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Facial Amphiphilicity-Induced Polymer Nanostructures for Antimicrobial Applications

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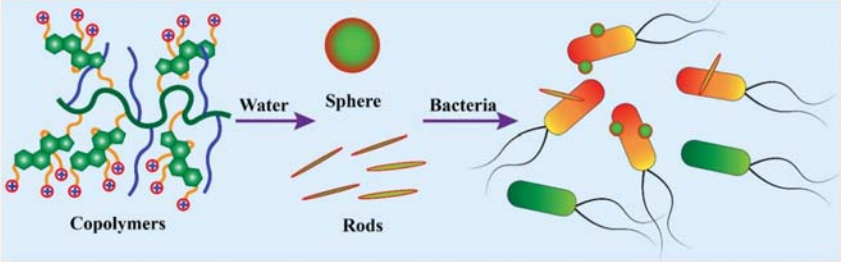

ABSTRACT: New antimicrobial agents are needed to address ever-increasing antimicrobial resistance and a growing epidemic of infections caused by multidrug resistant pathogens. We design nanostructured antimicrobial copolymers containing multicyclic natural products that bear facial amphiphilicity. Bile acid based macromolecular architectures of these nanostructures can interact preferentially with bacterial membranes. Incorporation of polyethylene glycol into the copolymers not only improved the colloidal stability of nanostructures but also increased the biocompatibility. This study investigated the effects of facial amphiphilicity, polymer architectures, and self-assembled nanostructures on antimicrobial activity. Advanced nanostructures such as spheres, vesicles, and rod-shaped aggregates are formed in water from the facial amphiphilic cationic copolymers via supramolecular interactions. These aggregates were particularly interactive toward Gram-positive and Gram-negative bacterial cell membranes and showed low hemolysis against mammalian cells.

KEYWORDS: *antimicrobial nanostructures, facial amphiphilicity, self-assembly, bile acids, gradient copolymers, charge density, biocompatibility*

INTRODUCTION

Antibiotic resistance is among the world's most urgent public health problems. According to Centers for Disease Control (CDC), at least 2 million people get infected, and at least 23 000 people die every year in the United States as a result of bacterial infections, especially those caused by multidrug resistant (MDR) bacteria.[1−3] The ever-increasing crisis of bacterial resistance to traditional antibiotics is a puzzling issue in battling infectious diseases.[1,4] Multidrug resistant Gram-negative bacteria are among the most dangerous.[5] The presence of double membranes in Gram-negative bacteria acts as an impermeable barrier to many antibiotics.[6]

Natural host defense peptide (HDP) mimics show broad-spectrum antimicrobial activities that typically involve a membrane-disruptive mechanism and are more difficult for bacteria to build resistance.[7−9] These peptides/polymers form α-helical- or β-sheet-like structures upon electrostatic interaction with negatively charged cell membranes, followed by insertion of their hydrophobic residues into the nonpolar membrane core, resulting in membrane permeabilization. These structures are globally segregated with cationic moieties on one side and lipophilic groups at the other side, also referred to as facial amphiphilicity, which facilitates effective insertion into bacterial membranes.[2,7−14] Extensive research has been done to develop facial amphiphilic antimicrobial polymers by the groups of DeGrado,[15−18] Gellman,[19] Tew,[20,21] Kuroda,[22] and Tang[23,24] Hedrick and Yang and many others developed polycations carrying quaternary ammonium and guanidium groups that exhibit broad-spectrum antimicrobial activities.[25−27] Several antimicrobial polymers with bulky hydrophobic structures (such as rosin acids) and antibiotic-metal bioconjugates are also developed.[28−31] Recently, we developed a new class of cationic polymers with strong antibacterial activity against Gram-negative bacteria, where each repeating unit possesses local facial amphiphilicity and promotes efficient interactions with bacterial cell membranes.[23]

