AMPs chemical properties (cationic and hydrophobicity), but also the distribution and patterns of these functional groups (segregated cationic and hydrophobic domains) expressed on the surfaces of active conformations in order to create novel antibiotics. To that end, we propose "macromolecular antibiotics" as a new antimicrobial strategy (**Fig. 5**); synthetic polymer platforms are promising and advantageous candidates to mimic the AMP cationic amphiphilic structures retaining the diverse compositions, shapes, and architectures. Indeed, while the majority of the discussion in this article focused on simple antimicrobial random copolymers with linear chains, the approach has been recently

extended to include block copolymers with specific sequences of cationic and hydrophobic monomers,[99, 100] branched/star-shaped and comb-like copolymers,[101-106] single-chain polymer nanoparticles,[107] and polymers with facially cationic amphiphilic moieties in the side chains[108]. While these studies are still based on single polymer chains, the platforms have been further extended to higher dimensional or ordered structures including assemblies and micelles of polymer chains[109-111], nanoparticles[112] and gels[113, 114]. Detailed discussion is beyond the scope of this article, and thus refer the readers to other excellent reviews more focused on those topics.[52, 63-66, 68, 70, 115-121] Although these new platforms have demonstrated promising results, there is a challenge to present the cationic and hydrophobic domains on the surface of polymer conformations and macromolecules in a manner similar to AMPs as there is more limited control over patterning due to the increased polydispersity in polymeric materials. This challenge provides future opportunities to create new antimicrobials to address the antibiotic resistance issue as well as understand the role of amphiphilic structures of AMPs and AMP-mimics on their antibiotic functions.

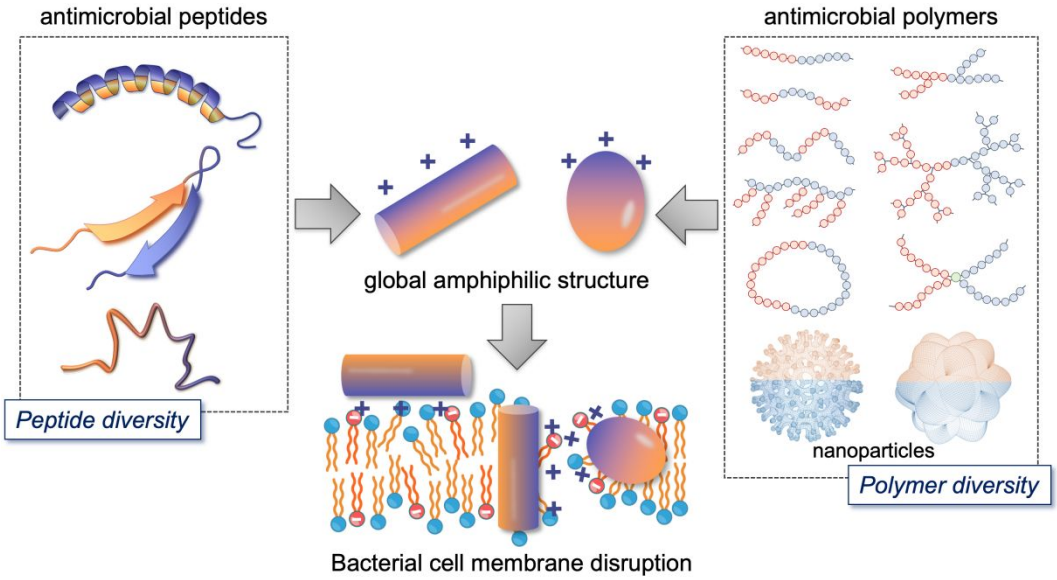

Figure 5. Design of macromolecular antibiotics. The AMP forms several types of secondary structure (α-helices, β-sheets, and globules) to provide global amphiphilicity to selectively bind to bacterial membrane and perturbate it. Similarly, the polymers with global amphiphilicity in their macroscopic molecular structure show antimicrobial activity.

4. Conclusions and Remarks

Membrane-active polymer therapeutics are emerging materials to address drug resistant bacteria. Synthetic polymers provide a diversity of chemical functionalities, design flexibility, and cost-effective production. However, there are still unsolved questions to produce practical and approved therapeutics, including (i) selectivity over

normal cells, (ii) determination of mechanism(s), (iii) development of resistance with long term usage, and (iv) side effects in the body. We need to understand underlying mechanisms and develop the design principle to teach synthetic polymers as to recognize and distinguish cellular membranes, that allows us to prepare "order made" platform for suitable design to attack specific target of bacteria, rather than conventional "trial and error" approach.

