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Synthesis of Antibacterial Glycosylated Polycaprolactones Bearing Imidazoliums with Reduced Hemolytic Activity

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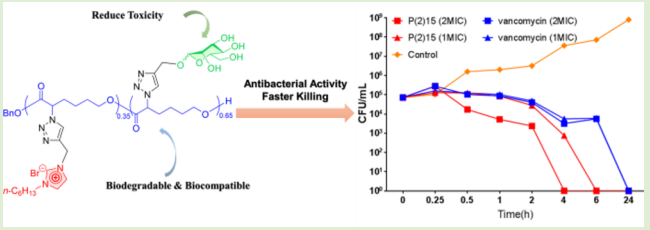
Supporting Information

ABSTRACT: Most synthetic antimicrobial polymers are not biodegradable, thus limiting their potential for large-scale applications in personal care disinfection and environmental contaminations. Poly(ε-caprolactone) (PCL) is known to be both biodegradable and biocompatible, thus representing an ideal candidate biopolymer for antimicrobial applications. Here we successfully grafted alkylimidazolium (Im) onto PCL to mimic the cationic properties of antimicrobial peptides. The poly(ε-caprolactone)-*graft*-butylimidazolium had only moderate MICs (32 μg/mL), reasonably good red blood cell selectivity (36) and relatively good fibroblast compatibility (81% cell viability at 100 μg/mL), indicating that combining the hydrophobic PCL backbone with the most hydrophilic butylimidazolium gives a good balance of MIC and cytotoxicity. On the other hand, the PCL-*graft*-hexylimidazolium and -octylimidazolium demonstrated better MICs (4−32 μg/mL), but considerably worse cytotoxicity. We postulated that the worse hydrophilicity of hexylimidazolium and octylimidazolium was responsible for their higher cytotoxicity and sought to moderate their cytotoxicity with different sugar compositions and lengths. Through our screening, we identified a candidate polymer, $P(C6Im)_{0.35}CL$-*co*-$P(Man)_{0.65}CL$, that demonstrated both superior MIC and very low cytotoxicity. We further demonstrated that our biopolymer hit had superior antimicrobial kinetics compared to the antibiotic vancomycin. This work paves the way forward for the use of biodegradable polyesters as the backbone scaffold for biocompatible antibacterial agents, by clicking with different types and ratios of alkylimidazolium and carbohydrate moieties.



INTRODUCTION

Antimicrobial peptides (AMPs) generally possess amphiphilic properties, arising from both hydrophilic and hydrophobic side chains, which are responsible for their activities.[1] Synthetic polymers which mimic the amphiphilic structures of AMPs, have attracted great attention as a new class of disinfectants because of their ease of synthesis and low-cost of production in large quantities.[2−4] The antimicrobial polymers, inspired by the cationic amino acids residues in AMPs, generally have cationic groups to facilitate their adsorption to negatively charged bacterial membranes.[5,6] For example, DeGrado and co-workers synthesized a biomimetic poly(arylamide) with amphiphilic structures bearing a primary amine, which showed broad antibacterial activity.[7,8] Also, many other polymers have been synthesized and tested for activities against different pathogens, for example, polynorbornenes,[9−12] polycarbonates,[13−17] poly(β-lactam),[18−20] polypeptides[21,22] and polyacrylate derivatives.[23−25] However, most synthetic antimicrobial polymers consist of carbon-based backbones that are not biodegradable, thus limiting their potential in clinical applications. Aliphatic polyesters constitute a class of biodegradable polymers and their roles as scaffolds for biodegradable antibacterial polymers have been rarely investigated.[13,17,26,27] Moreover, antibacterial polycarbonates have been well studied, which presented a good example of degradable antibacterial polymers. In comparison with polycarbonates, PCL (poly(ε-caprolactone)) has more efficient degradation behavior,[28−30] and little has been studied using PCL as the backbone for antibacterial polymers. Besides, some reported polyesters have the ability of self-degradation despite their relatively high hemolytic activity.[31,32] Intriguingly, cationic coumarin polyesters and polyurethanes were reported to exhibit selective activity against Gram-negative bacteria which is very surprising compared with other cationic polymers.[33,34] The backbone of the cationic antibacterial polymers is known to profoundly affect the resultant biological properties. Therefore, these interesting and attractive results had inspired us to design new antibacterial polymers based on

