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Polycarbonates with Potent and Selective Antimicrobial Activity toward Gram-Positive Bacteria

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Abstract

The resistance developed by life-threatening bacteria toward conventional antibiotics has become a major concern in public health. To combat antibiotic resistance, there has been a significant interest in the development of antimicrobial cationic polymers due to the ease of synthesis and low manufacturing cost compared to host-defense peptides (HDPs). Herein, we report the design and synthesis of amphiphilic polycarbonates containing primary amino groups. These polymers exhibit potent antimicrobial activity and excellent selectivity to Gram-positive bacteria, including multi-drug resistant pathogens. Fluorescence and TEM studies suggest that these polymers are likely to kill bacteria by disrupting bacterial membranes. These polymers also show low tendency to elicit resistance in bacteria. Their further development may lead to new antimicrobial agents combating drug-resistance.

Graphical Abstract

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ASSOCIATED CONTENT

Supporting Information

NMR spectra of polymers. The Supporting Information is available free of charge on the ACS Publications website.

INTRODUCTION

Bacterial infections pose a great threat to the public health.¹ The World Health Organization (WHO) recently acknowledged that certain bacterial pathogens have acquired significant resistance to most of the commercially available antibiotics.² In hospitals about 50–60% of the infections are caused by the lack of the sterility of medical devices used during surgeries, especially due to the contamination of notorious methicillin-resistant *staphylococcus aureus* (MRSA) strains. The Center for Disease Control (CDC) has reported that in the United States 2 million people develop antibiotic resistance after antibiotic treatment, and 23,000 people die due to the lack of proper treatment annually. Therefore, there is an escalating demand for the development of new antibacterial agents to combat the emerging resistance.³

One promising approach to circumvent bacterial resistance is to develop derivatives of host-defense peptides (HDPs). Although antimicrobial mechanisms of HDPs are still in debate, it is widely accepted that these cationic peptides fold into discrete secondary structures such as α -helices or β -sheets upon binding to bacterial membranes, on which distinct hydrophobic and cationic patches form on the peptides.⁴ The cationic groups of HDPs bind to the negatively charged bacterial cell surface by electrostatic attraction, while the hydrophobic patch interacts with hydrophobic lipid bilayer of the bacteria, leading to penetration of the peptides. It is known that eukaryotic cells mainly contain zwitterionic phospholipids such as phosphatidylcholine, phosphatidylethanolamine and sphingomyelin on their outer leaflet of membranes, whereas the negative charged lipids are largely sequestered in the inner leaflet of the membranes. As a result, these cells generally carry a net neutral charge on their surface. In contrast, both Gram-positive and Gram-negative bacteria bear a negative charge on their membrane surface due to the presence of phospholipids including phosphatidylglycine, phosphatidylserine and cardiolipin, making them more selective to cationic HDPs over mammalian cells due to charge attraction.⁵ In addition, Gram-positive bacteria have a thick peptidoglycan layer embedded with teichoic and lipoteichoic acids,⁶ whereas lipopolysaccharides (LPS) are an important component on the outer membranes of Gram-negative bacteria. These molecules all further contribute to the overall negative charge on the bacterial membranes. Another factor aiding to the selectivity of HDPs towards bacterial cells is that bacteria lack cholesterol in their membranes, whereas mammalian cells have 25% cholesterol or more in their membranes, which stabilizes their membrane integrity.⁷ As such, disruption of bacterial membranes is relatively less challenging. The disruption of the bacterial cell membranes causes the leakage of the cytoplasmic contents and ultimately cell death.⁸ As this biophysical interaction lacks defined membrane proteins and other targets, development of resistance is believed to be more challenging.⁹ Therefore,