In this review, we discussed the membrane disruption as the primary mechanisms of antimicrobial polymers. While the bacterial cell membrane is a promising target with low propensity for resistance development, we cannot ignore the possibility if we apply the antimicrobial polymers regularly and on a widespread scale. If such problematic situations arise, a combined and/or sequential application of antibiotics with a different antimicrobial mechanism in combination with the polymers may result in synergistic or additive enhancement of activity. Additionally, it is known that some AMPs act by interacting with intracellular targets such as DNA and may exploit multiple modes of action simultaneously,[46] which might contribute to their low likelihood of resistance development in bacteria. Indeed, it has been reported that cationic polymers entered bacteria and caused precipitation in the cytosol.[80] Our polymer design as membrane-disrupting agents may need to be "revisited" to investigate these mechanisms. In addition, the traditional design of antimicrobial polymers has been based on a binary system of cationic and hydrophobic monomers, which captures the essential functionalities of AMP's cationic amphiphilicity. While this minimalist approach provides simple and practical molecular designs of antimicrobial polymers, natural peptide sequences contain a variety of amino acids other than cationic and hydrophobic groups. This suggests that our current polymer design may not take a full advantage of evolutionarily optimized AMP's functions. Recently several laboratories reported antimicrobial polymers with amino acid functionalities including indole (tryptophan),[122] 4-hydroxyphenyl (tyrosine),[123] or serine (hydroxyl)[124, 125]. This new approach may be able implement the inherent functionalities of AMPs to the polymers by design, which may lead to more potent antimicrobial activity and selectivity as well as ultimately mimicry of the AMP's biological function as an immune modulator in the defense system of the body.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