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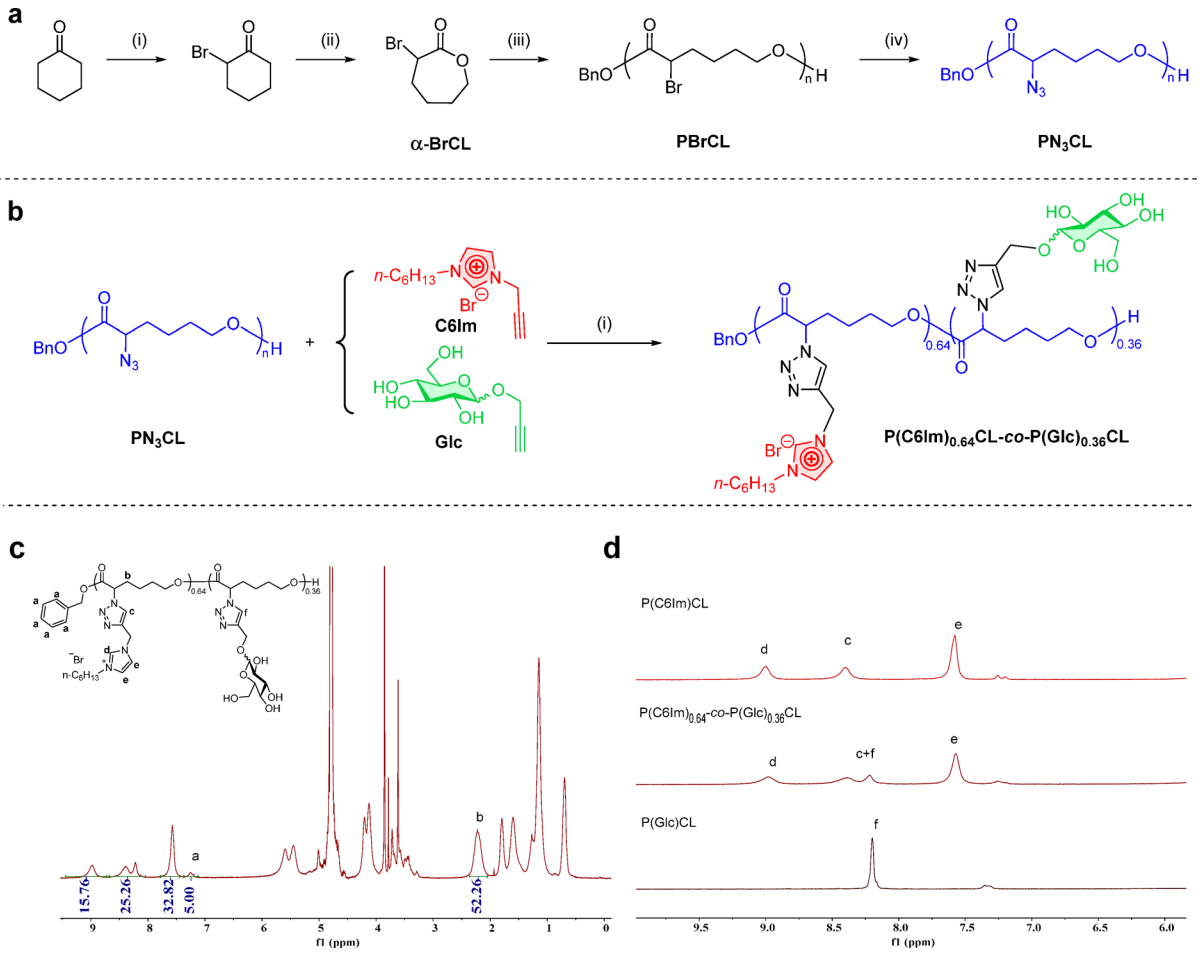

Figure 1. Design and synthesis of new antibacterial agent with biodegradable poly(ε-caprolactone) (PCL). (a) Synthesis of PCLs: (i) NBS, NH_4OAc, Et_2O, rt, 30 min; (ii) *m*-CPBA, DCM, rt, 24 h; (iii) BnOH, $Sn(Oct)_2$, 80 °C, 48 h; (iv) NaN_3, DMF, rt, overnight. (b) Synthesis of $P(C6Im)_{0.64}$-*co*-$P(Glc)_{0.36}CL$ in a one-pot reaction: (i) CuI, Et_3N, DMF, 40 °C, overnight (Glc = glucose). (c) Calculation of the ratio of grafted imidazolium and carbohydrate by the integrals in ^1H NMR, $Ratio_{(C6Im/Glc)} = I_d/(I_{c+f} - I_d)$, which should be 0.64 and 0.36 for $P(C6Im)_{0.64}$-*co*-$P(Glc)_{0.36}CL$ (predicted ratio is 70:30). (d) Characteristic signals in resulting PCLs: signals d and e are from imidazolium ring while c and f are from the formed triazole rings.

polycaprolactones. As a well-known biodegradable polyester, PCL has attracted considerable attention for the past decades, but the major drawback is the lack of side functional groups which can be solved by introducing functional groups to the α-position of the carbonyl.[35,36] Interestingly, functionalized ε-caprolactone has been prepared. It has been successfully polymerized and modified by click reactions, thus implying it is a great candidate substrate for producing biodegradable antimicrobial polyesters.[35,37,38] Furthermore, imidazolium salts have gained considerable attention in antimicrobial applications owing to their relative hydrophilicity, permanent charges and stability.[39−41]

Furthermore, like natural AMPs, most cationic polymers are hemolytic or cytotoxic to human cells,[42] limiting their further applications. Thus, studies have been widely pursued to manipulate the antimicrobial activity and biocompatibility of the synthetic polymers through various factors such as charge density,[9,43] molecular weight,[12,44] type of active moiety[45,46] and alkyl chain length.[23,24,47] In particular, the balance between hydrophilicity and hydrophobicity of the entire polymer is most crucial to the selectivity. This can be done by adding alkyl chains to increase hydrophobicity or adding biocompatible compounds such as poly(ethylene glycol)[48,49] and sugars[50−53] to increase the hydrophilicity. Noteworthily, the effect of glycounits was studied on antimicrobial polyacrylates systematically but with only one sugar.[53]