HDPs and their derivatives have been extensively explored for antimicrobial development.^{10,10b} However, HDPs have intrinsic drawbacks such as tedious and costly synthesis and purification. The pharmacokinetic properties and chemical instability of the peptides are other obstacles that hamper therapeutic applications of HDPs.⁷ Moreover, HDPs exhibit moderate selectivity and are reported to be toxic toward mammalian cells.¹ One of the successful examples of HDPs is magainin II,¹¹ which exhibits broad-spectrum but weak antimicrobial activity against bacteria. The synthetic analog of magainin, Pexiganan (also known as MSI-78) has shown much improved activity and entered Phase III clinical trials for the treatment of diabetic foot ulcers. However, it failed eventually due to its moderate in vivo efficacy and high cost.¹² Due to drawbacks of HDPs, considerable effort has been extended to develop cationic antimicrobial polymers that mimic the function of HDPs, in the hope to supplement the potential application of HDPs. Compared to HDPs, polymers have some apparent advantages. The procedure for the preparation of polymers is generally very straightforward. Most of the polymer synthesis involves one pot polymerization reaction, which makes it easy to scale up to obtain products in large quantity.¹³ In addition, polymers are cost-effective. Examples of antimicrobial polymers include poly (α -amino acid)s,¹³ metallopolymer,¹⁴ nylon-3 polymers,¹⁵ polyacrylates,¹⁶ polyvinyl pyridines,¹⁷ polystyrenes,¹⁸ polycarbonates,¹⁹ etc. Similar to HDPs, these polymers generally exert their activity by acting on bacterial membranes.

Yang and Hedrick et al recently reported cationic antimicrobial polycarbonates containing quaternary ammonium salts.^{20,21} These quaternary ammonium moieties were introduced through post-modification.²² Our previously findings suggest that antimicrobial agents having primary amino groups could have potent antimicrobial activity.^{10,10a} It is thus intriguing to study antibacterial activity of polycarbonates bearing same groups. Herein, we report the design and investigation of antimicrobial polycarbonates containing primary amino groups. These polycarbonates eliminate the post-modification step in their preparation. Surprisingly, they display potent and selective antimicrobial activity against clinically relevant Gram-positive bacteria, and show virtually no toxicity to blood cells under the tested condition.

MATERIALS AND METHODS

Synthesis of the monomer 1

The 5-methyl-2-oxo-1,3-dioxane-5-carbonyl chloride (MDC) was prepared according to the previous reported work by Yang and Hedrick.²² To synthesize the monomer **1**, MDC (6.8 g, 38 mmol) was dissolved in 30 mL DCM in a 100 mL round bottom flask, to which a solution of Boc-protected ethanolamine (6.15 g, 38 mmol) and TEA (7.85 mL, 57 mmol) in 10 mL DCM was added drop wise. The reaction was allowed to continue for 4 h in an ice bath. The solution was washed with 1 N HCl (100 mL $\times$ 3), water (75 mL $\times$ 3), brine (50 mL $\times$ 1), and then dried over sodium sulfate. The solvent was removed in vacuo to give a yellow colored oil, which was further purified by flash chromatography (ethyl acetate/hexane 2:1) to give the final product monomer **1** (6.9 g, 22 mol, 60 %) as a white solid.

ssSynthesis of the monomer 2

The monomer **2** was prepared according to the previous reported protocol by Yang and Hedrick.²²

Synthesis of polycarbonate polymers

All the polymers were prepared in a similar fashion using ring opening polymerization²¹ (Scheme 3 and Table 1). In order to synthesize Boc-protected random copolymer polycarbonate **P6'** which was formed by the mixed monomers of **1** and **2**, the initiator benzyl alcohol (0.05 g, 0.46 mmol) was dissolved in 10 mL of DCM in a N² purged round bottom flask. Hydrophobic monomer **2** (2.31 g, 9.0 mmol, 20 eq) and hydrophilic monomer **1** (2.8 g, 9.0 mmol, 20 eq) were added to the flask together (Scheme 3A), followed by addition of 1-(3,5-bis(trifluoromethyl)-phenyl)-3-cyclohexyl-2-thiourea catalyst (TU) (0.34 g, 0.9 mmol, 2 eq) and (1,8-diazabicyclo [5.4.0] undec-7-ene (DBU) (0.14 g, 0.9 mmol, 2 eq). The reaction was allowed to stir for 4 h under nitrogen, and then quenched benzoic acid (1.1 eq, 0.06 g, 0.5 mmol). The other Boc-protected random copolymers were prepared in the similar way.