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Notes and references

1. G. Zilahi, A. Artigas and I. Martin-Loeches, *Annals of Intensive Care*, 2016, **6**, 11.
2. L. B. Nellums, H. Thompson, A. Holmes, E. Castro-Sanchez, J. A. Otter, M. Norredam, J. S. Friedland and S. Hargreaves, *Lancet Infectious Diseases*, 2018, **18**, 796-811.
3. P. Dadgostar, *Infection and Drug Resistance*, 2019, **12**, 3903-3910.
4. I. C. G. i. D. Resistance, *No Time to Wait: Securing the future from drug-resistant infections -Report to the Secretary-General of the United Nations*, 2019.
5. O. World Health, *Global action plan on antimicrobial resistance*, World Health Organization, Geneva, 2015.
6. U. S. D. o. H. a. H. Services, 2019.
7. J. M. A. Blair, M. A. Webber, A. J. Baylay, D. O. Ogbolu and L. J. V. Piddock, *Nature Reviews Microbiology*, 2015, **13**, 42-51.
8. S. Santajit and N. Indrawattana, *Biomed Research International*, 2016, **2016**, 8.
9. S. Baker, N. Thomson, F. X. Weill and K. E. Holt, *Science*, 2018, **360**, 733-738.
10. U. Ndagi, A. A. Falaki, M. Abdullahi, M. M. Lawal and M. E. Soliman, *Rsc Advances*, 2020, **10**, 18451-18468.
11. C. H. Wang, Y. H. Hsieh, Z. M. Powers and C. Y. Kao, *International Journal of Molecular Sciences*, 2020, **21**, 18.
12. V. L. Simpkin, M. J. Renwick, R. Kelly and E. Mossialos, *Journal of Antibiotics*, 2017, **70**, 1087-1096.
13. A. Brauner, O. Fridman, O. Gefen and N. Q. Balaban, *Nature Reviews Microbiology*, 2016, **14**, 320-330.
14. E. Peterson and P. Kaur, *Frontiers in Microbiology*, 2018, **9**, 21.
15. T. S. Crofts, A. J. Gasparrini and G. Dantas, *Nature Reviews Microbiology*, 2017, **15**, 422-434.
16. J. C. Kester and S. M. Fortune, *Critical Reviews in Biochemistry and Molecular Biology*, 2014, **49**, 91-101.
17. C. W. Hall and T. F. Mah, *Fems Microbiology Reviews*, 2017, **41**, 276-301.
18. V. Lazar, I. Nagy, R. Spohn, B. Csorgo, A. Gyorkei, A. Nyerges, B. Horvath, A. Voros, R. Busa-Fekete, M. Hrtyan, B. Bogos, O. Mehi, G. Fekete, B. Szappanos, B. Kegl, B. Papp and C. Pal, *Nature Communications*, 2014, **5**, 12.
19. R. A. Fisher, B. Gollan and S. Helaine, *Nature Reviews Microbiology*, 2017, **15**, 453-464.
20. V. L. Healy, I. A. D. Lessard, D. I. Roper, J. R. Knox and C. T. Walsh, *Chemistry & Biology*, 2000, **7**, R109-R119.
21. L. Fernandez and R. E. W. Hancock, *Clinical Microbiology Reviews*, 2012, **25**, 661-+.
22. S. Garneau-Tsodikova and K. J. Labby, *Medchemcomm*, 2016, **7**, 11-27.
23. M. S. Wilke, A. L. Lovering and N. C. J. Strynadka, *Current Opinion in Microbiology*, 2005, **8**, 525-533.
24. I. Bjedov, O. Tenaillon, B. Gerard, V. Souza, E. Denamur, M. Radman, F. Taddei and I. Matic, *Science*, 2003, **300**, 1404-1409.
25. A. Harms, E. Maisonneuve and K. Gerdes, *Science*, 2016, **354**, 9.
26. F. M. Vallette, C. Olivier, F. Lezot, L. Oliver, D. Cochonneau, L. Lalier, P. F. Cartron and D. Heymann, *Biochemical Pharmacology*, 2019, **162**, 169-176.
27. G. Kapoor, S. Saigal and A. Elongavan, *J Anaesthesiol Clin Pharmacol*, 2017, **33**, 300-305.
28. N. Q. Balaban, J. Merrin, R. Chait, L. Kowalik and S. Leibler, *Science*, 2004, **305**, 1622-1625.
29. E. Kussell, R. Kishony, N. Q. Balaban and S. Leibler, *Genetics*, 2005, **169**, 1807-1814.
30. M. Jamal, W. Ahmad, S. Andleeb, F. Jalil, M. Imran, M. A. Nawaz, T. Hussain, M. Ali, M. Rafiq and M. A. Kamil, *Journal of the Chinese Medical Association*, 2018, **81**, 7-11.
31. A. Penesyan, M. Gillings and I. T. Paulsen, *Molecules*, 2015, **20**, 5286-5298.
32. D. Sharma, L. Misba and A. U. Khan, *Antimicrobial Resistance and Infection Control*, 2019, **8**, 10.
33. Y. C. Huan, Q. Kong, H. J. Mou and H. X. Yi, *Frontiers in Microbiology*, 2020, **11**, 21.
34. M. Mahlapuu, C. Bjorn and J. Ekblom, *Critical Reviews in Biotechnology*, 2020, **40**, 978-992.
35. V. Patrulea, G. Borchard and O. Jordan, *Pharmaceutics*, 2020, **12**, 39.

