Emerging Antimicrobial Research against Superbugs: Perspectives from a Polymer Laboratory

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Infectious diseases caused by drug-resistant microorganisms have become a major contributor for human morbidity and mortality. To overcome such threats, we have developed various antimicrobial agents using natural product derivatives and metallopolymers. Abundant biomass such as resin acids can be utilized to prepare cationic polymers for inhibiting a variety of bacteria. These polymers have been used in solution as well as surfaces as antimicrobial materials with low cytotoxicity. In addition, a class of charged metallopolymers have been developed to kill superbugs such as MRSA.

INTRODUCTION

The natural evolution of organisms has made the biosphere exceptionally diverse. Although most of evolutionary events are not evidently visible to us, an observable environmental evolution is the adaptation of highly dynamic populations of microorganisms such as bacteria toward antibiotics. Constant exposure of bacteria to various classes of antimicrobials over the last few decades has made a deadly army of multidrug-resistant bacteria, also called superbugs, which threaten the wellbeing of humans worldwide. Most antimicrobial agents target specific sites in the pathogen cells, where many types of resist mechanisms are developed to evade them (Figure 1).

Despite increasing occurrence of resistance, the discovery of novel antimicrobial agents has a sluggish growth. Therefore, it is urgent to explore new approaches to solving this pressing issue. Among many targets of bacterial cells, the cell membrane is thought to be a promising candidate that would have less likelihood of developing resistance.[2] Hydrophobic alkyl chain containing cationic surfactants or detergents are membrane damaging agents that are widely used for sterilizing purposes. However, most of them show nonspecific toxicity towards mammalian cells and are inefficient to selectively destroy multidrug-resistant bacteria. In recent years, more attention is given to membrane-active cationic antimicrobial polymers that show excellent antimicrobial activity while maintaining negligible or no toxicity towards host cells.[1, 3-5]

In our research endeavors, we navigate two paths to develop novel and effective antimicrobial agents with low toxicity. In one approach, we utilize naturally occurring products such as resin acids to make cationic polymers with tunable amphiphilicity to selectively target bacterial membranes. On the other hand, we have developed synthetic cationic metallopolymers (metal-containing polymers) that could be conjugated with traditional antibiotics against multidrug resistant bacteria. This short review summarizes our own research efforts in innovating antimicrobial polymers from the point view of a polymer laboratory.

Antimicrobials by Natural Resin Acids

Resin acids are abundant natural products mostly produced by conifer trees in the form of oleoresin or rosin. The worldwide production exceeds 1 million tons per year.[6, 7] They consist of diterpene-based acids such as abietic, levopimaric, and pimaric acids. We have developed various types of resin acid-derived polymers that showed excellent biodegradability and biocompatibility as well as good mechanical properties.[8-10] The bulky hydrophenanthrene rings of resin acids serve as hydrophobic building blocks that can be incorporated into the hydrophobic core of bacterial lipid membranes. In one of the studies, abietic acid was

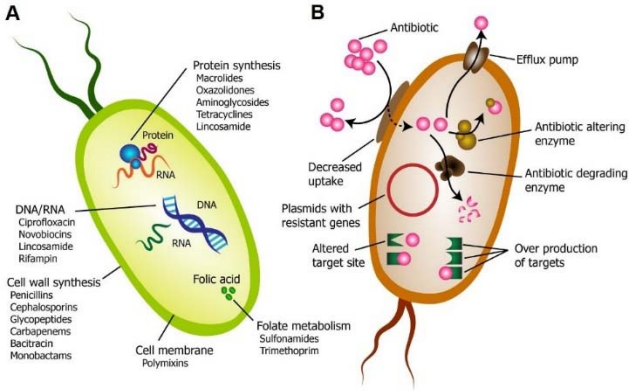
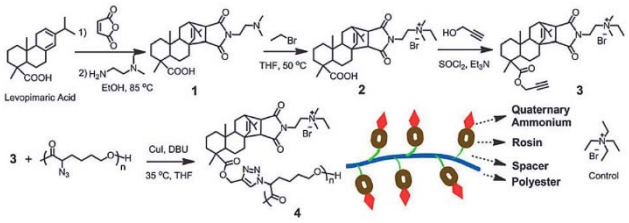


Figure 1. (A) Typical antibiotic target sites present in bacterial cells, and (B) mechanisms of antibiotic resistance. Reproduced with permission from Elsevier. Copyright © 2015 Elsevier Ltd.[1]

modified into a quaternary ammonium containing compound that showed outstanding activity against a range of bacteria and high selectivity against bacteria over mammalian cells.[11] As shown in Scheme 1, the synthesis involved a Diels–Alder reaction between levopimaric acid and maleic anhydride to produce maleopimaric acid, followed by amidation and quaternization reactions. This cationic compound was used to functionalize an azide-containing polycaprolactone polymer via azide-alkyne 'click' chemistry, which resulted in a cationic polymer.