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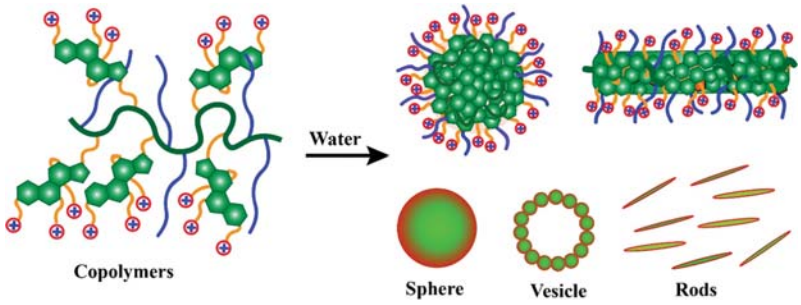
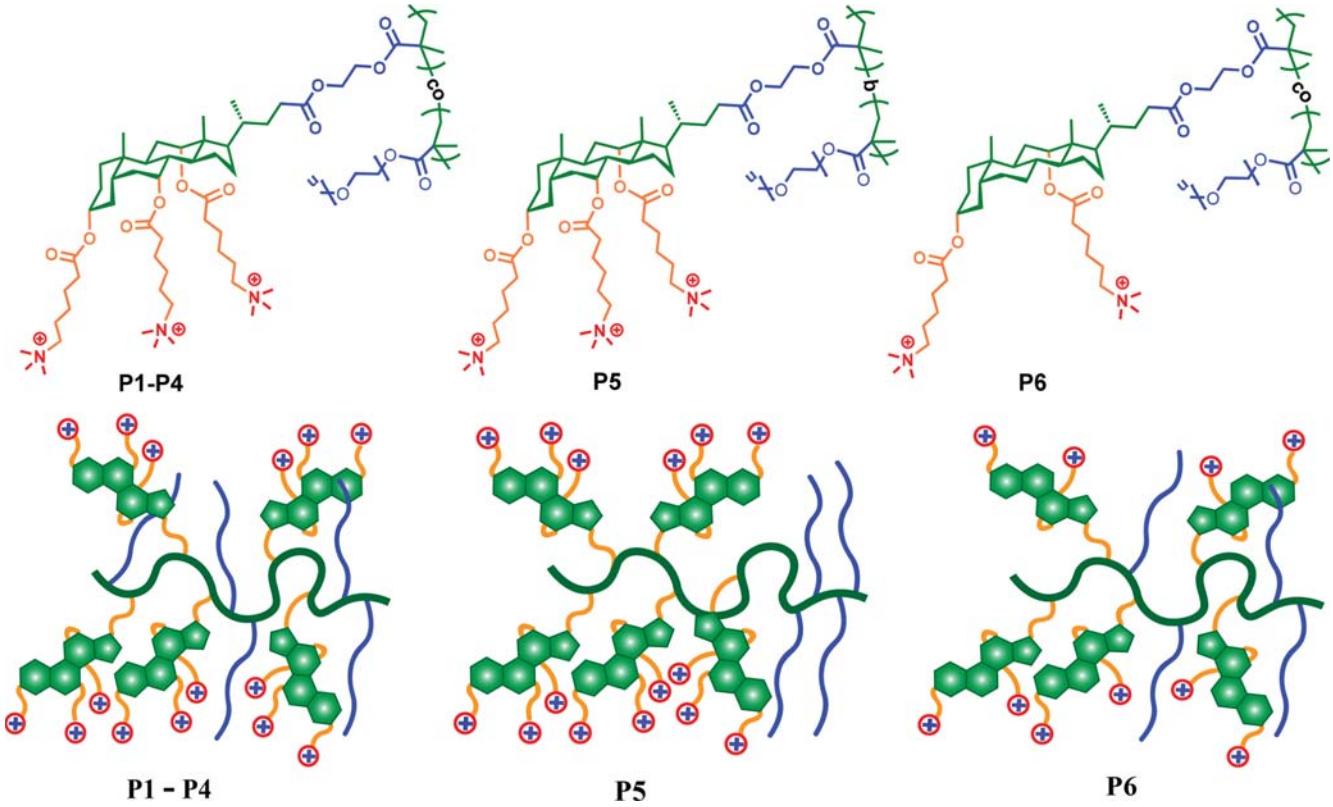


Figure 1. Multicyclic natural product-based cationic copolymers form sphere, rod, and vesicle type aggregates in water.

Scheme 1. Compositional Structures and Their Illustrations of Cholic Acid Based Gradient Copolymers (P1–P4), Diblock Copolymer (P5), and Deoxycholic Acid Based Copolymer (P6)[a]



[a]The cholic acid based gradient copolymers P1–P4 consist of 10, 30, 42, and 53 mol % PEG, respectively. The cholic acid containing diblock copolymer P5 has 43 mol % PEG, and deoxycholic acid based copolymers have 44 mol % PEG.

However, most of these HDP-mimicking antimicrobial polymers do not exhibit better selectivity toward bacterial cell membrane due to the effect of toxicity against mammalian cell.

Antimicrobial activity and biocompatibility of synthetic polymers are largely dictated by different structural parameters such as the balance of hydrophilic to hydrophobic moieties, facial amphiphilicity, molecular weight, nature of the charges, and polymer architectures.[23,32–38] To increase the selectivity of antimicrobial polymers toward bacteria, the effects of polymer architectures (such as homopolymers, block, statistical and gradient copolymers) have also been investigated.[39–41] The precise control on comonomer sequence plays an important role on the antimicrobial activity and hemocompatibility.[42] The effect of cationic and hydrophobic functionalities on antimicrobial polymers over their selectivity between bacteria and mammalian cells has been also investigated.[37] Increasing efforts have been devoted to the preparation of polymer nanoparticles or self-assembling materials with high antibacterial activity and improved biocompatibility against mammalian cells.[6,43–49]

Amphiphilic copolymers comprising hydrophilic and hydrophobic segments can self-assemble in water to form a wide variety of aggregates such as spheres, rods, and vesicles, where the hydrophobic portion forms the core and the hydrophilic segment rearranges toward the surroundings and stabilizes the aggregates.[50–52] These self-assembly behaviors largely depend on the polymer chain length, sequence, composition, and balance of hydrophobic and hydrophilic segments.[51–55] There are a wide variety of supramolecular interactions such as hydrophobic interactions, hydrogen bonding, van der Waals forces, and electrostatic interactions involved in the self-