36. E. M. Kohn, D. J. Shirley, L. Arotsky, A. M. Picciano, Z. Ridgway, M. W. Urban, B. R. Carone and G. A. Caputo, *Molecules*, 2018, **23**, 17.
37. K. D. Saint Jean, K. D. Henderson, C. L. Chrom, L. E. Abiuso, L. M. Renn and G. A. Caputo, *Probiotics and Antimicrobial Proteins*, 2018, **10**, 408-419.
38. H. K. Kang, C. Kim, C. H. Seo and Y. Park, *Journal of Microbiology*, 2017, **55**, 1-12.
39. K. A. H. Wildman, D. K. Lee and A. Ramamoorthy, *Biochemistry*, 2003, **42**, 6545-6558.
40. A. J. Mason, I. N. H. Chotimah, P. Bertani and B. Bechinger, *Molecular Membrane Biology*, 2006, **23**, 185-194.
41. M. Arias, K. B. Piga, M. E. Hyndman and H. J. Vogel, *Biomolecules*, 2018, **8**, 17.
42. Y. Liu, J. Shi, Z. Tong, Y. Jia, K. Yang and Z. Wang, *Microorganisms*, 2020, **8**.
43. M. A. Hitchner, M. R. Necelis, D. Shirley and G. A. Caputo, *Probiotics and Antimicrobial Proteins*, 15.
44. J. X. Yan, K. R. Wang, W. Dang, R. Chen, J. Q. Xie, B. Z. Zhang, J. J. Song and R. Wang, *Antimicrobial Agents and Chemotherapy*, 2013, **57**, 220-228.
45. L. D. Machado, E. V. B. de Carvalho, F. Silva, P. V. D. Cabreira and O. L. Franco, *Current Topics in Medicinal Chemistry*, 2017, **17**, 520-536.
46. Y. Y. Zhu, S. Mohapatra and J. C. Weisshaar, *Proceedings of the National Academy of Sciences of the United States of America*, 2019, **116**, 1017-1026.
47. C. B. Park, H. S. Kim and S. C. Kim, *Biochemical and Biophysical Research Communications*, 1998, **244**, 253-257.
48. C. F. Le, C. M. Fang and S. D. Sekaran, *Antimicrobial Agents and Chemotherapy*, 2017, **61**, 16.
49. A. K. Mishra, J. Choi, E. Moon and K. H. Baek, *Molecules*, 2018, **23**, 23.
50. M. M. Konai, B. Bhattacharjee, S. Ghosh and J. Haldar, *Biomacromolecules*, 2018, **19**, 1888-1917.
51. N. F. Kamaruzzaman, L. P. Tan, R. H. Hamdan, S. S. Choong, W. K. Wong, A. J. Gibson, A. Chivu and M. D. Pina, *International Journal of Molecular Sciences*, 2019, **20**, 31.
52. J. Tan, J. Tay, J. Hedrick and Y. Y. Yang, *Biomaterials*, 2020, **252**, 40.
53. R. M. Epand and R. F. Epand, *Biochimica Et Biophysica Acta-Biomembranes*, 2009, **1788**, 289-294.
54. V. Teixeira, M. J. Feio and M. Bastos, *Progress in Lipid Research*, 2012, **51**, 149-177.
55. G. van Meer, D. R. Voelker and G. W. Feigenson, *Nature Reviews Molecular Cell Biology*, 2008, **9**, 112-124.
56. S. Morla, *International Journal of Molecular Sciences*, 2019, **20**, 19.
57. A. A. Spector and M. A. Yorek, *Journal of Lipid Research*, 1985, **26**, 1015-1035.
58. J. A. Virtanen, K. H. Cheng and P. Somerharju, *Proceedings of the National Academy of Sciences of the United States of America*, 1998, **95**, 4964-4969.
59. P. A. Leventis and S. Grinstein, in *Annual Review of Biophysics, Vol 39*, eds. D. C. Rees, K. A. Dill and J. R. Williamson, Annual Reviews, Palo Alto, 2010, vol. 39, pp. 407-427.
60. I. R. Poxton, in *Molecular Medical Microbiology*, Second Edition edn., 2015, vol. 1, pp. 91-103.
61. A. Steimle, I. B. Autenrieth and J. S. Frick, *International Journal of Medical Microbiology*, 2016, **306**, 290-301.
62. C. Sohlenkamp and O. Geiger, *Fems Microbiology Reviews*, 2016, **40**, 133-159.
63. R. W. Scott and G. N. Tew, *Current Topics in Medicinal Chemistry*, 2017, **17**, 576-589.
64. H. Takahashi, G. A. Caputo, S. Vemparala and K. Kuroda, *Bioconjugate Chemistry*, 2017, **28**, 1340-1350.
65. C. Ergene, K. Yasuhara and E. F. Palermo, *Polymer Chemistry*, 2018, **9**, 2407-2427.
66. C. Ghosh, P. Sarkar, R. Issa and J. Haldar, *Trends in Microbiology*, 2019, **27**, 323-338.
67. D. S. S. M. Uppu, S. Samaddar, C. Ghosh, K. Paramanandham, B. R. Shome and J. Haldar, *Biomaterials*, 2016, **74**, 131-143.
68. X. K. Ding, A. Z. Wang, W. Tong and F. J. Xu, *Small*, 2019, **15**, 29.
69. Z. Hou, Y. V. Shankar, Y. Liu, F. Q. Ding, J. L. Subramanion, V. Ravikumar, R. Zamudio-Vazquez, D. Keogh, H. W. Lim, M. Y. F. Tay, S. Bhattacharjya, S. A. Rice, J. Shi, H. W. Duan, X. W. Liu, Y. G. Mu, N. S. Tan, K. C. Tam, K. Pethe and M. B. Chan-Park, *Acs Applied Materials & Interfaces*, 2017, **9**, 38288-38303.