Here, we report the synthesis of a new class of antibacterial compounds based on the biodegradable PCL backbone. Imidazolium was grafted to mimic the cationic properties of antimicrobial peptides whereas the alkyl chain on the imidazoliums and carbohydrates were used to balance the hydrophobicity/hydrophilicity of the resultant polymer. The synthesis of the polymers could be successfully achieved through ring-opening polymerization and subsequent click reaction between the azido PCL and alkyne-imidazoliums (Im) and alkyne-carbohydrates in a one-pot grafting reaction (Figure 1). Subsequently, the PCL-Im-carbohydrates grafts were tested for activity against both Gram-positive and Gram-negative bacteria, including up to 9 MRSA strains. In addition, to evaluate selectivity against bacteria, these polymers were also tested for toxicities against red blood cells and 3T3 cells.

EXPERIMENTAL SECTION

Materials. Chemicals and solvents are purchased from Alfa-Aesar, Sigma-Aldrich and VWR and used without further purification unless otherwise noted. Benzyl alcohol (Alfa-Aesar, 99%) was stirred with sodium at room temperature and distilled under nitrogen and stored

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in Schlenk flask in desiccator until further use. Anhydrous toluene (over sodium/benzophenone), tetrahydrofuran (THF, over sodium/benzophenone) and dichloromethane (DCM, over calcium hydride) were freshly distilled under nitrogen atmosphere before use. All the other anhydrous solvents were purchased from Sigma-Aldrich and used as received. Deuterated solvents are obtained from Cambridge Isotope Laboratories and used as received. Thin layer chromatography (TLC) with Merck TLC silica gel 60 F254 plate was used to monitor reaction. UV, potassium permanganate and iodine staining was used to visualize compounds on TLC plates. Regenerated cellulose dialysis tubing (MWCO 3500 Da) was purchased from Fisher Scientific. Deionized water was obtained from a Merck Millipore Integral 3 water purification system. For biological studies, NIH 3T3 fibroblasts were purchased from Millipore, Singapore. All broth and agar were purchased from Becton Dickinson Company (Franklin Lakes, US) and used as received. Bacteria strains used (*Escherichia coli* ATCC8739, *Staphylococcus aureus* ATCC29213, *Pseudomonas aeruginosa* PAO1, *Bacillus subtilis*, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* ATCC 19434 and drug-resistant *Staphylococcus aureus* MRSA BAA40, MRSA USA300) were purchased from ATCC. Drug-resistant *Staphylococcus aureus* MRSA 1−7 were clinical strains isolated from local hospital (TTSH).

Instruments for Characterization. ^{1}H and ^{13}C and 2D NMR spectra were recorded on Bruker Avance 300, Bruker Avance 400, Bruker AVIII 400 and JEOL JNM-ECA 400 spectrometers using deuterated solvents as reference. Mass spectra were obtained using an Agilent 6230 TOF LC/MS with an electrospray (ESI) source with purine and HP-0921 as an internal calibrates. Organic phase gel permeation chromatography (GPC) was carried out on a Shimadzu liquid chromatography system equipped with a Shimadzu refractive index detector (RID-10A) and two Agilent Polargel columns operating at 40 °C using DMF (with 1 wt % LiBr) or THF as the eluent at a flow rate of 1 mL/min using polystyrene kit as standard. Aqueous phase GPC was carried out on an Agilent liquid chromatography system equipped with an Agilent refractive index detector with two Shodex OHpak columns operating at 40 °C using 0.05 M NaCl solution as the eluent at a flow rate of 0.5 mL/min using pullulan kit as standard to determine M_n, M_w and polydispersity index (PDI = M_n/M_w).

Synthesis of α-Bromo-ε-caprolactone (α-BrCL). The α-bromo-ε-caprolactone (α-BrCL) was synthesized according to the literature procedure started from cyclohexanone.[54] Briefly, cyclohexanone (14.7 g, 0.15 mol) was dissolved in dry diethyl ether (150 mL) followed by the addition of *N*-bromosuccinimide (NBS, 28.0 g, 0.158 mol). The solution was stirred and ammonium acetate (1.16 g, 0.015 mol) was added portion-wise. After stirring for 0.5 h at room temperature, the solid was filtered off and the filtrate was washed with water, dried over Na_2SO_4, concentrated and subject to flash column chromatography, giving pale yellow liquid α-bromo-cyclohexanone (17.8 g, 67%). To the solution of α-bromo-cyclohexanone (10.0 g, 56 mmol) in anhydrous dichloromethane (DCM, 200 mL), 3-chloroperoxybenzoic acid (*m*-CPBA, 25.7 g, 85 mmol, ca. 70% with water) was added and the solution was stirred at room temperature for 24 h. The mixture was cooled to −20 °C and filtered. The filtrate was washed successively with $Na_2S_2O_3$ and saturated $NaHCO_3$ solution thoroughly until no acid could be detected by TLC. The organic layer was dried over Na_2SO_4, concentrated and subject to flash column chromatography, affording the desired monomer α-bromo-ε-caprolactone in 41% yield (4.4 g). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.85 (dd, *J* = 6.2, 3.6 Hz, 1H), 4.77−4.63 (m, 1H), 4.37−4.20 (m, 1H), 2.20−2.11 (m, 2H), 2.11−1.93 (m, 2H), 1.92−1.76 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.8, 69.7, 48.2, 31.8, 29.1, 25.2. MS (ESI) *m*/*z* calcd. for $C_6H_{10}{}^{79}BrO_2$ $[M + H]^+$ 192.99, found 192.96; calcd. for $C_6H_{10}{}^{81}BrO_2$ $[M + H]^+$ 194.98, found 194.96.

