Send Orders for Print-Reprints and e-prints to reprints@benthamscience.ae

Current Pharmaceutical Design, **2018**, *24*, **1-11** **1**

REVIEW ARTICLE

Antimicrobial Synthetic Polymers: An Update on Structure-Activity Relationships

Cansu Ergene and Edmund F. Palermo*

Materials Science and Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180

ARTICLE HISTORY

Received: January 23, 2018
Accepted: February 11, 2018

DOI:
10.2174/1381612824666180213140732

Abstract: The rising incidence of antibiotic-resistant infections, combined with a declining number of new antibiotic drug approvals, has generated an alarming therapeutic gap that critically undermines public health. Host Defense Peptides (HDPs), sometimes referred to as "Nature's Antibiotics", are short chain, amphiphilic and cationic peptide sequences found in all multicellular organisms as part of their innate immunity. While there is a vast diversity in terms of HDP sequence and secondary structure, they all seem to share physiochemical characteristics that can be appropriated for macromolecular design by the synthetic polymer chemist. Over the past decade, remarkable progress has been made in the design and synthesis of polymer-based materials that effectively mimic HDP action – broad-spectrum antibacterial potency, rapid bactericidal kinetics, and minimal toxicity to human cells – while offering the additional benefits of low cost, high scalability, and lower propensity to induce resistance, relative to their peptide-based counterparts. A broad range of different macromolecular structures and architectures have been explored in this design space, including polynorbornenes, poly(meth)acrylates, poly(meth)acrylamides, nylon-2 polymers, and polycarbonates, to name a just few. Across all of these diverse chemical categories, the key determinants of antibacterial and hemolytic activity are the same as in HDPs: net cationic charge at neutral pH, well-balanced facial amphiphilicity, and the molecular weight of the compounds. In this review, we focus in particular on recent progress in the polymethacrylate category first pioneered by Kuroda and DeGrado and later modified, expanded upon and rigorously optimized by Kuroda's and many other groups. Key findings and future challenges will be highlighted.

Keywords: **A**ntimicrobial, hemolytic, polymer, peptide, host defense, amphiphilic, cationic.

1. INTRODUCTION

Antibiotic drug resistant pathogens have engendered an accelerating global health threat [1-5]. Multidrug-resistant (MDR) strains were first discovered in the clinic[2], but soon spread in the community [6] and through the food chain [7]. The Centers for Disease Control and Prevention (CDC) reported that more than 2 million people annually experience infections associated with antibiotic-resistant bacteria in United States and at least 23,000 cases are fatal [1, 8]. By 2050, persistent infections worldwide are projected to approach 10 million deaths annually if current trends continue unabated [9]. The alarmingly rapid proliferation of bacterial resistance, combined with a persistent decline in the number of new antibiotic drug approvals, generates an urgent need to develop a new and effective antimicrobial arsenal against which pathogenic bacteria are unable to develop resistance.

Host defense peptides (HDPs) are evolutionarily ancient weapons of eukaryotic innate immunity and are produced by all multicellular organisms [10-13]. These so-called "Nature's antibiotics" exert rapid, broad spectrum activity against pathogenic microorganisms, including Gram-negative and Gram-positive bacteria, fungi, viruses and parasites with little propensity to induce available modes of bacterial resistance [14]. Furthermore, HDPs exhibit low toxicity towards human cells at therapeutic levels [15] and alert host cells to initiate immediate inflammatory and innate responses [16]. These features of HDPs are all significant targets for activity in future antibiotics toward overcoming the resistance problem. Synthetic peptides that exert similar activity are also known, and are included in the broader category of Antimicrobial Peptides (AMPs). Though occasionally used interchangeably, the term HDP refers to naturally occurring components of innate immunity and the category of AMP includes both the HDPs and their synthetically designed analogues.

There are thousands of known AMPs. Although highly diverse in terms of sequence and secondary structure, they possess some common physiochemical characteristics: cationic amphiphilicity [10, 11, 14]. Their cationic amphiphilic nature arises from the prevalence of positively charged amino acids at neutral pH (net charge of +1-10) along with a high content (30-60%) of hydrophobic residues [10, 11, 14]. They are generally low-molecular-weight (10-50 amino acids) sequences. With regard to secondary structure, AMPs have been categorized into four major groups: β-sheet, α-helical, cyclic and disordered random coils. Regardless of secondary structure, these diverse AMPs all tend to fold into *segregated domains* whereby cationic residues cluster together in a distinct domain, while hydrophobic residues cluster in another (Fig. **1**). This so-called "facial amphiphilicity" is typically triggered upon binding to biomembranes even if it is not evident in monomeric solution [14, 17].

Unlike conventional antibiotics, which are specifically designed to inhibit a targeted vital life process in the microbial cell [18], AMPs putatively attack the integrity of biomembranes via electrostatic attraction and hydrophobic interactions, resulting in disruption of the membrane barrier function [19]. Bacterial membranes, composed of anionic phospholipids as well as surface-displayed acidic polymers (lipopolysaccharides in Gram-negative, and wall-associated teichoic acids in Gram-positive) bear a net negative charge on their outer leaflets. In contrast to prokaryotic cells, the external surface of mammalian cells present a much lower negative charge density due to the abundance of Zwitterionic phospholipids in their outer leaflets, which bind less avidly to AMPs [14, 17]. While the mechanism of AMP action has been widely investigated and remains a topic of debate [17], the majority of mechanistic studies suggest that the bactericidal action of AMPs involves

*Address correspondence to this author at the Materials Science and Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180;
E-mail: palere@rpi.edu

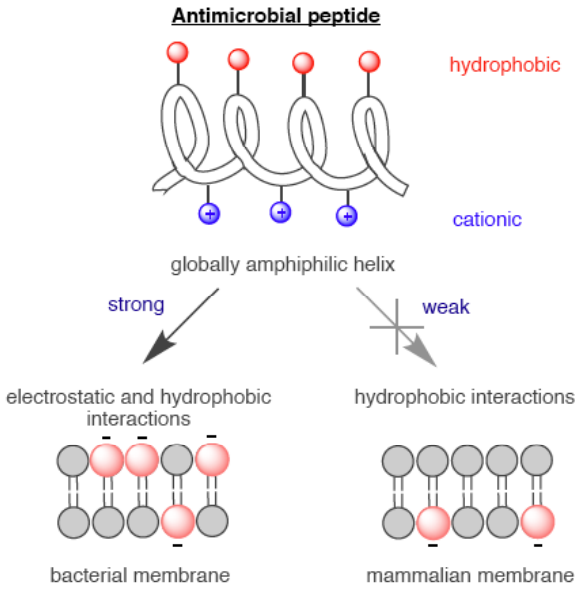

Fig. (1). Schematic representation of a cationic antimicrobial peptide folding into a facially amphiphilic helix upon binding to anionic biomembranes.

electrostatic attraction between cationic peptide and polyanionic bacterial membranes. Columbic attraction thus causes the peptides to accumulate on the outer leaflets of bacterial biomembranes, followed by the insertion into cell membrane via membrane thinning, transient pore-formation and disruption of the barrier function (or translocation through the cytoplasmic membrane and attack on intracellular targets) which may ultimately induce bacterial cell death [14].

Although naturally occurring peptides display therapeutic potential as prototypes for the design of novel antimicrobial sources, their topical use in biomedicine has been constricted by poor chemical and pharmacokinetic stability, high manufacturing cost and unknown systemic toxicity [14, 20]. To overcome these obstacles, researchers have devoted more attention to synthesize non-natural mimics of bioactive peptides. First of all, Wade *et al.* showed that all-D enantiomer magainin and cecropin analogs exerted the same level of biological activity compared to natural all-L peptides, but are not proteolytically degraded [21]. In another study, peptides were observed to adopt globally amphiphilic helices upon contact to lipid membranes, even without stable helix formation [22]. In that light, molecular inspiration by the physicochemical properties of AMPs is revealed as the key determinant in antimicrobial design rather than precise sequence, stereochemistry or stable secondary structure [23]. Accordingly, all these facts favored the production of synthetic antimicrobial peptides: β-peptides [24], α/β peptides [25] and peptoids [26], which exerted outstanding antimicrobial performance. Despite their membrane-disrupting behavior and resistance to proteolytic degradation, time- and cost-intensive preparation of peptidomimetics still hinders their widespread pharmaceutical and biomedical use.

Synthetic polymers have long been used to kill bacteria on contact. Polymeric disinfectants emerged concurrently with the discovery of AMPs in the 1980s, although initially there was little communication between these two disciplines. In contrast to AMPs, polymer disinfectants can be inexpensively produced on the commercial scale with a flexible framework for chemical modification, although they are typically toxic to human cells as well as bacteria [23]. Polymer disinfectants are high-molecular-weight synthetic polymers that contain quaternary ammonium salts (QAS) as the cationic moiety and long alkyl chains (C_8-C_{12}) as the hydrophobic component [27]. A variety of structures including poly(styrene)s, poly(vinylpyridine)s, poly(vinyl alcohol)s, and polymethacrylates have shown antibacterial activity [28]. In spite of their extensive solid state applications as biocides [29, 30] or antimicrobial coatings [31], unfortunately clinical and biomedical applications of these polymers is unwarranted due to prohibitively high toxicity to human cells [32]. Toxicity of these macromolecules, relative to AMPs, can be ascribed to their high molecular weight, high cationic charge density, and excessively hydrophobic alkyl chains. In principle, however, the toxicity of these polymers can be tuned by carefully adjusting the design parameters (MW, charge, hydrophobicity) and performing structure-activity correlations. To that end, chemists have harnessed the natural design principle of host defense peptides with inexpensive methods of polymer chemistry, enabling cost-effective and biocompatible ***peptide-mimetic antimicrobial polymers***. The first example of such materials was reported by Tew and DeGrado in 2002; they designed facially amphiphilic arylamide molecules that mimic the physical and biological features of AMPs [33]. In 2005, Kuroda and DeGrado demonstrated cationic amphiphilic polymethacrylates could exert potent antimicrobial activity [34]. Gellman and co-workers showed that nylon-type synthetic polymers are also effective as fully synthetic antimicrobials [35].