The antimicrobial activities of the resin acid-derived cationic compounds and polymers were investigated against a range of pathogenic and non-pathogenic microorganisms. The results obtained for the disk-diffusion assays are shown in Figure 2.



Scheme 1. Synthesis of quaternary ammonium-containing resin acid-derived antimicrobial compounds and polymers. Reproduced by permission of The Royal Society of Chemistry.[11]

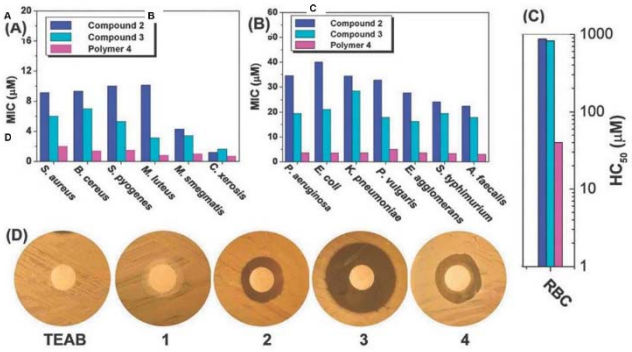


Figure 2. Resin acid-derived antimicrobial materials against various pathogens tested via a disk-diffusion method. Minimum inhibitory concentrations for (A) Gram-positive and (B) Gram-negative bacteria; (C) Images of agar plates obtained from the disk-diffusion assay. TEAB = tetraethylammonium bromide. Reproduced by permission of The Royal Society of Chemistry.[11]

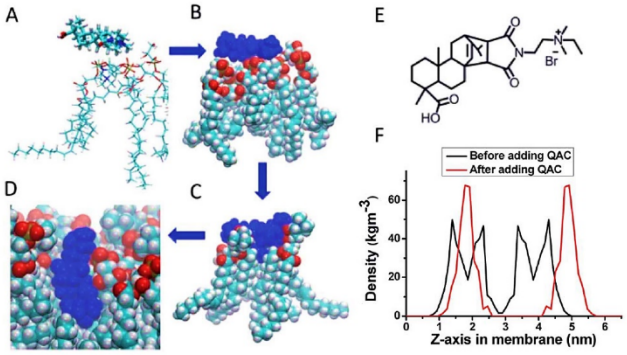


Figure 3. (A – D) Molecular dynamics simulations snapshots of four stages depict cationic compound binding to lipid molecules in the model anionic membrane; (E) the resin-acid derived compound; (F) Plot of partial densities of lipid phosphate head groups along the Z - axis, before and after addition of the compound to the system. Reproduced by permission of The Royal Society of Chemistry.[12]

In a more recent study, the antimicrobial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA), a complex of multidrug-resistant Gram-positive bacterial strains, was evaluated.[12] The results were unexpectedly promising with minimum inhibitory concentrations as low as 5.0 μg/mL for the cationic compound and 9.9 μg/mL for the polymer. The biocompatibility of these resin-acid derived materials was tested against mouse red blood cells and splenocytes. The results proved that the cationic materials are largely non-toxic against mammalian cells. Molecular dynamics simulations (Figure 3) and dye-leakage assays indicated a membrane-lysing effect. We believe that this is induced by the unique fused ring structures of resin acids that may be the underlying action for selective lysis of bacterial cells over mammalian cells.