70. S. Alfei and A. M. Schito, *Polymers*, 2020, **12**, 47.
71. Z. X. Zhou, D. F. Wei, Y. Guan, A. N. Zheng and J. J. Zhong, *Materials Science & Engineering C-Materials for Biological Applications*, 2011, **31**, 1836-1843.
72. J. N. Pendleton, S. P. Gorman and B. F. Gilmore, *Expert Review of Anti-Infective Therapy*, 2013, **11**, 297-308.
73. S. Hong, H. Takahashi, E. T. Nadres, H. Mortazavian, G. A. Caputo, J. G. Younger and K. Kuroda, *Plos One*, 2017, **12**, 17.
74. S. Q. Liu, R. J. Ono, H. Wu, J. Y. Teo, Z. C. Liang, K. J. Xu, M. Zhang, G. S. Zhong, J. P. K. Tan, M. Ng, C. Yang, J. L. Chan, Z. K. Ji, C. Bao, K. Kumar, S. J. Gao, A. Lee, M. Fevre, H. H. Dong, J. Y. Ying, L. J. Li, W. M. Fan, J. L. Hedrick and Y. Y. Yang, *Biomaterials*, 2017, **127**, 36-48.
75. W. Y. Lou, S. Venkataraman, G. S. Zhong, B. S. Ding, J. P. K. Tan, L. Xu, W. M. Fan and Y. Y. Yang, *Acta Biomaterialia*, 2018, **78**, 78-88.
76. W. N. Jiang, X. M. Xiao, Y. M. Wu, W. W. Zhang, Z. H. Cong, J. J. Liu, S. Chen, H. D. Zhang, J. Y. Xie, S. Deng, M. Z. Chen, Y. Wang, X. Y. Shao, Y. D. Dai, Y. Sun, J. Fei and R. H. Liu, *Biomaterials Science*, 2020, **8**, 739-745.
77. S. Chen, X. Y. Shao, X. M. Xiao, Y. D. Dai, Y. Wang, J. Y. Xie, W. N. Jiang, Y. Sun, Z. H. Cong, Z. Q. Qiao, H. D. Zhang, L. Q. Liu, Q. Zhang, W. J. Zhang, L. Zheng, B. R. Yu, M. Z. Chen, W. G. Cui, J. Fei and R. H. Liu, *Acs Infectious Diseases*, 2020, **6**, 479-488.
78. I. Sovadinova, E. F. Palermo, M. Urban, P. Mpiga, G. A. Caputo and K. Kuroda, *Polymers*, 2011, **3**, 1512-1532.
79. K. X. Zhang, Y. Du, Z. Y. Si, Y. Liu, M. E. Turvey, C. Raju, D. Keogh, L. Ruan, S. L. Jothy, S. Reghu, K. Marimuthu, D. Pratim, O. T. Ng, J. R. Mediavilla, B. N. Kreiswirth, Y. R. Chi, J. H. Ren, K. C. Tam, X. W. Liu, H. W. Duan, Y. B. Zhu, Y. G. Mu, P. T. Hammond, G. C. Bazan, K. Pethe and M. B. Chan-Park, *Nature Communications*, 2019, **10**, 14.
80. W. Chin, G. S. Zhong, Q. Q. Pu, C. Yang, W. Y. Lou, P. F. De Sessions, B. Periaswamy, A. Lee, Z. C. Liang, X. Ding, S. J. Gao, C. W. Chu, S. Bianco, C. Bao, Y. W. Tong, W. M. Fan, M. Wu, J. L. Hedrick and Y. Y. Yang, *Nature Communications*, 2018, **9**, 14.
81. K. Chindera, M. Mahato, A. K. Sharma, H. Horsley, K. Kloc-Muniak, N. F. Kamaruzzaman, S. Kumar, A. McFarlane, J. Stach, T. Bentin and L. Good, *Scientific Reports*, 2016, **6**, 13.
82. T. D. Michl, B. Hibbs, L. Hyde, A. Postma, D. T. T. Tran, A. Zhalgasbaikyzy, K. Vasilev, L. Meagher, H. J. Griesser and K. E. S. Locock, *Acta Biomaterialia*, 2020, **108**, 168-177.
83. 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, *Angewandte Chemie-International Edition*, 2013, **52**, 674-678.
84. H. Takahashi, E. T. Nadres and K. Kuroda, *Biomacromolecules*, 2017, **18**, 257-265.
85. J. M. Ageitos, A. Sanchez-Perez, P. Calo-Mata and T. G. Villa, *Biochemical Pharmacology*, 2017, **133**, 117-138.
86. A. Peschel and H. G. Sahl, *Nature Reviews Microbiology*, 2006, **4**, 529-536.
87. E. A. Groisman, *Journal of Bacteriology*, 2001, **183**, 1835-1842.
88. D. I. Andersson, D. Hughes and J. Z. Kubicek-Sutherland, *Drug Resistance Updates*, 2016, **26**, 43-57.
89. W. F. Walkenhorst, *Biochimica Et Biophysica Acta-Biomembranes*, 2016, **1858**, 926-935.
90. J. J. L. Cascales, S. Zenak, J. G. de la Torre, O. G. Lezama, A. Garro and R. D. Enriz, *Acs Omega*, 2018, **3**, 5390-5398.
91. D. Pranantyo, L. Q. Xu, Z. Hou, E. T. Kang and M. B. Chan-Park, *Polymer Chemistry*, 2017, **8**, 3364-3373.
92. L. Lin, P. Nonejuie, J. Munguia, A. Hollands, J. Olson, Q. Dam, M. Kumaraswamy, H. Rivera, R. Corriden, M. Rohde, M. E. Hensler, M. D. Burkart, J. Pogliano, G. Sakoulas and V. Nizet, *Ebiomedicine*, 2015, **2**, 690-698.
93. J. R. Smith, K. E. Barber, A. Raut, M. Aboutaleb, G. Sakoulas and M. J. Rybak, *Journal of Antimicrobial Chemotherapy*, 2015, **70**, 1738-1743.
94. D. Gritsenko, M. Fedorenko, J. J. Ruhe and J. Altshuler, *Clinical Therapeutics*, 2017, **39**, 212-218.