Synthesis of Poly(α-azido-ε-caprolactone) (PN_3CL). The poly(α-azido-ε-caprolactone) (**PN_3CL**) was synthesized according to the reported procedure with some modifications.[37,38] To the solution of α-bromo-ε-caprolactone (8.7 g, 45 mmol) in anhydrous toluene (20 mL), benzyl alcohol (BnOH, 162 mg, 1.5 mmol) and tin(II) 2-ethylhexanoate ($Sn(Oct)_2$, 648 mg, 1.6 mmol) were added sequentially. The mixture in the Schlenk flask was subject to three freeze−pump−thaw cycles, sealed under nitrogen and stirred at 80 °C for 48 h before quenched with highly diluted HCl in methanol. The resulted mixture was diluted with DCM and precipitated with hexane, centrifuged and dried under vacuum, giving the poly(α-azido-ε-caprolactone) (**PBrCL**) in 83% yield (7.2 g). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37 (m, *Ar*−*H* from Bn−), 5.21 (s, PhCH_2−), 4.53−3.88 (m, −OCH_2− and −CH(Br)−), 3.66 (t, −CH_2OH), 2.24−1.91 (m, −CH(Br)CH_2−), 1.85−1.37 (m, −CH(Br)$CH_2CH_2CH_2$−). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.7, 65.5, 45.7, 34.3, 27.8, 23.8. $M_{n,NMR}$ = 4.8 kDa, $M_{n,GPC}$ = 4.4 kDa, PDI = 1.29. To a solution of **PBrCL** (7.5 g, 39 mmol of repeating unit) in DMF (120 mL) was added sodium azide (NaN_3, 5.1 g, 78 mmol), and the mixture was stirred at room temperature overnight before concentrated under vacuum. The concentrated solution was diluted with toluene followed by centrifugation to remove the insoluble solid. The supernatant was concentrated and dried in vacuo afterward to afford the final desired **PN_3CL** (5.6 g, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 (m, *Ar*−*H* from Bn−), 5.22 (s, PhCH_2−), 4.40−4.11 (m, −OCH_2−), 3.97−3.78 (m, −CH(N_3)−), 3.67 (t, −CH_2OH), 1.94−1.68 (m, −CH(Br)$CH_2CH_2CH_2$−), 1.62−1.44 (m, −CH(Br)$CH_2CH_2CH_2$−). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 65.4, 61.9, 30.9, 28.1, 22.3. $M_{n,NMR}$ = 4.0 kDa, $M_{n,GPC}$ = 4.6 kDa, PDI = 1.30.

General Procedure for Modification of PN_3CL with Grafting Compounds. The modification of **PN_3CL** was modified from literature procedure.[37] All the modification reactions were conducted using a similar procedure; therefore, the procedure of modification with 1-propargyl-3-butyl-imidazolium bromide is taken as an example herein. To the solution of **PN_3CL** (129 mg, 1.0 equiv. azide group) and 1-propargyl-3-butyl-imidazolium bromide (212 mg, 1.05 equiv) in DMF (5 mL), anhydrous triethylamine (8.4 mg, 0.1 equiv) was added and the mixture was subject to two freeze−pump−thaw cycles before the addition of CuI (15.8 mg, 0.1 equiv). The mixture was subject to another two freeze−pump−thaw cycles before sealed under argon and stirred at 40 °C overnight. The resulted mixture was diluted with water and transferred into dialysis tubing directly followed by dialyzed against highly diluted EDTA solution (0.25 mg/L) for 2 days and deionized water for another 1 day. Puffy solid could be obtained after lyophilization.

Degradability Examination. The degradation ability of polymers was studied using the selected sample as a model. Polymer was incubated in phosphate-buffered-saline (PBS, PH = 7.4) at 37 °C. Aliquots were taken out at certain time intervals and monitored directly by GPC.

Minimum Inhibitory Concentration (MIC) Determination. Minimum inhibition concentrations (MICs) were measured following standard broth dilution method with minor modification.[55] Bacterial cells were grown overnight in Mueller−Hinton broth (MHB, Difco, Becton, Dickinson and Company) at 37 °C. The bacteria were 1:100 subcultured in MHB to a midexponential phase and diluted to 5×10^5 CFU·mL^{-1} in fresh MHB. Stock solutions of PCLs were prepared in the MHB medium at a concentration of 1024 μg·mL^{-1}. The solutions were 2-fold serially diluted in MHB medium, and 50 μL of each dilution was placed in each well of 96-well microplates (Nunc, ThermoScientific) followed by the addition of 50 μL of the bacterial suspension. The plate was mixed in a shaker incubator for 10 min before incubated at 37 °C for 18 h, and the absorbance at 600 nm was measured with a microplate reader (TECAN, infinite F200). A positive control without polymer and a negative control without bacteria were included. MIC was determined as the lowest concentration of the compound that inhibited the growth of bacteria by more than 90%. All tests were done in three independent tests with duplicate per test.