In the previous studies, the cationic charge was designed to locate at the periphery of a polymer. The effect of the cationic moiety located between the polymer backbone and the hydrophobic rosin moiety on antimicrobial efficacy was also investigated.[13] These novel copolymers (PDMAEM-*g*-rosin) with controlled molecular weight and narrow molecular weight distribution were prepared via reversible-addition fragmentation transfer (RAFT) polymerization and functionalized using quaternization reactions. These cationic polymers exhibited effective antibacterial activities against *Escherichia coli* and *S. aureus* (Figure 4) with the activities dependent on both the degree of quaternization of rosin group, the molecular weight of copolymers as well as the conformation of hydrophobic group. In a control study, PDMAEMA-*g*-eicosane having a linear alkyl chain instead of the rosin moiety was used for the antimicrobial assays. It showed no activity against *E. coli* and only exhibited weak activity against *S. aureus*, suggesting that the activity of PDMAEMA-*g*-rosin was due to not only the hydrophobicity of rosin, but also its unique fused-ring structure.

Antibacterial, antibiofilm, and biocompatible properties of surface-immobilized, quaternary ammonium-containing, resin acid-derived compounds and polycations were recently reported by our group.[14] As shown in the Figure 5, the cationic compound and its methacrylate monomer was prepared to modify surface of a substrate.

Copper-catalyzed azide-alkyne 1,3-dipolar cycloaddition click reaction and surface-initiated atom transfer radical polymerization were respectively used as chemical tools to graft compounds and

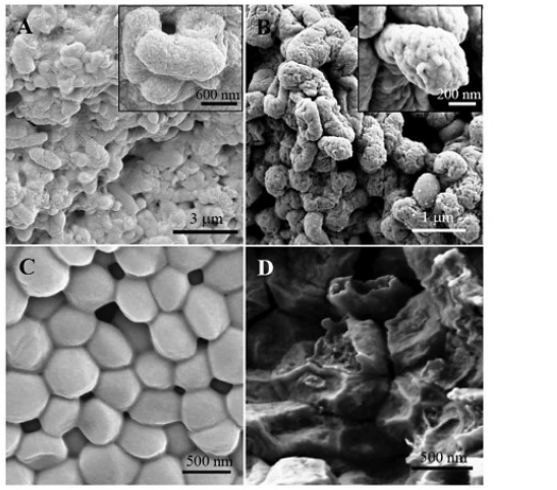


Figure 4. FE-SEM images of *E. coli* (A, B) and *S. aureus* (C, D) bacterial cells before (left) and after (right) treated with PDMAEM-g-rosin copolymers. Loss of cellular integrity indicated the damages to the cells resulted from the polymers. Reproduced by permission of The Royal Society of Chemistry.[13]

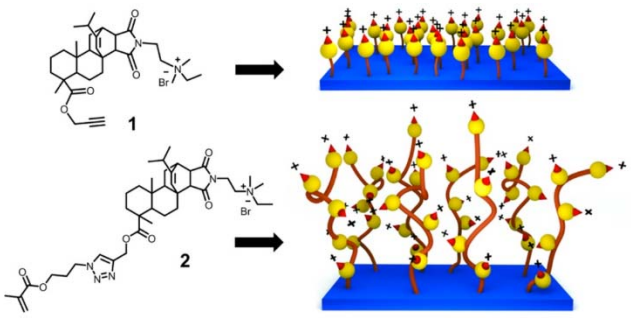


Figure 5. Surface modification with resin acid derived cationic compounds and polymers. Reprinted with permission.[14] Copyright (2015) American Chemical Society.

polymers from the substrate surface. Antibacterial and antibiofilm activities were tested against Gram-positive *S. aureus* and Gram-negative *E. coli* (Figure 6).

In addition to damaging bacteria that attach to the surfaces, these modified surfaces damaged planktonic bacterial cells in solution that came in contact with them. Furthermore, the biofilm growth on these cationic surfaces was considerably reduced as demonstrated by CDC bioreactor assays. An improved proliferation of human fibroblast cells on the surfaces indicated good biocompatibility of these coating materials.

Antimicrobials by Metal-Containing Polymers

Another major initiative in our laboratory is focused on synthetic metallopolymers in both synthetic methodology and medicinal chemistry. We discovered that cationic metallocene-containing polymers and their bioconjugates with conventional antibiotics are a new class of antimicrobial agents against multidrug-resistant bacteria.[15] Due to the exceptional ability of cationic cobaltocenium moieties to complex with carboxylate anions, cationic cobaltocenium-containing methacrylate polymers were prepared and complexed with various *β*-lactam antibiotics, including penicillin, ampicillin, amoxicillin, and cefazolin via ion-pairing complexation (Figure 8). These conjugated metallopolymers were tested against MRSA. Disc-diffusion assays indicated that metallopolymers at a concentration of 1-2.2 μM and most of the antibiotics alone showed very little inhibition, while their bioconjugates exhibits significantly enhanced inhibition against different strains of MRSA. Mechanistic investigation revealed that these conjugated metallopolymers show high efficiency in reducing *β*-lactamase activity and lyse bacterial cells effectively. The *β*-lactamase enzyme and cell walls of the bacteria are attacked by the metallopolymers, and at the same time, the conjugated antibiotics are protected via forming ion-pairs with cationic cobaltocenium-containing polymers (Figure 7).