Recent advances in polymer chemistry have paved the way for tailoring the physiochemical characteristics of macromolecules with ever-increasing sophistication. Tuning the molecular weight, dispersity, copolymer composition, sequence distribution, and chain architecture, with a remarkable degree of precision, are now common features of the polymer chemistry toolbox. In the context of antimicrobials, these modern synthetic tools have the potential to rapidly advance our understanding of the complex interplay between salient physiochemical features of macromolecules and their resultant biological activities.

One of the most widely employed strategies to prepare AMP-mimetic polymers is the statistical copolymerization of two monomers, one bearing a hydrophobic side chain and the other bearing a (protected) cationic group, followed by a deprotection step. The structures of each individual monomer, their feed ratios, and the ratio of initiator or chain transfer agent to monomer (and thus MW) are readily tunable. This method enables rapid, scalable, and inexpensive synthesis of a combinatorial library in which molecular weight, comonomer identity, and copolymer feed ratios are systematically varied. High-throughput microplate assays (turbidity-based, colorimetric, or fluorometric) are preformed to screen the antibacterial activity against a broad spectrum of bacteria, as well as toxicity assays against human cells. The measure of antibacterial activity is the Minimal Inhibitory Concentration (MIC), *i.e.* the lowest polymer concentration which completely inhibits the growth of the bacteria (for this reason, a *lower value of MIC* implies *better antimicrobial activity*). Toxicity to human red blood cells (RBCs), is quantified as the HC_{50}, or the concentration of polymer that induces 50% release of hemoglobin (for this reason, a *higher value of* HC_{50} implies *better hemo-compatibility*). In this review, we refer to "potent" antibacterial activity as an MIC value on the order of 10 μg/mL, whereas "non-hemolytic" refers to an HC_{50} value in the range of ≥ 1000 μg/mL. The wealth of data generated from these experiments provides a basis for understanding the complex relationships between the structure features and resulting biological activity of AMP-mimetic polymers. Indeed, studies of this sort have generated polymer candidate structures that possess the desired combination of potent antibacterial efficacy and minimal toxicity to human cells.

In this review, we discuss state-of-the-art methods that have been employed to optimize macromolecular structures for high antibacterial activity with low toxicity to human cells. We focus specifically on polymethacrylates, a prototypical antibacterial platform that has been used by several groups recently. For reviews on other structural design platforms, including the polynorbornenes, nylons, polycarbonates, and many others, the reader is directed to

the numerous existing reviews of this highly active field [28, 36-40]. Here, we summarize the most important results from prior work specifically on the polymethacrylate platform, dating back to 2005, and we provide an update on the most recent studies that have expanded the cannon of structural complexity within this category. Finally, we look forward to new opportunities and make note of significant challenges in this exciting field of macromolecular science.

2. STRUCTURAL DETERMINANTS OF ANTIMICROBIAL AND HEMOLYTIC ACTIVITY

2.1. Amphiphilic Balance

A fundamental design criterion for synthetic antimicrobial polymers requires a finely tuned balance of cationic charge and hydrophobic content. Polymers with too high a density of cationic charges and very low hydrophobicity will exhibit little propensity to insert into the hydrophobic core of the phospholipid bilayer, although they may bind to anionic components of the outer leaflet electrostatically. Also, excessively cationic polymers induce hemagglutination of RBCs, which is a major risk for internal medicine. At the opposite extreme end of composition, highly hydrophobic polymers with low cationic content exhibit high toxicity to both bacterial and human cell membranes and thus lack any appreciable selectivity. Of course, excessively hydrophobic polymers will also lack solubility in aqueous media and thus exert no significant activity against bacteria or human cells. In regard to all factors above, synthetic polymers should be carefully designed and synthesized to achieve the optimal balance between cationic charge and hydrophobicity, thus leading to potent antibacterial activity with low toxicity to host cells (Fig. **2**).

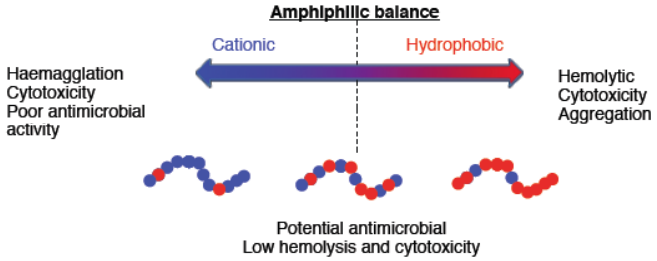

Fig. (2). Optimization of ratio between cationic and hydrophobic residues is the key parameter to develop antimicrobial polymers with cell-type selectivity. Reproduced from [51].

2.2. Hydrophobic Groups

The structure of the hydrophobic side chains strongly impact observed activities. Alkyl chains of varying length, as well as aryl groups, have been widely employed, either as pendant groups in the side chains of a copolymer, or as "spacer groups" connecting the polymer backbone to the cationic group. In 2005, Kuroda and DeGrado first introduced amphiphilic polymethacrylates as antimicrobial substances [34] and these have since been extensively optimized by Kuroda and many others (Fig. **3**) [41-53]. The antimicrobial activity was quantified using a turbidity-based growth inhibition microplate assay on 96-well plates that is still widely employed today. Their first-generation formulations were random copolymers composed of 2-aminoethylmethacrylate and butyl methacrylate with varying feed ratios. The number average molecular weights were controlled by the use of a thiol-based chain transfer agent. For copolymer compositions from 0 to 0.3 mole fraction butyl side chains ($f_{butyl} > 0.3$), they observed increasing antibacterial activity against *E. coli* with increasing f_{butyl}. Increasing the hydrophobic content beyond this ratio led to loss of the antibacterial activity, presumably due to aggregation or collapse of the non-polar

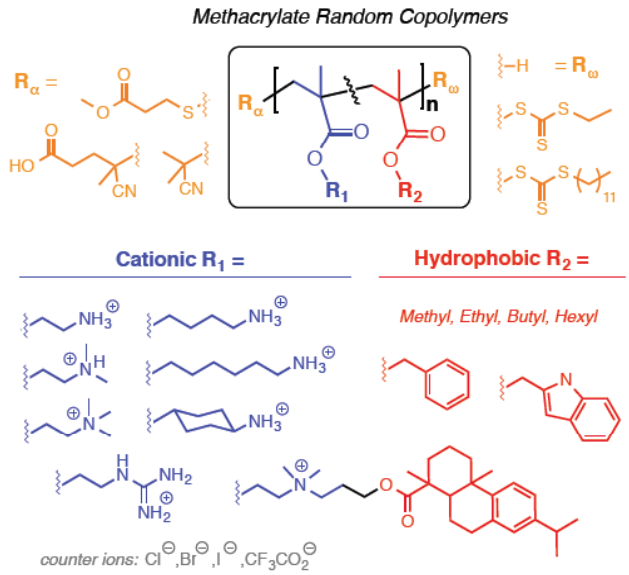

Fig. (3). The diversity of structures in the polymethacrylate design platform. Several groups have tuned the activity of polymers by adjusting the identity of the cationic charges and hydrophobic groups.

macromolecules in solution, which reduces the driving force for insertion into biomembranes. The copolymers in this initial work suffered from substantial toxicity to human red blood cells (RBCs). The authors addressed this limitation in a subsequent paper, in which it was demonstrated that less intensely hydrophobic comonomers such as methyl- or ethyl methacrylate yielded polymers which retained their antimicrobial activity but dramatically reduced the toxicity to RBCs [54].

In 2009, Palermo and Kuroda elucidated the role of the cationic group structure in methacrylate copolymers with varied content of hydrophobic alkyl groups (methyl, ethyl and butyl) to evaluate antimicrobial and hemolytic activities [52]. In general, hydrophilic polymers containing less than a 0.2 mole fraction of methyl groups (f_{methyl}), did not display any significant antibacterial efficacy against *E. coli* up to MIC of 2000 μg/mL. In primary amine functionalized polymers, antibacterial activity was enhanced by two orders of magnitude as f_{methyl} was increased to about 0.5. At this ratio of methyl groups, the polymers displayed little hemolytic activity and were in fact the most cell-type selective formulations in that study. Interestingly, polymers with tertiary amine groups in the side chains (instead of primary amines) revealed a parabolic trend in MIC which gives the lowest value at $f_{methyl} = 0.5$. The loss of activity above 0.5 may be explained by excessive hydrophobicity that causes aggregation of polymers and reduction in the proportion of cationic groups required to mediate Coulombic interaction with anionic biomembranes. Furthermore, replacing the modestly hydrophobic methyl groups with longer butyl chains led to enhancement of bactericidal activity, but also dramatically aggravated hemolytic toxicity. Eventually, they determined the best candidate with the highest selectivity index (the ratio of HC_{50}/MIC) was a random copolymer bearing 47% hydrophobic methyl groups, 53% cationic primary ammonium groups and a DP of about 10-15 repeat units. In another study of Kuroda *et al.*, primary amine based methacrylate copolymers containing a variety of alkyl groups and benzyl units were synthesized with multiple hydrophobicity composition [54]. Similarly, they reported increased hemolytic activity with increasing lipophilic content either by number of groups, or size of the group, or both. They concluded that the use of modestly hydrophobic methyl methacrylate enables the same level of antibacterial potency as a lower mole fraction of more hydrophobic butyl

groups; however, the methyl groups gave markedly lower hemolytic toxicity.