For the synthesis of Boc-protected di-block polymers **P4'**, the two monomers **1** and **2** were added in two batches separately in order to form two segments in the polymers (Scheme 3B). In brief, the Boc protected monomer **1** was first added to the reaction vessel in the presence of TU and DBU in 20 mL DCM and the reaction was allowed to run for 4 h under nitrogen. Subsequently, the hydrophobic monomer **2** was added and the reaction was allowed to continue for another 4 h. The reaction was quenched by benzoic acid at the end.

Dialysis of polymers P1'-P8'

The polymers were dissolved in 2 mL methanol and dialyzed against methanol (dialysis tubing MWCO=3000) for three days, with methanol being replaced twice a day.¹³ After dialysis, the solvent was evaporated to yield the products as colorless sticky oils, which were characterized by ¹H NMR (supporting information).

Preparation of polymers P1-P8

Polymers **P1'-P8'** were treated with 50 % TFA in 10 mL DCM for 2 h (Scheme 3). The solvent was removed in vacuo and the residue was dissolved in DMSO and dialyzed against methanol for three days. After methanol was removed, the samples were dissolved in 5 mL of water and lyophilized to give the final polymers as colorless sticky oils.

MIC (Minimum Inhibitory Concentration)/Antimicrobial Activity

Minimum inhibitory concentration (MIC) was used to measure the efficacy of the synthesized polycarbonates against bacteria.²³ MIC is defined as the lowest concentration of the compound by which it completely inhibits the growth of the bacteria for a period of 20 h. Three clinically relevant Gram-positive strains, *Methicillin-resistant S. epidermidis* (MRSE, RP62A), *Vancomycin-resistant E. faecalis* (VREF, ATCC 700802), and *Methicillin-resistant S. aureus* (MRSA, ATCC 33591) were used in the assay. Briefly, a single colony was isolated from the agar plate and allowed to grow in 4 mL TSB solution overnight in a

shaking incubator at 37 °C. The culture was diluted by 100-fold and the diluted culture was shaken for 6 h in order for the bacteria to grow to the mid-logarithm phase. In a 96-well plate 50 µL of the polymer solution in 2-fold serial dilutions (50 µg/mL to 0.8 µg/mL) were added to the wells. Next, aliquots of the bacterial solution (50 µL, 1×10^6 CFU/mL) were added to those polymer solutions, respectively. The plate was incubated at 37 °C for 20 h, and the absorbance was read at 600 nm wavelength on a Biotek Synergy HT microtiter plate reader. Results were repeated at least three times in duplicates each time.²⁴

Hemolytic Activity Study

To determine the selectivity of the polymers, the compounds were incubated with the human red blood cells and the HC^{50} for the compounds was calculated. HC^{50} is defined as the concentration that causes 50% hemolysis of the human red blood cells (hRBCs).^{25,26,27} In this assay, freshly drawn blood was centrifuged, and erythrocytes were separated and washed a couple of times with PBS buffer, and the supernatant was removed. The polymer samples (50 µL) of various concentrations were placed in a 96 well plate using 2-fold serial dilution technique. The erythrocytes were diluted to a final concentration of 5% (v/v) in PBS buffer. 50 µL of the abovementioned diluted erythrocyte solution were added to the serial-diluted polymer solutions and incubated for 1 h at 37 °C. The 96 well plate was centrifuged at 3500 rpm for 10 min, and 30 µL supernatant liquid was transferred to a new 96 well plate containing 100 µL of PBS in each vial. The absorbance of these solutions was taken at a wavelength of 540 nm. For this assay PBS was used as negative control and 0.1% Triton x-100 was used as positive control. Hemolysis is calculated using the formula below:

$$\% \text{hemolysis} = \left[\frac{\text{Abs of Sample} - \text{Abs of PBS negative control}}{\text{Abs of positive Control} - \text{Abs of PBS negative control}} \right] \times 100$$

