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Feature article

Controlling macromolecular structures towards effective antimicrobial polymers

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ABSTRACT

Drug resistance of pathogenic bacteria is a major global problem leading humanity towards a pre-antibiotic era. Decline in the discovery of novel antibiotics and the lack of a resilient platform to develop novel antimicrobial agents worsens the situation. Amphiphilic antimicrobial polymers, which have roots coming from antimicrobial peptides, show promise as potent antimicrobials having low susceptibility for developing resistance, unlike small molecular antibiotics. This feature article highlights recent advances in the fabrication of membrane-active antimicrobial polymers. The design of various types of macromolecular architectures with control of structural parameters such as hydrophobicity/hydrophilicity balance, molecular weight, and ionic groups will be emphasized in order to achieve strong antimicrobial activities while minimizing toxicity to mammalian cells. Advanced polymeric assemblies with well-defined nanostructures including core/shell shaped nano-objects and polymeric vesicles are also discussed. Lastly, current challenges and future directions in the field of antimicrobial polymers for ensuing practical biomedical applications are presented.

1. Introduction

Antimicrobial chemotherapy has revolutionized modern medicine in many aspects and has significantly reduced ailments and death from infectious diseases. Many classes of antibiotics that are clinically used today were discovered during the golden era of antibiotic discovery from 1940s to 1960s [1,2]. Molecular targets of pathogens, which are absent or significantly different from human cells such as cell wall, 60S ribosomes, cell membranes, genetic materials and biosynthetic pathways, are utilized to design antimicrobial agents (Fig. 1A). Environmental pressure from the action of antibiotics combined with short life cycles and lateral gene transfer mechanisms have resulted in rapid appearance of resistant pathogenic populations of microorganisms [3]. For example, widespread outbreaks of penicillin-resistant *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) infections occurred just a few years after the introduction of β-lactam antibiotics, penicillin and methicillin.

Resistance mechanisms include efflux pumps, chemical modifications such as phosphorylation, acetylation or hydrolysis, altering target and reprogramming biosynthesis, most of which are against small molecule antimicrobials (Fig. 1B) [1,6]. The most prominent issue is the expeditious growth of acquired resistance in bacteria that cause major healthcare crisis. For example, since the introduction of the first β-lactam antibiotics, the number of unique β-lactamase enzymes has grown from zero to over 1000 [6]. Decades of use and misuse of antibiotics, combined with a forty year lull in the pipe line of novel antimicrobial agents, have consequences of a global superbug threat that could lead human civilization to a pre-antibiotic era. The devastating nature of the increasing resistance to available antibiotics is a global concern at high priority. Antibiotic resistance seems inevitable. Therefore, it is essential to continuously develop antibiotics with novel modes of action to face the evolving resistance [7].

Antimicrobial polymers are a class of novel antimicrobial agents that is fueled by the combined knowledge on antimicrobial peptides (AMPs) [8] and polymer disinfectants that have emerged as two distinct fields since the 1980s [9]. There are several books, a variety of reviews and highlights on antimicrobial polymers published over the past few years that give broader and diverse perspectives [5,9–25]. However, there has been a rapid expansion of novel antimicrobial polymers and related research in the last decade (Fig. 2), which has not been reviewed frequently.

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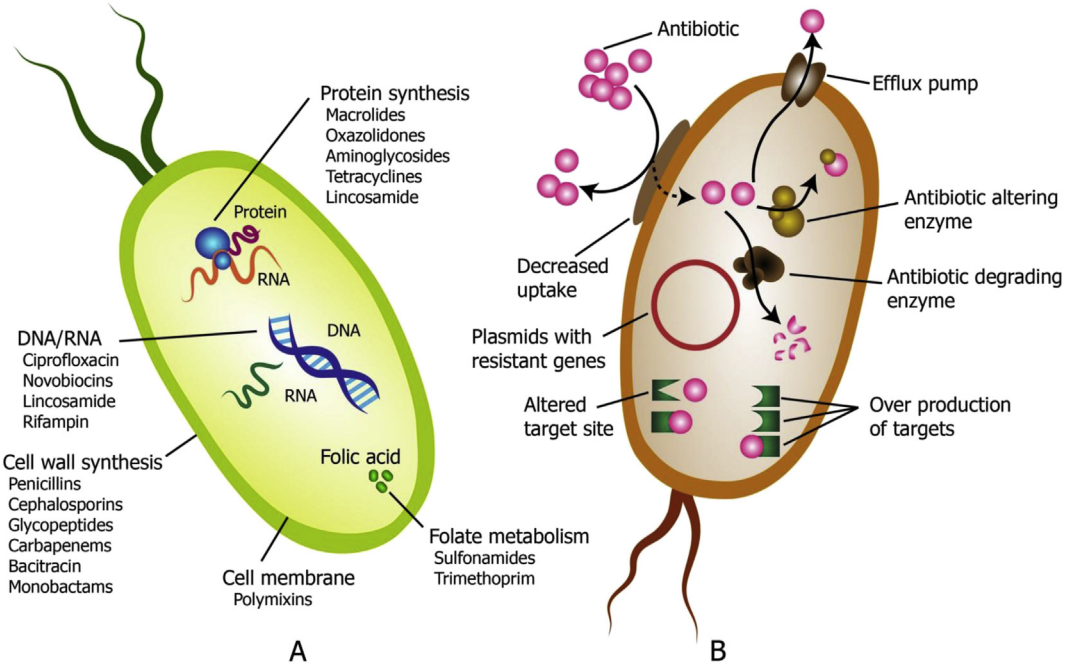


Fig. 1. (A) Typical antibiotic target sites present in bacterial cells and; (B) mechanisms of antibiotic resistance. Adapted with permission from Refs. [4,5].

Hence this feature article focuses on the most recent advances in membrane-active cationic polymeric antimicrobials and our perception about modulating the structural features of these materials to optimize their potential as clinically relevant materials. However, we do not intend to provide an exhaustive review of all aspects of antimicrobial polymers. The emphasis will be placed on research related to antimicrobial polymers and assemblies in solution. The discussion will start by looking at the feasibility of antimicrobial polymeric macromolecules as an innovative platform to address the current healthcare crisis pertaining to infectious diseases. Then the emphasis will be moved to the current understandings and most recent experimental explorations about polymer architectures and macromolecular structural determinants to improve the properties of antimicrobial polymers with regard to antimicrobial activities and biocompatibilities. We will discuss research on nano assemblies of antimicrobial polymers. Synthetic strategies, including polymerization techniques, post-polymerization modifications and self-assembling procedures, will be briefly mentioned in prominent case studies. Lastly, the current challenges in the field and future directions will be deliberated with the hope for further expansion of antimicrobial polymer research.

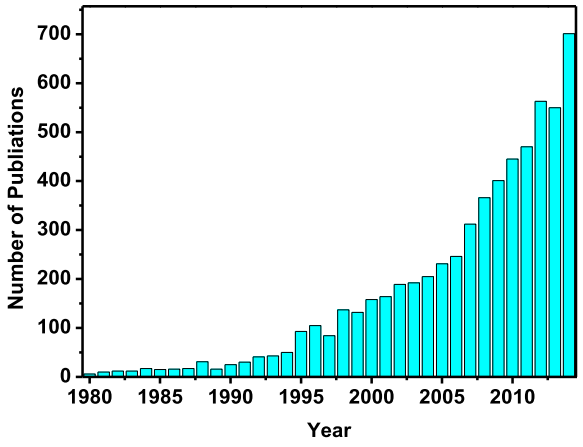


Fig. 2. Number of publications containing "antimicrobial polymer" from 1980 to 2014, searched via SciFinder.

2. Tailoring next-generation antibiotics

It is increasingly recognized that microbial membranes provide an effective target for the development of de novo designed antimicrobial agents. Recent understanding of the innate immunity mediated by macromolecules highlights the importance of short amphiphilic peptides that modulate host defenses against microbial pathogens [26]. Also known as antimicrobial peptides, these molecules are produced by almost all forms of life [27]. AMPs are potent, broad-spectrum antimicrobials that act as the first line of defense against a wide range of invading pathogens including bacteria, protozoa, yeast, fungi and viruses by rapid and direct killing as well as several other means of modulating host immune systems [28,29]. Several decades of studies have revealed more than two thousand AMPs with diverse sequences of amino acids and a range of structures. (Readers are directed to the comprehensive AMP database curated by Wang and co-workers (http://aps.unmc.edu/AP/main.php) [30].) However, all AMPs show a common characteristic: the presence of an amphiphilic structure (in some literature, "amphipathic" is often used). The optimal amphiphilicity, which comes from cationic amino acids (e.g. lysine, arginine) and hydrophobic residues (e.g. isoleucine, valine), enables AMPs to fold into cationic and facially amphiphilic secondary structures. This feature permits AMPs to strongly interact with biological membranes. Interestingly, receptor-mediated antimicrobial activity is generally absent in AMPs. For instance, it was shown that all-D synthetic enantiomer homologous of magainins and cecropins have similar potency to all-L natural peptides [31]. This non-specific property has shown to be a class of promising anti-infective agents that are assumed to defer long-term resistance development compared to small molecule antibiotics.

2.1. Mechanisms of action

Essentially, all types of living cells comprise a cytoplasmic membrane made of lipid bilayers that serves as a protective barrier to separate and protect the cell from its surrounding environment. In addition, being a 'semi-permeable membrane,' it acts as a gateway regulating the transport of substances to and from the intracellular space. Therefore, the cytoplasmic membrane has a vital task for the survival of the cell. Most widely accepted mechanism of antimicrobial action of AMPs is direct microbial killing by the disruption, reorganization or pore formation of cell membranes, resulting in the leakage of cellular contents and eventual cell death. Although still under debate, several models such as "barrel stave," "toroidal pore" and "carpet model" have been proposed to explain the membrane damaging interaction of AMPs with lipid bilayers (Fig. 3) [32]. All these model studies have in common the fact that amphiphilic secondary structures of AMPs can lead to cell death by rapid leakage of cellular contents that is beyond the control of the cell.

The fundamental mode of microbicidal activity of synthetic antimicrobial polycationic agents is also found to be similar to that of AMPs [34]. It is interpreted in terms of a sequence of essential processes [35]: (1) It initiates by the adsorption on microbial cell surface. This utmost important step is also the basis of selectivity towards microbes; (2) Then the polycations diffuse through the cell wall and/or (3) interact with the cytoplasmic membrane; (4) This interaction may irreversibly damage the integrity of the cell membrane; (5) subsequently result in the release of cytoplasmic components including K^+ ions, DNA/RNA; and (6) finally lead to cell death. In addition to the membrane disruption by the integration of cationic polymers with lipid membranes, they may also destabilize the membrane surface by displacing divalent cations such as Ca^{2+} associated with the membrane phospholipids.

Lipid membrane interactions with polymers or nanoparticles are widely discussed recently. There are several reviews about polymer-membrane interactions [36]. Many research groups investigated the mechanistic aspects of antimicrobial polymers using molecular dynamics simulations (MDS) and other biophysical means. Kuroda, Vemparala and co-workers carried out impressive investigations on mechanistic aspects of the interaction of random methacrylate polymers with model bacterial membranes [37,38]. It was found that cationic polymers assemble into micellar aggregates in water phase where hydrophobic groups were buried inside and cationic arms extending out (Fig. 4). The polymer aggregates reached anionic membrane via attractive electrostatic interactions between the cationic groups on the polymer and the anionic lipid heads of the membrane. At the vicinity of the membrane, the polymer adopted an extended structure, leading to increased interactions with the membrane surface. Later, individual polymer chains dissociated from the aggregate when the polymer-membrane interaction became stronger than the polymer−polymer interactions. Those polymers partitioned onto the membrane, acquired a facially amphiphilic conformation with cationic and hydrophobic groups that were clearly separated and sustained throughout the simulation. This caused inhomogeneity in membrane thickness, which is regarded as a possible trigger for cellular leakage.

Although the membrane-destabilizing model is widely accepted now, there remains the curiosity for the investigations of other possible polymer−cell interactions. Baulin and co-workers investigated the translocation behavior of amphiphilic polymers through lipid bilayers using MDS [39]. According to the simulations, fully hydrophilic polymer formed random coils in solution and was rejected from the bilayer while completely hydrophobic polymer turned into a globular structure, trapped inside the hydrophobic core of the bilayer in a quasi-two-dimensional solvent of tails. However, it was expected to observe an in-between transition from the above two extremes. Interestingly, the partially hydrophobic polymer with the optimum amphiphilic balance eventually translocated through the lipid bilayer and enhanced local solvent permeability. Therefore, antimicrobial polymers with precise hydrophobic matching with the solvent and the membrane can be expected to translocate through microbial membranes. On the other hand, polymers with higher degrees of chemical

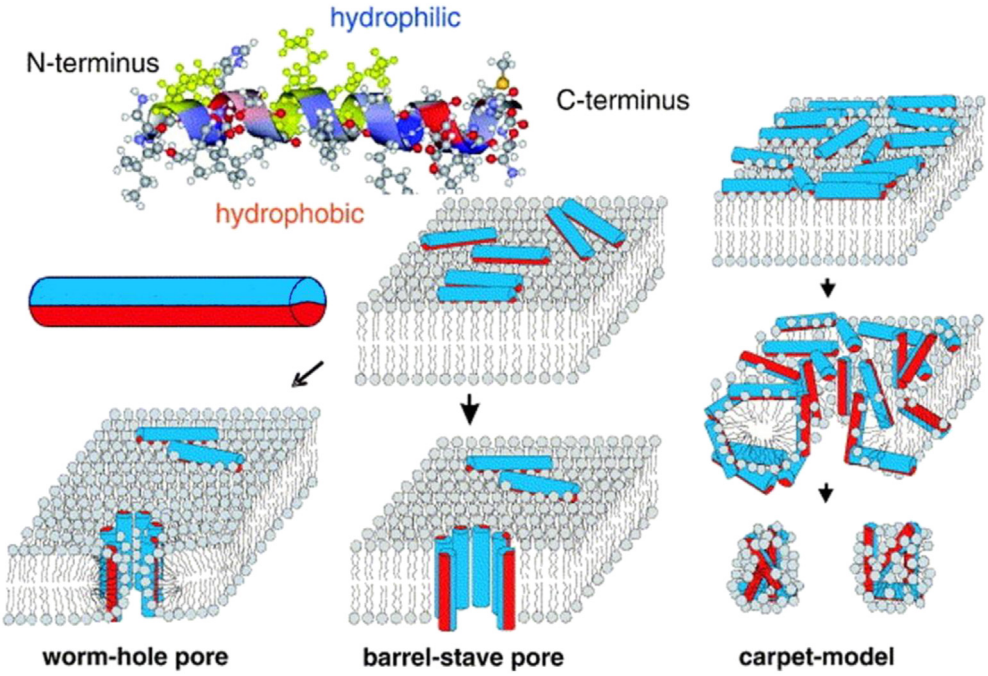


Fig. 3. Mechanisms of action for membrane-active AMPs that form facially amphiphilic structures. Top left: the α-helical conformation of Magainin. Reprinted and modified with permission from Ref. [33].

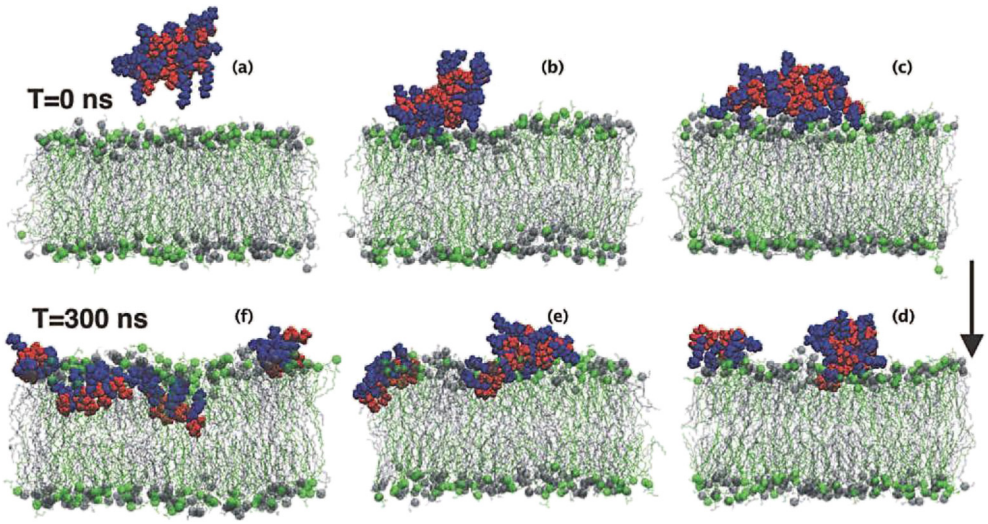


Fig. 4. MDS snapshots of amphiphilic random copolymer-lipid membrane interactions. Reprinted with permission from Ref. [38].

heterogeneity (e.g. random copolymers) can cause localization effects at the membrane surface that reduce polymer translocation. However, this may maintain induced permeability and result in pore forming effects.

Kuroda, Wong, L. Yang and co-workers conducted a mechanistic comparison between AMPs and synthetic mimics of antimicrobial peptides (SMAMPs), with regard to the cationic and hydrophobic content [40]. They found out that both systems can form similar amounts of negative Gaussian curvatures (NGCs). However, the amount of cationic and hydrophobic content required by the SMAMPs to exert the same levels of membrane deformation was significantly higher than that of AMPs. This may be a consequence of chemical heterogeneity (random sequences) and varying conformations (random coils) present in the random copolymers.

Gellman and co-workers developed a family of nylon-3 polymers which are found to be a class of very promising, broad-spectrum antimicrobial materials. These polymers are among the first synthetic antimicrobial macromolecules that displayed pronounced antimicrobial activity while maintaining good biocompatibility. In a recent study, Wong, Gellman et al. found out two interdependent mechanisms of antimicrobial activity in nylon-3-based polymers [41]. At low concentrations, the polymers were able to permeate bacterial membranes by generating NGCs and subsequent binding to intracellular DNA to cause cell death without lysing the cells. However, membrane lysis was possible at higher concentrations. Vesicle dye leakage, bacterial permeation assays, and bactericidal assays with small-angle X-ray scattering were utilized to observe such phenomena. It was concluded that nylon-3-based polymers demonstrate concentration dependent antimicrobial mechanisms of action.