95. D. Pletzer, S. C. Mansour and R. E. W. Hancock, *Plos Pathogens*, 2018, **14**, 14.
96. R. Namivandi-Zangeneh, Z. Sadrearhami, D. Dutta, M. Willcox, E. H. H. Wong and C. Boyer, *Acs Infectious Diseases*, 2019, **5**, 1357-1365.
97. S. L. Hanna, J. L. Huang, A. J. Swinton, G. A. Caputo and T. D. Vaden, *Biophysical Chemistry*, 2017, **227**, 1-7.
98. M. Magana, M. Pushpanathan, A. L. Santos, L. Leanse, M. Fernandez, A. Ioannidis, M. A. Giulianotti, Y. Apidianakis, S. Bradfute, A. L. Ferguson, A. Cherkasov, M. N. Seleem, C. Pinilla, C. de la Fuente-Nunez, T. Lazaridis, T. Dai, R. A. Houghten, R. E. W. Hancock and G. P. Tegos, *Lancet Infect Dis*, 2020, **20**, e216-e230.
99. Y. Oda, S. Kanaoka, T. Sato, S. Aoshima and K. Kuroda, *Biomacromolecules*, 2011, **12**, 3581-3591.
100. A. Kuroki, P. Sangwan, Y. Qu, R. Peltier, C. Sanchez-Cano, J. Moat, C. G. Dowson, E. G. L. Williams, K. E. S. Locock, M. Hartlieb and S. Perrier, *Acs Applied Materials & Interfaces*, 2017, **9**, 40117-40126.
101. M. S. Chen, M. Hu, D. L. Wang, G. J. Wang, X. Y. Zhu, D. Y. Yan and J. Sun, *Bioconjugate Chemistry*, 2012, **23**, 1189-1199.
102. E. H. H. Wong, M. M. Khin, V. Ravikumar, Z. Y. Si, S. A. Rice and M. B. Chan-Park, *Biomacromolecules*, 2016, **17**, 1170-1178.
103. C. Yang, W. Y. Lou, G. S. Zhong, A. Lee, J. Y. Leong, W. Chin, B. S. Ding, C. Bao, J. P. K. Tan, Q. Q. Pu, S. J. Gao, L. Xu, L. Y. Hsu, M. Wu, J. L. Hedrick, W. M. Fan and Y. Y. Yang, *Acta Biomaterialia*, 2019, **94**, 268-280.
104. W. Zheng, M. Anzaldua, A. Arora, Y. J. Jiang, K. McIntyre, M. Doerfert, T. Winter, A. Mishra, H. R. Ma and H. J. Liang, *Biomacromolecules*, 2020, **21**, 2187-2198.
105. P. R. Judzewitsch, N. Corrigan, F. Trujillo, J. T. Xu, G. Moad, C. J. Hawker, E. H. H. Wong and C. Boyer, *Macromolecules*, 2020, **53**, 631-639.
106. M. Rauschenbach, S. B. Lawrenson, V. Taresco, A. K. Pearce and R. K. O'Reilly, *Macromolecular Rapid Communications*, 7.
107. 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 Infectious Diseases*, 2017, **3**, 237-248.
108. M. A. Rahman, M. Bam, E. Luat, M. S. Jui, M. S. Ganewatta, T. Shokfai, M. Nagarkatti, A. W. Decho and C. B. Tang, *Nature Communications*, 2018, **9**, 10.