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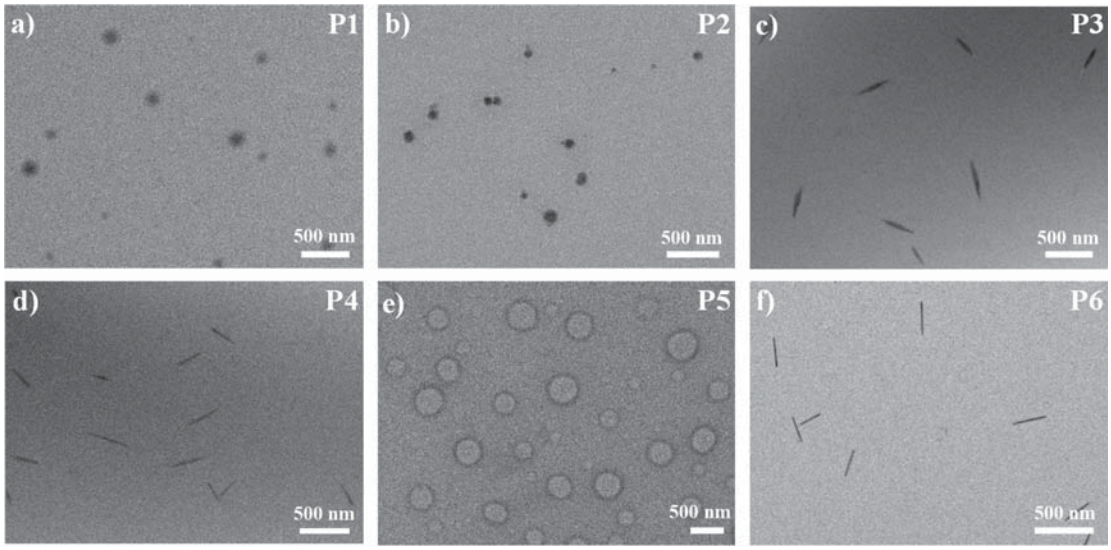

Figure 2. TEM images of copolymers (a) P1, (b) P2, (c) P3, (d) P4, (e) P5, and (f) P6. TEM images were taken from water solutions at a concentration of 5 mg/mL after aging for 24 h at 37 °C.

assembly of amphiphilic copolymers. The self-assembly process and morphologies formed by these weak interactions, can be substantially influenced by environmental conditions. Recently, we developed facial amphiphilicity-induced self-assembly (FAISA) of amphiphilic copolymers toward controlled nanostructures such as spheres, rods, and vesicles.[56] While our prior work initially focused on the effect of local facial amphiphilicity in cationic homopolymers on antimicrobial efficacy,[23] herein we hypothesized that nanostructured polymers with facial amphiphilicity and biocompatibility can be a promising agent for combating MDR bacteria because the formation of nanostructures significantly increases the local mass and cationic charge density of macromolecules. These factors could possibly result in the enhanced ability for continuous disruption of bacterial membranes particularly Gram-negative bacteria, while simultaneously demonstrating low toxicity against red blood cells (RBCs).

Specifically, we chose a multicyclic facial amphiphilic bile acid and a neutral polyethylene glycol (PEG) component to make amphiphilic copolymers that can form nanosized particles. These amphiphilic copolymers formed spheres, rods, and vesicles (Figure 1). The antimicrobial activity of different nanosized aggregates were also studied. One of the bile acid derivatives named cholic acid was chosen because the presence of large cross-sectional hydrophobic multicyclic hydrocarbons on the convex β-face, and multiple cationic charges on the concave α-face) provides local facial amphiphilicity.[57,58] We synthesized quarternary ammonium charge (QAC) containing cholic acid based facial amphiphilic copolymers with a tunable PEG component to balance hydrophilicity and hydrophobicity. To explore the effect of charge density and the level of facial amphiphilicity of the nanosized aggregates, another bile acid derivatives named deoxycholic acid was also chosen. The facial amphiphilicity, biocompatibility, and self-aggregation of bile acid and PEG components make them attractive as antimicrobial nano-objects. The antimicrobial activity of different polymer architectures such as diblock, and gradient copolymers was investigated.

RESULTS AND DISCUSSION

Synthesis of Amphiphilic Copolymers. Given the presence of a bulky size hydrophobic diterpenoid structure, bile acid based cationic copolymers can self-assemble into nanostructures in a selective solvent. To tune the balance of hydrophilicity and hydrophobicity in these copolymers, a series of amphiphilic copolymers bearing hydrophobic multicyclic units and hydrophilic PEG were prepared. A cholic acid based methacrylate monomer, (2-methacryloyloxy)ethyl cholate (MAECA), and PEG-containing methacrylate monomer (PEGMA, M_n = 500 Da) were copolymerized via reversible addition−fragmentation chain transfer (RAFT) polymerization and followed by postmodification to yield quaternary ammonium-containing copolymers, according to a method recently reported by us (Schemes 1 and S1).[23] Interestingly, these copolymers (P1−P4) have a gradient composition and can self-assemble in water.[56] In addition to gradient copolymers, a diblock copolymer (P5) with 43% mol PEGMA was synthesized in order to investigate the effect of polymer architecture on the antimicrobial activity. The preparation of P5 is shown in the reaction in Scheme S1c, where sequential RAFT polymerization was used, and subsequent postpolymerization modification was carried out to obtain the quaternized block copolymer. Deoxycholic acid based copolymer (leveled as P6) with 56% mol MAEDA was also synthesized to study the charge density effect on antimicrobial activity.

Self-Assembly Behaviors. All copolymers self-assembled into different morphologies in deionized water at 37 °C, which were visualized by transmission electron microscopy (TEM). Copolymers with 90 and 70 mol % cholic acid (P1 and P2) produced spherical structures with an average diameter of ∼150−350 nm. On the other hand, copolymers P3 and P4 with 58 and 47 mol % cholic acid exhibited rodlike structures, as shown in Figure 2. The diblock copolymer P5 formed a large vesicle-like morphology. Deoxycholic acid based copolymer P6 with two charges at each multicyclic unit formed rodlike structures. The hydrodynamic diameter (D_h) and zeta potential of these nanostructures were measured by dynamic light scattering (DLS). All copolymers showed the D_h

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ACS Appl. Mater. Interfaces XXXX, XXX, XXX−XXX