2.2. Molecular basis of selectivity

The basis of the selectivity of AMPs or polymer mimics towards bacterial or fungal cell membranes comes from the fundamental difference in the cell membrane lipid composition and surface components (Fig. 5) [42]. Typically, the cytoplasmic membrane leaflets of mammalian cells are asymmetric in terms of charge. For example, the outer leaflet of human erythrocyte membrane is composed of neutral (zwitterionic) lipids such as phosphatidylcholine, sphingomyelin and phosphatidylethanolamine, while the inner leaflet bears negative charge coming from phosphatidylserine [23]. In contrast, the presence of phosphatidylserine, phosphatidylglycerol or cardiolipin in microbial cell membrane outer leaflets, make the outer surface appealing to cationic molecules such as AMPs. In addition, bacterial and fungal cells have additional cell envelope components essentially, the cell wall that provides sufficient mechanical strength to endure changes in osmotic pressure imposed by the environment. In Gram-positive bacteria, teichoic acids, which are linked to either the peptidoglycan cell wall or to the underlying cell membrane, impart net negative charges because of the presence of phosphate moieties in their structure. Gram-negative bacteria have an additional outer membrane bearing phospholipids and lipopolysaccharides. The lipopolysaccharides impart a strongly negative charge to cell surface. Fungal cell walls are comprised of glycoproteins and polysaccharides, mainly glucan and chitin that are extensively cross-linked together to form a complex network [43]. The phosphodiester linkages in these glycoproteins result in additional negative charges to the fungal cell surface [44].

Also the presence of different classes of sterols or analogous molecules in mammalian cells and pathogens may modulate the selectivity of AMPs. For example, cholesterol in mammalian cell membranes, which is absent in prokaryotes, has a significant protective mechanism against AMPs [45]. It comes from the fact that cholesterol controls the fluidity of the lipid bilayer by forming rigid and thick ordered domains (liquid ordered phase) that may resist membrane curvature induced by AMPs [46–48]. Hence the AMP dosage requirement gets to a higher threshold, limiting its action on mammalian cell membranes [49].

2.3. Potential for resistance development

Typically, the relative chance of microbes to develop resistance to an agent depends on the target specificity of the antimicrobial mechanism of action [50]. This is the obvious fact for the rapid resistance development against antibiotics since they are highly specialized to attack a specific microbial target. In contrast, polycations are mostly nonspecific in their action on microbes. However, it is unrealistic to expect that microbial pathogens are unable to develop resistance against these macromolecules. It should be noted that there are few reports indicating bacterial resistance development against AMPs [51,52] and synthetic polymers [53]. Nevertheless, widespread and rapid resistance development towards membrane active, cell lysing antimicrobial macromolecules may be unlikely compared to small molecule antimicrobials

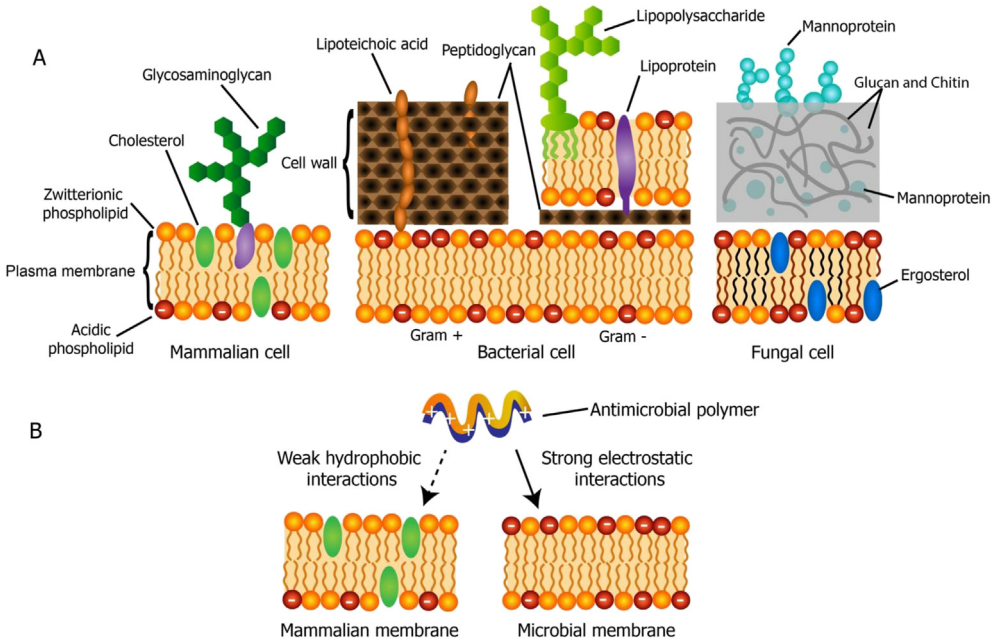


Fig. 5. Origin of the cell selectivity of cationic antimicrobial macromolecules. (A) Illustration of the cross sections of microbial and mammalian cell envelopes. Mammalian cell membrane surface (left) is largely neutral compared to bacterial (middle) or fungal (right) cell membranes. (B) Selective interactions between cell membranes and cationic polymers. Adapted with permission from Refs. [19,42].

mediated with specific receptor sites [54]. It has been observed that there is a much greater number of passes required to induce resistance in bacteria against AMPs or synthetic mimics of AMPs under *in vitro* experiments [55,56]. On the other hand, it is ambiguous how *in vivo* conditions, where a multitude of defense agents and mechanisms are present in the host organism, may define the microbial adaptations against cationic macromolecules [57]. These features may be the reason why AMPs have been actively present in biological systems as effective defensive macromolecules in many forms of life for millions of years.

2.4. Membrane-active macromolecules

Synthetic mimics of AMPs are rapidly expanding, indicating that AMPs have formed a better platform to develop a class of next generation antimicrobial therapeutics. Although many types natural AMPs and mimics of AMPs such as synthetic AMPs [58], β-peptides [59] [60], peptoides [61] and AApeptides [62] have been developed with comparable or even better activities than the natural versions. However, in most situations costly synthetic approaches, fast proteolytic degradation, low bioavailability or toxicity limit their widespread clinical applicability [63,64].

The ability to modulate the structural features, low cost synthesis, potent biological activity and stability make synthetic antimicrobial polymers favorable over other analogs of AMPs. Compared with conventional antibacterial agents of low molecular weight, polymeric antibacterial agents have advantages such as non-volatilization, inability to permeate the skin, longer circulatory time and reduced residual toxicity to the environment. The general term 'antimicrobial polymers' include several classes of materials such as cationic polymers, biocide-releasing polymers and antibiotic-conjugated polymers. Synthetic polymer disinfectants with cationic functionalities that emerged simultaneously with AMPs show strong biocidal activities. These macromolecules usually have cationic functionality such as quaternary ammonium groups, and hydrophobic alkyl moieties and have been mostly derived from poly(styrene)s, poly(vinylpyridine)s, poly(vinyl alcohol)s, poly(methacrylate)s, etc. [24]. However, earlier versions of polycationic biocides showed significant toxicity to human cells. This property could be only in line with their targeted application, which is in the solid state as potent disinfectants or biocidal coatings. Therefore the improvement of both antimicrobial and biocompatible polymers is essential to enable widespread systemic or topical clinical use of these macromolecules.

2.5. Definitions and units on antibacterial and hemolytic properties

There are several important measurements used to quantify the antimicrobial activity of polymers. The minimum concentration of antimicrobial agent needed to inhibit bacterial growth known as minimum inhibitory concentration (MIC) is the standard measurement to find the antimicrobial potency of the material. MIC is mostly reported as μg/mL, μM or ppm units. The minimum bactericidal concentration (MBC) is the concentration at which all the microorganisms are eradicated. Preliminary data on the biocompatibility is obtained by hemolytic activity assays against mammalian erythrocytes. Therefore 50% hemolytic concentration (HC_{50}) is defined as the minimum concentration of the material that results in 50% of red blood cells (RBCs) to get lysed when exposed to the agent. Preferably the MIC of an antimicrobial agent should be much lower compared to HC_{50} to be a useful therapeutic agent. The ratio of HC_{50}/MIC is known as the selectivity index (SI) that gives a measure of the selectivity of the antimicrobial agent towards microorganisms instead of mammalian cells. Therapeutic index (TI) is the safety window of a therapeutic agent. It represents as a ratio of the lethal dose of a drug for 50% of population (LD_{50}) divided by the minimum effective dose for 50% of the population (ED_{50}).

3. Macromolecular structure parameters

3.1. Macromolecular architectures

There should be careful considerations to rationally design the structural parameters of antimicrobial polymers to develop

systems with better selectivity indices. Targeted applications would be for systemic or topical treatments against infectious microorganisms or for modification of polymeric medical devices such as sutures or medical implants. Unlike small molecule antimicrobial agents, there are numerous types of polymer architectures such as homopolymers, random copolymers, block copolymers, telechelic or zwitterionic and branched polymers that can be wisely utilized to modulate the biological activities (Fig. 6). An important understanding can be gained from the fact that facial amphiphilicity and hydrophobic content are strongly correlated with antimicrobial activity and selectivity of AMPs. Therefore an in-depth analysis of such structural determinants is necessary to develop antimicrobial polymers in terms of optimal hydrophobicity/hydrophilicity balance, molecular weight, cationic units, counterions and charge density, in addition to different polymer architectures to achieve strong antimicrobial activities with high selectivity indices.

Due to electrostatic repulsions coming from cationic residues, some AMPs, for instance magainin and indolicidin, adopt random coil structures in aqueous solutions. However, they can readily adopt facially amphiphilic secondary structures via different conformations such as α-helical structures upon binding to lipid membranes [5]. In addition, it has been demonstrated that an induced helical formation improves the bioactivity while defects in helical formation lead to decreased activity.

However, it is not the helix-induced rigid, distinct and facially amphiphilic secondary structure leading to the antimicrobial properties, but the globally amphiphilic secondary structure that forms upon contact with a polyanionic cell membrane surface (Fig. 7) [65]. This averts the requirement to adopt a defined amphiphilic secondary structure as observed for many AMPs and also explains why randomly sequenced polycations are membrane active. However, it should be noted that although a priori facially amphiphilic structure is not necessarily required, such conformations are energetically favorable for a stronger macromolecule-cell membrane interaction. Applicability of flexible polymer architectures permits scientists unrestrained possibilities to explore various kinds of macromolecular architectures to produce cationic antimicrobial polymers.

3.1.1. Random copolymers

First, it should be noted that in this section copolymers with unspecified sequences are referred to as “random” copolymers, in order to signify against other macromolecular architectures and

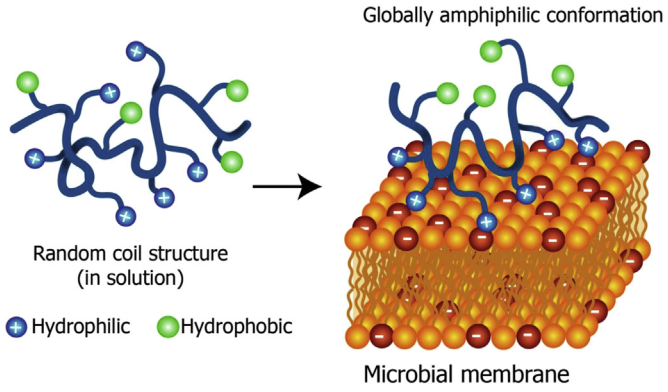


Fig. 7. Representative globally amphiphilic polymeric structures. Polymer in solution has a random coil structure which turn into a globally amphiphilic conformation upon contact with a cell membrane.

does not necessarily imply truly random copolymers. Random copolymers are the most widely reported polymeric architecture to prepare antimicrobial polymers. They can be facilely synthesized by combining a non-polar monomer with a cationic monomer. These “segregated” structures have charged and hydrophobic moieties that are ‘randomly’ isolated along the polymer backbone. Utilizing an assortment of structurally different monomers that have a range of hydrophobicities or by adjusting feed ratios, the amphiphilicities of the copolymers can be controlled. Another strategy is the post-polymerization modification of a homopolymer via introducing cationic biocide functionality or modifying the hydrophobicities of some repeat units. The advantage of the latter approach is that a variety of cationic groups can be introduced, and the degree of modification can be well-controlled while the polymer backbone remains persistent. This allows for accurate structure-property evaluations by using a common base polymer structure and molecular weight to generate a lineage of derivatives having comparable structures [66]. Antimicrobial random polymers mostly include functionalized poly(methacrylate)s, poly(methacrylamide)s, poly(β-lactam)s, poly(norborene)s and poly(carbonate)s.

Unlike cationic amine groups, guanidine moieties introduce intriguing behaviors to antimicrobial polymers. Haeussler et al. scrutinized the effects of guanylated poly(methacrylate)s towards antimicrobial and hemolytic activities [67]. They synthesized

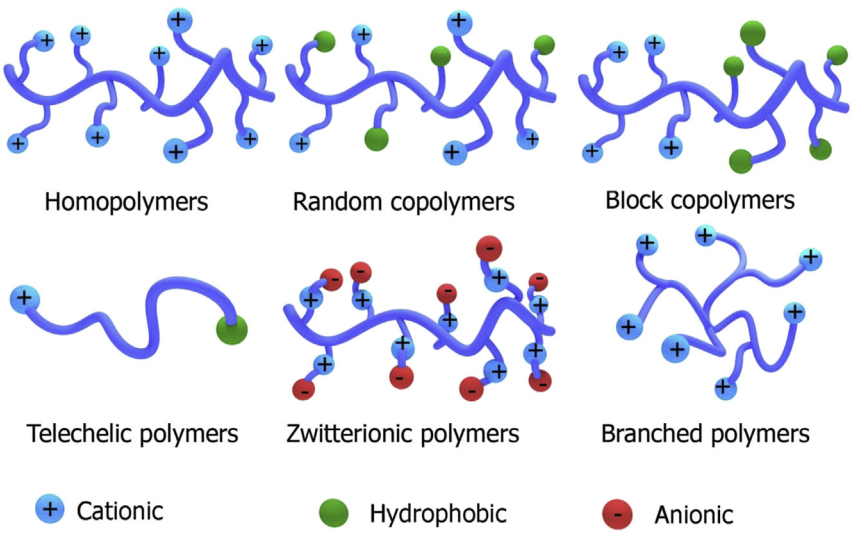


Fig. 6. Commonly employed polymeric architectures to prepare antimicrobial polymers.

poly(methacrylate)s decorated with amine or guanidine cations and hydrophobic side chains (Table 1A) by reversible addition–fragmentation chain transfer (RAFT) polymerization. Interestingly, guanidine copolymers were more active against microbes compared to the amine analogs and were less hemolytic. In addition, guanidine polymers were able to sustain activity in tryptic soy broth (TSB) with fetal bovine serum (FBS) while amine analogs lost their activity. Inspired by the naturally occurring, tryptophan-rich cationic antimicrobial materials, Haeussler and co-workers elegantly designed novel poly(methacrylate) random copolymers [68]. They synthesized methacrylate monomers that mimic the chemical structures of tryptophan, lysine and arginine (Table 1B). Low levels of indole content were required to optimize antimicrobial potency and minimize toxicity of the two series of amine and guanidine antimicrobial copolymers.

Antifungal polymers have been gaining more interest recently. Achieving similar activities against fungi compared to bacteria is somewhat difficult due to the similarities in fungal and mammalian cells, as both are eukaryotes. However, Gellman, Masters and co-workers found out that a group of nylon-3 copolymers (Table 1C) were highly active against *Candida albicans* while maintaining low hemolytic activity [69]. These sequence-random poly(β-peptide)s are structurally very similar to AMPs since they contain protein like polymer backbone. Typically, these polymers are prepared by anionic ring-opening polymerization of appropriate β-lactam monomers. Therefore this class of macromolecules combines AMP structural similarities while providing the ease of synthesis. McBride, Gellman et al. investigated the activity of another class of nylon-3 copolymers against *Clostridium difficile*, an important endospore-forming anaerobic bacterial pathogen [70]. The polymers effectively inhibited the growth of vegetative cells as well as spore outgrowth.

Keul, Möller and co-workers, designed cationic antimicrobial poly(ethylene imines)s with three distinct microstructures [71]. They incorporated hydrophobic, cationic or amphiphilic couplers to poly(ethylene imines)s to generate the microstructures. Polymers with cations directly attached to the hydrophobe were more effective against bacteria, compared to segregated polymers and polymers with cation and hydrophobe linked with an alkyl chain. In a more recent study, they synthesized azetidinium functionalized poly(vinyl amine) copolymers (Table 1D) via a one-pot post-polymerization modification approach using suitable functional couplers [72]. When the hexyl chain in the hydrophobic group was replaced by a decyl chain, antimicrobial activity against *S. aureus* and *Escherichia coli* increased significantly. They observed certain threshold percentages of cationic and hydrophobic content that achieved best antibacterial activity. Antimicrobial poly(acrylate) copolymers with superior antibacterial activity were developed by Yang and co-workers [73]. They used monomers with various spacers linking primary ammonium groups (Table 1E) to modulate the activity.

Toxicity of cationic polymers can result in adverse effects to host cells. To circumvent off-target effects, 'smart' antimicrobial random copolymers were developed by Yang and co-workers [74] that can be activated by acids. They incorporated methacrylic acid in addition to cationic 2-aminoethyl methacrylate and hydrophobic methacrylate. At physiological pH conditions net neutral polymers were generated and had diminished hemolytic activity. However, under acidic conditions the polymers were active against bacteria. In another study, antimicrobial random copolymers with improved selectivities were developed by them. They used hydrophilic and cationic methacrylate repeating units in the polymers (Table 1F) [75]. Kuroda et al. designed novel cationic antimicrobial polymers that can self-degrade via intramolecular amidation reactions [76]. They used metal-catalyzed simultaneous chain- and step-growth radical polymerization of *tert*-butyl acrylate and 3-butenyl 2-chloropropionate to prepare polymers (Table 1G). The degradation of primary amine-containing polymers in aqueous media resulted in oligomers that were poorly antimicrobial. This new concept is useful to prepare antimicrobial macromolecules that have lower residual toxicities.