Drug Resistance Assay

MICs of the samples were obtained by the method described above. After determination of the MIC, the bacterial solution from the well that contained the polymer at one-half concentration of the MIC value was used to dilute to 1×10^6 CFU/mL. Next, 50 µL of this bacterial solution was added to 50 µL of 2-fold serial-diluted polymer samples, and the new MIC was measured. This assay was repeated for 14 passages. If the polymer had virtually the same MIC after every passage it indicated that the polymer did not developed resistance in the bacteria.²⁸

Fluorescence Microscopy

DAPI (4', 6-Diamidino-2-phenylindole dihydrochloride) and PI (Propidium iodide) were used in the assay.^{29,29b} DAPI is the dye that stains all dead and living bacteria, whereas the PI dye only stains the dead bacteria with damaged membranes as itself is not cell permeable and it has to interact with the nucleic acids of the bacteria and fluoresce in bright red color. Briefly, the bacteria were allowed to grow to the mid logarithmic phase and then incubated with the polymer **P6** (10 µg/mL) at 37 °C for 3 h. The solution was centrifuged at 10,000 g for 10 min in an Eppendorf tube. The supernatant was removed and the bacterial pellets were washed with PBS three to four times. PI (5 µg/mL) was added and incubated for 15 min in dark at 0 °C. The excess of the dye was removed by PBS washes (×3). Next, the cells

were incubated with DAPI (10 µg/mL in water) for 15 min in dark at 0 °C and excess of the dye was removed, followed by PBS washes (×3). The bacteria were then examined under oil-immersion objective (100×) by using the Zeiss Axio Imager Z1 optical microscope.³⁰

Time Kill Assay

This assay determines bacterial killing kinetics for the polymer. Different concentrations of the polymer were incubated with bacterial suspension, at time intervals of 0 min, 10 min, 30 min, 1 h, and 2 h. At these time points, 100 µL of the solution were taken and diluted 10² to 10⁴ times and then spread on respective agar plates for incubation at 37 °C. After 20 h, the bacterial colonies were counted. The assay was repeated at least three times.³¹

Dead Bacteria TEM

The control and the polymer treated bacterial samples were made in the similar way as in fluorescence microscopy assay. The samples were spread on 200-mesh copper grids and were left for 1 h for adsorption onto the grid, and stained by 1% uranyl acetate for 30 sec. The grids were analyzed at 60 kV with FEI Morgagni 268D TEM instrument.^{29b}

RESULTS AND DISCUSSION

Synthesis of cationic polycarbonate polymers containing primary amino groups

Yan and Hedrick et al developed antimicrobial polycarbonates containing quaternary ammonium salts²⁰ that show good activity towards a range of Gram-positive bacteria. However, the formation of quaternary ammonium salts involves post-modification. Based on our previous findings, we envisioned that polycarbonates with primary amino groups should also be active and maybe even more selective toward bacteria. As such, we designed and synthesized a series of such type of polymers (**P1-P8**) and investigated their antibacterial activity. The synthesis is straightforward as described earlier. The polymers were characterized by NMRs of the polymers P1'-P8' which contain boc groups. (Table 1, Figure 1, and supporting information).