The Locock group investigated cationic polymethacrylates with increasing hydrophobic content, keeping the molecular weight constant [55]. With increasing mole fraction of hydrophobic comonomer, they recorded diminished activity against *S. epidermidis* for amine-based polymers, whereas guanidine analogs showed little or no change in MIC values. However, both polymers induced increased hemolysis as f_{methyl} increased. The same group designed a library of cationic polymethacrylates with indole groups inspired by the hydrophobic tryptophan residues found in many AMPs [56]. Unfortunately, increasing the indole content lowered antimicrobial efficacy and enhanced human RBC lysis. Still, they suggested that potentially promising antimicrobial polymers with minimal cytotoxicity could be designed with appropriately low indole content.

Palermo *et al.* reported random copolymers of ethyl methacrylate (EMA) and 2-aminoethylene, 4- aminobutylene, and 6-aminohexylene to evaluate the impact of "spacer length" on antimicrobial and hemolytic performance [45]. They reported excellent antimicrobial activity of the copolymers containing 4-aminobutylene side chains with minimal hemolysis compared to those with shorter (2-aminoethylene) or longer (6-aminohexylene) spacer groups. Thus, they concluded that the optimal combination of antimicrobial potency and low toxicity to RBCs could be achieved by tuning the number of carbons in the side chain that spatially separate the cationic charge from the backbone, using an analogy to the "snorkel" effect discussed in regards to transmembrane helical peptides. This hypothesis was in accord with observation by molecular dynamics (MD) simulations on phospholipid bilayers.

Yang and co-workers produced a series of primary amine functionalized random poly(acrylate)s via free radical copolymerization of a monomer having 2-carbon spacer arm (M2) and a 6-carbon spacer arm monomer (M6) with varying mole fractions of each [57]. Homopolymers with the short alkyl arm (M2) were ineffective against Gram-negative and Gram-positive bacteria up to high polymer concentration (1000 μg/mL) while that of long-alkyl spacer arm length (M6) was highly biocidal and hemolytic. Interestingly, copolymers with increasing content of the 6-carbon monomer showed promising antibacterial activity and low hemolysis.

Hedrick and co-workers utilized biodegradable poly (carbonate)s having quaternary ammonium functionality with hydrophobic tails of various alkyl chain length (methyl, ethyl, hexyl), as well as pyridinium and imidazolinium (Fig. **4**) [58]. The polymer with four carbon (butyl) tails at the cationic substitutes was reported as the best candidate among those studied with different alkyl chain lengths due to its profound selectivity, by an impressive factor of >256 for *E. coli* and >1026 for *S. aureus*. Furthermore, cationic poly(carbonate)s with aromatic units at the tail showed high antibacterial efficacy as well as low toxicity against rat RBCs. Recently, Cai and co-workers reported amphiphilic carbonate *block copolymers* bearing primary amine groups with great selectivity for bacteria cells [59]. The diblock and random copolymers exhibited potent biological activity against three different Gram-positive bacteria and little hemolysis up to 1 mg/mL. The block copolymers self-assemble into dynamic biodegradable micelles with excellent potential as antibacterial nanomaterials.

Gellman and co-workers elucidated the influence of cyclic and acyclic hydrophobic groups on biological performance of nylon-3 random copolymers [60]. Copolymers with cyclohexane subunits showed enhanced antimicrobial activity with low hemolytic behavior compared to acyclic homologous. They speculated that the alteration in local backbone flexibility might result in activity differences between the cyclic and acyclic polymers.

Tang and co-workers developed polymers of *N,N*-dimethylaminoethyl methacrylate (DMAEMA) quaternized with a

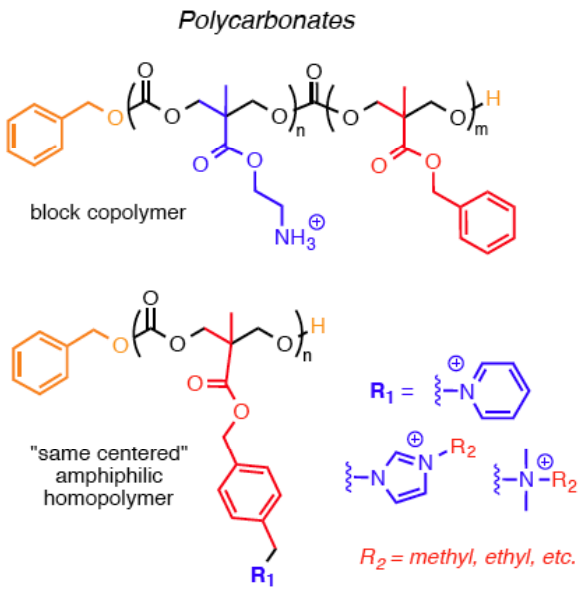

Fig. (4). The polycarbonates are biodegradable variants of the conventional polymethacylate design platform. Structure activity relationships in these interesting materials have been extensively studied.

derivative of dehydroabietic acid (from natural rosin) as the hydrophobic residue [61]. Whereas the starting polymer of DMAEMA was inactive against *E. coli* and *S. aureus*, the rosin-functionalized variant was a potent antibacterial agent. Interestingly, relative to polymers of DMAEMA quaternized with a linear alkyl chain having same number of carbon atoms as the rosin group, the amphiphilic polymers bearing rosin pendant groups displayed the most efficacious biological activity against *E. coli* and *S. aureus*. Thus, naturally occurring compounds from sources such as rosin may enable "green" and renewable antimicrobial polymer variants, thus reducing dependence on petrochemical feedstocks.

In summary, the overall hydrophobicity of the polymer must exceed a certain threshold to achieve activity, but increasing the hydrophobicity beyond this threshold will aggrevate the hemolytic toxicity. Thus, all reports agree on the one unifying principle for the design of an effective antimicrobial polymer with low hemolytic activity: target the lowest possible hydrophobicity required to confer potent antibacterial activity, but no more than that amount.

2.3. Cationic Groups

Both cationic and hydrophobic functionalities work in concert to confer membrane-disrupting ability in polymeric antimicrobials. Naturally occurring peptides are rich in the basic amino acids lysine and arginine, which are highly protonated at physiological pH. Thus, AMPs typically bear a net positive charge in the range of +2 to +9 at pH 7.4. The identity of the cationic source, the charge density, and the spatial arrangment of charges within the polymeric architecture all significantly influence antimicrobial performance. This is because the major driving force for binding between negatively charged microbial cell surface and cationic molecule is mediated by Coulombic attraction forces. A diverse range of cationic moieties, including quaternary ammonium, pyridium, imidazolium [62], thiazolium, triazolium [63], phosphonium [64], sulfonium or guanidinium groups, have been employed to that end. Still, the majority of antimicrobial polymer systems contain pendant ammonium groups, whose structure is reminiscent of lysine residues found abundantly in HDPs.

In 2009, Palermo and Kuroda synthesized methacrylate copolymers with primary, tertiary and quaternary ammonium groups to evaluate the impact of cationic moiety on antimicrobial and

hemolytic activity (Fig. **5**) [52]. Here, primary amines were mean to represent synthetic mimics of lysine residues in HDPs, whereas the quaternary units represented polymer disinfectants that typically employ QAS as a pH-independent cationic functionality. Clearly, the primary ammonium-functionalized polymers (with modestly hydrophobic methyl methacrylate comonomer) gave the best combination of low MIC and high HC_{50} values. Amphiphilic copolymers with primary or tertiary amines completely inhibited *E. coli* growth with little or no hemolytic activity. In contrast, the equivalent polymeric system with QAS pendant groups lacked substantial antimicrobial activity even at high concentrations. Upon increasing the overall hydrophobicity of the QAS polymers by replacing alkyl groups from methyl to butyl, these macromolecules showed biocidal activity, *i.e.* it resulted in high bactericidal and hemolytic toxicity.