3.1.2. Amphiphilic homopolymers

The next strategy is to prepare homopolymers using monomers that house both hydrophobic units and hydrophilic cationic groups together. In reference to the polymer backbone, several structural differences can be defined as reported by Hedrick, Yang and co-workers in their recent review [19]. One type of homopolymers has a brush-like architecture in which cationic groups and hydrophobic groups spread out from the same repeating unit along the polymer backbone. Such polymers contain "facially amphiphilic" repeat units with respect to the polymer backbone (Table 2A and E). The second type is a "same-centered" repeat unit structure, where the hydrophobic moiety directly accompany the charged moiety, thus the functional groups are not spatially separated over the backbone (Table 2B and C). With regard to random copolymers where hydrophobic and cationic moieties are linked to different repeating units that are randomly distributed, homopolymers show amphiphilic balance at the monomer level.

Tew and coworkers prepared a series of antimicrobial poly(norbornene) homopolymers derived from facially amphiphilic monomers (Table 2A) [77]. Hemolytic activities of the polymers increased with increasing alkyl chain lengths. In another study, they conducted a direct comparison between poly(norbornene)s derived from facially amphiphilic monomers and similar copolymers made from segregated monomers [83]. The results show a significant improvement of activity in the first system with facially amphiphilic repeat units. This indicates that local amphiphilic balance at the monomer level has a considerable effect on the biological activity of amphiphilic homopolymers. Poly(carbonate) based biodegradable antimicrobial polymers were prepared by Hedrick and coworkers. The "same-centered" homopolymers (Table 2B) showed outstanding results with high antibacterial activities against MRSA, vancomycin-resistant *Enterococcus* (VRE), carbapenem-resistant *Acinetobacter baumannii* and fluconazole-resistant *Cryptococcus neoformans* [78]. They employed several cationic appendages to control the amphiphilic balance. Consequently, polymers with butyl group attached to the quaternary ammonium turned out to be a highly efficacious and nonhemolytic antimicrobial agent.

Tang and co-workers developed a class of novel antimicrobial homopolymers using renewable biomass. In one study, they synthesized resin acid-derived cationic poly(ε-caprolactone) homopolymers using ring-opening polymerization (ROP) and click chemistry (Table 2C). The polymers had promising antibacterial activities against a wide range of bacteria including Community-acquired (CA) methicillin-sensitive *S. aureus*, CA-MRSA and hospital-acquired MRSA (HA-MRSA). Interestingly, the polymers had outstanding *in vitro* and *in vivo* biocompatibilities [79,84]. Recently, Kuroda and co-workers investigated cationic poly(methacrylate)s as topical antimicrobial agents against *S. aureus* nasal colonization [53]. The polymers (Table 2D) were prepared via RAFT polymerization of *N*-(*tert*-butoxycarbonyl)aminoethyl methacrylate and subsequent deprotection with trifluoroacetic acid. The polymers reduced the number of viable *S. aureus* cells in the nasal environment of cotton rats, illustrating the potential as topical anti-infective agents.

Novak and co-workers investigated the antimicrobial and hemolytic activity of a family of cationic helical polymers derived from polycarbodiimides [80]. They incorporated guanidium groups

Table 1
Antimicrobial random copolymers.

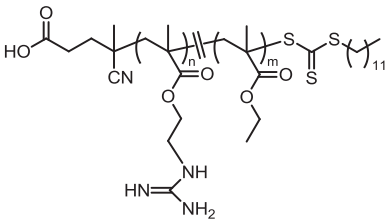
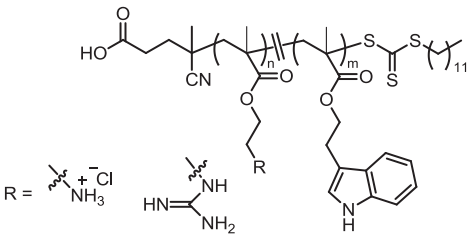
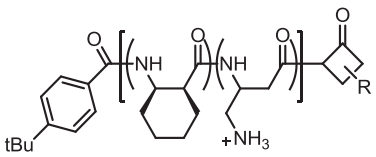
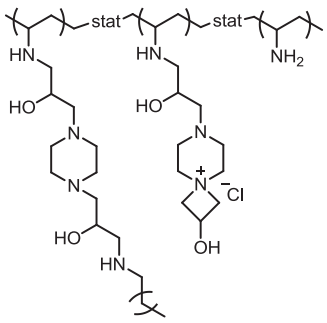
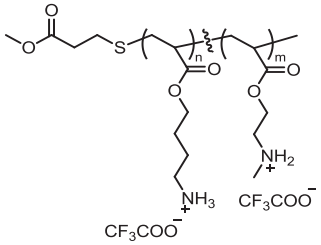
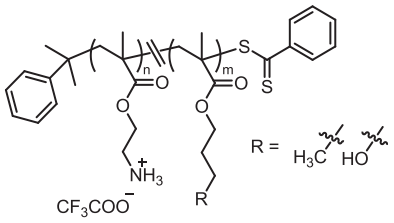
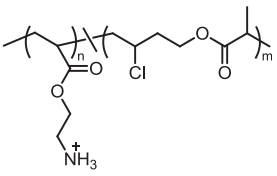
	Representative chemical structure	Test subject	MIC or HC_{50} (μg/mL)	Ref.
1A	 $M_{n,NMR}$ = 3,360 g/mol	*S. aureus* *S. epidermidis* *E. coli* *C. albicans*	94 10 >1,500 32	[67]
1B	 $M_{n,NMR}$ = 5,300 g/mol	*S. epidermidis* MRSA	12 47	[68]
1C	 $M_{n,GPC}$ = 3,500 g/mol	*C. albicans* RBC	3.1 >400	[69]
1D	 $M_{n,GPC}$ = 482,000–367,000 g/mol	*S. aureus* *E. coli* RBC	3–100 10–100 23–41	[72]
1E	 $M_{n,GPC}$ = 6,200–7,800 g/mol	*S. aureus* RBC	16–104 >1,619	[73]
1F	 $M_{n,GPC}$ = 3,200–38,000 g/mol	*S. aureus* *B. subtilis* *P. aeruginosa* *E. coli* RBC	8–32[a] 8[a] 4–32[a] 3–4[a] 2–512[a]	[75]

Table 1 (*continued*)

	Representative chemical structure	Test subject	MIC or HC_{50} (μg/mL)	Ref.
1G	 $M_{n,NMR}$ = 4,800 g/mol	*E. coli* RBC	104 >500	[76]

[a] MBC is given.

to the polymers prepared by coordination-insertion polymerization of carbodiimide monomers with different alkyl chain lengths (Table 2E). Helical handedness did not influence antibacterial activity proving the non-specific activity of the polymers. Surprisingly, the polycations mostly precipitated RBCs without hemolysis.

Many types of antimicrobial polymers based on poly(phenylene ethynylene) cationic conjugated polyelectrolytes were investigated by Whitten and co-workers [85]. In a recent study, antifungal activity of poly(phenylene ethynylene) polyelectrolytes (Table 2F) and oligo-phenylene ethynylenes were investigated [81]. The polycations effectively inhibited the fungus *Saccharomyces cerevisiae*, an opportunistic human pathogen, both in the dark and under ultraviolet (UV)/visible light irradiation. Fungal spores were completely inactivated under light conditions. Under UV/visible light irradiation these conjugated macromolecules generate corrosive singlet oxygen species ($^1O_2$) and subsequent secondary reactive oxygen species that rapidly damage microorganisms. A new family of hemocompatible and antifungal nylon-3 polymers (Table 2G) was added to the genre of antimicrobial polymers developed by Gellman and co-workers [82]. These cationic homopolymers were identified as a strong candidate against *C. albicans* with SI > 130.

3.1.3. Block copolymers

Compared to other polymeric systems, block copolymers are typically prepared by living polymerization techniques. Block copolymers exhibit strong partitioning between hydrophobic and hydrophilic regions along the polymer backbone, hence providing another approach to control the amphiphilic balance. Unlike homopolymers or random copolymers, block copolymers form self-assembled nanostructures. Such examples will be discussed in a separate section. There are relatively few reports related to antimicrobial block copolymers in solution. This may be due to the low critical micelle concentration (CMC) values of amphiphilic block copolymers. Most reported work focused on antimicrobial block copolymers are related to antimicrobial micelles or nanoobjects [86–88], antimicrobial fibers [89] and surface coatings [90,91].

Chojnowski et al. compared the antimicrobial activity of block and statistical all-siloxane copolymers containing quaternary ammonium salts [92]. Interestingly, there were no clear differences of antibacterial activity observed between the two polymeric systems. A series of amphiphilic block copolymers of poly(vinyl ether) derivatives (Table 3A) were prepared by Kuroda and coworkers via living cationic polymerization [93]. They observed similar antibacterial activity of block copolymers to analogous random copolymers against *E. coli*. However, block copolymers did not show hemolytic activity even up to 1000 μg/mL, while random copolymers showed strong hemolytic activity at much lower concentrations. As an explanation for this behavior, they proposed the formation of single molecule cationic particles from block copolymers that prevent the hydrophobic core reaching out to the red blood cell membrane.

Liu and coworkers, prepared random and diblock copolymers of 2-(*N*,*N*-dimethylamino)ethyl methacrylate and butyl methacrylate prepared by atom transfer radical polymerization (ATRP) (Table 3B) [94]. Although the antibacterial activities against *S. aureus* and *E. coli* were similar, the diblock copolymers had much lower hemolytic activity compared to the random copolymers. Therefore, the selectivities of the diblock copolymers were superior to the analogous random copolymers with similar compositions and molecular weights. These investigations demonstrate that partitioning of the functionalities (i.e. cationic groups that modulate the cell selectivities and hydrophobic groups that damage the membrane) on the macromolecule may improve cell selectivities.

A recent study about amphiphilic block copolymers was conducted by Fernández-García et al. [82]. They used ATRP to prepare well-defined block and statistical copolymers of butyl methacrylate, 2-(dimethylamino)ethyl methacrylate and a glycomonomer (Table 3C). The polymers were active against a range of microorganisms including *S. aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Candida parapsilosis*. The incorporation of certain amount of carbohydrate pendant groups lowered the hemolytic activity substantially while maintaining the antimicrobial activity.

3.1.4. Telechelic polymers

In recent years, there is a considerable interest in telechelic polymer architecture in designing antimicrobial macromolecules. Telechelic polymers contain reactive end groups that come from the initiator or the terminating or chain transfer agents, and are typically synthesized via controlled/living polymerization methods. These reactive groups have the capacity to allow additional polymerization or other reactions that can be used to prepare more advanced architectures including block and graft copolymers, star, hyperbranched or dendritic polymers and macrocycles [95]. Telechelic polymers composed of non-biocidal repeating units are particularly appealing. Complete degradation of such polymers would result in inactive compounds that are favorable in reducing resistance development and contamination of the environment.

Poly(oxazoline)s are useful for polymer therapeutics due to their biocompatibility, better solubility, variation of size, architecture as well as chemical functionality [96]. Tiller and coworkers have largely contributed to the area of telechelic antimicrobial polymers. They prepared telechelic poly(oxazoline)s with biocidal end groups and an NH_2 function at the starting end (satellite groups (SGs)) of the polymer (Table 4A) [97]. Cationic ring-opening polymerization was used to prepare the polymers. SGs were incorporated as initiators, and biocidal functionality was achieved by terminating with tertiary amines. It is intriguing that the SG, although located far from the biocidal group, has a significant effect on the antimicrobial activity of the polymer.

They investigated telechelic antimicrobial function of poly(-oxazoline)s to reveal the influence of different non-active SGs distal

Table 2
Antimicrobial homopolymers.

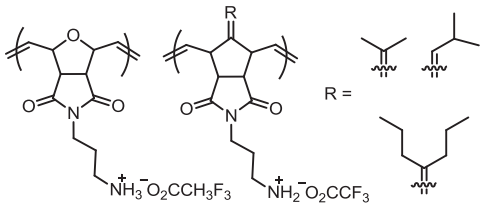
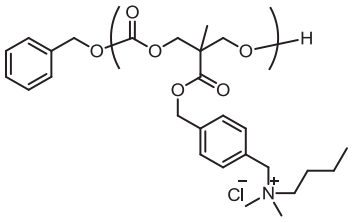
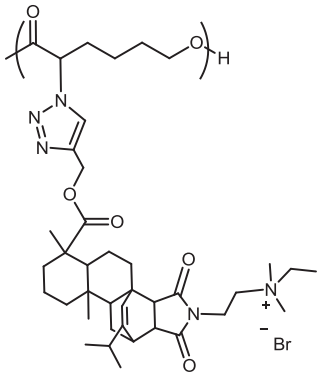
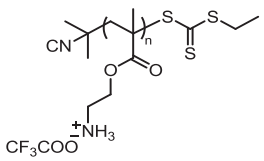
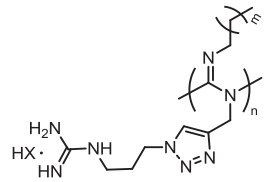
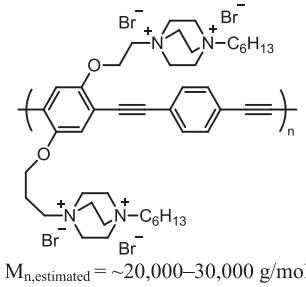
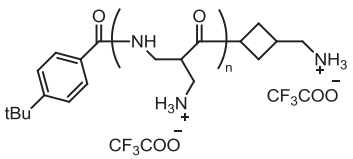
	Representative chemical structure	Test subject	MIC or HC_{50} (μg/mL)	Ref.
2A	 $M_{n,GPC}$ = 1,600–137,500 g/mol	*B. subtilis* *E. coli* RBC	25–>500 25–>500 <1–>4,000	[77]
2B	 $M_{n,NMR}$ = 7,100–25,000 g/mol	MRSA VRE *A. baumannii* *C. neoformans* RBC	7.8 3.9 62.5 31.3 >4,000	[78]
2C	 $M_{n,NMR}$ = ~84,000 g/mol	CA-MSSA CA-MRSA HA-MRSA RBC	8.8 9.9 14 >492	[79]
2D	 $M_{n,NMR}$ = 2,100–3,200 g/mol	*S. aureus* RBC	42–63 >1,000	[53]
2E	 $M_{n,GPC}$ = ~1,400–24,000 g/mol	*S. aureus* RBC	64–128 >3,000 μM	[80]
2F	 $M_{n,estimated}$ = ~20,000–30,000 g/mol	*S. cerevisiae*	>6-log reductions	[81]

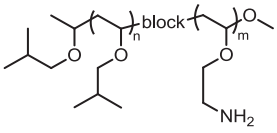
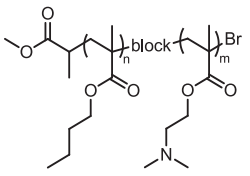
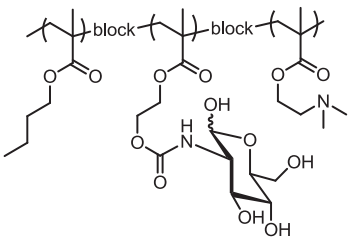
Table 2 (*continued*)

	Representative chemical structure	Test subject	MIC or HC_{50} (μg/mL)	Ref.
2G	 $M_{n,GPC}$ = ~3,400 g/mol	*C. albicans* RBC	3.1 (MIC_{50}) >400 (HC_{10})	[82]

to the cationic end groups on the antimicrobial effect (Table 4B) [98]. They found that the antimicrobial activity can be controlled over several orders of magnitude, illustrating the fact that hydrophilic SGs with necessary length activate the biocidal group while nonbasic hydrophilic groups deactivate it. Critical micelle concentrations decreased abruptly with increasing chain lengths of the SG. This may have significantly influenced the antibacterial activity for some polymers.

Tiller et al. also demonstrated that the antimicrobial activity of poly(2-methyloxazoline) can be switched via the satellite group modification (Fig. 8) [99]. Cationic ring-opening polymerization of 2-methyl-2-oxazoline was used to prepare the polymer while termination with the biocidal group *N*,*N*-dimethyldodecylamine introduced antimicrobial activity (Table 4C). Introduction of a hydrolyzable ester satellite group permitted the bioswitchable antimicrobial activity. It was apparent that hydrophobic SGs greatly activate the distal biocidal group, while hydrophilic SGs deactivate it. The polymer was specific for Gram-positive bacteria and showed high hemocompatibility. As a matter of fact, the selectivity at 120 was very high towards *S. aureus*. Upon degradation by lipase, the antimicrobial activity against *S. aureus* decreased by 30-fold, and the HC_{50} of the hydrolyzed polymer increased by 5-fold.

Table 3
Antimicrobial block copolymers.

	Representative chemical structure	Test subject	MIC or HC_{50} (μg/mL)	Ref.
3A	 $M_{n,NMR}$ = 7,100–25,000 g/mol	*E. coli*	1.6–2.4[a]	[93]
3B	 $M_{n,NMR}$ = 4,000–5,000 g/mol	*S. aureus* *E. coli*	34–67 54–97	[94]
3C	 $M_{n,NMR}$ = 9,100 and 19,830 g/mol	*S. aureus* *S. epidermidis* *P. aeruginosa* *C. parapsilosis* RBC	16–64 16–32 250 8–16 >2,500	[82]

[a] Biocidal concentration for 99.9% killing.

Although different from typical telechelic polymers, end group functionalization is used to control biological activities of other types of polymers as well. Gellman at al. functionalized the N- and C-terminals of nylon-3 random copolymers (Table 4D) [100]. They found that there is a significant difference in the two types of polymer terminus in terms of introducing a hydrophobic group. The N-termini modification generated a polymer that had better selectivity, while the C-termini modified polymer suffered from increased hemolysis. The impact of end-groups on the activity and cytotoxicity of cationic methacrylate polymers was investigated by Griesser and co-workers [101]. They used RAFT polymerization to prepare poly(-methacrylate) random copolymers which bear either amine or guanidine pendant groups, while incorporating different R- and Z-end-groups (Table 4E). Presence of a carboxylic group on R terminus resulted in a lower level of hemolysis compared to isobutyronitrile group. In addition, longer alkyl chains in the Z-group improved the antimicrobial profile. These studies establish the concept of controlling antimicrobial and hemolytic activities of antimicrobial polymers via the chemical modifications at the chain ends.

3.1.5. Zwitterionic polymers

Zwitterionic polymers are a class of novel charged polymeric antimicrobials that bear an equal number of anionic and cationic groups in their polymer chains. The ionic nature of zwitterionic materials enables the adjustment of polymer charge density, pH sensitivity, counterion association via switching between cationic and zwitterionic states.