Antimicrobial activity of the polymers

In order to understand the effect of sequence composition on the antimicrobial activity of the polymers, we synthesized both random (in which hydrophilic and hydrophobic monomers were randomly arranged) and diblock (in which there are defined hydrophobic and hydrophilic segments) polycarbonate copolymers. These amphiphilic polycarbonate polymers (**P1 – P8**) were tested for their antimicrobial activity against three different Gram-positive bacterial strains, Methicillin-resistant *S. epidermidis* (MRSE, RP62A), Vancomycin-resistant *E. faecalis* (VREF, ATCC 700802), and Methicillin-resistant *S. aureus* (MRSA, ATCC 33591), all of which are clinically relevant threatening strains (Table 2). The polymer **P1**, containing only hydrophilic amino groups but no hydrophobic groups, failed to show any activity against the bacterial strains under tested concentrations. In contrast, the other polymers containing both hydrophilic and hydrophobic groups all show good antibacterial activity. This data clearly demonstrates that although positive charges are necessary for initial recognition and selective association with bacteria, cationic groups alone are not sufficient for bacterial killing. Hydrophobic groups are critical for membrane

interaction and disruption, and ultimate death of the bacteria.³² The diblock copolymers **P2** and **P4**, differing in the number of hydrophobic groups, show similar activity against three different Gram-positive bacterial strains. We hypothesized that diblock copolymers may form stable nanomicelle structures in solution, in which their hydrophobic groups are sequestered, leading to comparable antimicrobial activity. To test our hypothesis, we synthesized the random polymer **P3** that contains same number of hydrophobic and hydrophilic groups as **P4**. Consistent to our hypothesis, the antibacterial activity of **P3** was better than **P4**. Although the activity against VREF remained the same for both **P3** and **P4**, **P3** exhibited good activity of 2.2 μM and 2.6 μM against MRSA and MRSE, which is 2-fold more potent than **P4** toward these two strains. We thus focused on the study of activity of random polymers, and further investigated how the ratio of hydrophobic and hydrophilic groups affect the activity of this type of polymers. With the same number of hydrophilic groups, increasing the number of hydrophobic groups enhanced antimicrobial activity, as seen for polymers **P3**, **P5** and **P8**. Containing 20 hydrophobic groups, **P8** exhibited an excellent activity of 0.7 μM , 1.4 μM and 2.8 μM against MRSA, MRSE and VREF respectively. Similar trend was also revealed with the change of positively charged groups, which led to the discovery of the most potent polymer **P6**. Containing 20 hydrophilic and 20 hydrophobic groups, the random copolymer **P6** showed potent activity against all three bacterial strains, with the activity of 0.17 μM , 0.55 μM and 0.55 μM against MRSA, MRSE and VREF respectively. It is reasonable because the sequences containing more hydrophobic and hydrophilic groups have stronger interaction with bacterial membranes. The findings again suggested that both cationic and hydrophobic groups are essential for the development of antimicrobial polymers, and their arrangement in the sequences plays a very important role. It should be noted that **P6** is much more active compared with magainin II (Table 2), a well-known HDP, augmenting the promise of this class of polycarbonates for antimicrobial applications.

Hemolysis is the lysis of red blood cells, and it is the mostly identified side effect of HDPs and polymers.¹⁹ Thus, hemolytic assay is the common method to measure the toxicity of HDPs and polymers including polycarbonates.^{20,21} One can evaluate the potential application of cationic polymers by comparing their antimicrobial activity and hemolytic activity.

We next conducted the hemolytic assay to evaluate the selectivity of the polymers (Table 2). Surprisingly, none of the polymers was toxic at the tested condition even up to 1 mg/mL. The most potent polymer **P6** displayed a selectivity of at least 600 fold for MRSA. The results indicate that these polymers are highly selective toward bacteria and thus hold great promise for the development of potent antibacterial agents.

Morphology of polymers

As we predicted that the antimicrobial activity of the polymers is related to their nanomorphology, we next conducted Transmission Electron Microscopy (TEM) experiment to test our hypothesis. As expected, most of the polymers formed micelles in water with the size ranging from 80 to 200 nm (Figure 2). We particularly compared the morphology of **P3**, **P4** and **P6**. These micelles show hydrophobic cores and hydrophilic coronas. The size of the