Table 1. CMC, MIC, HC_{50}, and Selectivity of Bile Based Cationic Copolymers

			minimum inhibitory concentration (MIC) (μg/mL)[a]						
polymers	bile acid (mol %)	CMC (μg/mL)	*E. coli* (ATCC-11775)	*P. aeruginosa* (ATCC-10145)	*E. coli* (ATCC-BAA-197)	*S. aureus* (ATCC-33591)	HC_{50} (μg/mL)	HC_{50}/MIC *E. coli* (ATCC-11775)	HC_{50}/MIC *S. aureus* (ATCC-33591)
P1	90	1.2	12.8	10.2	12.8	25.6	744	58	29
P2	70	1.0	18.6	25.6	19.2	18.6	>1000	>54	>54
P3	58	1.5	25.6	38.4	25.6	12.8	>1000	>39	>78
P4	47	1.0	51.2	102.4	51.2	38.4	>1000	>19	>26
P5	57	NT	51.2	102.4	51.2	>102.4	662	13	6
P6	56	NT	51.2	38.4	51.2	>102.4	411	8	4

[a]MIC was determined by a broth microdilution method. NT = Not tested.

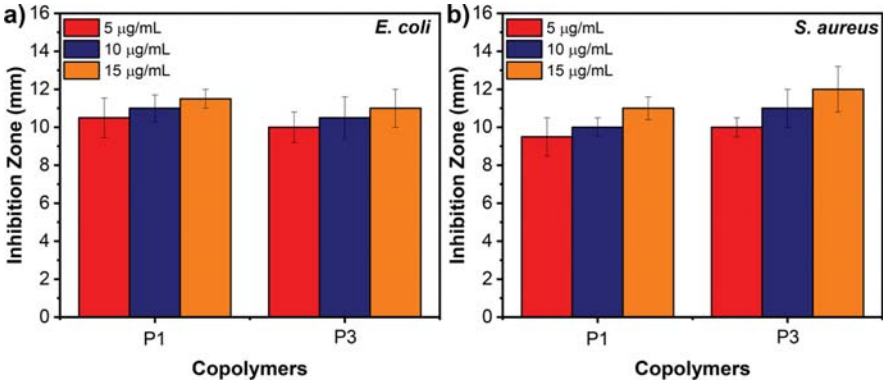

Figure 3. Inhibition zone measurements of copolymers P1 and P3 were determined by the disc diffusion method against (a) *E. coli* and (b) *S. aureus*. The samples were dissolved in DMSO at concentrations of 5, 10, and 15 μg/mL.

in the range of 180−400 nm, confirming the formation of aggregates in water (Figure S1). The positive zeta potentials were in the range of 45−70 mV (Table S1), indicating that outer surfaces of aggregates are covered by the QAC groups. The zeta potential values decreased with the increase of PEG fraction.

The critical micelle concentration (CMC) of cholic acid based copolymers was measured following a previously reported method.[24] Pyrene was used as a hydrophobic fluorescent probe. The ratio of intensities of vibronic bands at 390 and 374 nm (I_{390}/I_{374}) increased sharply with the increase of polymer concentrations, indicating the pyrene encapsulation into the hydrophobic core of polymer micelles (Figure S6). The CMC for the copolymers was determined to be in the range of 1−1.5 μg/mL. These results indicate that the hydrophobicity of cholic acids plays an important role in the formation of nanoaggregates.

Antimicrobial Activities. Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* were chosen for the investigation of antimicrobial activities of bile acid based cationic copolymers. Recently, we demonstrated that bile acid based facially amphiphilic homopolymers are potent antimicrobial agents, especially toward Gram-negative bacteria.[23] Here, we evaluated the antimicrobial activity of different nanostructured aggregates prepared from bile acid based amphiphilic copolymers. All copolymer aggregates were incubated at 37 °C and then evaluated for in vitro antimicrobial activity by determining their minimum inhibitory concentrations (MICs). The MICs of all copolymers were determined by a broth microdilution method following our previous report, as shown in Table 1.[23] The spherical shaped nanoparticles formed by P1 and P2 copolymers appeared to be highly efficient in inhibiting the growth of Gram-negative bacteria compared to other copolymers. The MIC values of P1 copolymer against *E. coli* and *P. aeruginosa* are 12.8 and 10.2 μg/mL, respectively. The rodlike aggregates formed by the copolymer P3 showed strong antimicrobial activity against Gram-positive bacteria, whereas P4 exhibited less activity against *S. aureus*. The MIC value against *S. aureus* decreased with subsequent increases of the PEG fraction in the copolymers up to 42 mol %, above which the activity decreased. The copolymers with lower PEG fractions exhibited better potency against Gram-negative bacteria and showed less activity against Gram-positive bacteria. All MIC values are significantly higher than the CMCs, suggesting that it is the aggregates instead of individually solvated polymer to kill bacteria. The MIC results (Table 1) demonstrated that increasing the amount of neutral PEG ratio in the copolymers resulted in a loss of antibacterial activity, which was also observed by others.[59] Consequently, the selective interactions with bacterial membranes largely depends on the delicate balance of hydrophobicity and hydrophilicity of antimicrobial polymers. We also observed that the double headed QAC charge-containing copolymer (P6) with the same PEG feed ratio was less sensitive toward Gram-positive and Gram-negative bacteria than P3 with three headed QAC charges. The copolymers with higher charge densities could lead to more effective interactions with bacterial membranes, and thus enhanced antimicrobial behavior. Under the same fraction of cholic acid, the gradient copolymers showed higher sensitivity against both Gram-positive and Gram- negative bacteria than the block copolymer.