Hemolysis Studies. Fresh human blood was collected from a health donor (IRB-2015-03-040) and used within the same day. Human blood was drawn directly into K2-EDTA-coated Vacutainer tubes to prevent coagulation of blood and stored at 4 °C for 30 min. 1 mL of blood was mixed with 9 mL of PBS and centrifuged at 1,000 rpm for 5 min. Supernatant was discarded, and red blood cells

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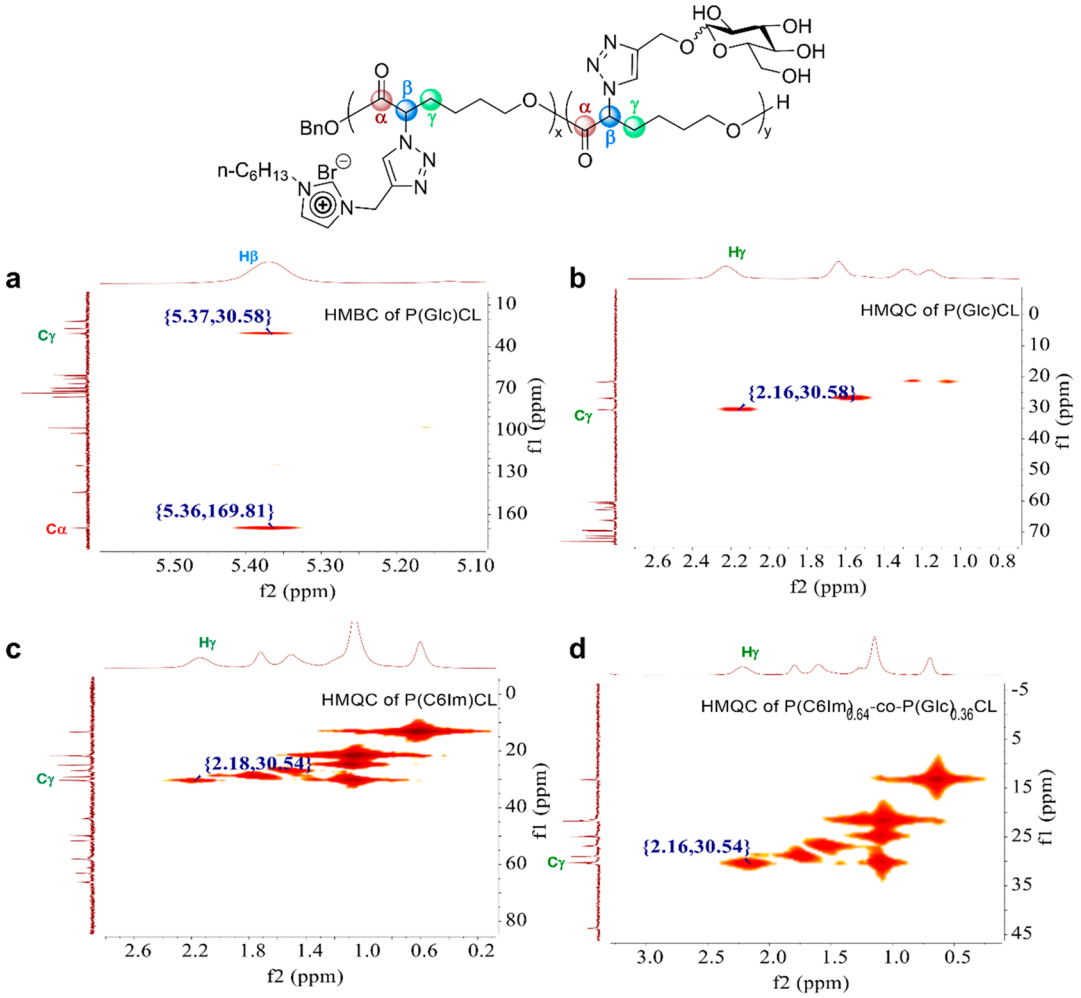

Figure 2. Confirmation of the characteristic protons from the main chain of PCL with 2D NMR spectra. (a) Confirmation of C_γ from the main chain. (b) Confirmation of H_γ from the main chain (c,d). Confirmation that the H_γ is isolated from other signals in imidazolium bearing PCLs.

(RBCs) were collected. The RBCs were washed with PBS three times and resuspended to a final concentration of 5% (v/v) in PBS. A 2-fold dilution series of polymer in PBS solution was prepared, 50 μL red blood cell suspension was mixed with 50 μL polymer solution in each well and incubated for 1 h at 37 °C in an inoculation shaker with continuous shaking at 150 rpm. The 96-well plates were centrifuged at 1,000 rpm for 10 min. After centrifugation, 80 μL centrifuge supernatant samples were transferred to a new 96-well plate and diluted with 80 μL PBS, and hemolytic activity was calculated by measuring absorbance at 540 nm using a 96-well plate spectrophotometer (Benchmark Plus, BIO-RAD). PBS buffer (pH 7.4) was used as a negative hemolysis control, and Triton X-100 (0.1% v/v in PBS) was used as a positive control. The percentage of hemoglobin release was calculated from the following equation: Hemolysis (%) = [(Op − Ob)/(Ot − Ob)] × 100% where Op is the absorbance for the polymer, Ob is the absorbance for the negative control (PBS), and Ot is the absorbance for the positive control of Triton X-100. All data were obtained from the mean value of three replicates.