particle increases as the hydrophobic and hydrophilic entities increase, as observed for **P3** and **P6** (Figure 2A and 2B). The diblock copolymer **P4** (Figure 2C) was found to be larger than the random copolymer **P3** (Figure 2A) which comprised of same number of hydrophobic and hydrophilic entities. This may be because **P4** forms more stable core-shell micelle structure due to its diblock structure, and therefore more sequences can self-assemble together to form large-sized micelles. In the contrast, although **P3** also contains both hydrophobic and hydrophilic groups, their random arrangement renders less ability of the sequences to assembly into defined nanostructure. This is also demonstrated by their zeta potentials (Table 3). **P4** has a zeta potential of 58.98 mv, which is much larger than **P3** and **P6**. This is consistent to our hypothesis, that random polymers are more active to bacteria than diblock polymers as they can quickly dissociate into smaller aggregates or single molecules, and as a result, they effectively interact and disrupt bacterial membranes.

Antimicrobial mechanism

The mode of action of the polymers against bacteria was initially evaluated by fluorescence microscopy using double staining method (Figure 3).³³ The most potent sequence **P6** was selected for the study. The control, which was just MRSA bacteria themselves, show blue fluorescence under DAPI channel (**a1**). They were not observed under PI channel (**a2**) because their membranes were intact, and therefore they could not be stained by DAPI. In contrast, after MRSA were treated with **P6**, they were stained by both PI and DAPI (**b1, b2**), and observable under both channels, indicating MRSA membranes were disrupted.

The mechanism of action was further supported by TEM (Figure 4), under which the morphology of untreated MRSA and the polymer **P6** treated MRSA was revealed. The control (bacteria without treatment) show spherical shape with intact cell membranes (Figure 4A), a typical morphology of MRSA. However, after the treatment of bacteria with **P6**, most bacteria lost their spherical morphology. Instead, the leakage of the contents from the bacterial cells was observed (Figure 4B). These results suggest that bacterial membranes were disrupted by the polymer **P6**.

Based on antimicrobial activity and nanomorphology of the polymers, we propose their mechanism of action (Figure 5). The polymers are initially believed to exist in micelle conformation in solution. As polymers approach the bacterial surface, due to change in the electrostatic interactions, the polymers lose their stability and start to dissociate into small entities or free polymer chains, which could bind to the bacterial membranes more effectively. The free polymer chain penetrates the surface of the bacteria due to its amphipathic nature and ultimately disrupts the bacterial membrane, leading to bacterial cell death. It is anticipated that the mechanism of action might be more complex as many other proteins, carbohydrates and lipids are present. It is plausible that the presence of other biological molecules may further destabilize the micelle structures of polymers, leading to enhanced activity in bacterial eradication.

Time-kill assay

To determine the time of action and efficacy of the polymers, the most potent polymer **P6** was chosen for time kill study (Figure 6). Cell viability was determined by the colony

county method on agar plate at regular intervals of 30 min, 1 h, 2 h, 3 h, 6 h, 9 h, 12 h and 24 h. At the concentration of 50 µg/mL and 25 µg/mL, the bacteria were completely eradicated after 6 h, suggesting that the polymer has the bactericidal mechanism and the action is rapid.

Drug resistance study

The TEM and florescence microscopy suggest the polymers act by membrane disruption mechanism on the bacteria. Under this mechanism, it is accepted that the bacteria are less prone to develop resistance. To evaluate the probability of the polymer to induce resistance in bacteria, drug resistance study was conducted for the most potent compound **P6** against MRSA. As shown in the Figure 7, the MIC values were virtually constant even after 14 passages, indicating that the bacteria do not develop resistance readily towards the polymer.

CONCLUSION

We reported the development of potent and highly selective antimicrobial polycarbonate polymers containing primary amino groups. Although they do not show strong activity toward Gram-negative bacteria, they display remarkable antimicrobial activity toward multi-drug resistant Gram-positive bacteria. Our results suggest that amphiphilic nature is necessary for bacterial killing, and random block polymers are more potent than diblock polymers, possibly due to stable nanostructures of diblock polymers which prevent them from interacting with bacterial membranes more effectively. The fluorescence microscopy and the TEM data suggest that these polymers have the mechanism of action of bacterial membrane disruption. Remarkably, these polymers are highly selective towards bacterial cells and show no discernable hemolytic activity. In vivo study of these polymers on the mouse model to evaluate their efficacy is currently underway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
 ^1H NMR (400 MHz, CDCl_3) of **P6'**.