The block copolymer P5 exhibited the weakest activity against bacteria, likely due to the formation of larger aggregates in water. The antimicrobial activity of copolymers was further

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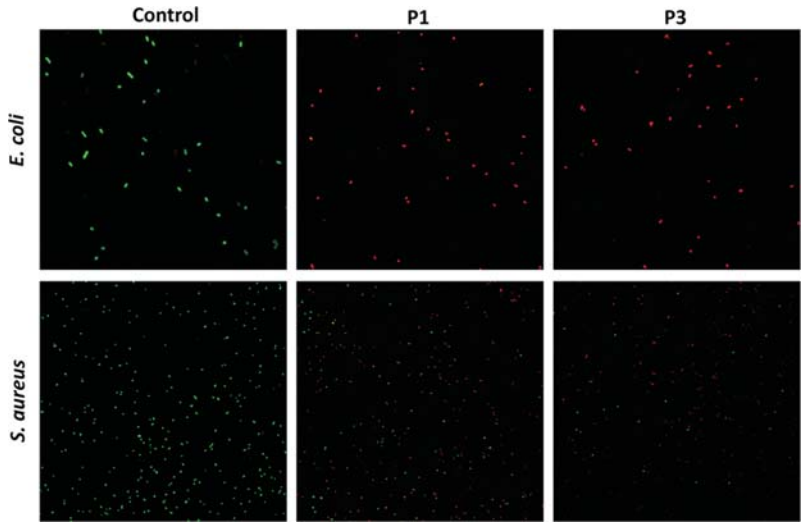

Figure 4. CLSM images of *E. coli* and *S. aureus* under control, P1, and P3 treatments with a concentration 2× the MIC. The concentration of bacteria was 1.0×10^6 CFU/mL. Bacterial solutions without copolymer were used as the control.

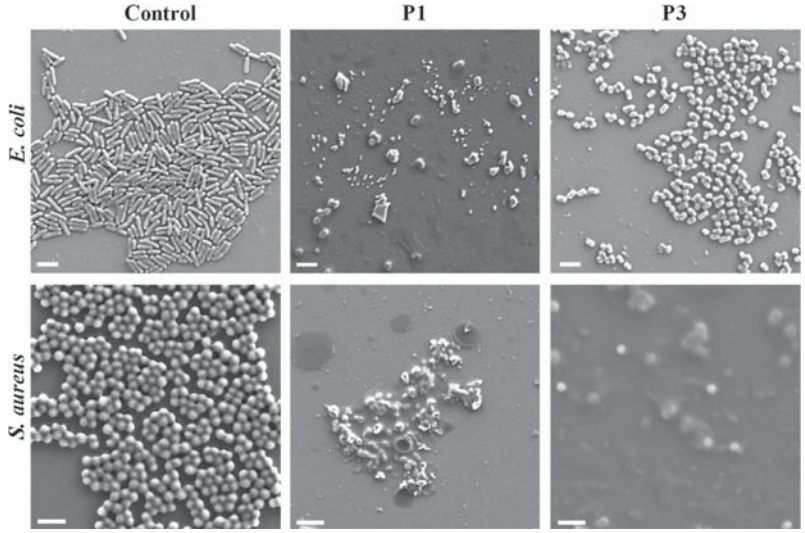

Figure 5. SEM images of *E. coli* and *S. aureus* under control, P1, and P3 treatment with a concentration 2× the MIC. The concentration of bacteria was 1.0×10^6 CFU/mL. Bacterial solutions without copolymer were used as the control.

explored against clinically isolated MDR bacteria such as *E. coli* (ATCC-BAA-197). The MIC results indicated the similar activity of all copolymers. Overall, the higher MIC values for copolymers with the larger size of aggregates may be due to the impeding penetration through the bacterial cell membrane. Another reason could be the shielding of QAC groups by the PEG corona, reducing the targeting ability of copolymers.

As all copolymers formed aggregates in water, we also studied the antimicrobial activity of the copolymers after fully dissolved in solution. DMSO is a good solvent for these types of copolymers and does not show any toxicity against bacteria up to 30 μg (Figure S5). P1 and P3 copolymers were dissolved in DMSO and tested for their antimicrobial activities against one Gram-positive, *S. aureus* (ATCC 33591), and two different Gram-negative bacteria such as *E. coli* (ATCC 11775) and *P. aeruginosa* (ATCC-10145), by the conventional agar disc diffusion assay (Figures 3 and S4). These copolymers inhibit the growth of bacteria and form a clear zone around the disc. The inhibition zone indicates the ability of the test samples to kill the bacteria. Interestingly the large inhibition zones of P1 and P3 copolymers against bacteria indicated that the copolymers were active against all bacterial strains (Figures 3 and S3). Therefore, the antimicrobial activity largely depends on a number of factors such as polymer compositions, charge density, and hydrophobic–hydrophilic balance, on top of the self-assembled nanostructures.