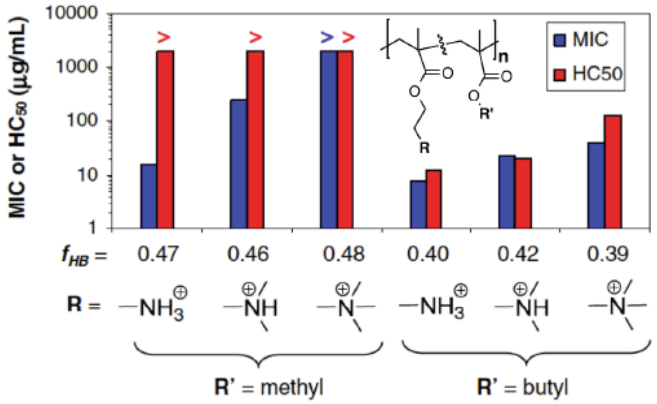

Fig. (5). Effect of cationic group structure. MIC values against *E. coli* and HC_{50} of amphiphilic polymethacrylates with primary, tertiary, and quaternary ammonium groups. Bars with a ">" indicate that MIC or HC_{50} > 2000 µg/mL. Reproduced from [51].

Many groups have observed similar trends. For example, the antibacterial activity of a series of polyacrylates bearing tertiary amine side chains was investigated before and after the quaternization of corresponding polymers [65]. This study reported reduced antibacterial action of quaternized polymers towards Gram-negative bacteria, attributed to loss in hydrophobicity required to damage bacterial membranes. Furthermore, The Morgan and McCormick groups designed a series of primary- and tertiary amine functionalized methacrylamide homopolymers and statistical copolymers via aqueous RAFT polymerization [66]. Relative to primary amine functionalized polymers, the tertiary amine groups were less effective against *E. coli* and *B. subtilis*, possibly due to steric hindrance of these bulky groups, which may mitigate the cation-microbial cell interactions on the molecular level (*e.g.* reduced hydrogen bond strength). Interestingly though, Alvarez-Paino *et al.* reported improved antimicrobial ability after quaternization of tertiary amines in methacrylate copolymers [67]. These results clearly indicate that the number of cationic groups and the chemical structure of these groups are important determinants of biological activity. Compared to QAS groups, protonated amine groups generally displayed better antibacterial activity and lower hemolytic toxicity. These findings suggest that perhaps the combination of electrostatic attraction forces and hydrogen bonding generate stronger interactions between primary amine groups and bacterial phospholipids.

While most studies on antimicrobial polymers have concentrated on ammonium groups as the cationic moiety, there are several examples of *guanidinium*-functionalized polymethacrylates, inspired by the arginine residues found abundantly in certain HDPs. In general, peptides with a high proportion of arginine exhibit better antimicrobial activity than corresponding lysine-rich peptides [68] and they can act as cell-penetrating agents that translocate across biomembranes and possibly attack intracellular targets [69, 70]. The guanidinium group features strong resonance stabilization, delocalizing the positive ions around three nitrogen atoms [71]. The pK_a value of arginine is ~12.5 which is substantially higher than that of lysine, ~10.5. Thus, polymers containing multiple guanidine groups in proximity are expected to have higher degrees of protonation at physiological pH. Also, the bidentate nature of the hydrogen bonding between guanidine and phospholipid further enhances the interaction. Similar effects were observed in synthetic antimicrobial guanidinylated polymers. [72] Locock *et al.* synthesized a series of primary amine-based polymethacrylates and converted these into guanidine-functionalized polymers to allow a direct comparison [55, 73]. They reported that polymers with pendant guanidine groups gave lower haemotoxicity and stronger antimicrobial activity against *S. epidermidis*, *S. aureus*, *E. coli* and *C. albicans* compared to primary amine-based polymers. Ikeda *et al.* synthesized acrylate homopolymers and copolymers with acrylamide containing pendant biguanide units, and these also showed promising antimicrobial activity against a broad spectrum of pathogens [27].

The Fernández-García group reported non-hemolytic, antimicrobial activity of polymethacrylates by quaternization of *azole*-functionalized methacrylate polymers (Fig. **6**) [63]. Polymethacrylates bearing triazoles and thiazoles were alkylated with butyl iodide with controlled degrees of quaternization (DQ) from 10-100%. Interestingly, they observed very potent antibacterial activity (MIC < 10 µg/mL) against *P. aeruginosa* and *S. aureus* for DQ > 50% and remarkable non-hemolytic activity (HC_{50} > 5000 µg/mL), which gives a very high ratio of selectivity. The bactericidal kinetics gave a 3-log reduction in CFU/mL in 15 min. Furthermore, cell morphologies revealed by SEM showed a loss of smoothness in cell surfaces and extensive bacterial cell aggregation after treatment with these novel polymers, which supports the hypothesis of a membrane-disrupting mechanism of action.

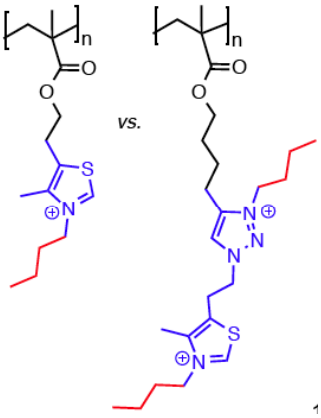

Fig. (6). Polymethacrylates bearing azole units quaternized with butyl iodide.

Imidazole side chains, inspired by histidine-rich HDPs, are another example of the source of cationic charge used in certain antimicrobial polymers. Hedrick and co-workers synthesized biodegradable cationic polycarbonates containing propyl and hexyl side chains quaternized with imidazoles [74]. Those polymers presented a potential antimicrobial activity against broad spectrum of pathogens with selectivity through red blood cells. In addition, imidazolium-based poly (ionic liquids) (PILs) were developed by Zheng and Yang [62]. Both mono- and bis-imidazolium functionalized polymers displayed high antibacterial properties to *S. aureus* and *E. coli*.

There are examples of cationic polymers that employ heteroatoms other than nitrogen. For example, Kurihara *et al.* synthesized copolymers of *N*-isopropylacrylamide (NIPAAm) with methacryloyloxyethyl trialkyl *phosphonium* chlorides (METRs) containing alkyl chains of varying length [75]. Phosphonium-based cationic copolymers revealed more potent antibacterial efficacy with longer alkyl tails.

In addition to the type of cationic source, density, location and spatial arrangement of positive charges on polymer chains affect biological activity. Tew's group studied poly(norbornene)s with one, two or three primary amine substituents on each repeating unit, to examine the effect of increasing cationic charge density [76]. They found a decrease in hemolytic activity of a hydrophobic polymer while retaining antimicrobial performance towards *E. coli* and *S. aureus*. Yang *et al.* also reported lower MIC (minimum inhibitory concentration required to prevent bacterial growth) values for higher charge density (bis-cations out-performed mono-cations) of imidazolium-based PILs [62]. In a similar vein, Palermo and Kuroda tested cationic antimicrobial poly(methacrylate)s in media where the pH was varied between 6 and 8. They pointed out that increasing the density of amine groups in a polymer chain reduces the apparent pK_a of the polymer due to electrostatic repulsion between neighboring charges, hence reducing the extent of ionization of amine groups relative to their monomeric counterparts. This is an important consideration since changes in pH can alter the amphiphilic balance. In a recent study by Kuroda's group, amphiphilic random methacrylate copolymers were tested under various pH 7.4 (represents infected skin condition) and pH 5.5 - 6.5 (represents healthy skin conditions) with drug-resistant strains of *S. aureus* [77]. Polymers showed pH-dependent antibacterial activity which is highly active at neutral pH, but ineffective under acidic environment. According to zeta potential measurements, bacterial cell surfaces were found to be less negatively charged in the lower pH media. This mitigates the binding of positively charged polymer to the membrane. They also noted that a change in pH of test media affects the amphiphilic balance of the polymer that may become more hydrophobic at higher pHs, favoring membrane disruption. Encouragingly, there was not any substantial hemolytic activity against human RBCs or cytotoxicity to human dermal fibroblast across the pH range studied.

It has been also found that bactericidal and hemolytic activities are affected by the spatial arrangement of cationic and lipophilic moieties on polymer backbone (Fig. **7**). A series of amphiphilic pyridinium-methacrylate copolymers differing in the coordination of charge and alkyl tail, either all on the same center or spatially separated, were studied by Sen and co-workers to access toxicity [78]. Despite higher antimicrobial efficacy of polymers with different centered groups, macromolecules with cationic pyridines and lipophilic tail on the same center exhibited non-hemolytic activity. Punia and co-workers also showed the effect of structural arrangement of cationic and lipophilic residues along the backbone [79]. They reported highly antibacterial, non-hemolytic PEGylated acrylate copolymers with hydrophobic hexyl and cationic group on the same repeating unit. However, in the case of terpolymers (positively charged and lipophilic moieties on separate centers), the incorporation of PEG side chains did not mitigate the RBC toxicity even up to 40% PEG content.