Figure 2.
TEM micrographs showing the morphology of the polycarbonate nanomicelles.

Figure 3.
Fluorescence micrographs of MRSA treated with 10 $\mu\text{g/mL}$ of **P6**.

Figure 4.
TEM micrographs of Control MRSA (A) and MRSA treated with 10 µg/mL of P6 (B).

Figure 5.
Proposed mechanism of action of the polymers on Gram-positive bacteria.

Figure 6.
Time kill study of **P6** for MRSA.

Figure 7.
Drug Resistance Study of **P6** for MRSA.

Scheme 1.
Synthesis of the monomer **1**.

Scheme 2.
Synthesis of the monomer **2**.

Scheme 3.

Synthesis of the amphiphilic polycarbonates. **A**, synthesis of random polymer P6' and P6, in which the monomer 1 and 2 were added in one batch; **B**, synthesis of di-block polymer P4' and P4, in which the monomer 1 and 2 were added in two batches.

Table 1

Structures of the synthesized polymers.

Compound	Type of co-polymer	Hydrophobic units	Hydrophilic units	Molecular weight	
				Theoretical	Observed ^a
P1	Single	0	20	4168	4.1×10^2
P2	Di-block	10	15	5653	5.6×10^2
P3	Random	10	10	4638	4.6×10^2
P4	Di-block	10	10	4638	4.6×10^2
P5	Random	15	10	5888	5.9×10^2
P6	Random	20	20	9168	9.0×10^2
P7	Random	15	15	6903	6.9×10^2
P8	Random	20	10	7138	7.1×10^2

^abased on NMR integration of boc-protected polymers P1'-P4'.

Table 2

Antibacterial activity of polycarbonates. Magainin II,²⁹ a host-defense peptide, was included as a positive control.

Compound	Type of co-polymer	Hydrophobic units	Hydrophilic units	$\mu\text{g/mL}$ (μM)			Hemolysis (HC_{50}) ($\mu\text{g/mL}$)	Selectivity Index ($\text{HC}_{50}/\text{MIC}$ of MRSA)
				MRSA	MRSE	VREF		
P1	Single	0	20	>50	>50	>50	>1000	>20
P2	Di-block	10	15	25 (4.47)	25 (4.47)	25 (4.47)	>1000	>60
P3	Random	10	10	10 (2.2)	12 (2.6)	25 (5.45)	>1000	>100
P4	Di-block	10	10	25 (5.44)	20 (4.35)	25 (5.44)	>1000	>40
P5	Random	15	10	10 (1.7)	20 (3.4)	20 (3.4)	>1000	>100
P6	Random	20	20	1.6 (0.17)	5.0 (0.55)	5.0 (0.55)	>1000	>625
P7	Random	15	15	10 (1.45)	20 (2.89)	20 (2.89)	>1000	>100
P8	Random	20	10	5 (0.7)	10 (1.4)	20 (2.8)	>1000	>200
Magainin II	HDP			16 (40)	>50	>50	>1000	>62.5

Table 3

Size and Zeta potential of polycarbonates.

Compound	Type of co-polymer	Hydrophobic units	Hydrophilic units	Z-Average (d.nm)	Zeta Potential (mV)
P1	Single	0	20	255.7	44.2
P2	Di-block	10	15	154.0	54.7
P3	Random	10	10	81.20	26.1
P4	Di-block	10	10	212.0	58.9
P5	Random	15	10	209.0	31.7
P6	Random	20	20	288.4	16.1
P7	Random	15	15	328.7	9.70
P8	Random	20	10	435.1	7.73