Cytotoxicity Assays. The mammalian cell biocompatibility study was tested toward 3T3 fibroblast cell using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in colorimetric assay. 3T3 cells (ATCC) were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin/streptomycin). The cells in tissue culture flask were cultured at 37 °C in a humidified incubator with 5% CO_2 until 80% confluence was reached. 3T3 cells were harvested from the confluence flask by trypsinization. Cell number was determined using a hemocytometer and 10^4 cells/well were seeded into a 96-well tissue culture plate and incubated at 37 °C in a humidified incubator with 5% CO_2 for 24 h. Polymer in culture medium solutions at 100 μg/mL and 200 μg/mL were added into the 96-well plate seeded with cells and incubated at 37 °C in a humidified incubator with 5% CO_2 incubator for 24 h. Cells incubated with only DMEM were used as positive nontoxic controls. Afterward, the culture medium containing polymer was removed, and each well was washed with PBS prior to the addition of MTT solution (1 mg·mL^{-1} in DMEM). After another 4 h of incubation, the MTT solution was aspirated and 100 μL dimethyl sulfoxide (DMSO) was added into each well, and the plate was shaken at 150 rpm for 10 min, after which the absorbance of each well was measured at 570 nm using a microplate reader spectrophotometer (BIO-RAD, Benchmark Plus). The cell viability results were expressed as percentages relative to the absorbance obtained in the control experiment.

$$\%\text{Cell viability} = \frac{\text{Average abs of treated cells}}{\text{Average abs of controls}} \times 100\%$$

Bacteria Killing Kinetics. Time kill study was conducted by incubating bacteria with different concentrations of polymer/antibiotics and determining CFU·mL^{-1} at various time points. Bacterial cells were grown overnight in Mueller−Hinton broth medium at 37 °C. After subculturing to a midexponential phase, bacteria were diluted to 5 × 10^5 CFU·mL^{-1} in fresh MHB. Polymer or antibiotic were added to 1000 μL bacteria in MHB suspension in Eppendorf tubes to achieve a final polymer/antibiotic concentration of 4 × MIC, 2 × MIC, 1 × MIC and 0.5 × MIC respectively. Bacteria in MHB suspension without addition of polymer were used as positive control.

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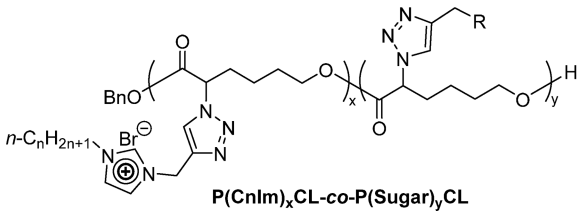
Table 1. Antibacterial, Hemolytic Activity and Mammalian Cell Biocompatibility of Resulting PCLs



Entry	Sample	MIC (μg/mL)				Selectivity[b]		Cell viability (%), 3T3	
		S. aureus 29213	MRSA BAA40	*E. coli* 8739	*P. aeruginosa* PAO1	HC_{50}[a] (μg/mL), RBC	HC_{50}/MIC	100 μg/mL	200 μg/mL
P1	P(C4Im)CL	32	32	32	128	1158	36	81	27
P2	P(C6Im)CL	4	4	8	32	687	172	20	6
P3	P(C8Im)CL	8	8	16	32	332	42	18	9
P4	P(C10Im)CL	32	16	64	128	24	1	4	4
P(3)5	$P(C8Im)_{0.63}CL$-*co*-$P(Glc)_{0.37}CL$	8	8	32	32	149	19	31	6
P(3)6	$P(C8Im)_{0.48}CL$-*co*-$P(Glc)_{0.52}CL$	8	16	32	64	346	43	32	5
P(3)7	$P(C8Im)_{0.37}CL$-*co*-$P(Glc)_{0.63}CL$	16	32	16	256	386	24	48	7
P(3)8	$P(C8Im)_{0.2}CL$-*co*-$P(Glc)_{0.8}CL$	128	256	512	>512	1361	11	98	95
P(2)9	$P(C6Im)_{0.76}CL$-*co*-$P(Glc)_{0.24}CL$	4	8	16	64	139	35	33	6
P(2)10	$P(C6Im)_{0.64}CL$-*co*-$P(Glc)_{0.36}CL$	8	8	16	64	337	42	38	6
P(2)11	$P(C6Im)_{0.5}CL$-*co*-$P(Glc)_{0.5}CL$	16	32	64	256	1497	47	80	35
P(2)12	$P(C6Im)_{0.38}CL$-*co*-$P(Glc)_{0.62}CL$	32	32	128	512	1796	56	84	45
P(2)13	$P(C6Im)_{0.29}CL$-*co*-$P(Glc)_{0.71}CL$	64	128	256	>512	>12500	>195	92	72
P(2)14	$P(C6Im)_{0.33}CL$-*co*-$P(Gal)_{0.67}CL$	32	64	64	256	1380	43	85	51
P(2)15	$P(C6Im)_{0.35}CL$-*co*-$P(Man)_{0.65}CL$	64	64	128	512	>12500	>195	90	82
P(2)16	$P(C6Im)_{0.33}CL$-*co*-$P(GlcNAc)_{0.67}CL$	64	64	64	512	5782	90	96	93
P17	P(Glc)CL	>512	>512	>512	>512	>12500	–	99	96

[a]Values obtained from the plotted hemolysis curve. [b]Calculated based on the MIC values of *S. aureus* 29213.