The kinetics of antibacterial activity of P1 and P3 copolymers were investigated to determine how rapid the copolymers can kill bacteria. *S. aureus* and *E. coli* were selected for the time killing assay. The aqueous solution of copolymers (30 μL) with a concentration of 2× MIC was added to 96-well plates. Then, 170 μL of bacteria solution in TSB medium (OD600 = 1.00) was added to each well. The OD600 values of the bacterial solution were monitored from 0 min to 6 h at 30 min intervals. As shown in Figure S2, the copolymers started to kill bacteria instantaneously, leading to the significant reduction of bacteria concentration. The OD600 values decreased from 1.0 to 0.6 after 1 h, while the OD600 values of bacteria in the control group increased to 1.2. The copolymers P1 and P3 showed significant antimicrobial

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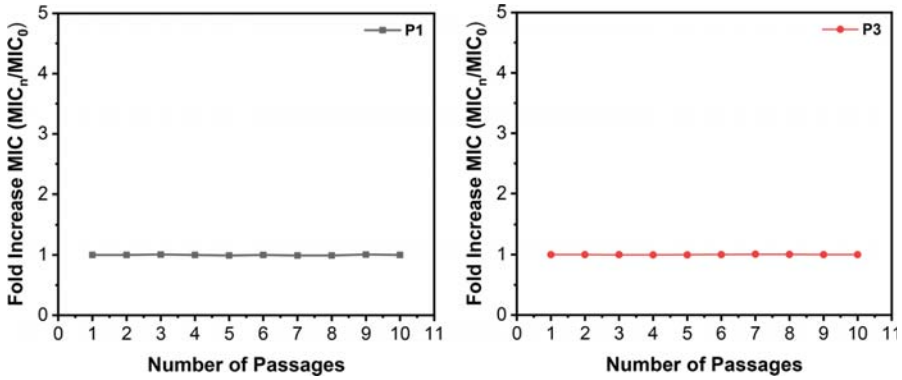

Figure 6. Drug resistance study of P1 and P3 copolymers against *E. coli* exposed to multiple sub-MIC treatments.

efficiency between 2 and 4 h, while OD600 values reached to zero for both *S. aureus* and *E. coli*. With a concentration of just two times greater than the MIC, all bacteria were completely killed within this short period of time. When the concentration was increased to four times than the MIC, it could kill all bacteria approximately within 1 h.

The inhibition effects of copolymers P1 and P3 against *E. coli* and *S. aureus* were further visualized by confocal laser scanning microscopy (CLSM). The bacteria were first stained with LIVE/DEAD BacLight dye, and then the viability was visualized via CLSM (Figure 4). The bacterial membrane permeability changed before and after treatment with P1 and P3. The 2× MIC concentration of copolymers was used for this assay. Control bacteria showed green-colored cells, disclosing that most cells were alive with intact bacterial cell membranes, as shown in Figure 4. On the other hand, only dead bacteria generated red fluorescence as the propidium iodide (PI) in the BacLight dye can penetrate only cells with compromised membranes.[47] The confocal images indicated that P1 and P3 can kill almost all of the *E. coli* but not all of the *S. aureus*. The copolymer P3 is more potent than P1 against *S. aureus* as observed. These results also demonstrated that the bile acid based cationic copolymers are more active against Gram-negative bacteria and generate substantial bacterial membrane disruption.

Morphological changes of bacterial cells using scanning electron microscopy (SEM) could provide insights on the antimicrobial mechanism of action of these polymeric nanoparticles. *E. coli* and *S. aureus* remained unbroken with smooth surfaces and displayed an integrated rod or spherical morphology in control bacteria solution, as shown in Figure 5, whereas copolymer-treated cells were significantly fragmented and damaged from their original morphology. We observed that P1 and P3 aggregates disrupted the bacterial membranes and produced numerous cell fragments. The SEM images also suggested that the copolymers killed the bacteria by a mechanism of membrane disruption. The damaged bacterial cell membranes also suggest that the multiple nanoaggregates interact with the same bacteria cell membranes simultaneously and cause the bacteria death.

Bacteria are able to develop resistance against conventional antibiotics or small molecular drugs through repeated use of drugs or extended contact of bacteria with nonlethal dose. It is well-known that peptide-mimicking antimicrobial polymers are less prone to induce resistance due to their membrane disruptive mechanism. In order to demonstrate the potential of the cholic acid based cationic copolymers, we studied the antimicrobial resistance for two cholic acid based cationic copolymers, P1 and P3, against *E. coli*. At a sub-MIC level, *E. coli* was exposed to the copolymers for multiple times, and the MIC was determined for every consecutive exposure/passages. No significant increase in the MIC values was observed after 10 passages, as shown in Figure 6. These results demonstrated that development of resistance against cholic acid based cationic copolymer nanostructures is difficult for *E. coli*. In comparison, some of common antibiotics, e.g., ciprofloxacin, showed an increase of the MIC value after a few passages with *E. coli* as the target bacteria.[27,38]

Hemolytic Activities. The hemolysis activity of bile acid derived cationic copolymers was assessed by determining hemoglobin release from mouse red blood cells (RBCs) at numerous concentrations. Recently, we found out that bile acid based cationic homopolymers exhibited some toxicity because bile acid derivatives are intrinsically hydrophobic due to the presence of a large four fused-ring structure.[23] To overcome these issues, neutral PEG was incorporated into the copolymers to increase the hydrophilicity of bile acid based copolymers. The results showed that increasing the composition of neutral PEG reduced hemolysis activity. The HC_{50}, the concentration that causes 50% hemolysis of RBCs, was measured for all copolymers (Figure 7). The HC_{50} values for copolymers P1−P4 are 744, >1000, >1000, and >1000 μg/mL, respectively, demonstrating that the PEG can increase the biocompatibility by increasing the hydrophilicity of the copolymers. On the other hand, diblock copolymer P5 with

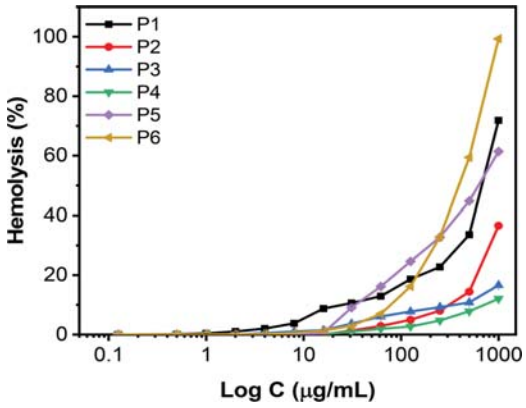

Figure 7. Hemolysis activity of copolymers measured by hemoglobin release from mouse RBCs at various concentrations (error bars are in the data points, but very small).