2.4. Neutral hydrophilic groups

Another method to alleviate the hemolytic activity of antimicrobial polymers is to incorporate electrically neutral, hydrophilic moieties to side chains in order to accomplish the desired amphiphilic balance (Figure **8**). In addition to the cationic and hydrophobic residues found in most HDPs, hydrophilic and neutral residues are also a substantial fraction of the amino acid sequences. This third component of the HDP composition has not been incorporated into many prior antimicrobial synthetic polymers, but may play a useful role. Accordingly, introducing uncharged hydrophilic groups such as, polyethylene glycol, sugar or Zwitterionic units into amphiphilic macromolecules is an attractive strategy to achieve biocompatible antimicrobial properties. In 2007, Youngblood and co-workers copolymerized quaternized poly(vinylpyridine) with hydroxyethyl methacrylate and PEG methyl ether methacrylate [80]. Polymers bearing hydrophilic units showed diminished hemolytic activity with maintained or even enhanced antibacterial performance. Yang and co-workers converted hydrophobic groups of primary amine functionalized polymethacrylates into hydrophilic moieties to improve biocompatibility [81]. Indeed, hydrophilic-and-cationic polymers, compared to hydrophobic-and-cationic parents, demonstrated both membrane active ability against bacteria and reduction in hemolysis, in doing so, strongly enhanced selectivity. Punia and Yang utilized amphiphilic acrylic copolymers with hexamethyleneamine and poly(ethylene glycol) side chains which exhibited >100-fold selectivity towards *E. coli* over RBCs while cationic homopolymers were highly antibacterial and hemolytic [82]. The cytotoxicity of triblock copolymers of methacrylates was investigated by Fernández-García [67]. Their group developed quaternary ammonium based methacrylates with hydrophobic poly(butylmethacrylate) and hydrophilic 2-(D-glucosamin-2-*N*-yl) carbonylethyl methacrylate segments of different mole fractions. They found that the introduction of carbohydrate pendant units reduced cytotoxicity while retaining potent antimicrobial activity. The hydrophilic modification trend additionally applied to polynorbornene systems by incorporation of PEG and sugars at the side chains [83]. Although antimicrobial activity of polymers was reduced slightly with increasing hydrophilicity, the hemolytic activity was drastically lowered. In summary, three-component "*terpolymers*" containing hydrophobic, cationic, and neutral hydrophilic substituents are highly attractive candidates for biocompatible antibacterial materials.

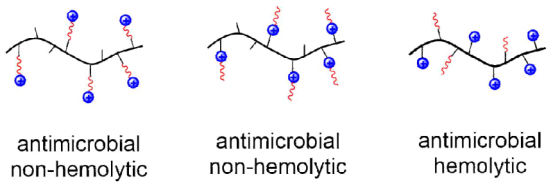

Fig. (7). Effect of spatial arrangement of cationic and hydrophobic groups on antibacterial and hemolytic performance. Reproduced from [78].

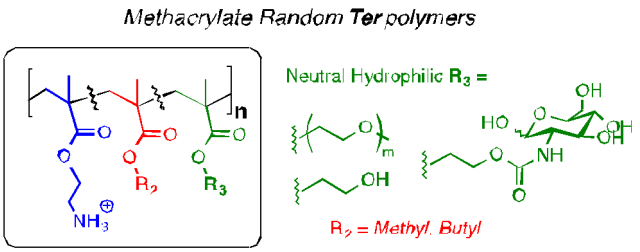

Fig. (8). Random terpolymers of methacrylates bearing cationic, hydrophobic, and neutral hydrophilic components. These terpolymers exert excellent antibacterial activity combined with reduced hemolytic toxicity.

2.5. Molecular Weight

Controlled "living" free radical polymerization methods (including Reversible Addition Fragmentation Transfer (RAFT), Atom Transfer Radical Polymerization (ATRP), and others) enable precise tuning of the biological activity of antimicrobial macromolecules by precisely modulating the average chain length, with narrow dispersity, as an additional design parameter. Higher molecular

weight was initially considered a desirable trait in polymer disinfectants to achieve antimicrobial potency based on the hypothesis that it would provide more cationic and hydrophobic sites per chain to interact with and disrupt the bacterial cell membrane. Also, it was thought that antimicrobial efficacy would be enhanced via irreversible complexation of a polycation with polyanion, in contrast to reversible complexes of a polyanion with poorly charged agents. On the other hand, there are several key factors that clearly disfavor increasing molecular weight: limited solubility and diffusion through the bacterial cell wall, aggregation in biological media, and the propensity of high MW polycations to exert toxicity against human cells. Naturally occurring HDPs are typicaly quite small in size, varying in the range of 2-8 kDa, with little hemolytic toxicity. Thus, peptide-mimetic antimicrobial polyemrs tend to be rather short in chain length, with degrees of polymerization (DP) typically in the range of 10-20 repeat units.

The polymethacrylates of Kuroda and DeGrado, with varying molecular weights in the range of 1.3-10.1 kDa, were screened to elucidate the effect of chain length on biological activity [34]. Low MW copolymers exhibited more favorable selectivity indices (HC_{50}/MIC) regardless of copolymer compositions, as compared to high MW analogues. Interestingly, they showed that the antibacterial activity of the cationic homopolymer (composed of 100% aminoethyl methacrylate, $f_{HB} = 0$) was enhanced with increasing polymer chain length [54]. In general, they observed increased hemolytic behavior of polymers with increasing MW, perhaps due to the hydrophobicity of the polymer backbone.

Several other reports have confirmed a strong correlation between MW and antibacterial and hemolytic activity. For example, Locock *et al.* synthesized cationic amphiphilic polymethacrylates by RAFT with various molecular weights but similar lipophilicity levels [55]. The MIC values against *S. epidermidis* exhibited MW-independent profile for amine polymers, while guanidines showed a clear increase in antibacterial action with decreasing MWs, possibly due to the nature of guanidine groups which may enable translocation across biomembranes and intracellular targeting. Importantly, they showed that increasing chain length dramatically enhanced the hemolysis for both ammonium and guanidine based polymers. The effect of varying the chain length of nylon-3 polymers on biological activity was studied by Gellman's group [84]. They did not find any consistent impact of molecular size on MIC values taken from four different bacterium species, but the larger MW polymers were much more hemolytic. Tang and co-workers elucidated the molecular weight effect on cationic polymethacrylates bearing hydrophobic bulky rosin moieties while keeping the degree of quaternization constant [61]. Lower MW polymers exhibited more powerful antimicrobial efficacy against both Gram-positive and Gram-negative strains. A similar trend against *S. aureus* for shorter polymer chains was also noted by Yang and Hedrick on amphiphilic poly(carbonate)s [85], however, large molecular weight polymers were more effective against Gram-negative bacteria such as, *E. coli* and *P. aeruginosa*.

2.6. Counter-anions

The identity of counter anions has been shown to impact the activity of polymers, although this topic has received relatively less attention. The Cooper group observed more profound biocidal efficacy in quaternary ammonium poly(propyleneimine) dendrimers with bromide anions compared to those with chloride [86]. Furthermore, counter-anion dependence of poly [tributyl(4-vinyl benzyl) phosphonium] on antibacterial activity was studied by Kanazawa and co-workers [87]. They ranked antimicrobial performance of polymers with different anions in an order of chloride > tetrafluoride perchlorate > hexafluorophosphate. In contrast, Panarin *et al.* did not find any significant effect of different counter anion including chloride, bromide and iodide on antibacterial activity of vinylamine and methacrylate homopolymers with QAS [88]. The counter anion effect can be explained with regards to solubility and ion-pair formation. If the anion possesses a slightly hydrophobic character, it would decrease the solubility of the polymer in the test media and affect the amphiphilic balance. Secondly, if the anion generates a stronger ion-pair with the polymer cation, it will mask the efficacy of cations which mediate polymer-biomembrane attraction [36]. Thus, consideration of the subtle but important counterion parameter ought to be included as a molecular design principle for antibacterial polymers.

2.7. End Groups

Most studies have focused attention on the nature of the polymer main chain and the hydrophobic and cationic functional groups in the side chains, ignoring the end groups. Although the properties of most high MW polymers do not depend at all on the end group identity, it is intuitively apparent that end group effects may significantly impact the activity of relatively low molecular weight antimicrobial polymers (DP = 10-20 units in many cases). Interestingly, there are a few studies that examine the effect of end-groups on antimicrobial activity. In 2014, Locock and co-workers synthesized RAFT-derived cationic polymethacrylates with primary amine or guanidine groups pendant in the side chains, while altering the R- and Z-end groups [89]. For amine polymers, a change in R-group from cyanovaleric acid to isobutyronitrile did not substantially impact antimicrobial efficacy, however, biological activity especially against *C. albicans* increased in guanidine polymers with reduction in cytotoxicity. Moreover, Z-end groups of both amine and guanidine polymers were modified with either a dodecyl or an ethyl end group. In both scenarios, amphiphilic polymers with longer alkyl substituent demonstrated better killing performance against vancomycin- and methicillin-resistant strains of *S. aureus* (MRSA) and *C. albicans* due to the enhanced membrane insertion and permeabilization ability with similar hemolytic activity. To probe the influence of hydrophobic end groups, they also tested corresponding polymers without lipophilic unit at the Z-terminus, which surprisingly led to a reduction in antimicrobial activity, perhaps due to micellization or other aggregation process of these end-functionalized macromolecular amphiphiles.

In summary, the end groups are an often neglected aspect of the polymer design and deserve further examination in future work, especially for the case of low MW oligomeric compounds, which is indeed the case for many of these materials.