109. L. H. Liu, K. J. Xu, H. Y. Wang, P. K. J. Tan, W. M. Fan, S. S. Venkatraman, L. J. Li and Y. Y. Yang, *Nature Nanotechnology*, 2009, **4**, 457-463.
110. W. Z. Yuan, J. R. Wei, H. Lu, L. Fan and J. Z. Du, *Chemical Communications*, 2012, **48**, 6857-6859.
111. Y. J. Xi, T. Song, S. Y. Tang, N. S. Wang and J. Z. Du, *Biomacromolecules*, 2016, **17**, 3922-3930.
112. S. J. Lam, E. H. H. Wong, N. M. O'Brien-Simpson, N. Pantarat, A. Blencowe, E. C. Reynolds and G. G. Qiao, *Acs Applied Materials & Interfaces*, 2016, **8**, 33446-33456.
113. H. Du, G. Y. Zha, L. L. Gao, H. Wang, X. D. Li, Z. Q. Shen and W. P. Zhu, *Polymer Chemistry*, 2014, **5**, 4002-4008.
114. C. Zhou, V. X. Truong, Y. Qu, T. Lithgow, G. D. Fu and J. S. Forsythe, *Journal of Polymer Science Part a-Polymer Chemistry*, 2016, **54**, 656-667.
115. X. Y. Zhou and C. C. Zhou, *Progress in Chemistry*, 2018, **30**, 913-920.
116. A. C. Engler, N. Wiradharma, Z. Y. Ong, D. J. Coady, J. L. Hedrick and Y. Y. Yang, *Nano Today*, 2012, **7**, 201-222.
117. J. Chen, F. Y. K. Wang, Q. M. Liu and J. Z. Du, *Chemical Communications*, 2014, **50**, 14482-14493.
118. V. W. L. Ng, J. M. W. Chan, H. Sardon, R. J. Ono, J. M. Garcia, Y. Y. Yang and J. L. Hedrick, *Advanced Drug Delivery Reviews*, 2014, **78**, 46-62.
119. M. R. E. Santos, A. C. Fonseca, P. V. Mendona, R. Branco, A. C. Serra, P. V. Morais and J. F. J. Coelho, *Materials*, 2016, **9**, 33.
120. W. Ren, W. R. Cheng, G. Wang and Y. Liu, *Journal of Polymer Science Part a-Polymer Chemistry*, 2017, **55**, 632-639.
121. C. Krumm and J. C. Tiller, in *Bio-inspired Polymers*, The Royal Society of Chemistry, 2017, pp. 490-522.

122. K. E. S. Locock, T. D. Michl, N. Stevens, J. D. Hayball, K. Vasilev, A. Postma, H. J. Griesser, L. Meagher and M. Haeussler, *Acs Macro Letters*, 2014, **3**, 319-323.
123. L. P. Datta, D. Dutta, A. Chakraborty and T. K. Das, *Biomaterials Science*, 2019, **7**, 2611-2622.
124. S. Chakraborty, R. H. Liu, Z. Hayouka, X. Y. Chen, J. Ehrhardt, Q. Lu, E. Burke, Y. Q. Yan, B. Weisblum, G. C. L. Wong, K. S. Masters and S. H. Gellman, *Journal of the American Chemical Society*, 2014, **136**, 14530-14535.
125. H. Mortazavian, L. L. Foster, R. Bhat, S. Patel and K. Kuroda, *Biomacromolecules*, 2018, **19**, 4370-4378.