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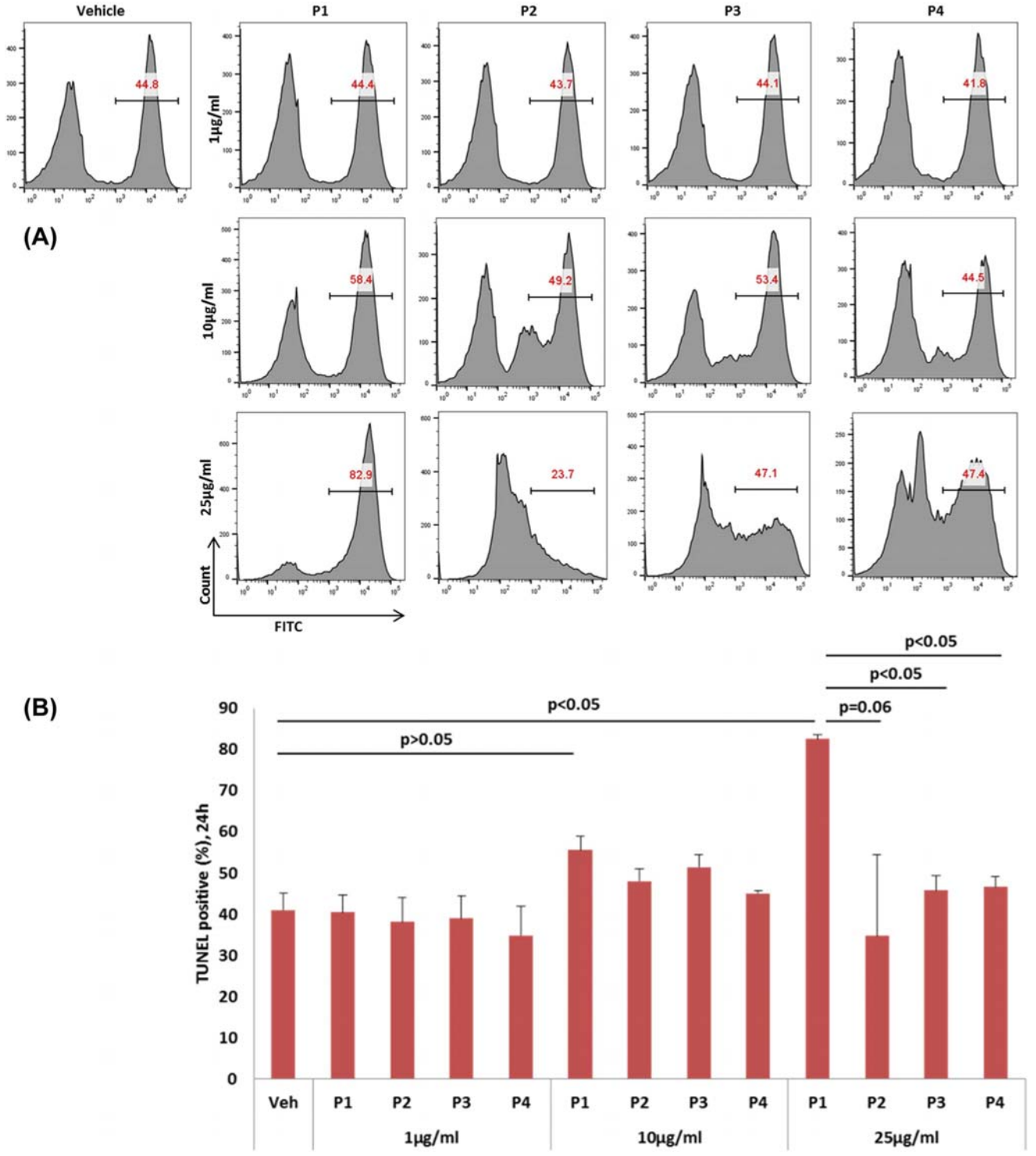

Figure 8. Freshly isolated splenocytes from mice were cultured with copolymers at various concentrations (A, left label), for 24 h, and TUNEL assay was performed to detect apoptosis of cells by flow cytometry. The first panel shows TUNEL positive percentage in vehicle treated cells as a negative control for the assay. P1−P4 indicates the copolymers used for the test. Three different concentrations were tested for the assay. The highest concentration is based on the corresponding MICs obtained on bacteria. The values indicate TUNEL positive percentages. Panel B shows the mean ± SEM of TUNEL positive percentages of samples in triplicate as bar graph. *P* values were obtained by using Student's *t* test.

40 mol % PEG composition showed a lower HC_{50} value compared to P3. These results indicated the importance of charge segregation upon the interaction with RBCs. The hemolytic activity of P6 is comparatively high due to higher hydrophobicity with a lower charge density.