3. MICROBIAL BIOFILM APPLICATIONS

Biofilms, the most prevalent survival mode for microbes, are formed when a quorum of microbes accumulate on a surface, encased and protected by a extracellular matrix [90, 91]. This protective layer not only assists pathogens to accrete, but also blocks the diffusion of antimicrobials, hence, remarkably increasing the resistence of cells to antibiotic treatment and immune response [92]. As a consequence, MIC and MBC values for biofilm cells have be reported at 10 to 1000 times higher than those of planktonic cells [93, 94]. Biofilm growth on surfaces is the direct cause of deleterious, even fatal outcomes in medicine, dentistry, water treatment, food processing and other fields. In fact, almost 80% of all medical infections are associated with biofilm formation, reported in a public announcement by the US National Institutes of Health [95, 96]. Biofilms are responsible for a broad spectrum of persistent infections including mainly urinary tract infections, chronic wounds and cystic fibrosis-associated lung infections [91]. Furthermore, biofilms are also known as important colonizers of medical devices such as, urinary, venous and arterial catheters, orthopedic prostheses and joints, shunts, stents, contact lenses, dentures and so on [97].

Biofilm formation on medical devices is an enormously costly problem in the clinic. Such complications extend the period of treatment, and in some cases require additional surgery to replace

in-dwelling devices. As a result, new solutions to prevent biofilm formation, or to demolish established biofilms, are urgently needed. Recently, Traven and co-workers determined the efficacy of guanidinylated polymethacrylates in the treatment of biofilms containing *S. aureus* and *C. albicans* fungus [98]. According to confocal microscopy image analysis, the polymers were effective against polymicrobial biofilms. They induced 94% and 80% reduction in *S. aureus* and *C. albicans* cell viability within the biofilm matrix, respectively. It is worth noting that the biofilms were exposed to commercial antibiotics at ten-fold higher concentration than the polymers – highlighting the exemplary performance of these materials relative to small molecule drugs.

Kuroda and co-workers tested cationic methacrylate homopolymers bearing primary amine groups (PE_0) and random copolymers with both cationic and hydrophobic residues (PE_{31}, f_{ethyl} = 0.31) on *S. mutans* biofilms [99]. The MIC values against planktonic cells were 52 and 8 μg/mL for PE_0 and PE_{31}, respectively. Polymers also prevented biofilm formation at concentrations in the range of 6-8 μg/mL, whereas MBIC value for cholorhexidine was 1 μg/mL. However, while cholorhexidine was inactive to remove *S. mutans* biofilms, the methacrylate polymers were found to be highly effective at eradication of biofilms after 1 day of maturation. Also, these cationic homopolymers are non-hemolytic and non-cytotoxic to human cells up to 1000 μg/mL, unlike the hydrophobic copolymer and cholorhexidine, which both exhibited marked toxicity.

There is still much to be done in the field of antimicrobial polymers towards long-term prevention of biofilms. These materials can indeed kill bacteria on contact by a mechanism of cell membrane disruption; however, the cellular debris that result from lysis will often adhere to the surface, fouling the active layer and allowing biofilm formation to proceed after long exposure times and repeated microbial challenges. Thus, new methods are urgently needed to address this significant challenge.

CONCLUSIONS AND FUTURE OUTLOOK

The practically limitless combinations of structure in (co)polymer design allow us to expand the possibilities of more novel antimicrobial compounds with well-tuned biological activity. One of the macromolecular approaches might involve copolymers with increasingly sophisticated structure and chain architecture. In 2011, Kuroda *et al.* reported amphiphilic block and random copolymers of poly (vinyl) ether with similar chain length and monomer fraction to test antibacterial and hemolytic performance [100]. Diblock and random copolymers presented similar anti- *E. coli* activity, however the diblock variants were much less hemolytic relative to random copolymers. They speculated that the difference was based on single-chain conformation of the polymers in water. Block copolymers might generate intramolecular aggregates with a hydrophobic core shielded with cationic residues, which reduces hydrophobic interactions between polymer and RBC membrane, hence, diminishing hemolytic activity. In contrast, random copolymers might form random coils or slightly shrunk conformation in low hydrophobic content, which tends to bind RBC membranes, resulting in cell lysis (Fig. **9**). Thus, it can be concluded that copolymer sequence and architecture (block, random, gradient, graft, branched, dendritic etc.) may be crucial design parameters that are worthy of increased attention.

With an eye toward utilizing antimicrobial polymethacrylates in the clinical setting, Kuroda and his group determined *in vivo* antibacterial efficacy of methacrylate copolymers in a rodent model for *S. aureus* nasal infection [101]. They found stronger inhibitory performance against Gram-positive bacteria than Gram-negative ones. These polymers were also almost non-hemolytic and did not present substantial cytotoxicity towards mammalian cells. Cationic homopolymers indicated potent anti-*S. aureus* efficacy in fetal bovine serum (FBS) relative to that of antibiotic mupirocin. Finally, they investigated significant reduction in the amount of viable cells in nasal environment of cotton rats which exhibits promising topical use of the polymer to treat S. *aureus* infections in the future.

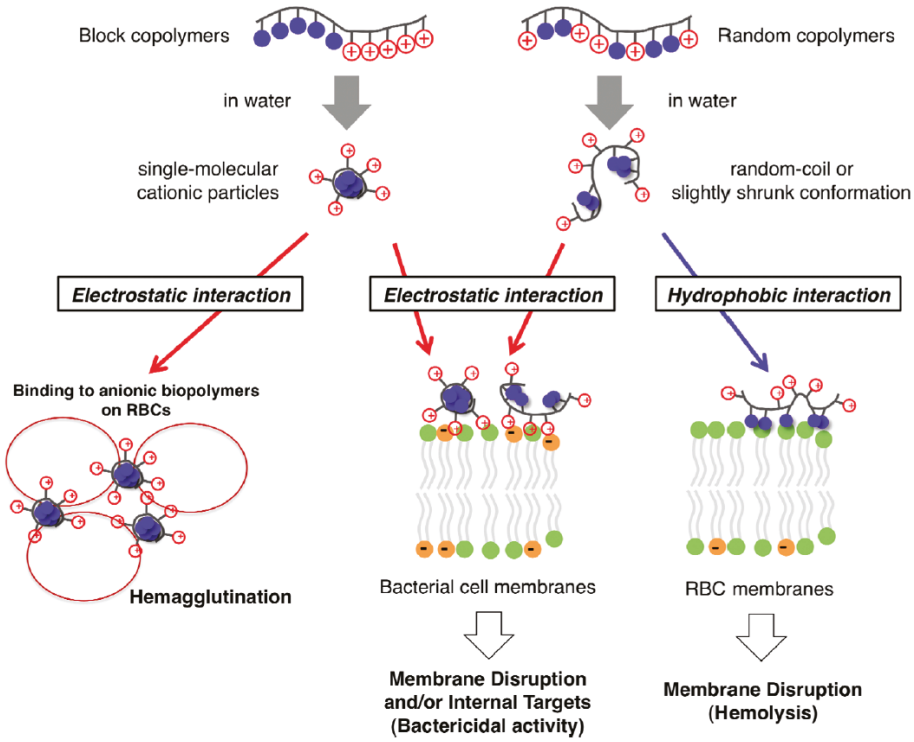

Fig. (9). Schematic representation of a hypothesis regarding the antibacterial and hemolytic activity mechanisms of diblock and random copolymers. Reprinted from [100].

Over the past decade, an impressive body of work has been devoted to understanding and optimizing the structure, activity, and mechanism of action in antimicrobial polymers. Within this broad category, methacrylate-based copolymers have played a significant role. Much has been learned about the complex interplay between physiochemical properties (charge, hydrophobicity, molecular weight) as well as tuning more subtle effects (end groups, counter ions, spacer groups) towards a thorough understanding of the structure-property relationships. In this review, we summarize the most recent findings and look towards the future of development in this exciting field of macromolecular bioscience.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

EFP gratefully acknowledges funding from the National Science Foundation (CAREER-DMR-BMAT-1653418), and a 3M Non-Tenured Faculty Award. CE was supported in part by a Presidential Graduate Research Fellowship from Rensselaer Polytechnic Institute.