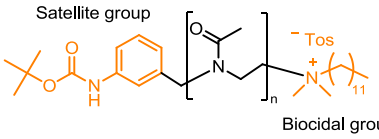
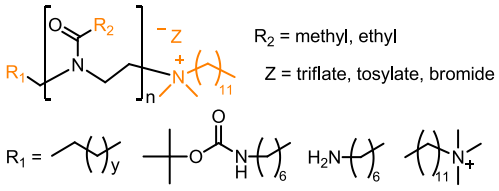
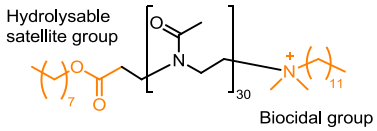
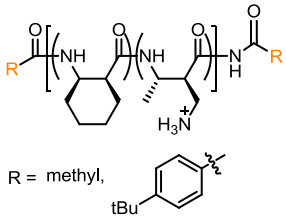
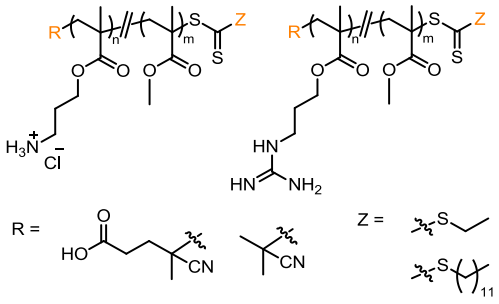
Lowe and co-workers studied the antimicrobial activities of a series of statistical copolymers derived from 2-(dimethylamino) ethyl methacrylate with four different hydrophobic comonomers (ethyl, butyl, cyclohexyl, and octyl methacrylates) [102]. They used conventional free radical copolymerization and post polymer modifications to yield corresponding poly(sulfopropylbetaine) derivatives (Table 5A). Some of the copolymers exhibited bacteriostatic activity against *S. aureus* and *E. coli*. Antimicrobial activity improved with the increasing hydrophobic content.

Zwitterionic poly(oxanorbornene) antimicrobial polymers (Table 5B) were prepared by Tew et al. to examine the effect of covalently connected 'intramolecular counterion', which would not be able to diffuse away from the polymer backbone [103]. The zwitterionic polymer sample was less active compared to the analogous diamine monomer, and they assumed that this was due to ion pair formation.

Yang and co-workers synthesized well-defined PEGylated-polymers with various tertiary amines via RAFT polymerization [104]. They introduced various alkyl halides to produce quaternized polymers. When they introduced a zwitterion to the polymer (Table 5C), antimicrobial activity was lost. These observations may be due to the fact that zwitterion reduces the general cationic nature of the polymer. However, zwitterionic polymers are very effective for non-fouling applications.

Jiang and co-workers have extensively contributed to the field of antimicrobial and non-fouling zwitterionic polymers over the past

Table 4
Telechelic antimicrobial polymers.

	Representative chemical structure	Test subject	MIC or HC_{50} (μg/mL)	Ref.
4A	 $M_{n,GPC} = \sim 2{,}000 - 12{,}600$ g/mol	*S. aureus*	200–>1,000	[97]
4B	 $M_{n,GPC} = \sim 2{,}200 - 12{,}800$ g/mol	*S. aureus* *E. coli*	6–>5,000 μM 53–>5,000 μM	[98]
4C	 $M_{n,NMR} = 3{,}100$ g/mol	*S. aureus* *E. coli* *B. subtilis* *S. epidermidis* *S. mutans* RBC	40 1,250 40 40 156 >4,730	[99]
4D	 $M_{n,GPC} = \sim 3{,}900$–$6{,}100$ g/mol	*S. aureus* *E. coli* *B. subtilis* *E. faecium* RBC	25–400 12.5–200 3.13–12.5 12.5–100 >800	[100]
4E	 $M_{n,NMR} = 3{,}700 - 4{,}000$ g/mol	Vancomycin and methicillin resistant *S. aureus* *S. epidermidis* *C. albicans*	16–128 16–31 32–256	[101]

few years. They have published a concise review about the zwitterionic polymers [105]. Most of the zwitterionic polymer systems have been tested for their surface bound bactericidal or non-fouling activity. Direct bactericidal property was achieved by reversible lactonization [106] or side-chain ester hydrolysis (Fig. 9) [107].

3.1.6. Branched antimicrobial polymers

Antimicrobial macromolecules with dendrimer, hyperbranched, brush or star structures have also been studied although they do not necessarily mimic the fundamental structural features on most AMPs. Dendrimers are monodisperse globular shaped macromolecules that have been widely studied in biomedical applications including drug delivery, imaging, and antimicrobial. There are many comprehensive reviews [108–111]. The highly branched, three dimensional architecture of dendrimers can be tailored to produce substantially functionalized macromolecules with tunable internal cavities, surfaces, sizes and molecular weights [110]. Inherently polyvalent compact structure with the availability of many end groups offer high local concentrations of active groups that can act in a synergistic fashion. Although the diffusion of dendrimers is limited, initial adsorption and binding to cell membranes is stronger than linear polymers. Therefore

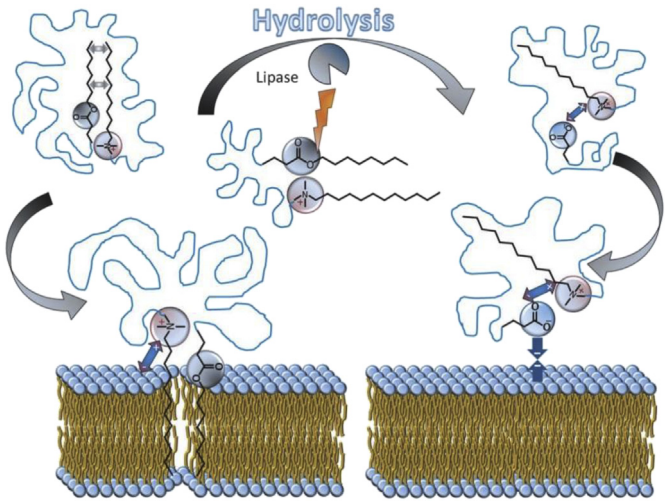


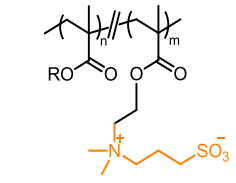
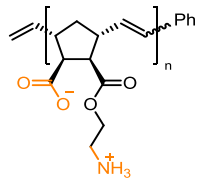
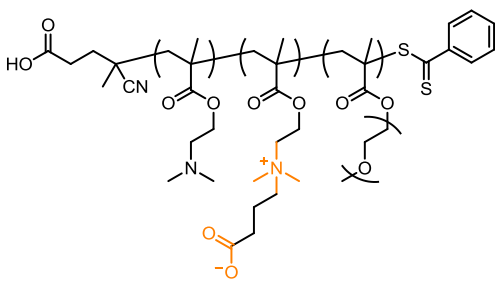
Fig. 8. Schematic representation of membrane-active bioswitchable biocidal telechelic polymers. Reprinted with permission from Ref. [99].

branched macromolecules are expected to show higher level of membrane disrupting activity (Fig. 10).

Typically, dendrimers are synthesized by either a divergent or a convergent approach [110]. At each stage of synthesis, identified as generations (G), dendrimers increase the number of functional groups, the size, and the molecular weight. There are several classes of dendrimers that can be applied as antimicrobials. Those include glycodendrimers, cationic dendrimers, anionic dendrimers, and peptide dendrimers [109]. Antimicrobial dendrimers also show a size and hydrophobic alkyl chain length dependence, similar to most conventional liner polymer antimicrobials. Cooper et al. investigated PPI dendrimers with terminal quaternary ammonium C_{12} hydrophobes. The influence of generation number on antimicrobial activity had a trend of G5 > G4 > G1 > G2 > G3 [112]. This indicates the requirement for an optimization of two opposing factors, cationic functionality and overall size of the dendrimer.

Common cationic dendrimer biocides are prepared by functionalizing end groups of dendrimers with quaternary ammonium salts to produce cationic dendrimers [115]. Poly(amidoamine) (PAMAM), poly(propylenimine) (PPI) and poly(carbosilane) (PCS) based dendrimers are widely investigated as antimicrobials (Fig. 11). Schoenfisch and co-workers developed a class of novel dual action PAMAM antimicrobial dendrimers [113]. They synthesized nitric oxide-releasing quaternary ammonium functionalized G1 and G4 PAMAM dendrimers (Fig. 11A). Both individual and dual action dendrimers demonstrated activity against *S. aureus* and *P. aeruginosa* that was found to be dependent on the type of bacteria, dendrimer generation and quaternary ammonium alkyl appendage length. Longer alkyl chains (octyl and dodecyl) improved antimicrobial activity. Lisowska et al. investigated the antibacterial and antifungal activity of PPI G4 dendrimers unmodified or modified by maltose [116]. PPI dendrimers had various levels of cationic charges on the surface depending on the medium. It was found that unmodified PPI dendrimer was efficient against *S. epidermidis* and *C. albicans* at higher concentrations. Maltose modification improved the activity against *S. aureus* and improved

Table 5
Zwitterionic antimicrobial polymers.

	Representative chemical structure	Test subject	MIC or HC_{50} (μg/mL)	Ref.
5A	 R = C_2H_5, C_4H_9, C_6H_{11}, C_8H_{17} $M_{n,GPC}$ = ~ 235,000 g/mol	*S. aureus* *E. coli*	1,250–1,750 1,125–2,000	[102]
5B	 M_n = 3,000 – 10,000 g/mol	*S. aureus* *E. coli*	50–>200 >200	[103]
5C	 $M_{n,GPC}$ = ~ 20,000 g/mol	*B. subtilis* RBC	>200 >500	[104]

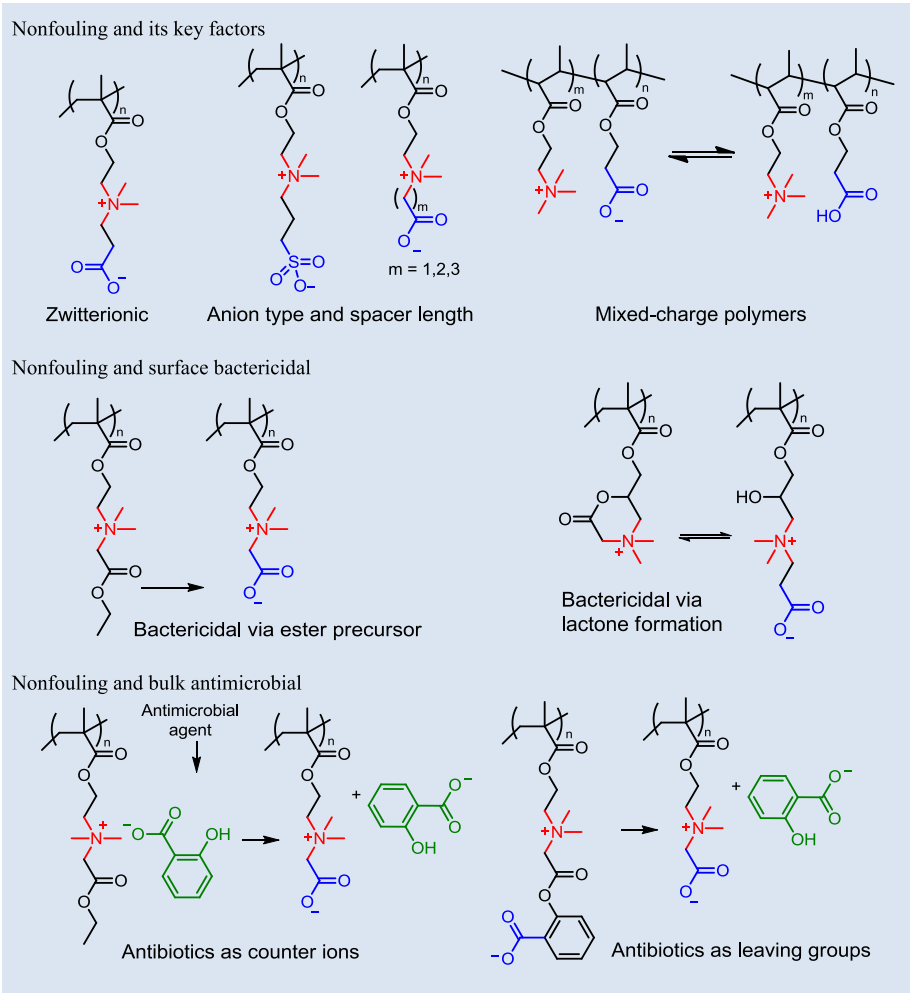


Fig. 9. Antimicrobial and nonfouling applications of various types of zwitterionic polymers in solution and surfaces. Reproduced with permission from Ref. [105].

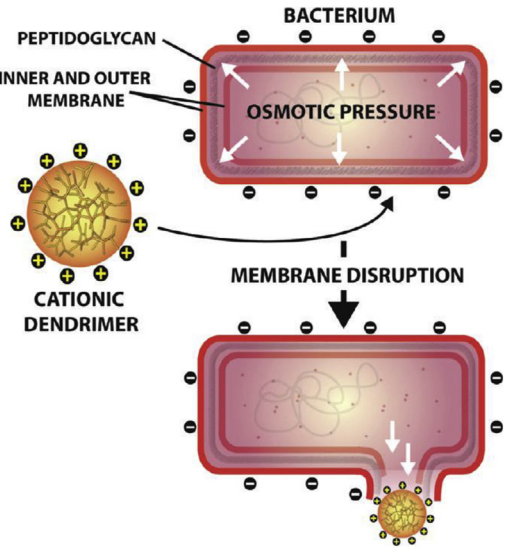


Fig. 10. Proposed mechanism of action of cationic dendrimers. Reprinted with permission from Ref. [109]. Copyright (2012) American Chemical Society.

biocompatibility. In an earlier report, Cooper and co-workers demonstrated enhanced antibacterial properties of quaternized PPI dendrimers [117].

Silicon-containing antimicrobial agents are interesting due to the presence of organic-inorganic hybrid materials. Silicon provides low hydrophilic characters. Gómez and co-workers compared the antimicrobial activity of dendrimer and hyperbranched materials with carbosilane skeleton and terminal quaternary ammonium groups (Fig. 11B) [114]. Both materials had similar MICs against *S. aureus* and *E. coli* in the range of 4–16 μg/mL. Mata, Gómez and co-workers recently published their investigation of carbosilane cationic dendrimers synthesized via thiol-ene click chemistry [118]. They prepared three types of dendrimers. Two of them had surface charges as primary ammonium or quaternary ammonium moieties while the third one had internalized quaternary ammonium moieties with ethylalcohol appendages. They observed the loss of antimicrobial activity with increasing generations as well as increasing size of substituents on nitrogen atoms. Interestingly, the presence of sulfur instead of silicon close to the nitrogen atom, enhanced the antibacterial activity.

Kuroda et al. reported structure–activity relationships in the antimicrobial activity of linear and branched poly(ethylene imine)s [119]. It was found that low molecular weight polymers were active against *S. aureus* and *E. coli*. In addition, branched molecules had lower cytotoxic effects on mammalian cells. Izzo et al. designed novel star-like heteroarms polymer containing cationic and

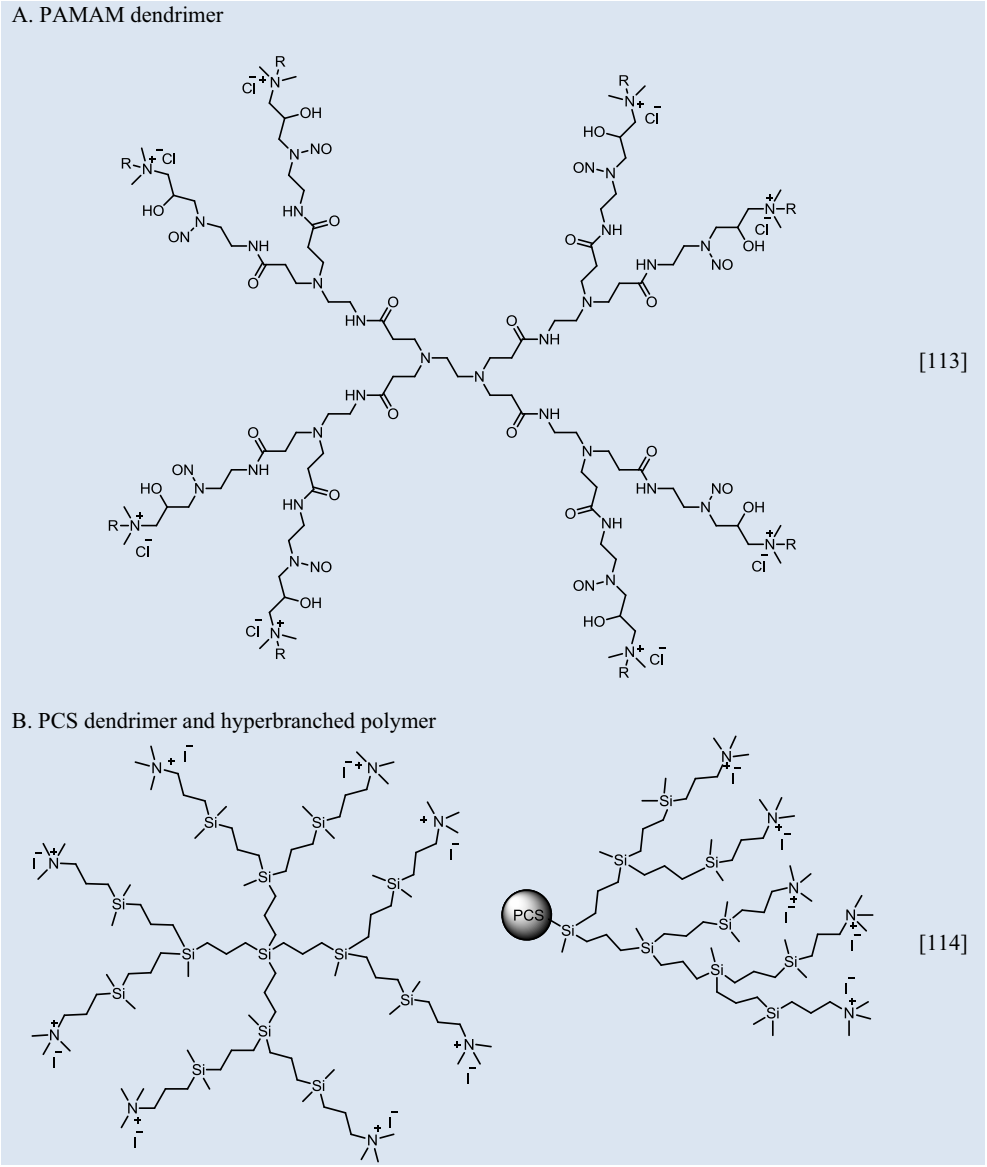


Fig. 11. Antimicrobial cationic dendrimers and hyperbranched polymers. Refs. [113,114].

hydrophilic groups [120]. They prepared three series of polymers with linear, two-arm and four-arm architectures where poly(-ethylene glycol) was the starting block and the second block had methyl methacrylate and nonquaternized 2-(dimethylamino)ethyl methacrylate. The films prepared using these polymers demonstrated strong antibacterial effects. Polymers with PEG attached to two arms showed finest results. Gao, Yang and co-workers investigated 8-arm poly(glycidyl methacrylate)s with quaternary ammonium functionality [121]. The polymers were active against *S. aureus* and *E. coli* with the lowest MICs of 16 μg/mL and 64 μg/mL respectively.