These antibiotic-metallopolymer bioconjugates demonstrated high resistance toward *β*-lactamase-induced hydrolysis and significantly improved efficacy against various strains of MRSA cells compared to conventional antibiotics. In addition, these metallopolymers, at higher concentrations (≥ 5 μM), also showed excellent antimicrobial activities against different strains of MRSA by selectively disrupting bacterial cell membranes. Although these cationic metallopolymers exhibited excellent efficiency against multidrug-resistant MRSA, they showed negligible hemolytic effects on red blood cells and minimal *in vitro* and *in vivo* toxicity.

In another study, we synthesized metallopolymer-containing hydrogels (hydrogels are water swelling polymeric networks) for healthcare applications.[16] Cationic cobaltocenium-containing hydrogels were tested for antimicrobial activities against various bacteria, including drug-resistant strains. These hydrogels also had the similar ability to disrupt negatively charged cell walls of bacteria. We found that the metallopolymer hydrogel at a concentration of 20 mg/mL showed inhibition of the growth of Gram-negative *E. coli* (90% inhibition), Gram-positive *S. aureus* (80% inhibition), and hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA, 80% inhibition). In addition, these hydrogels were also capable of absorbing antibiotics from contaminated water, due to the ability of cobaltocenium moieties to bind with *β*-lactam antibiotics.

CONCLUSIONS

In summary, we demonstrated that pathogenic bacteria including multidrug resistant superbugs can be effectively eliminated by multiple strategies that we developed in a polymer laboratory. More research will be carried out toward clinical applications of these next-generation antimicrobial biomaterials.

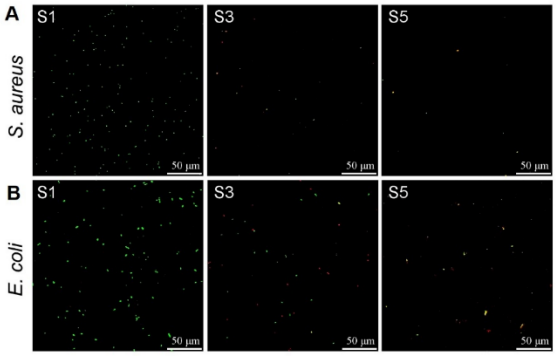


Figure 6. Antimicrobial assay results. Stained (Live/Dead stain) surfaces after 24 h incubation with (A) *S. aureus* and (B) *E. coli.* Green cells indicate live bacteria colonizing the surface, while dead cells appear in red color. S1: pristine surface; S3: compound 1-grafted; and S5: polymer-grafted surface. Reprinted with permission.[14] Copyright (2015) American Chemical Society.

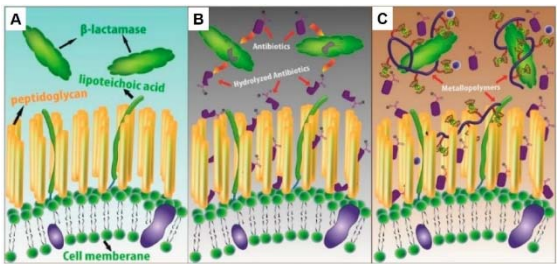


Figure 7. Illustration of several key interactions involving *β*-lactamase and *β*-lactam antibiotics. (A) MRSA cell surface; (B) typical *β*-lactamase hydrolysis of *β*-lactam antibiotics; and (C) Proposed interactions between *β*-lactam antibiotic−metallopolymer bioconjugates, *β*-lactamase, and cell wall. Reprinted with permission.[15] Copyright (2015) American Chemical Society.