REFERENCES

[1] Kupferschmidt K. Resistance fighters. Science 2016; 352(6287): 758-61.
[2] Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. Nat Med 2004; 10(12)(Suppl.): S122-9.
[3] Levy SB. The challenge of antibiotic resistance. Sci Am 1998; 278(3): 46-53.
[4] Neu HC. The crisis in antibiotic resistance. Science 1992; 257(5073): 1064-73.
[5] Rossolini GM, Arena F, Pecile P, Pollini S. Update on the antibiotic resistance crisis. Curr Opin Pharmacol 2014; 18: 56-60.
[6] Furuya EY, Lowy FD. Antimicrobial-resistant bacteria in the community setting. Nat Rev Microbiol 2006; 4(1): 36-45.
[7] Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. Call of the wild: Antibiotic resistance genes in natural environments. Nat Rev Microbiol 2010; 8(4): 251-9.
[8] Centers for Disease Control and Prevention (CDC). Antibiotic / Antimicrobial Resistance. In: ed.^eds.
[9] World Health Organization (WHO). Antimicrobial Resistance: Global Report on Surveillance 2014. In: ed.^eds., 2014.
[10] Zasloff M. Antimicrobial peptides of multicellular organisms. Nature 2002; 415(6870): 389-95.
[11] Hancock RE, Lehrer R. Cationic peptides: A new source of antibiotics. Trends Biotechnol 1998; 16(2): 82-8.
[12] Ganz T. Chemistry. Rings of destruction. Nature 2001; 412(6845): 392-3.
[13] Kelley KJ. Using host defenses to fight infectious diseases. Nat Biotechnol 1996; 14(5): 587-90.
[14] Hancock RE, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol 2006; 24(12): 1551-7.
[15] Aoki W, Kuroda K, Ueda M. Next generation of antimicrobial peptides as molecular targeted medicines. J Biosci Bioeng 2012; 114(4): 365-70.
[16] Mookherjee N, Hancock RE. Cationic host defence peptides: innate immune regulatory peptides as a novel approach for treating infections. Cell Mol Life Sci 2007; 64(7-8): 922-33.
[17] Brogden KA. Antimicrobial peptides: Pore formers or metabolic inhibitors in bacteria? Nat Rev Microbiol 2005; 3(3): 238-50.
[18] Kohanski MA, Dwyer DJ, Collins JJ. How antibiotics kill bacteria: from targets to networks. Nat Rev Microbiol 2010; 8(6): 423-35.
[19] Hancock RE, Diamond G. The role of cationic antimicrobial peptides in innate host defences. Trends Microbiol 2000; 8(9): 402-10.
[20] Marr AK, Gooderham WJ, Hancock RE. Antibacterial peptides for therapeutic use: obstacles and realistic outlook. Curr Opin Pharmacol 2006; 6(5): 468-72.
[21] Wade D, Boman A, Wåhlin B, *et al.* All-D amino acid-containing channel-forming antibiotic peptides. Proc Natl Acad Sci USA 1990; 87(12): 4761-5.
[22] Shai Y. Mode of action of membrane active antimicrobial peptides. Biopolymers 2002; 66(4): 236-48.
[23] Kuroda K, Caputo GA. Antimicrobial polymers as synthetic mimics of host-defense peptides. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2013; 5(1): 49-66.
[24] Porter EA, Wang X, Lee HS, Weisblum B, Gellman SH. Non-haemolytic beta-amino-acid oligomers. Nature 2000; 404(6778): 565-5.
[25] Schmitt MA, Weisblum B, Gellman SH. Interplay among folding, sequence, and lipophilicity in the antibacterial and hemolytic activities of alpha/beta-peptides. J Am Chem Soc 2007; 129(2): 417-28.
[26] Patch JA, Barron AE. Helical peptoid mimics of magainin-2 amide. J Am Chem Soc 2003; 125(40): 12092-3.
[27] Ikeda T, Yamaguchi H, Tazuke S. New polymeric biocides: synthesis and antibacterial activities of polycations with pendant biguanide groups. Antimicrob Agents Chemother 1984; 26(2): 139-44.
[28] Kenawy R, Worley SD, Broughton R. The chemistry and applications of antimicrobial polymers: A state-of-the-art review. Biomacromolecules 2007; 8(5): 1359-84.
[29] Tashiro T. Antibacterial and bacterium adsorbing macromolecules. Macromol Mater Eng 2001; 286: 63-87.
[30] Dizman B, Elasri MO, Mathias LJ. Synthesis and antibacterial activities of water-soluble methacrylate polymers containing quaternary ammonium compounds. Journal of Polymer Science Part a-. Polym Chem 2006; 44: 5965-73.
[31] Tiller JC, Liao CJ, Lewis K, Klibanov AM. Designing surfaces that kill bacteria on contact. Proc Natl Acad Sci USA 2001; 98(11): 5981-5.
[32] Gelman MA, Weisblum B, Lynn DM, Gellman SH. Biocidal activity of polystyrenes that are cationic by virtue of protonation. Org Lett 2004; 6(4): 557-60.
[33] Tew GN, Liu D, Chen B, *et al.* De novo design of biomimetic antimicrobial polymers. Proc Natl Acad Sci USA 2002; 99(8): 5110-4.
[34] Kuroda K, DeGrado WF. Amphiphilic polymethacrylate derivatives as antimicrobial agents. J Am Chem Soc 2005; 127(12): 4128-9.
[35] Mowery BP, Lee SE, Kissounko DA, *et al.* Mimicry of antimicrobial host-defense peptides by random copolymers. J Am Chem Soc 2007; 129(50): 15474-6.
[36] Ganewatta MS, Tang CB. Controlling macromolecular structures towards effective antimicrobial polymers. Polymer (Guildf) 2015; 63: A1-A29.
[37] Tew GN, Scott RW, Klein ML, Degrado WF. De novo design of antimicrobial polymers, foldamers, and small molecules: from discovery to practical applications. Acc Chem Res 2010; 43(1): 30-9.
[38] Munoz-Bonilla A, Fernandez-Garcia M. Polymeric materials with antimicrobial activity. Prog Polym Sci 2012; 37: 281-339.
[39] Ren W, Cheng W, Wang G, Liu Y. Developments in antimicrobial polymers. J Polym Sci A Polym Chem 2017; 55: 632-9.
[40] Huang KS, Yang CH, Huang SL, Chen CY, Lu YY, Lin YS. Recent Advances in Antimicrobial Polymers: A Mini-Review. Int J Mol Sci 2016; 17(9): 17.
[41] Takahashi H, Palermo EF, Yasuhara K, Caputo GA, Kuroda K. Molecular design, structures, and activity of antimicrobial peptide-mimetic polymers. Macromol Biosci 2013; 13(10): 1285-99.
[42] Hu K, Schmidt NW, Zhu R, *et al.* A critical evaluation of random copolymer mimesis of homogeneous antimicrobial peptides. Macromolecules 2013; 46(5): 1908-15.
[43] Palermo EF, Vemparala S, Kuroda K. Antimicrobial Polymers: Molecular design as synthetic mimics of host-defense peptides. In: Scholz C, Kressler J, ed.^eds., Tailored polymer architectures for pharmaceutical and biomedical applications, 2013; pp. 319-330.
[44] Mizutani M, Palermo EF, Thoma LM, Satoh K, Kamigaito M, Kuroda K. Design and synthesis of self-degradable antibacterial polymers by simultaneous chain- and step-growth radical copolymerization. Biomacromolecules 2012; 13(5): 1554-63.
[45] Palermo EF, Vemparala S, Kuroda K. Cationic spacer arm design strategy for control of antimicrobial activity and conformation of amphiphilic methacrylate random copolymers. Biomacromolecules 2012; 13(5): 1632-41.

[46] Sovadinova I, Palermo EF, Urban M, Mpiga P, Caputo GA, Kuroda K. Activity and mechanism of antimicrobial peptide-mimetic amphiphilic polymethacrylate derivatives. Polymers (Basel) 2011; 3: 1512-32.

[47] Zimmerman LB, Worley BV, Palermo EF, *et al.* Absorbance-based assay for membrane disruption by antimicrobial peptides and synthetic copolymers using pyrroloquinoline quinone-loaded liposomes. Anal Biochem 2011; 411(2): 194-9.

[48] Avery CW, Palermo EF, McLaughlin A, Kuroda K, Chen Z. Investigations of the interactions between synthetic antimicrobial polymers and substrate-supported lipid bilayers using sum frequency generation vibrational spectroscopy. Anal Chem 2011; 83(4): 1342-9.

[49] Palermo EF, Lee DK, Ramamoorthy A, Kuroda K. Role of cationic group structure in membrane binding and disruption by amphiphilic copolymers. J Phys Chem B 2011; 115(2): 366-75.

[50] Sovadinova I, Palermo EF, Huang R, Thoma LM, Kuroda K. Mechanism of polymer-induced hemolysis: nanosized pore formation and osmotic lysis. Biomacromolecules 2011; 12(1): 260-8.

[51] Palermo EF, Kuroda K. Structural determinants of antimicrobial activity in polymers which mimic host defense peptides. Appl Microbiol Biotechnol 2010; 87(5): 1605-15.

[52] Palermo EF, Kuroda K. Chemical structure of cationic groups in amphiphilic polymethacrylates modulates the antimicrobial and hemolytic activities. Biomacromolecules 2009; 10(6): 1416-28.

[53] Palermo EF, Sovadinova I, Kuroda K. Structural determinants of antimicrobial activity and biocompatibility in membrane-disrupting methacrylamide random copolymers. Biomacromolecules 2009; 10(11): 3098-107.

[54] Kuroda K, Caputo GA, DeGrado WF. The role of hydrophobicity in the antimicrobial and hemolytic activities of polymethacrylate derivatives. Chemistry 2009; 15(5): 1123-33.

[55] Locock KE, Michl TD, Valentin JD, *et al.* Guanylated polymethacrylates: A class of potent antimicrobial polymers with low hemolytic activity. Biomacromolecules 2013; 14(11): 4021-31.

[56] Locock KE, Michl TD, Stevens N, *et al.* Antimicrobial Polymethacrylates Synthesized as Mimics of Tryptophan-Rich Cationic Peptides. ACS Macro Lett 2014; 3: 319-23.

[57] Punia A, He E, Lee K, Banerjee P, Yang NL. Cationic amphiphilic non-hemolytic polyacrylates with superior antibacterial activity. Chem Commun (Camb) 2014; 50(53): 7071-4.

[58] Chin W, Yang CA, Ng VW, *et al.* Biodegradable broad-spectrum antimicrobial polycarbonates: Investigating the role of chemical structure on activity and selectivity. Macromolecules 2013; 46: 8797-807.

[59] Nimmagadda A, Liu X, Teng P, *et al.* Polycarbonates with potent and selective antimicrobial activity toward gram-positive bacteria. Biomacromolecules 2017; 18(1): 87-95.