3.2. Hydrophobic/hydrophilic balance

Undoubtedly the most important design parameter in the development of antimicrobial polymer is the amphiphilicity, also referred to as hydrophobic/hydrophilic balance. It is the fundamental factor directly linked with the activity and selectivity of these materials. AMPs have a surprisingly optimal amphiphilicity as a result of million years of experiments by Mother Nature, which are still going on. Although amphiphilic balance is influenced by many chemical and physical features of the molecule, scientists have been able to capture some essence of these features to design synthetic antimicrobial polymers. For example highly hydrophilic or dense cationic polymers may have the ability to bind to polyanionic cell surfaces [122]. However, without enough hydrophobic groups they may not insert into the bilayer core that is required to damage the membrane. At the same time, largely hydrophobic polymers may be toxic to all types of cells. There may be also some solubility issues in biological media. Therefore hydrophobicity of the polymers can be considered as the main determinant of hemolysis or toxicity [123].

When stationed inside the core of cell membrane, the hydrophobic components of polymers cause reorganization and disintegration of the membrane. Partition coefficient is an important parameter to evaluate the hydrophobicity of polymers that significantly correlate with the membrane activity. It is assumed that polymer-membrane binding can be depicted in terms of partitioning of hydrophobic content of polymers from an aqueous phase to an organic phase. The water–octanol partition coefficient of

polymers is reported in several recent studies as a measure of overall amphiphilic balance [75,124]. It is observed that the amphiphilicity of polymers is influenced by the chemical structure, size or length of hydrophobic group, as well as its location on the polymer chain.

3.2.1. Alkyl spacers and tails

It is obvious that the penetration and burial of antimicrobial polymers into cell membranes depend on the alkyl chain length, because the cationic functionalities tend to complex with phospholipid head groups while the hydrophobic backbone or other moieties favor the membrane core. In 'same-centered' polymer structures, at least one substituent is a longer alkyl chain that acts as a spacer to connect the cationic group to the polymer backbone or a tail conjugated to a positive charge. The length of this alkyl group has a significant effect on the biological activity. The increase of alkyl chain length buildups hydrophobicity so that the polymer backbone can effectively penetrate into the hydrophobic core of the membrane lipid bilayer.

Kurihara and coworkers prepared thermosensitive antimicrobial polymers by copolymerization of *N*-isopropylacrylamide with methacryloyloxyethyl trialkyl phosphonium chlorides with different alkyl tail lengths [125]. They observed improving antibacterial activities of copolymers with increasing alkyl tail lengths on the phosphonium group. A series of amphiphilic pyridinium copolymers were prepared by Sen and coworkers to compare the spatial relationship between the positive charge and the pendent alkyl tail as well as the effect from the tail length [126]. The polymers were prepared using vinylpyridine and methacrylate monomers via free radical polymerization. The polymers having same centered structure were modified with tails from C_2 to C_{10}. They observed a parabolic shape for the MIC against the alkyl chain length, where the lowest MIC for *E. coli* was obtained for C_4, and C_6 for *Bacillus subtilis*. Hemolytic activity increased with longer alkyl tails up to C_8. A similar trend was observed for the segregated approach. However, it was observed that the same centered polymers had lower membrane disrupting capabilities while the spatially separated centers resulted in a higher membrane-disrupting ability, as evident from the increased antibacterial and hemolytic activities (lower selectivities). In addition, they observed strong cellular agglutination of RBCs caused by spatially separated polymers, which may be a leading factor for hemolysis. The underlying structure–activity relationship may be due to the fact that the hydrophobic groups can form aggregates when the copolymer has a segregated or "separate centered" structure. These groups can donate the hydrophobic component into the cell membranes without any selectivity. On the other hand, "same centered" or "facially amphiphilic" structures may prevent such aggregations at single molecule level and that may be the reason for their better selectivities.

Kuroda and coworkers controlled the antimicrobial and hemolytic activities of amphiphilic random copolymers by modulating the spacer length in side chain [37]. They prepared a series of random copolymers with ethyl methacrylate and 2-aminoethylene, 4-aminobutylene, or 6-aminohexylene groups where the copolymers with 4-aminobutylene cationic side chains showed the optimal activity. 2-aminoethylene side chains resulted in lower activity while the more elongated hexylene side chains showed potent antimicrobial activity with stronger hemolytic activities as well.

Yang and Hedrick et al. demonstrated that biodegradable broad-spectrum poly(carbonate)s had a comparable antimicrobial tendency on alkyl chain length [78]. They synthesized the poly(-carbonate) polymers via organocatalytic ring-opening polymerization and functionalized with quaternary ammonium groups of various pendant structures (e.g., alkyl, aromatic, imidazolinium) (Fig. 15G). They observed an optimal alkyl chain length at four carbons (butyl) for the series of polymers quaternized with *N*,*N*-dimethyl alkylamines with various alkyl chain lengths. Interestingly, the poly(carbonate)s with butyl substituents had remarkable selectivity > 256 against *E. coli* and 1026 against *S. aureus*. In addition, cyclic and aromatic substituent containing quaternary ammonium polymers had better selectivities as well. Activity-alkyl chain length dependence was observed for the "same centered" quaternary ammonium and PEG copolyoxetanes prepared by Wynne et al. [127]. However, the alkyl chain here was not the spacer linking the backbone and the quaternary ammonium, but an alkyl tail extending out from the quaternary ammonium group. They synthesized the copolymers via cationic ring-opening polymerization, resulting in 1:1 ratio of repeating units. The quaternary alkyl chain length was varied from 2 to 16 carbons. The most effective antimicrobial polymer had quaternary alkyl chains of 8 carbons, resulting in MICs of 4 μg/mL for *E. coli*, 2 μg/mL for *S. aureus* and 24 μg/mL for *P. aeruginosa*.

Alkyl spacer length dependent activity was also observed for the antimicrobial poly(acrylate) copolymers developed by Yang and coworkers [73]. They used monomers having 2-carbon or 6-carbon spacers linking primary ammonium groups (Table 1C). Interestingly, antimicrobial activity substantially increased with increasing amount of the long-alkyl monomer. However, the hemolytic activity was very low in the copolymer. Homopolymer of short-alkyl monomer was low hemolytic while that of long-alkyl monomer was very high.

3.2.2. Cycles and fused cycles

For a majority of antimicrobial polymers, linear alkyl chains are used as the hydrophobic units. However, there are only few reports focused on cyclic or fused cyclic structures as hydrophobic groups in antimicrobial polymers. Gellman and coworkers investigated the effects of cyclic and acyclic hydrophobic subunits on the biological

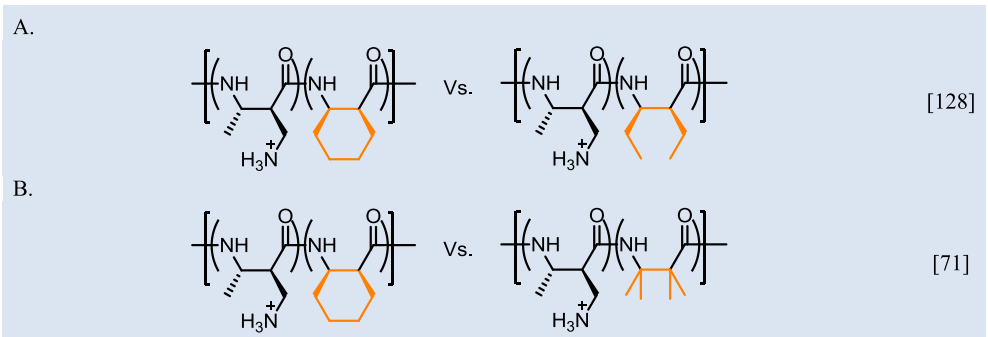


Fig. 12. Cyclic vs acyclic hydrophobic subunits in nylon-3 copolymers. Refs. [71,128].

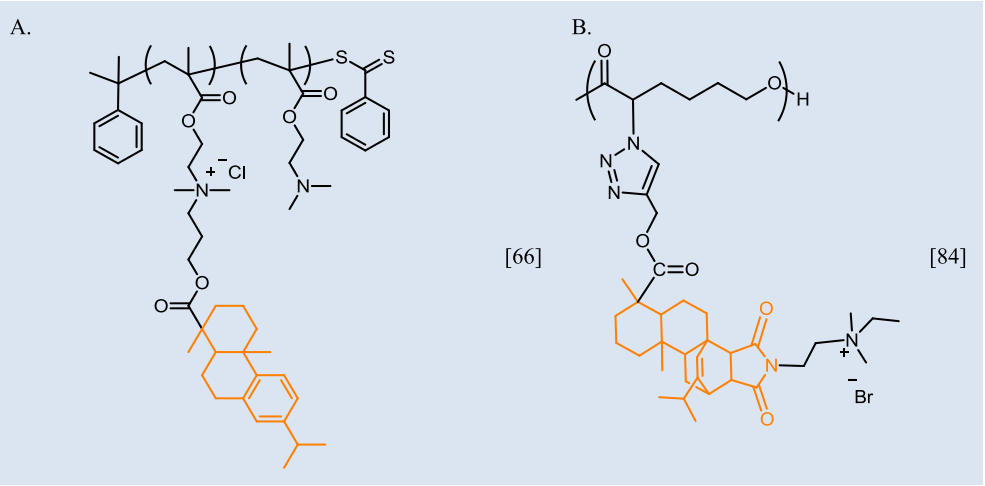


Fig. 13. Antimicrobial polymers with fused-ring structures as hydrophobic components. Refs. [66,84].

properties of nylon-3 random copolymers (Fig. 12A) [128]. The copolymer with cyclohexane hydrophobic groups indicated improved antimicrobial efficacy, with low hemolytic activity relative to analogous acyclic subunits. They assumed that the major contribution for the observed properties comes from changes in local backbone flexibility between cyclic and acyclic hydrophobic nylon-3 polymers. Conversely, a more recent study on binary nylon-3 random copolymers with isomeric or nearly isomeric cyclic and acyclic hydrophobic subunits demonstrated an opposite phenomenon. The copolymer without a cyclic constraint on the backbone (Fig. 12B right) displayed superior properties. This may be due to the shorter alkyl chain lengths in the hydrophobic subunit.

Tang et al. utilized resin acids with bulky hydrophenanthrene rings as hydrophobic substituents (Fig. 13) for their antimicrobial polymers [66,84]. They prepared poly(*N*,*N*-dimethylaminoethyl methacrylate) via RAFT polymerization and partially quaternized the polymer using rosin ester chloride (Fig. 13A). The polymers were highly active against *E. coli* and *S. aureus*. Interestingly, the starting polymer and the polymer quaternized with a linear alkyl chain with the same number of carbon atoms as the rosin group did not show significant antibacterial properties. In another study, they attached the quaternary ammonium group to the periphery of rosin moiety (Fig. 13B). Molecular dynamics simulations revealed a strong preference of hydrophobic fused ring structure to penetrate and dock into the hydrophobic core of the lipid bilayer of a model system [79]. Also these systems demonstrated superior antimicrobial activities even against MRSA and strong biocompatibilities both *in vitro* and *in vivo*. It is believed that the natural hydrophenanthrene ring structure has a better compatibility with the hydrophobic core of the lipid membrane in a similar fashion to steroids present in cell membranes.

3.3. Molecular weight effect

Unlike the small molecules, antimicrobials macromolecules provide the freedom for chemists to optimize the biological activities by varying the molecular weight of macromolecules. The contact-active mechanism of polycations suggests higher molecular weight requirements to have better selectivity since it relies on increased initial electrostatic attractions, while better antimicrobial activity comes from increased hydrophobic active sites that penetrate into the lipid bilayer. In addition, favorable antimicrobial effect comes from the fact that the complexation of a polycation with a polyanion is essentially irreversible, while complexes of a polyion

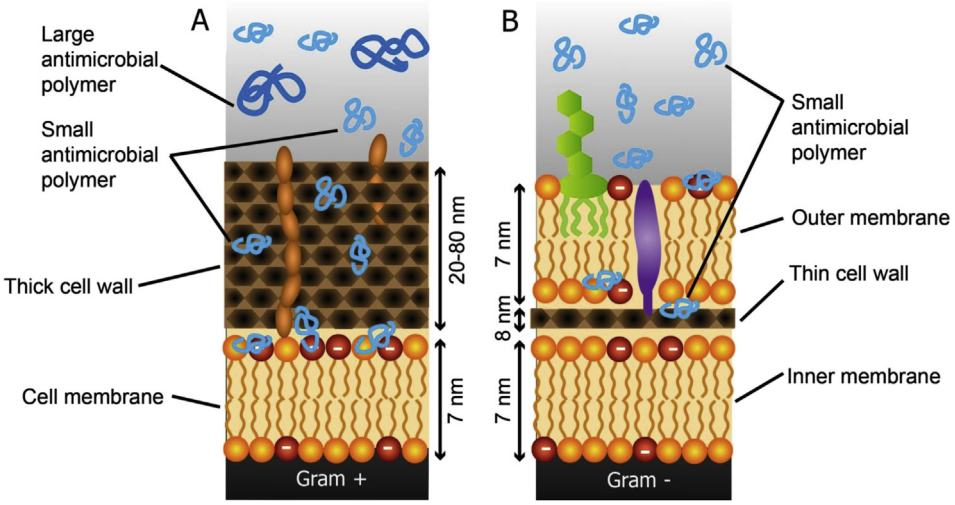


Fig. 14. Illustration of the cell membrane and cell-wall morphology of bacteria and their interactions with antimicrobial polymers. (A) Gram-positive bacteria. High molecular weight polymers encounter a "sieving effect" from the cell wall. (B) Outer membrane of Gram-negative bacteria limit antimicrobial polymers reaching the inner membrane. Reproduced with permission from Ref. [131].

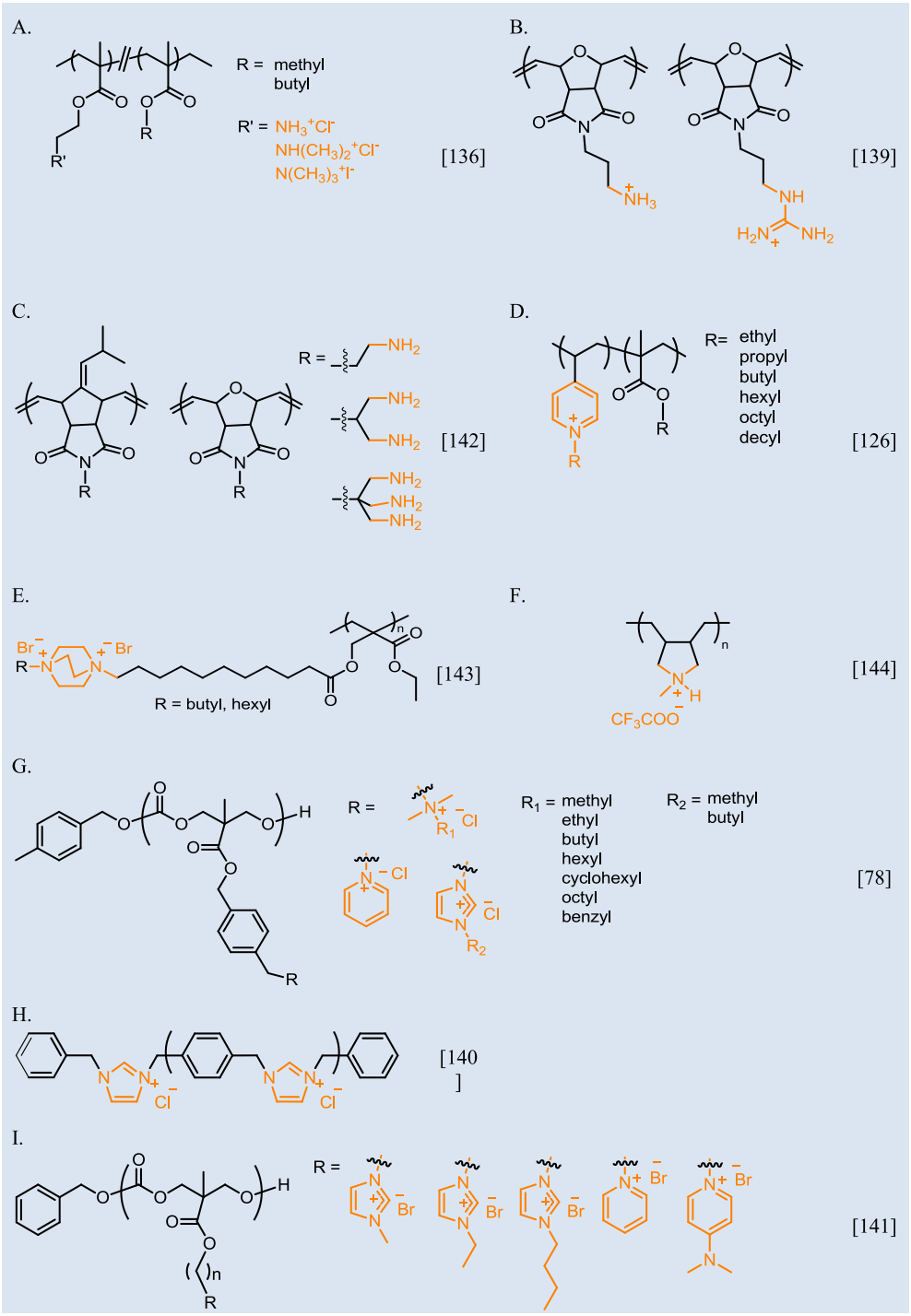


Fig. 15. Representative chemical structures of various ammonium groups integrated in antimicrobial polymers. Refs. [78,126,136,139,140–144].

with species with low amount of charges are mostly reversible [66]. However, with increase of molecular weight, there comes into play other limiting parameters such as solubility, diffusion and aggregation in the biological media, barriers such as bacterial cell wall, etc.