The bacteria suspensions with polymers were incubated in an inoculation shaker at 37 °C with continuous shaking. Aliquots were taken at different time intervals (0, 0.25, 0.5, 1, 2, 4, 6, and 24 h) and 10-fold serial diluted in PBS for plating. The diluted aliquots were plated on LB agar and incubated at 37 °C and CFU of each sample was determined after 20 h. The killing model for the comparison with polymer degradation was carried out in PBS with same conditions except no medium. Two independent experiments with duplicate for each test were performed for each polymer/pathogen combination, and the resulting average values are plotted on $CFU{\cdot}mL^{-1}$ against time.

RESULTS AND DICUSSION

Synthesis of Poly(azido-ε-caprolactone) (PN_3CL) with Clickable Side Groups. The functionalizable monomer of α-bromo-ε-caprolactone was synthesized using previously reported procedure[54] and functionalizable poly(ε-caprolactone) were synthesized by reported procedure with some modifications (Figure 1).[37,38] Cyclohexanone was converted into α-bromocyclohexanone by bromination with N-bromosuccinimide (NBS) followed by Baeyer–Villiger oxidation with 3-chloroperoxybenzoic acid (*m*-CPBA) as the oxidant, producing the monomer α-bromo-ε-caprolactone (α-BrCL) in moderate yield (41%). With the monomer in hand, the poly(α-bromo-ε-caprolactone) (PBrCL) was prepared by ring-opening polymerization using benzyl alcohol (BnOH) as initiator and tin(II) 2-ethylhexanoate ($Sn(Oct)_2$) as catalyst. The antibacterial activities and toxicity of cationic polymers have been found to be influenced by molecular weights. Polymers with moderate molecular weights have generally been found to be active against bacteria with moderate toxicities.[21,42,56] Thus, various functionalized polycaprolactone derivatives with moderate molecular weight of about 4 to 5 kDa were targeted by controlling the feeding ratio and a single peak was observed in GPC for each polymer, indicating a well-controlled polymerization. For example, a PBrCL of 4.8 kDa (DP of 25) was obtained by controlling the feeding ratio of benzyl alcohol and monomer at 1:30. Considering the 83% yield, we can conclude that the targeted molecular weight was successfully obtained in a controlled manner (Supporting Information, Figure S1 and S2). Subsequently, the bromo substituents on PBrCL were converted into azido groups by reaction with sodium azide (NaN_3) in DMF at room temperature. The resulting poly(α-azido-ε-caprolactone) (PN_3CL) was characterized with ^1H NMR and the results indicated the complete conversion of bromo to azido groups (Supporting Information, Figure S1).

Conjugation of Poly(α-azido-ε-caprolactone) with Alkyne-Imidazoliums and Alkyne-Carbohydrates via CuAAC Click Chemistry. The postmodification of the obtained PN_3CL was conducted using CuAAC click reaction via a modified literature procedure.[37] Briefly, the click reaction was carried out utilizing the CuI/triethylamine combination in anhydrous and degassed DMF at a slightly elevated temperature of 40 °C because of poor solubility of cationic compounds and carbohydrates at room temperature. Initially, as a first trial, a functionalized PCL modified with 1-propargyl-3-hexyl-imidazolium bromide (C6Im) and prop-2-ynyl-D-glucopyranoside (Glc) was synthesized successfully in a one-

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Table 2. Antibacterial Activity of Selected Samples against other Gram-Positive Strains Including Resistant Strains

	MIC[a] (μg/mL)										
Entry	*B. subtilis* 6633	*E. faecalis* 29212	*E. faecium* 19434	MRSA USA300	MRSA 1	MRSA 2	MRSA 3	MRSA 4	MRSA 5	MRSA 6	MRSA 7
P(3)6	8	16	8	8	8	8	16	8	16	16	8
P(3)7	32	32	32	32	16	32	64	16	64	32	32
P(2)11	8	64	16	32	16	16	128	16	32	32	32
P(2)12	16	64	32	32	16	16	128	16	32	64	32
P(2)13	64	256	64	128	64	64	256	64	128	128	128
P(2)14	16	64	32	32	32	32	128	32	32	64	64
P(2)15	32	128	32	64	64	64	256	32	64	64	64
P(2)16	32	128	32	64	32	32	128	32	64	64	64

[a]*B. subtilis, E. faecalis, E. faecium*, MRSA BAA40, MRSA USA300 were purchased from ATCC. MRSA 1−7 were clinical strains isolated from local hospital (TTSH).