The selectivity of copolymers for bacterial cells over mammalian cells was calculated by the ratio of HC_{50} values to MIC values (HC_{50}/MIC) (Table 1). All cholic acid based cationic copolymers displayed very negligible hemolysis at their respective MIC values, indicating excellent selectivity toward a broad range of pathogenic microbes over mammalian

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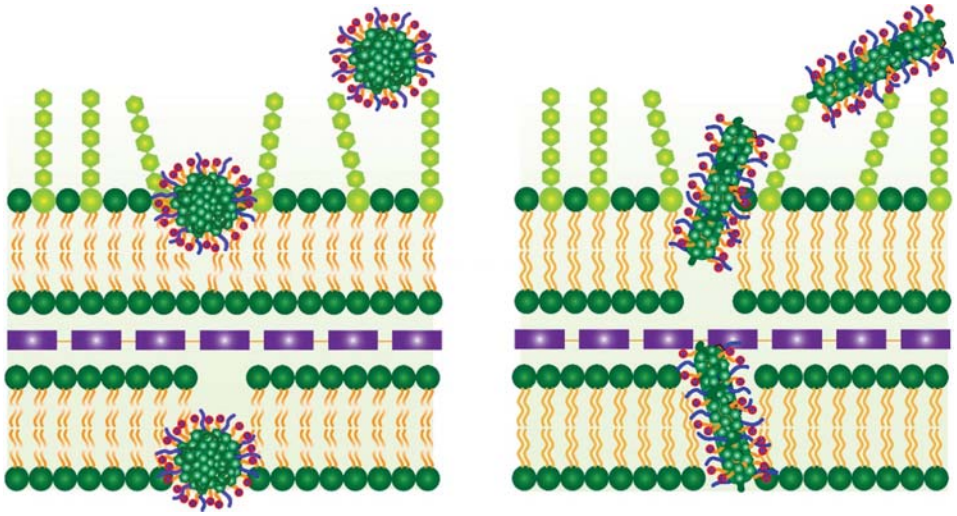

Figure 9. Proposed mechanism of action of bile acid based amphiphilic copolymers derived spherical and rodlike aggregates on the Gram-negative bacterial cell membrane.

cells. The hemolysis and antimicrobial activity results of the copolymers suggested that the selectivity increased with the increasing PEG ratio in the gradient copolymers. The selectivity decreased with the lowering of charge density, e.g., P6. The gradient copolymers exhibited higher selectivity toward bacterial cell membrane compared to diblock copolymers. The selectivity results suggested that polymer architectures also play a vital role in designing potential antimicrobial polymers and their nanostructure. The sequence control comonomer, facial amphiphilicity, and morphology of core−shell nanosized particles altogether play a rule to afford high selectivity toward the bacterial cell membrane.

Apoptosis in Immune Cells. The main aim for performing the apoptotic cell death assay was to determine whether the copolymers had toxic effects on immune cells. As indicated in Figure 8, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay indicated that all polymers at 1 μg/mL did not induce apoptosis in the splenocytes when compared to the vehicle-treated cells. At 10 μg/mL concentration, however, the polymers showed a slight increase in the percentage of TUNEL positive cells. We increased the concentration of copolymers up to 25 μg/mL based on the observed MIC values on bacterial strains. At this concentration, only P1 showed a very high percentage of TUNEL positive cells, whereas we did not observe much difference in the percentage of TUNEL positive cells following treatment with other copolymers when compared to vehicle-treated cells. TUNEL assay is a sensitive technique to detect apoptosis as it indicates DNA fragmentation which is a marker of cell death. Our observations, which the cells did not undergo death at 1 μg/mL, implied that these polymers are safe at this concentration for immune cells. Furthermore, even upon increasing the concentration of polymers to 25 μg/mL, most copolymers except for P1 did not induce profound cell apoptosis, again indicating that these PEG copolymers are safe at a concentration similar to MICs. However, we understand that it will be important to check the toxicity by performing in vivo tests and analyze the effects on various organs and tissues as the copolymers might have different effects based on cell type.

Mechanism of Action. A plausible mechanism is proposed for the action of spherical and rodlike aggregates upon contact with Gram-negative bacteria, which is shown in Figure 9. The cationic nanostructures attach to the surface through adsorption and diffuse through the outer cell membrane. Then cationic facial amphiphilic moieties are attached to the cytoplasmic membrane and disrupt the membrane. The bile acid based local facial amphiphilic structures would coordinate with each other through the outer membrane and further facilitate the entire macromolecule to penetrate through the plasma membrane. The loss of cytoplasmic membrane components leads the cell death. The nanostructures with high surface area and high charge density are critical for efficient adsorption onto cells.

CONCLUSIONS

In summary, we reported facial amphiphilicity-induced nanostructures formed by amphiphilic copolymers of bile acid and neutral PEG fractions, which showed a broad spectrum of antimicrobial activity against both Gram-positive and Gram-negative bacteria. Spheres and rod-shaped aggregates were particularly interactive toward Gram-positive and Gram-negative bacterial cell membranes and showed low hemolysis against mammalian cells. Incorporation of PEG improved the biocompatibility of bile acid based cationic copolymers. From this study, the monomer distribution along the copolymers appeared to have an impact on antibacterial activity as gradient copolymers showed better antimicrobial activity than block copolymers. Three QAC charged cholic acid containing copolymers showed better efficacy than double-headed deoxycholic acid containing copolymers did. These self-assembled macromolecular structures may open a new pathway to develop the next-generation antimicrobial agents to treat bacterial infectious diseases.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.9b19712.

Materials and detailed experimental methods; procedures for CMC and MIC measurements, disc diffusion assay, drug resistance study DLS, and zeta potential data (PDF)

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Notes

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ACS Appl. Mater. Interfaces XXXX, XXX, XXX−XXX