Synthetic antimicrobial materials that mimic the small AMPs generally show better antimicrobial properties with minimum hemolytic activities. Typically AMPs consist of small number of amino acid residues (12–100 aa) with molecular weights in the range of 2–8 kg/mol. A similar trend is observed for antimicrobial polymers where pronounced antimicrobial efficacy is generally with molecular weights ranging from 2 to 12 kg/mol. The molecular weight effect in methacrylate based system was studied by Kuroda et al. [124]. It showed the increase of antimicrobial and hemolytic properties with increase of molecular weight up to 10,100 g/mol. However, Gellman et al. did not observe similar effect in the nylon-3 polymers with regard to chain length [129]. Significant hemolysis was observed above ~30 repeating units where minimum hemolytic concentration went below MIC.

Tang et al. studied the molecular weight dependence for poly(methacrylate) system with bulky rosin group as the hydrophobic moiety [66]. The degree of quaternization was kept similar while

the molecular weights were changed. Low molecular weight polymers showed higher antibacterial activities than their high molecular weight counterparts. Also this dependence of molecular weight was somewhat characteristic to the cell envelope structure of bacteria. For example, MIC became abruptly high for Gram-positive bacteria with increasing molecular weight. Tew et al. made facially amphiphilic oxanorbornene monomer derived polymeric systems by ring-opening metathesis polymerization. They observed a "sieving effect" for high molecular weight polymers against Gram-positive bacteria (Fig. 14.) [130,131]. In addition, double membrane structure of Gram-negative bacteria prevented the polymers reaching the inner membrane at proper concentrations. Therefore the Gram-positive bacterial were more susceptible to the agents. Poly(oxonorbornene)-based antimicrobial polymers were found to be promisingly human cell compatible [132]. This "doubly selective" system, with the ability to kill bacteria over mammalian and one bacterial type over the other, may be a fascinating example for targeted therapy.

Yang and Hedrick et al. studied the molecular weight effect on antimicrobial poly(carbonate)s [133]. They also observed similar trends with Gram-positive activity for low molecular weight polycarbonates and the reverse trend for Gram-negative bacteria such as *E. coli* and *P. aeruginosa*. Tang and coworkers prepared antimicrobial poly(caprolactone) homopolymers containing cationic rosin groups, which showed strong bactericidal activities even at high molecular weights, e.g. 74,000 g/mol [84]. This intrinsic property may be attributed to the strong bactericidal property of cationic rosin units as well as their locality as a 'same-centered' polymer architecture.

3.4. Ionic/polar group effect

All membrane-active macromolecules require some sort of amphiphilic functionality. In natural AMPs it is fulfilled by amino acid residues such as lysine, arginine or histidine that are protonated at physiological pH, generating a net positive charge on the peptide, typically in the range of +2 and +9 [134]. Since the driving force for the selective interaction between antimicrobial macromolecules and the microbe cell surface is largely based on electrostatic interactions, it is obvious that the type of charge, the charge density and the charge location would have a strong impact on the activity profile of the macromolecules. Therefore, careful manipulation is necessary because the addition of charged groups along the polymers usually affect the overall hydrophobicity, due to the interplay of charge and hydrophobicity. Most antimicrobial polymeric systems, contain permanent cationic moieties largely composed of ammonium groups. There are relatively fewer studies that employ alternative cations such as phosphonium, sulfonium systems or metal ions.

3.4.1. Ammonium

The ammonium group is the most widely studied source of cationic charge due to its resemblance to AMPs, in addition to the ease of synthesis. In AMPs, the cationic charge comes mostly from primary amines (lysine) or guanidium moiety (arginine) due to their greater abundance in such peptides. In contrast, most polymeric antimicrobials employ quaternary ammonium groups as cations, primarily because the antimicrobial activity is independent from the pH of the media. Synthetic polymers with quaternary nitrogen atoms are widely explored and well-reviewed [135]. Kuroda and coworkers studied the impact of the type of amine groups such as primary, tertiary, or quaternary ammonium on antimicrobial and hemolytic activity (Fig. 15A) [136,137]. Interestingly, the poly(methacrylate) copolymers bearing primary and tertiary amine groups were able to completely inhibit *E. coli* growth with almost no hemolytic activity. In contrast, the analogous system with quaternary ammonium groups did not show antimicrobial properties until higher concentrations. The same quaternary ammonium polymer showed significant hemolytic activity and some antimicrobial activity upon increasing the hydrophobic content by changing alkyl chains from methyl to butyl moieties. Water–octanol partition coefficients demonstrated that the overall amphiphilicity does not depend only on the number of cationic groups but also their chemical structure. This indicates the fact that the dissociation constants of the charged group have a strong influence on the polymer amphiphilicity. Specifically, amphiphilic polymers containing permanent quaternary ammonium groups are more hydrophilic than the analogous polymers with exchangeable cationic groups formed by the protonation of amine groups. In other terms, it can be envisioned that the permutable nature of the protonation of amine groups leads to a greater selectivity because of the variable nature of amphiphilicity. In addition, it is assumed that protonated ammonium groups form stronger complexes with phosphate lipid heads via a combination of hydrogen bonding and electrostatic force, opposed to quaternary ammonium groups.

Cation structure dependent antimicrobial activity was also observed by Morgan et al., for their primary and tertiary amine-containing poly(methacrylamide) homopolymers and statistical copolymers prepared via aqueous RAFT polymerization [138]. They found that copolymers consisting largely of primary amines were most effective against both strains of *E. coli* and *B. subtilis*. Tertiary amines with methyl or ethyl groups had limited activities, indicating steric hindrance that dwindles polymer bacterial interactions.

In another study, Tew et al. investigated the consequences of guanidium functionality instead of ammonium groups in a poly(-norobornene) system (Fig. 15B) [139]. Unlike the poly(-norobornene) antimicrobials with primary amine groups, the guanidium functionalized polymer was strongly antibacterial against Gram-negative and Gram-positive bacteria as well as non-hemolytic against human red blood cells. The mechanistic investigations revealed that the guanidine functionalized polymer caused little or no membrane disruption. However, the antimicrobial activity likely occurred via a different mechanism with intracellular targets such as anionic macromolecules including essential membrane proteins or RNA/DNA.

Mathias et al. prepared water soluble antimicrobial polymers containing pendant quaternary ammonium moieties based on 1,4-diazabicyclo-[2.2.2]-octane (Fig. 15E) [143]. They were moderately effective against bacteria. Zhang, Fan and co-workers synthesized a novel main-chain imidazolium oligomer material as a promising broad-spectrum antimicrobial agent (Fig. 15H) [140]. The oligomer was strongly active against most pathogenic bacteria including MRSA and VRE with MICs in the range of 1.5–31.25 μg/mL. Interestingly, the cationic oligomers were mostly non-hemolytic. The selectivity indices were over 3000. *In vivo* infection activity was also tested using mice, which revealed a high therapeutic index (LD_{50}/ED_{50}) of 37 against *S. aureus* infection.

Hedrick, Yang and coworkers investigated the impact of nitrogen-containing heterocycles as quaternizing agents on the antimicrobial and hemolytic activity of antimicrobial polycarbonates [141]. They synthesized biodegradable cationic polycarbonates containing propyl or hexyl side chains quaternized with various nitrogen-containing heterocycles, including imidazoles and pyridines (Fig. 15I). The polymers were broad spectrum activity while stronger activities against Gram-positive bacteria were observed. Hexyl side chains improved the properties. Interestingly, *N*-heterocycle quaternized polymers had enhanced antimicrobial activity compared to their analogs quaternized with trimethylamine. In addition, among imidazole-containing polymers, 1-

butylimidazole had better antibacterial activities. A greater inhibitory efficiency was observed when pyridine was replaced by 4-(dimethylamino)pyridine.

3.4.2. Phosphonium

Phosphonium containing polymers are widely explored in fields such as ionic liquids and nucleic acid delivery. However, their antimicrobial potency remains relatively untapped. Endo and coworkers studied various phosphonium salts containing biocides, which were active against a range of bacteria [145]. It was found that phosphonium-containing biocides had better bactericidal activity and killing rates compared to analogous ammonium containing systems. Recent reports suggest that phosphonium groups possess the additional advantage of lower toxicity towards mammalian cells as well as thermal stability [146,147].

Quaternary phosphonium grafted on an insoluble 'gel-type' styrene divinylbenzene copolymer was prepared by Dehelean et al. [148]. They reported that ethyl phosphonium grafted polymers had better activity than phenyl phosphonium polymers. Quaternary ammonium and phosphonium salts based on random copolymers of glycidyl methacrylate and 2-hydroxyethyl methacrylate were synthesized via free radical polymerization and post-polymerization modification by Kenawy and coworkers [35]. Compared to triphenylphosphonium moiety, polycation with tributylphosphonium had the highest antimicrobial activity against a range of bacteria including *E. coli*, *P. aeruginosa* and *B. subtilis*, and the fungus *T. rubrum*. These polymers had better efficacy towards the fungus while the lowest activity was against Gram-positive bacteria. Nevertheless, an opposite observation was resulted from ammonium- or phosphonium-containing crosslinked copolymers investigated by Kenawy and co-workers [149]. Triphenylphosphonium salt of the modified copolymer was most effective against *C. albicans* and *S. aureus*. Fig. 16 illustrates representative chemical structures of phosphonium-containing polymers.

Ao et al. modified epoxied natural rubber with quaternary phosphonium groups and observed antibacterial activity of the polycationic materials [153]. Liang and coworkers prepared a class of new antimicrobial cationic polymers consisting of poly(-phenylene oxide) (PPO) backbone, from the quaternization reactions of the methyl-brominated PPO with tertiary amines or phosphines [152]. Interestingly, they also observed triphenylphosphonium-containing polymers to be active against both *E. coli* and *S. epidermidis*, while the quaternary ammonium polymers and tributylphosphonium polymers analogs were active against *S. epidermidis* only. The polymers were also found to be compatible with Hs68 fibroblasts. This difference may have attributed from the balanced hydrophobicity coming from the phenyl moieties that facilitate the interactions with more complicated Gram-negative cell surfaces.

Amine-, ammonium- and phosphonium-containing cationic poly(acrylamide) tripolymers (PPAD) were investigated by Zhao and coworkers [150]. Free radical solution polymerization was used to prepare polymers that demonstrated bactericidal and excellent virucidal activities. The polymers showed an inhibitory action against the adenovirus (ADV) infection on human embryonic kidney cell (Fig. 17). With the increase of phosphonium content, the MIC values decreased significantly, indicating the enhanced antibacterial activities.

3.4.3. Sulfonium

Sulfonium compounds are analogous to quaternary ammonium materials in terms of charge. However, their antimicrobial and hemolytic activities are not in line. In contrast to other cationic groups, very few studies have been performed on polymeric sulfonium compounds as antimicrobials and their biocompatibility. Endo and coworkers prepared poly(p-vinylbenzyl tetramethylenesulfonium tetrafluoroborate)s (Fig. 16C) with various molecular weights, to explore the effect of hetero atoms on the antibacterial activity of polymeric onium salts. The polymers were prepared by radical polymerization of vinylbenzyl tetramethylenesulfonium tetrafluoroborate. They tested the activity against *S. aureus* and *E. coli* and found higher antibacterial activity against Gram-positive bacteria [151]. Hirayama reported the antimicrobial and biocompatibility properties of sulfonium salts, illustrating that the sulfonium salts were much better in antibacterial activities with minimum toxicities in contrast to quaternary ammonium salts [154]. However, one limitation of sulfonium polycations is their low thermal stability.

3.4.4. Cationic metals

Organometallic polymers have been used in many areas in medicinal chemistry as anticancer drugs, enzyme inhibitors, targeting agents, contrasting agents, etc. [155]. Metal ions or nanoparticles could be located throughout polymer backbone or as

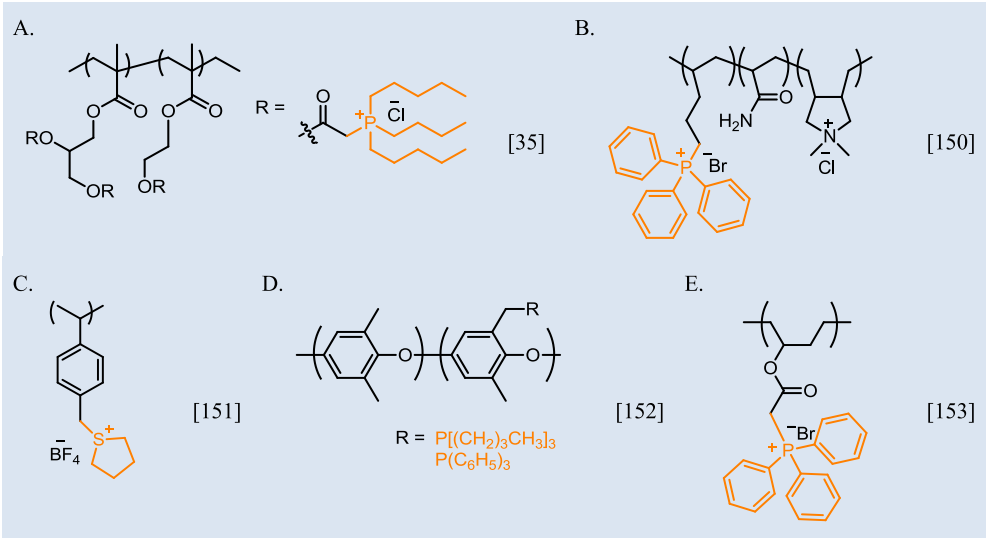


Fig. 16. Representative phosphonium- and sulfonium-containing antimicrobial polymer structures. Refs. [35,150–153].

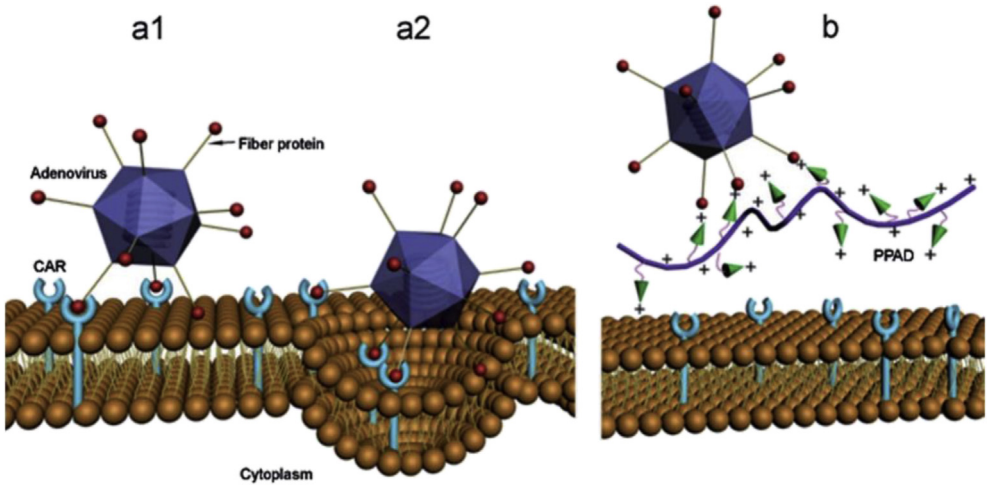


Fig. 17. A model for the role of PPAD in inhibiting ADV binding and entry into the host cell. Reprinted with permission from Ref. [150].

pendant groups. There is plenty of research on silver-containing antimicrobial polymers largely due to strong and broad spectrum antimicrobial activity of silver ions or silver nanoparticles [156]. Most silver-containing antimicrobial polymers consist of either elemental silver or as Ag^{+} releasing polymeric composite systems. However, organometallic antimicrobial polymers with cationic metal ion complexes and their application as biocompatible and membrane-active antimicrobial materials have not been explored well. Metal such as Mn, Co, Ni, Cu and Zn have been incorporated on to polymers. One limitation in such polymers would be the toxicity of most metal ions towards mammalian cells and low light-thermal stability. There are several reviews that describe about antimicrobial organometallic polymers [11,17].

Tang and coworkers recently reported a class of novel charged metallopolymers based on cationic cobaltocenium-containing polymers that were bactericidal and highly biocompatible [157]. Cobaltocenium-containing methacrylate polymers with hexafluorophosphate anion and their halide paired cobaltocenium-containing polymers were synthesized via RAFT polymerization and further ion exchange. In addition to inherent antimicrobial activity, these polymers formed conjugates with conventional antibiotics such as Penicillin G, Amoxicillin, Ampicillin and Cefazolin via ion-pair interactions (Fig. 18). It was found that metallopolymers attack both cell envelopes and β–lactamase enzymes. This novel activity inhibited β-lactamase degradation of the antibiotics and protected conjugated antibiotics via ion-pairing between polymers and antibiotics.

3.4.5. Uncharged polar or neutral groups

Another emerging trend to circumvent hemolytic activity of antimicrobial polymers is to introduce uncharged hydrophilic groups such as highly biocompatible poly(ethylene gylcol) (PEG). PEGylation is an attractive method to increase the hydrophilicity of polymers. Youngblood and coworkers identified the increased efficacy of quaternized poly(vinylpyridine) through copolymerization with hydroxyethyl methacrylate and poly(ethylene gycol) methyl ether methacrylate (Fig. 19A) [158]. It was attributed to the synergistic activity of poly(ethylene gycol) with the bactericidal quaternized poly(vinylpyridine). Tew et al. modified amphiphilic poly(norbornene) polymers through the integration of hydrophilic and biocompatible moieties such as sugar and poly(ethylene glycol) (Fig. 19B) [159]. It was found that increasing hydrophilicity reduced antibacterial activity. However, at the same time the hemolytic activity was much significantly lowered. The hemocompatibility of the Wynne and co-workers' copolyoxetanes (Fig. 19C) may be owing to the presence of PEG side chains [127]. The incorporation of certain amounts of carbohydrate pendants groups substantially increased the hemolytic concentration of the amphiphilic block copolymers (Fig. 19D) developed by Fernandez-García et al. [86]. However, the antimicrobial activity was sustained.