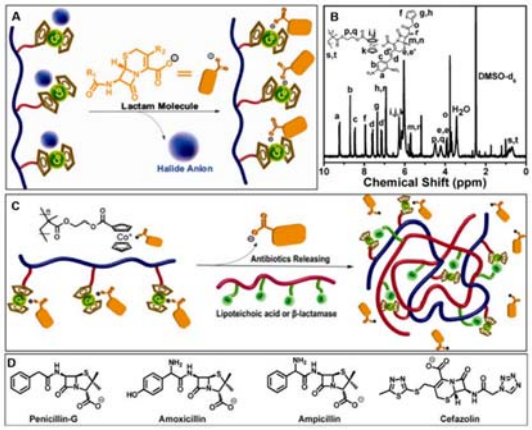


Figure 8. (A) Formation of ion-pairs between *β*-lactam antibiotics and cationic cobaltocenium-containing polymers; (B) Four *β*-lactam antibiotics used in this study; (C) Antibiotic release from antibiotic-metallopolymer ion-pairs via lipoteichoic acid or *β*-lactamases. Reprinted with permission.[15] Copyright (2015) American Chemical Society.

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Notes and References

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1. Ganewatta, M. S.; Tang, C., *Polymer* **2015,** *63*, A1-A29.
2. Mingeot-Leclercq, M.-P.; Decout, J.-L., *MedChemComm* **2016,** *7*, 586-611.
3. Rodríguez-Hernández, J., Polymers Against Microorganisms. In *Polymers against Microorganisms: On the Race to Efficient Antimicrobial Materials*, Springer International Publishing: Cham, 2017; pp 1-11.
4. Engler, A. C.; Wiradharma, N.; Ong, Z. Y.; Coady, D. J.; Hedrick, J. L.; Yang, Y.-Y., *Nano Today* **2012,** *7*, 201-222.
5. Santos, M.; Fonseca, A.; Mendonça, P.; Branco, R.; Serra, A.; Morais, P.; Coelho, J., *Materials* **2016,** *9*, 599.
6. Yao, K.; Tang, C., *Macromolecules* **2013,** *46*, 1689-1712.
7. Wilbon, P. A.; Chu, F.; Tang, C., *Macromol. Rapid Commun.* **2013,** *34*, 8-37.
8. Yao, K.; Wang, J.; Zhang, W.; Lee, J. S.; Wang, C.; Chu, F.; He, X.; Tang, C., *Biomacromolecules* **2011,** *12*, 2171-2177.
9. Wilbon, P. A.; Zheng, Y.; Yao, K.; Tang, C., *Macromolecules* **2010,** *43*, 8747-8754.
10. Ganewatta, M. S.; Ding, W.; Rahman, M. A.; Yuan, L.; Wang, Z.; Hamidi, N.; Robertson, M. L.; Tang, C., *Macromolecules* **2016,** *49*, 7155-7164.
11. Wang, J.; Chen, Y. P.; Yao, K.; Wilbon, P. A.; Zhang, W.; Ren, L.; Zhou, J.; Nagarkatti, M.; Wang, C.; Chu, F.; He, X.; Decho, A. W.; Tang, C., *Chem. Commun.* **2012,** *48*, 916-918.
12. Ganewatta, M. S.; Chen, Y. P.; Wang, J.; Zhou, J.; Ebalunode, J.; Nagarkatti, M.; Decho, A. W.; Tang, C., *Chem. Sci.* **2014,** *5*, 2011-2016.
13. Chen, Y.; Wilbon, P. A.; Chen, Y. P.; Zhou, J.; Nagarkatti, M.; Wang, C.; Chu, F.; Decho, A. W.; Tang, C., *RSC Adv.* **2012,** *2*, 10275-10282.
14. Ganewatta, M. S.; Miller, K. P.; Singleton, S. P.; Mehrpouya-Bahrami, P.; Chen, Y. P.; Yan, Y.; Nagarkatti, M.; Nagarkatti, P.; Decho, A. W.; Tang, C., *Biomacromolecules* **2015,** *16*, 3336-3344.
15. Zhang, J.; Chen, Y. P.; Miller, K. P.; Ganewatta, M. S.; Bam, M.; Yan, Y.; Nagarkatti, M.; Decho, A. W.; Tang, C., *J. Am. Chem. Soc.* **2014,** *136*, 4873-4876.
16. Zhang, J.; Yan, J.; Pageni, P.; Yan, Y.; Wirth, A.; Chen, Y.-P.; Qiao, Y.; Wang, Q.; Decho, A. W.; Tang, C., *Sci. Rep.* **2015,** *5*, 11914.