[60] Chakraborty S, Liu R, Lemke JJ, *et al.* Effects of cyclic vs. Acyclic hydrophobic subunits on the chemical structure and biological properties of nylon-3 Co-polymers. ACS Macro Lett 2013; 2(8): 753-6.

[61] Chen Y, Wilbon PA, Chen YP, *et al.* Amphipathic antibacterial agents using cationic methacrylic polymers with natural rosin as pendant group. RSC Advances 2012; 2: 10275-82.

[62] Zheng Z, Xu Q, Guo J, *et al.* Structure-antibacterial activity relationships of imidazolium-type ionic liquid monomers, Poly(ionic liquids) and poly(ionic liquid) membranes: Effect of alkyl chain length and cations. ACS Appl Mater Interfaces 2016; 8(20): 12684-92.

[63] Tejero R, López D, López-Fabal F, Gómez-Garcés JL, Fernández-García M. High efficiency antimicrobial thiazolium and triazolium side-chain polymethacrylates obtained by controlled alkylation of the corresponding azole derivatives. Biomacromolecules 2015; 16(6): 1844-54.

[64] Kenawy ER, Abdel-Hay FI, El-Shanshoury A, El-Newehy MH. Biologically active polymers. V. Synthesis and antimicrobial activity of modified poly(glycidyl methacrylate-co-2-hydroxyethyl methacrylate) derivatives with quaternary ammonium and phosphonium salts. J Polym Sci Part 2002; 40: 2384-93.

[65] Grace JL, Huang JX, Cheah SE, *et al.* Antibacterial low molecular weight cationic polymers: dissecting the contribution of hydrophobicity, chain length and charge to activity. RSC Advances 2016; 6(19): 15469-77.

[66] Paslay LC, Abel BA, Brown TD, *et al.* Antimicrobial poly(methacrylamide) derivatives prepared via aqueous RAFT polymerization exhibit biocidal efficiency dependent upon cation structure. Biomacromolecules 2012; 13(8): 2472-82.

[67] Álvarez-Paino M, Muñoz-Bonilla A, López-Fabal F, Gómez-Garcés JL, Heuts JP, Fernández-García M. Effect of glycounits on the antimicrobial properties and toxicity behavior of polymers based on quaternized DMAEMA. Biomacromolecules 2015; 16(1): 295-303.

[68] Zou G, de Leeuw E, Li C, *et al.* Toward understanding the cationicity of defensins. Arg and Lys versus their noncoded analogs. J Biol Chem 2007; 282(27): 19653-65.

[69] Abes R, Arzumanov A, Moulton H, *et al.* Arginine-rich cell penetrating peptides: design, structure-activity, and applications to alter pre-mRNA splicing by steric-block oligonucleotides. J Pept Sci 2008; 14(4): 455-60.

[70] Brock R. The uptake of arginine-rich cell-penetrating peptides: putting the puzzle together. Bioconjug Chem 2014; 25(5): 863-8.

[71] Sgolastra F, Deronde BM, Sarapas JM, Som A, Tew GN. Designing mimics of membrane active proteins. Acc Chem Res 2013; 46(12): 2977-87.

[72] Locock KE. Bioinspired polymers: Antimicrobial polymethacrylates. Aust J Chem 2016; 69: 717-24.

[73] Locock KE, Michl TD, Griesser HJ, Haeussler M, Meagher L. Structure-activity relationships of guanylated antimicrobial polymethacrylates. Pure Appl Chem 2014; 86: 1281-91.

[74] Ng VW, Tan JP, Leong JY, Voo ZX, Hedrick JL, Yang YY. Antimicrobial Polycarbonates: Investigating the Impact of Nitrogen-Containing Heterocycles as Quaternizing Agents. Macromolecules 2014; 47: 1285-91.

[75] Nonaka T, Hua L, Ogata T, Kurihara S. Synthesis of water-soluble thermosensitive polymers having phosphonium groups from methacryloyloxyethyl trialkyl phosphonium chlorides-N-isopropylacrylamide copolymers and their functions. J Appl Polym Sci 2003; 87: 386-93.

[76] Al-Badri ZM, Som A, Lyon S, Nelson CF, Nüsslein K, Tew GN. Investigating the effect of increasing charge density on the hemolytic activity of synthetic antimicrobial polymers. Biomacromolecules 2008; 9(10): 2805-10.

[77] Hong S, Takahashi H, Nadres ET, *et al.* A Cationic Amphiphilic Random Copolymer with pH-Responsive Activity against Methicillin-Resistant Staphylococcus aureus. PLoS One 2017; 12(1): e0169262.

[78] Sambhy V, Peterson BR, Sen A. Antibacterial and hemolytic activities of pyridinium polymers as a function of the spatial relationship between the positive charge and the pendant alkyl tail. Angew Chem Int Ed Engl 2008; 47(7): 1250-4.

[79] Punia A, Lee K, He E, *et al.* Effect of Relative Arrangement of Cationic and Lipophilic Moieties on Hemolytic and Antibacterial Activities of PEGylated Polyacrylates. Int J Mol Sci 2015; 16(10): 23867-80.

[80] Sellenet PH, Allison B, Applegate BM, Youngblood JP. Synergistic activity of hydrophilic modification in antibiotic polymers. Biomacromolecules 2007; 8(1): 19-23.

[81] Yang X, Hu K, Hu G, *et al.* Long hydrophilic-and-cationic polymers: A different pathway toward preferential activity against bacterial over mammalian membranes. Biomacromolecules 2014; 15(9): 3267-77.

[82] Punia A, Mancuso A, Banerjee P, Yang NL. Nonhemolytic and antibacterial acrylic copolymers with hexamethyleneamine and poly(ethylene glycol) side chains. ACS Macro Lett 2015; 4: 426-30.

[83] Colak S, Nelson CF, Nüsslein K, Tew GN. Hydrophilic modifications of an amphiphilic polynorbornene and the effects on its hemolytic and antibacterial activity. Biomacromolecules 2009; 10(2): 353-9.

[84] Mowery BP, Lindner AH, Weisblum B, Stahl SS, Gellman SH. Structure-activity relationships among random nylon-3 copolymers that mimic antibacterial host-defense peptides. J Am Chem Soc 2009; 131(28): 9735-45.

[85] Qiao Y, Yang C, Coady DJ, Ong ZY, Hedrick JL, Yang YY. Highly dynamic biodegradable micelles capable of lysing Gram-positive and Gram-negative bacterial membrane. Biomaterials 2012; 33(4): 1146-53.

[86] Chen CZ, Beck-Tan NC, Dhurjati P, van Dyk TK, LaRossa RA, Cooper SL. Quaternary ammonium functionalized poly(propylene imine) dendrimers as effective antimicrobials: structure-activity studies. Biomacromolecules 2000; 1(3): 473-80.

[87] Kanazawa A, Ikeda T, Endo T. Polymeric phosphonium salts as a novel class of cationic biocides. 2. Effects of couter anion and molecular-weight on antibacterial activity of polymeric phosphonium salts. Polym Chem 1993; 31: 1441-7.

[88] Panarin EF, Solovskii MV, Zaikina NA. G. E. Biological activity of cationic polyelectrolytes. Makromol Chem 1985; 9: 25-33.

[89] Michl TD, Locock KE, Stevens NE, *et al.* RAFT-derived antimicrobial polymethacrylates: elucidating the impact of end-groups on activity and cytotoxicity. Polym Chem 2014; 5: 5813-22.

[90] Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol 2010; 8(9): 623-33.

[91] Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: A common cause of persistent infections. Science 1999; 284(5418): 1318-22.

[92] Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents 2010; 35(4): 322-32.

[93] Smith K, Hunter IS. Efficacy of common hospital biocides with biofilms of multi-drug resistant clinical isolates. J Med Microbiol 2008; 57(Pt 8): 966-73.

[94] Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet 2001; 358(9276): 135-8.

[95] Römling U, Balsalobre C. Biofilm infections, their resilience to therapy and innovative treatment strategies. J Intern Med 2012; 272(6): 541-61.

[96] Davies D. Understanding biofilm resistance to antibacterial agents. Nat Rev Drug Discov 2003; 2(2): 114-22.

[97] Wu H, Moser C, Wang HZ, Høiby N, Song ZJ. Strategies for combating bacterial biofilm infections. Int J Oral Sci 2015; 7(1): 1-7.

[98] Qu Y, Locock K, Verma-Gaur J, Hay ID, Meagher L, Traven A. Searching for new strategies against polymicrobial biofilm infections: guanylated polymethacrylates kill mixed fungal/bacterial biofilms. J Antimicrob Chemother 2016; 71(2): 413-21.

[99] Takahashi H, Nadres ET, Kuroda K. Cationic amphiphilic polymers with antimicrobial activity for oral care applications: Eradication of S. mutans Biofilm. Biomacromolecules 2017; 18(1): 257-65.

[100] Oda Y, Kanaoka S, Sato T, Aoshima S, Kuroda K. Block versus random amphiphilic copolymers as antibacterial agents. Biomacromolecules 2011; 12(10): 3581-91.

[101] Thoma LM, Boles BR, Kuroda K. Cationic methacrylate polymers as topical antimicrobial agents against Staphylococcus aureus nasal colonization. Biomacromolecules 2014; 15(8): 2933-43.