In a recent study, Gellman et al. highlighted a similar trend in achieving biocompatibility [160]. They have optimized the selectivity of previously evaluated binary hydrophobic–cationic nylon-3 copolymers that showed strong antibacterial and hemolytic profiles. The homoglycine subunits and honoserine subunits were newly introduced to the polymers as neutral or hydrophilic repeating units (Fig. 19E). The partial replacement of hydrophobic subunits, cationic subunits, or both into a neutral or hydrophilic functionality led to a decline in hemolytic activity while antibacterial activity was maintained. Yang et al. demonstrated a method to improve biocompatibility via simply substituting the hydrophobic moiety of an antimicrobial polymer into a hydrophilic moiety [75]. Long hydrophilic and cationic poly(methacrylate) random copolymers (Fig. 19F) were significantly less hemolytic compared to the analogous hydrophobic-and-cationic poly(methacrylate)s.

In summary, it can be established that incorporation of hydrophilic biocompatible groups into cationic polymers would improve the biocompatibility of the macromolecule. However, a correct balance should be maintained to retain the antimicrobial activity.

3.4.6. Charge density and position

Although most polymeric antimicrobials contain a single cation per monomer, there is a possibility to incorporate extra cationic groups to fine-tune the amphiphilicity. Tew and coworkers prepared cationic antimicrobial poly(norbornene)s that carry one, two, and three charges per monomer repeat unit to investigate the effect of charge density on biological activities (Fig. 15C) [142]. For more hydrophilic polymers, increased amount of amine groups per repeat unit resulted in very little influence on antimicrobial activity against both *S. aureus* and *E. coli*, while the hemolytic activity significantly reduced. Although hydrophilic polymers remained non-hemolytic, enhanced activity against Gram-positive bacteria was observed with increased amount of amine groups.

The location of the cationic group on polymers has a pronounced effect on both antimicrobial and hemolytic activities. Tang et al. studied the effects of charge location over biological activity when the quaternary ammonium group was at the periphery, compared to when the quaternary ammonium was sandwiched

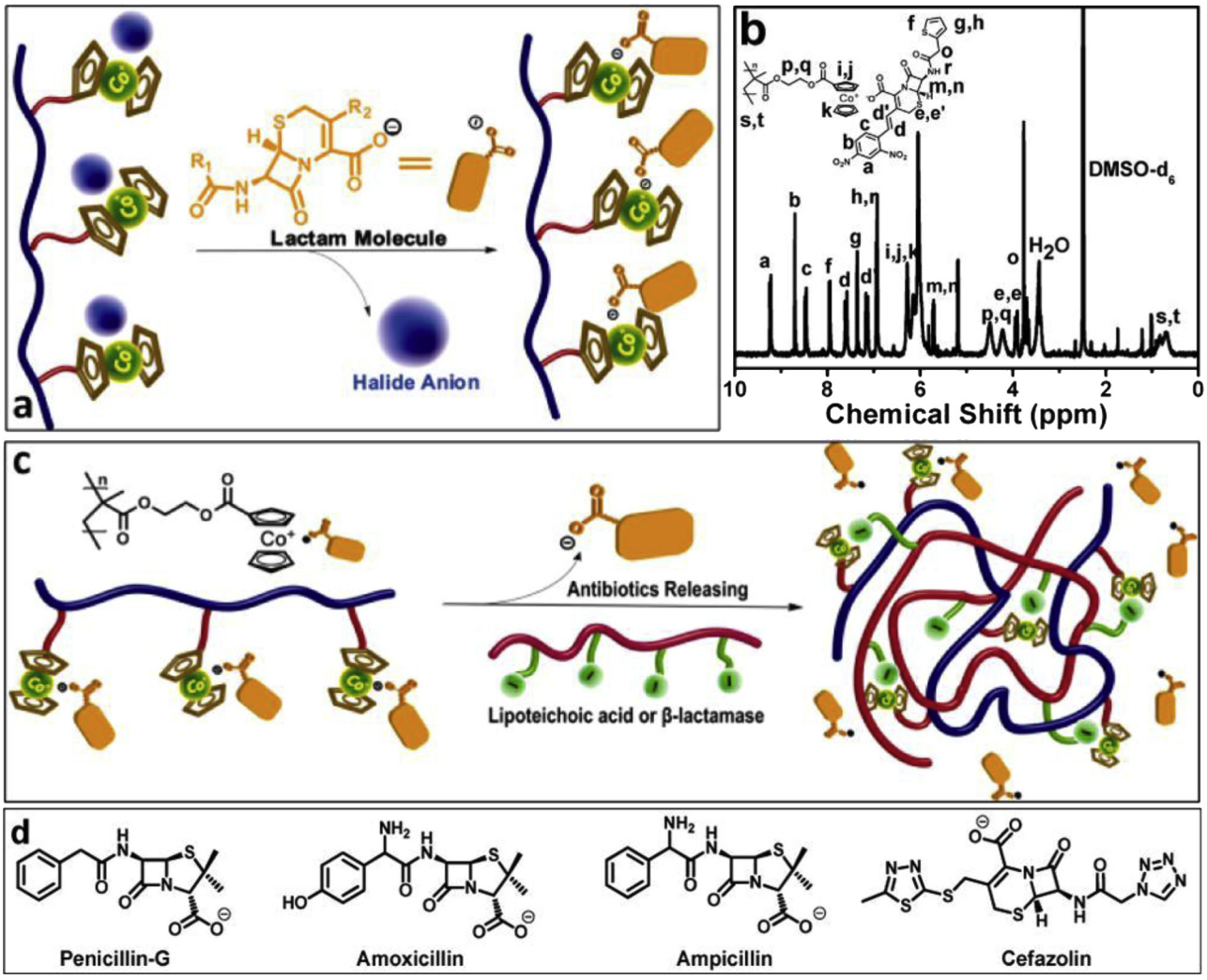


Fig. 18. Antimicrobial metallopolymers, the formation of ion-pairs between β-lactam antibiotics and cobaltocenium-containing polymers. Reprinted with permission from Ref. [157]. Copyright (2014) American Chemical Society.

between a bulky hydrophobic group and the polymer backbone [66,84]. When the cationic groups were located on the termini of the pendant hydrophobic moiety, they were able to act like small needles with pronounced antimicrobial activities. However, the polymer with positive charges sandwiched between two hydrophobic moieties, showed little or no activity. This was attributed to the increased steric hindrance lowering electrostatic interactions between the polymer and the cell membranes. In addition, they observed a dependence of antibacterial activity on the degree of quaternization (DQ). The MICs first decreased as a result of increased cationic functionality. However, the MICs increased with further increase of DQ. It is believed that the increase of DQ was bounded with incorporation of additional rosin moieties that could increase the overall hydrophobicity of polymers. This could result in partial aggregation of polymers and reduce solubility in biological media. Youngblood and co-workers demonstrated the steric effect at the quaternized group on the antimicrobial properties. They compared copolymers from 2-vinylpyridine (2VP) and poly(ethylene glycol) methyl ether methacrylate to the analogs of 4-vinylpyridine (4VP) copolymers [161]. There is more steric crowding at the cation of 2VP due to the close proximity to the polymer backbone. In general, 4VP copolymers were more active against microbes and had better selectivities.

Sampson and coworkers inspected the effect of the spatial separation between cationic groups towards antimicrobial activity [162]. Ring-opening metathesis polymerization was utilized to prepare a series of alternating copolymers, random copolymers and homopolymers containing trialkylammonium, ammonium, and guanidinium substitutents. They identified the requirement for an ordered microstructure toward optimal antimicrobial activity. The most successful polymer system had regularly spaced, featuring a 6–8 carbon stretch along the backbone spatially separating the side chains that presented positively charged groups. It was concluded that random copolymers with 8–10 Å backbone spacing between the functional groups may yield better properties.

The amount of quaternary ammonium groups along polymer chains also has a major effect on the biological activity of polymers. Wynne and coworkers explored this phenomena on their copolyoxetanes with quaternary ammonium and PEG-like side chains [163]. Along with most polycations, the copolyoxetanes had a strong polyvalent effect compared with the monovalent analogs. Water-soluble copolyoxetanes with C_{12} alkylammonium side chains had a systematic trend in composition versus antimicrobial activity against *E. coli*, *S. aureus* and *P. aeruginosa*, improving with the increase of mole percent of cationic repeating units up to 60%.

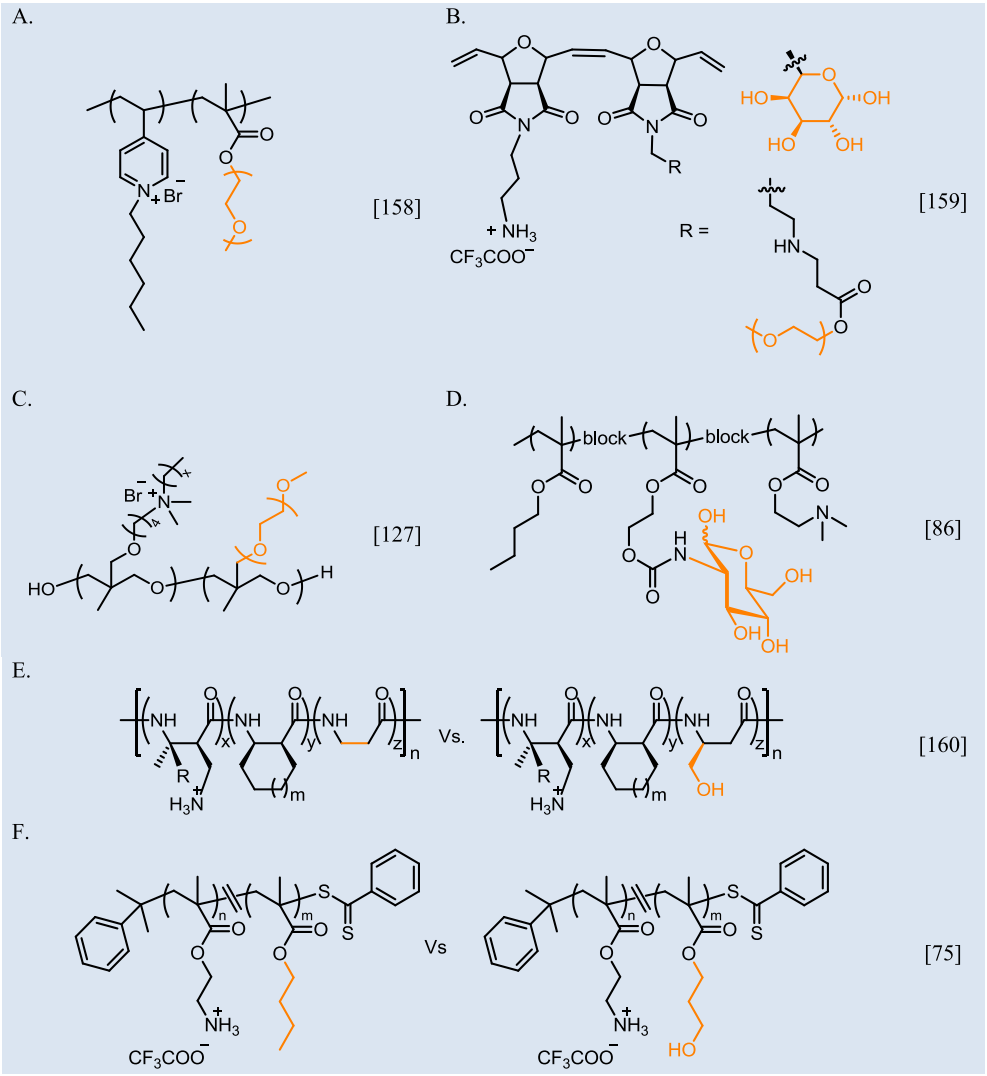


Fig. 19. Introduction of PEG, neutral or uncharged polar groups to improve hemocompatibility. Refs. [75,86,127,158–160].

However, the MICs gradually decreased over 60% of cationic groups, which may be a result of increasing alkyl chains to shield the charges or to decrease hydrophilic character.

3.4.7. *Counter anion influence*

Counter anions can influence cationic polymers in terms of solubility and ion-pair formation. If the counter anions introduce strong hydrophobicity, the solubility will be reduced in polar media. It should be noted that, there is an entropic gain when the counter anions get released at the time of polymer -membrane ion-pair formation. However, if the counter anions form tight ion pairs with polymer cations, it would result in unfavorable enthalphic penalties, in addition to masking of the cations that reduce initial polymer-membrane attractive forces. Counter anion effects on antimicrobial activity have been studied sparingly. Endo et al. examined the counter anion effects on poly[tributyl(4-vinylbenzyl)phosphonium] antibacterial activity against *S. aureus* [164]. Poor activity was observed when the counter anion tends to form a tight ion-pair with phosphonium ion, while it was high for anions facilitating ionic dissociation. This behavior was in concurrent with the solubility of polymers. The antibacterial activity was in the order of chloride > tetrafluoride > perchlorate > hexafluorophosphate, and was inversely related to the polymer-anion ion-pair tightness. Tew and co-workers investigated the impact of organic counter anions on the activity of poly(oxanorbornene)-based system [103]. The hydrophobic counter anions turned out to inactivate the antimicrobial polymers against microbes. It was found that the counter anions form tight ion pairs with the cationic amines, leading to a strong masking of overall cationic nature of polymers. In summary, one could claim that weaker ion-pairing and hydrophilic counter anions may improve the antimicrobial activity of cationic macromolecules.

4. Antimicrobial polymer assemblies

In contrast to monomeric units, antimicrobial polymers typically show enhanced activity due to increased local concentration of active groups. Self-assembled structures of polycations bring even more enhanced activity due to the fact that they can immensely localize mass and cationic charge of the amphiphilic macromolecules [165]. In addition, nanostructures have high surface area leading to higher activities. Therefore nanostructures of cationic antimicrobial macromolecules such as micellar nanoparticles, liposomes, bilayer fragments are quite attractive [166]. There is much attention about cationic polymers that can form secondary structures via non-covalent interactions such as hydrophobic and hydrogen bonding.

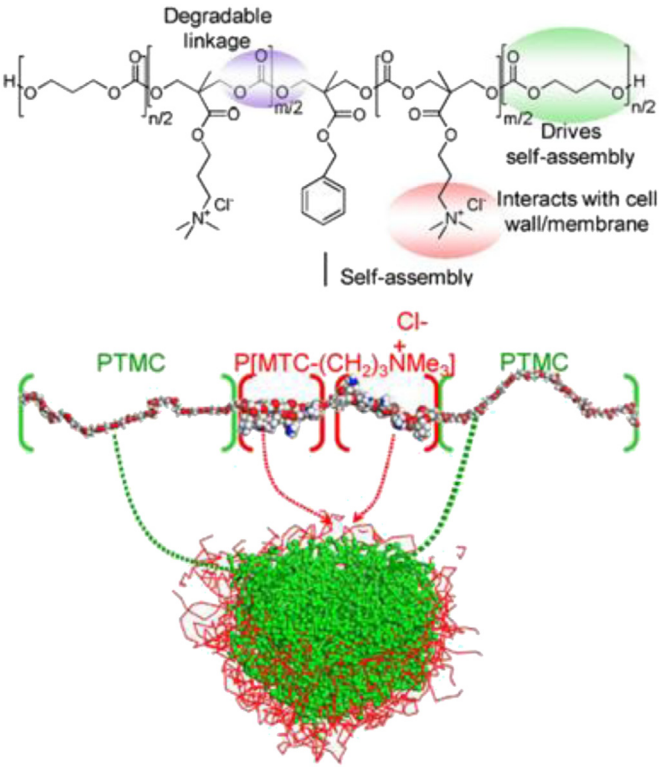


Fig. 20. Cationic amphiphilic polycarbonates that form micelles. Reprinted with permission from Ref. [88].

Core/shell polymer nanoparticles are among the most explored nanostructures in antimicrobial applications because they provide the highest surface to volume ratio, better stability in a range of environmental conditions, ease of synthesis and the ability to control size by the utilization of inorganic nanoparticles as a core substrate on which the polymer shell is grown. In depth discussion about such nanoparticles is beyond the scope of this feature article and hence the readers are routed for a recent review [167].

Amphiphilic block copolymer micelles are another class of widely studied antimicrobial nanostructures. Most amphiphilic block copolymers can self-assemble into core/shell nanoparticles for loading drugs or as drug carriers. Typically, amphiphilic block copolymer micelles could be potent against a broad spectrum of fungi and bacteria. In their outstanding quest on poly(carbonates), Hedrick, Yang and coworkers reported a class of novel biodegradable and *in vivo* antimicrobial cationic micellar nanoparticles (Fig. 20), which were appreciably active against a range of Gram-positive bacteria and a fungus with MIC values as low as ~39 μg/mL [88]. The cationic polycarbonate triblock copolymers (M_n = 6,200–9,200 g/mol) were prepared by metal free organocatalytic ring-opening polymerization of functional cyclic carbonates. Although these polymers that formed larger aggregates with an average diameter of ~400 nm did not show efficacy, it was believed that micelles with considerably smaller size (43–198 nm) damaged the cell wall. Interestingly, these polymers did not show significant activity below the critical micellar concentration, illustrating the importance of self-assembled nanostructures that collectively enhance the potency rather than discrete antimicrobial polymer chains.

In another study, Hedrick, Yang and coworkers developed highly dynamic, biodegradable micelles from poly(carbonate)s that demonstrated stronger bactericidal properties [133]. The micelles from random poly(carbonate)s showed stronger activity against Gram-negative bacteria, compared to block copolymers. The instability of micelles from random copolymers allowed them to better integrate with bacterial membranes such that the hydrophobic core can move into the hydrophobic lipid bilayer core at faster rates. This study confirmed the concept that in order to be highly potent, antimicrobial nanostructures should be stable enough to keep their integrity in solution, but disassemble rapidly upon contact with negatively charged bacterial cell membrane.

Another type of biodegradable cationic antimicrobial nanoparticles was synthesized by Hedrick and co-workers [168]. An assembly directing terephthalamide-bisurea core was used to initiate triblock copolymers of lactide and cyclic carbonates. These copolymers self-assembled into either spherical or rod-like three-dimensional cationic structures. Although the molecular weight was in the range of 11,800–13,400 g/mol, the nanostructures were small in diameter (rod shape ~10 nm and spherical shape ~20 nm). It may be the major reason for pronounced activity of these nanostructures, compared to other systems. Interestingly, both shapes were found to have similar size and charge density, while only the rod-like assemblies were effective against *C. albicans*.

The biopolymer chitosan is a widely used antimicrobial agent due to its biodegradability and nontoxicity. The antimicrobial properties are related to its polycationic structure. There are several interesting review articles on antimicrobial chitosan derivatives [169,170]. Chitosan nanoparticles can be prepared by ionic gelation

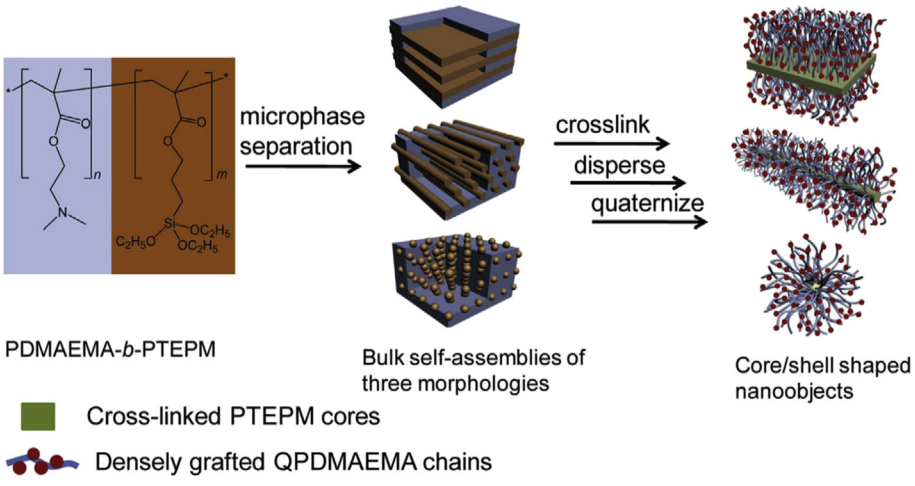


Fig. 21. Schematic representation of a synthetic procedure for shaped antibacterial nanoobjects. Reprinted with permission from Ref. [87].

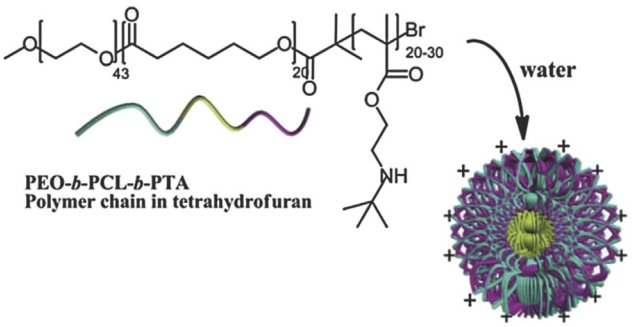


Fig. 22. Self-assembly of PEO-b-PCL-b-PTA triblock copolymer into antibacterial micelles. Reprinted with permission from Ref. [86].

of chitosan with different anions. There are many reports on antimicrobial chitosan nanoparticles [171] and nanofibers [172]. Nanoparticles made from low molecular weight chitosan showed better efficacy against microbes [173]. In addition, quaternary ammonium modified chitosan increased the solubility and antibacterial activity of nanoparticles.

Chen et al. fabricated antibacterial core/shell polymer nanoobjects with sheet-like, cylindrical, and spherical shapes (Fig. 21) [87]. The nanoobjects had crosslinked poly(siloxane) cores and densely grafted poly(ammonium) shells. They were prepared by dispersing cross-linked microphase separated materials of diblock copolymers, poly(2-(dimethylamino)ethyl methacrylate)-block-poly(3-(triethoxysilyl)propyl methacrylate) (PDMAEMA-b-PTEPM) and further quaternization with *n*-octyl bromide. Antibacterial activities of these nanoobjects were assessed against *E. coli*. All nanoobjects had similar bactericidal activity and were 10 fold better than corresponding quaternized homopolymers.

Water-dispersible, biodegradable, unique core/corona nanostructured polymer micelles with significant antibacterial activities were developed by Du and coworkers, utilizing amphiphilic ABC triblock copolymers comprising of poly(ethylene oxide)-*b*-poly-(ε-caprolactone)-block-poly[(2-*tert*-butylaminoethyl) methacrylate] (Fig. 22) [86]. The micelles had a hydrodynamic diameter of 23 nm or 34 nm. Below CMC, the antibacterial activity against *E. coli* or *S. aureus* was little or zero, but more effective above CMC, proving the activity enhancement was achieved via micelle formation.

Du et al. also studied antimicrobial activity of solution self-assembled polymer vesicles prepared by thermo- and pH-responsive diblock copolymers [174]. Interestingly, this diblock copolymer system, made of poly[2-(2-methoxyethoxy)ethyl methacrylate]-*b*-poly[2-(*tert*-butylaminoethyl) methacrylate], had secondary amines that can get protonated in the medium (Fig. 23). The vesicular structures had a mean diameter of 240 ± 50 nm and a membrane thickness of 21 nm. The hydrodynamic diameter was dependent on the pH of the media. These polymer vesicles were active against *E. coli* and *S. aureus* under physiological conditions in contrast to un-self-assembled individual polymer chains. In addition, these hollow structures are potentially promising as delivery vehicles for nano medicines.

The same diblock copolymer was self-assembled into an "armed" high-genus vesicle by a solvent switch method [175]. These high-genus vesicles were even better at antimicrobial activities and excellent in hemocompatibility. In addition, they used the vesicles to successfully deliver anti-cancer drug Doxorubicin, proving the system to be useful in antibacterial and anticancer therapeutic processes simultaneously. Xie and coworkers fabricated a covalent organic polymer with hollow structure via Sonogashira coupling from precursors containing positive charge [176]. Fluorene decorated with positive charge and 1,3,5-triethynylbenze

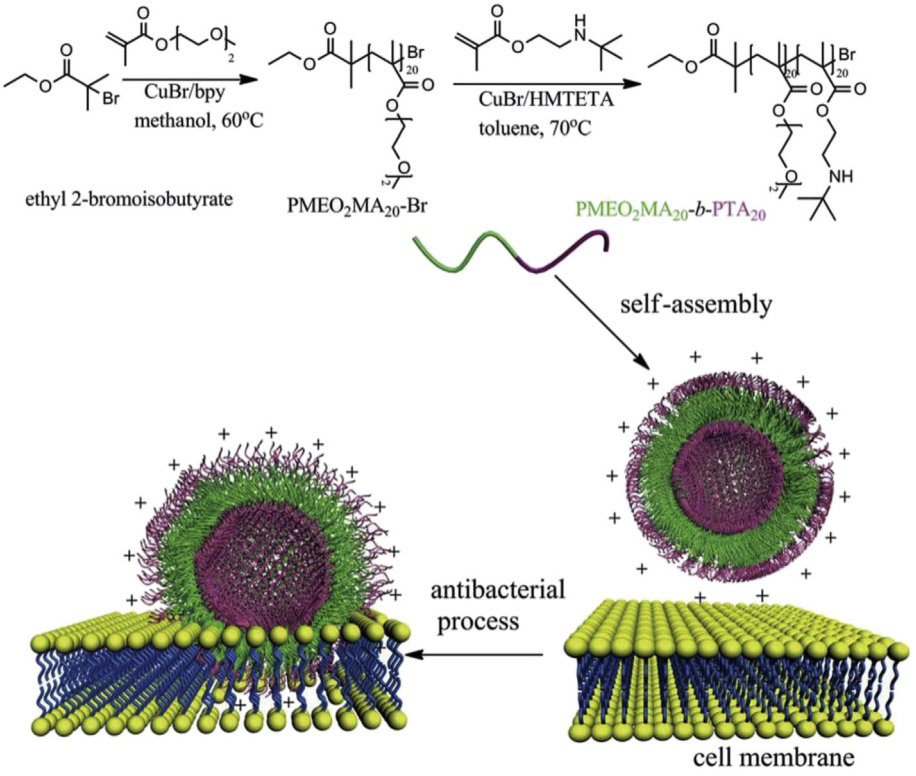


Fig. 23. Synthesis, self-assembly and antimicrobial mechanism of a diblock copolymer vesicle. Reprinted with permission from Ref. [174].

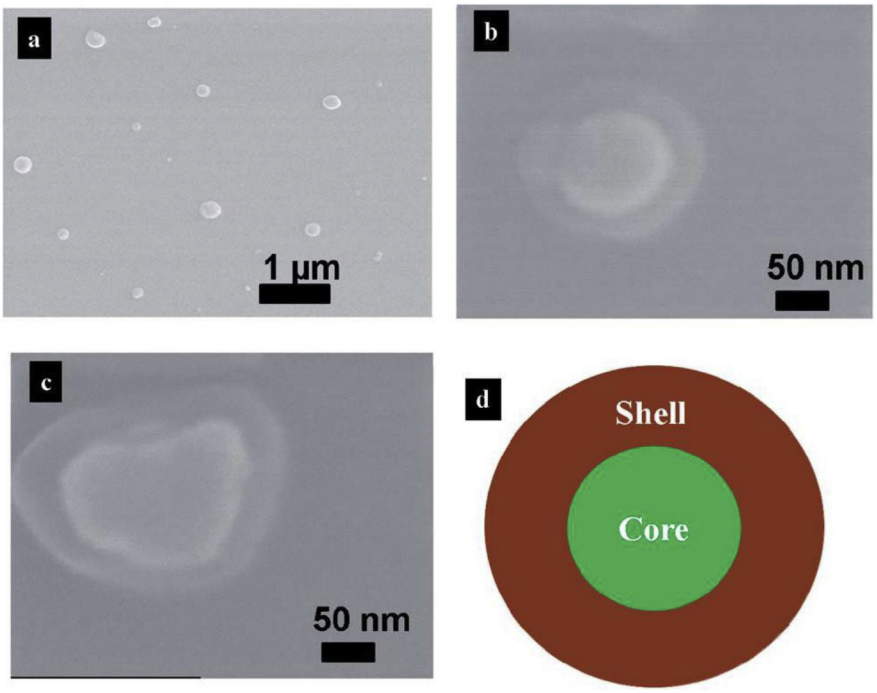


Fig. 24. SEM micrographs of biodegradable nanoparticles from PEG-poly(amino acid)s, and an schematic illustration. Reprinted with permission from Ref. [177].

was used as core structural units. The hollow structures were active against *E. coli* with an MIC of 0.25 mg/mL.

Cai and co-workers developed biodegradable nanoparticles from PEG-poly(amino acid)s [177]. They synthesized a series of PEGylated poly(amino acid)s via ring-opening polymerization of *N*-carboxyanhydrides with cationic and hydrophobic properties. The polymers formed nanoparticles with 50–200 nm under aqueous conditions (Fig. 24). The antibacterial activities of the nanoparticles were tested against a range of bacteria. It was found that they had broad-spectrum activity. PEGylation promoted the nanoparticle formation as well as the reduction of hemolytic activity.

Riberio et al. reported hybrid nanoparticles from cationic lipids and polymers [14]. They prepared bilayer fragments by a layer-by-layer deposition method. The antibacterial activity was pronounced against *P. aeruginosa* and *S. aureus*. They observed the antimicrobial effect dependent on the amount of positive charge on particles and independent of particle sizes [178]. In a more recent investigation, they characterized antimicrobial polymer particles of poly(-methylmethacrylate), which were prepared by emulsion polymerization in the presence of quaternary ammonium surfactants [179]. It was found that high surfactant concentrations yielded stable colloids with permanent cationic groups that were significantly antibacterial. Polymeric nanofibrous structures are also intriguing antimicrobial agents because their high aspect ratio and large surface area improve the interactions with cell surfaces. Three-dimensional scaffolds produced by polymeric nanofibers are widely used in tissue engineering with even greater control over cell incorporation and function. Electrospinning, self-assembly, phase separation, templating and several other techniques with varying degree of success have been applied to generate nanofibrous polymeric materials [167,180].

5. Conclusions and outlook

In this feature article, a wide range of AMP-mimicking cationic polymer systems are described as a promising platform for the development of next-generation antimicrobial agents. These synthetic polymers can be prepared with low cost at a large scale, and are more stable for *in vivo* applications in contrast to most other analogs of AMPs. Cell membrane disruption is widely considered as the major mechanism of action for cationic polymers, while several other possibilities are being unearthed slowly. Weak aggregations between polymer–polymer and cation–anion are important in terms of enthalpic and entropic penalties to gain stronger membrane–polymer interactions. The vast potential on the chemistry of antimicrobial polymers is not fully explored yet. The field is rapidly expanding. Over the course of the development of antimicrobial polymers, it has resulted in a diverse library of materials, improved synthetic methodologies, and better mechanistic studies that can be applied to other fields as well. These membrane-active polymers show potent effects against most bacteria, several fungal and few viral species, illustrating their broad-spectrum activity. The absence of specific molecular targets and rapid biocidal properties make antimicrobial polymers much more tolerant towards microbial resistance development compared to conventional antibiotics. These features favor the application of antimicrobial polymers as disinfectants in clinical settings, medical device coatings, topical antimicrobial agents, intravenous formulations for systemic treatments and many other industrial and domestic applications. However, more research is required to understand complex, long-term interactions of antimicrobial polymers with microorganisms as well as host animals. It can be concluded that antimicrobial polymers have varying potencies and behaviors in accordance to their macromolecular architectures such as homopolymers, random copolymers, block copolymers, telechelic polymers and zwitterionic polymers. In addition, the type of cation, charge location and cationic density also influence the antimicrobial activities. Incorporation of non-cationic hydrophilic groups as well as cyclic or heterocyclic hydrophobic moieties may improve biocompatibility. Higher ordered structures such as micelles and other nano-objects demonstrated intriguing antimicrobial effects.

Since the field is rapidly expanding and more scientists are getting involved, there is a greater need to compare the

antimicrobial and biocompatible properties in different polymeric systems. However, due to different conditions utilized in each laboratory, it is rather difficult to directly compare the MICs or HC values found in literature. For example, it has been observed by Gellman, Weisshaar and co-workers that there is a medium effect on MIC of nylon-3 polymers against *E. coli* [181]. Therefore it is essential to identify a proper medium to evaluate the antimicrobial activities of novel antimicrobial polymers. In addition, it would be beneficial to report MICs of new polymers together with the MIC value for a standard such as magainin or melittin. This could serve as a basis for normalization of biological profiles across different labs. Although many research groups reported *in vitro* antimicrobial and hemolytic activities, there is a lack of research where *in vivo* experiments that are carried out using infected animal models with topical infections or systemic infections [53]. This type of work could direct the field to prepare more biocompatible polymeric systems.

It is widely accepted that many synthetic cationic macromolecules are membrane-active antimicrobials. However, there is a great need to understand specific mechanisms of action because there is a plethora of diverse chemical structures involved with each system. Several biophysical methods are utilized to investigate the membrane disrupting mechanism including the measurement of dye-leakage from dye-filled synthetic liposomes [79,83,182,183], quantification of leaked cellular constituents (e.g. phosphorylated compounds) [14,184], fluorescence-based methods [84], and observation of morphologic changes by scanning electron microscopy [159] [66], transmission electron microscopy [144] and atomic force microscopy [185]. However, all of these methods assist to identify the membrane damaging effects of polymers. Unfortunately, there are almost no investigations on interactions of antimicrobial polymers with internal cellular components such as cell organelles, proteins and genetic materials. Such investigations are greatly essential to unveil other possible mechanisms of antimicrobial action carried out by cationic polymers. It would be fascinating to observe real-time antimicrobial effects on cell surfaces. This could be attainable by using labeled antimicrobial nanoparticles. In addition, it would be interesting to find out if synthetic antimicrobial polymers can induce oxidative stress on microbes as some antimicrobial peptides do [186].

Unlike the homogenous nature of helical forming AMPs, it is assumed that antimicrobial cationic polymers form random coils in solution that aid the antimicrobial activity. Therefore, it would be helpful to have in-depth investigations of polycation structures in solution, conformational preferences and different partitioning properties to completely unveil the mechanistic profiles of antimicrobial polymers. Although phosphonium and sulfonium containing polymers are known to be better (in limited cases) in biocompatibility and stability over ammonium-containing counterparts, there is less focus on novel systems with such cations. Cationic metal-containing polymers are at their infancy and worthy for more research, as their binding to cell membranes is largely unknown. In addition, polymers with cationic groups on polymer backbone are not well explored as antimicrobial materials. Such polymers may have different properties with regard to the partitioning into the biological membranes. Furthermore, macromolecular architectures such as star, comb, brush and cyclic polymers have not been actively explored as antimicrobial polymers. In addition, “Janus” architectures would be very interesting to study, since they allow a higher level of amphiphilicity manipulations. Therefore, we believe investigations on Janus particles [187] would open a new class of antimicrobial macromolecules. These structures may lead to interesting structure–activity relationships.

Microbial resistance spans over almost all antibiotics clinically used today. It is widely assumed that membrane-active antimicrobial polymers may enjoy longer effectiveness due to slow resistance development by microorganisms. However, it is wise to find in advance, the reservoir of resistance determinants of microbes, especially multidrug resistant bacterial species that may show clues about the future of resistance development against these novel materials [188,189]. Moreover microorganism responses induced by cationic polymers are pivotal to predict any resistance development. For example, it could be worth exploring antimicrobial polymer-induced DNA expressions, extracellular chemical production, effects on quorum sensing, etc.

Most antibiotics are released to the environment as active species at sublethal doses, resulting in accelerated evolution and spread of antibiotic resistance [190]. There is a possibility to evade microbial resistance towards cationic antimicrobial polymers by the introduction of degradability to these materials. Since antimicrobial activity of cationic macromolecules function as a collective feature, degradation of such molecules would result in inactive ingredients that may have no effect on resistance development. Therefore, considering the ecology of antibiotics, degradable macromolecules can be envisioned as a better choice compared to current small molecule antibiotics. Also this may improve biocompatibility with host organisms and have minimum footprint on the environment.

Finally, it is apparent that there is a tremendous opportunity for the development of novel antimicrobial materials using cationic macromolecules tailored to specific functional requirements through the use of a wide variety of building-block monomers, macromolecular architectures, and the profound knowledge gained during the past decades. This rapid and widely expanding research area may bring a new momentum to defeat or at least control the threat posed by resistant microorganisms.

Acknowledgments

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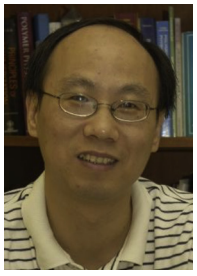

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