pot reaction (Figure 1). In order to investigate the structure−activity relationship especially the carbohydrate effects, a library of PCLs was synthesized. The signal of proton on triazole ring (8.2 ppm for sugars and 8.3 for imidazolium) is different from the signals of protons on the imidazolium ring (8.9 and 7.6 ppm), and thus the ratios of sugars and imidazoliums can be calculated from the integrals in ^{1}H NMR of resultant polymers. (This was calculated to possess 64% imidazolium and 36% glucoside and coded as P-$(C6Im)_{0.64}$CL-*co*-P$(Glc)_{0.36}$CL in Figure 1.) Also, some proton signals on the main chain of PCL are isolated from the others, which could be confirmed by 2D NMR and applied in calculating the click efficiency as well as molecular weight (Figure 2, full spectra see Supporting Information). The HMBC of P(Glc)CL indicates H_β coupled with the adjacent carbonyl C_α and C_γ, proving H_β is on the adjacent carbon C_β of carbonyl group. With the information from HMQC of P(Glc)CL, H_γ has coupled with only C_γ, proving it is the only proton connected with carbon C_γ. Furthermore, the H_γ only coupled with the C_γ in the HMQC of PCLs bearing imidazolium as well, indicating H_γ is the isolated proton signal in the main chain. The molecular weight of the polymers was calculated from both ^{1}H NMR and GPC, and the results are summarized in Table S1. (The GPC traces were unimodal (Figure S2).) Moreover, the actual content of resulting polymers is very close to the designed feeding ratio of grafting agents (Table S2).

Antibacterial Activities and Toxicity of the Resulting Poly(ε-caprolactone). The antibacterial efficacy of the resulting PCLs was first evaluated by employing both Gram-negative (*Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*)) and Gram-positive bacteria (*Staphylococcus aureus* (*S. aureus*) and methicillin-resistant *Staphylococcus aureus* (MRSA)). The minimum inhibitory concentration (MIC), or the lowest concentration of compound needed to prevent visible growth of bacteria, of various polymer derivatives were measured against the different bacterial strains. We measured hemolytic toxicity as the concentration causing 50% hemolysis of human red blood cells (HC_{50}). We measured the selectivity of the functionalized PCLs as the ratio between HC_{50} and MIC values (Herein, MIC values against *S. aureus* was used). Toxicity toward a typical mammalian cell (3T3 fibroblasts) at 100 and 200 μg/mL (several times the MICs) was also measured.

As imidazolium salts were reported as good antibacterial agents,[57,58] imidazolium salts with variable alkyl tail length were grafted onto the PCLs. The resultant PCL derivatives have good efficacy against both Gram-negative and Gram-positive bacteria (Table 1, P1−P4). Among these PCLs, polymers possessing imidazolium with intermediate length (6 or 8) carbon tails have better antibacterial activities than those possessing shorter and longer carbon chains (P1 and P4).

With the exception of P1, the other imidazolium functionalized PCLs had relatively high toxicity toward mammalian cells. The hemolytic activities of P1 to P3 were reasonable, with a selectivity index of 171 and 41 for P2 and P3, respectively. P4 is too hemolytic, likely because the side chain with 10 carbons is too long.

To determine whether increasing the hydrophilicity of the polymers can improve the toxicity profile of the polymers,[52] carbohydrates were introduced into two series with 8 and 6 alkyl side chains (i.e., P5 to P8 and P9 to P13, respectively) and their effects were systematically studied with different molar ratios and various carbohydrates.

Comparing P3 with their glycosylated derivatives (P5 to P8), the MIC values against Gram-negative strains increased significantly with increasing ratios of carbohydrate in the polymers whereas the MIC values went up less significantly for Gram-positive bacteria. It is probably due to the impermeability of the outer membranes of Gram-negative bacteria to large hydrophilic molecules (Table 1, P5−P8).[59,60] However, the C8-Im derivatives' hemolysis and cytotoxicity did not decrease until the sugar content is rather large (i.e., with P8).

On the other hand, for similar ratios of carbohydrate and imidazolium, PCLs bearing C6-Im has higher MICs than those possessing C8-Im despite their lower hemolysis and lower 3T3 toxicity (Table 1, P6 vs P11 and P7 vs P12). As with P3 derivatives (i.e., P5−P8), the MIC increased much less significantly with Gram-positive bacteria when the ratio of glycosylated substitution was increased. However, their toxicity to 3T3 cells, as well as their hemolytic toxicity, was significantly reduced. For example, P13 has fairly balanced profiles of bactericidal activities against Gram-positive bacterial and good selectivity indices for 3T3 and red blood cells. Additionally, more Gram-positive strains including *B. subtilis*, *Enterococcus faecalis*, *Enterococcus faecium* and MRSA USA300, together with a panel of other clinical MRSA strains from local hospitals, were used to evaluate selected compounds. Comparable results (Table 2) to those in Table 1 were obtained, indicating they (including P11 which has good 3T3 and red blood cell compatibility, together with MICs) are potentially bactericidal toward deadly MRSA strains. Thus, we can conclude, the alkyl side chain length, and content of imidazolium in these PCL-backbone polymers are crucial to the antibacterial activity. Interestingly, only the shorter C6-Im can achieve a more balanced MIC and selectivity values

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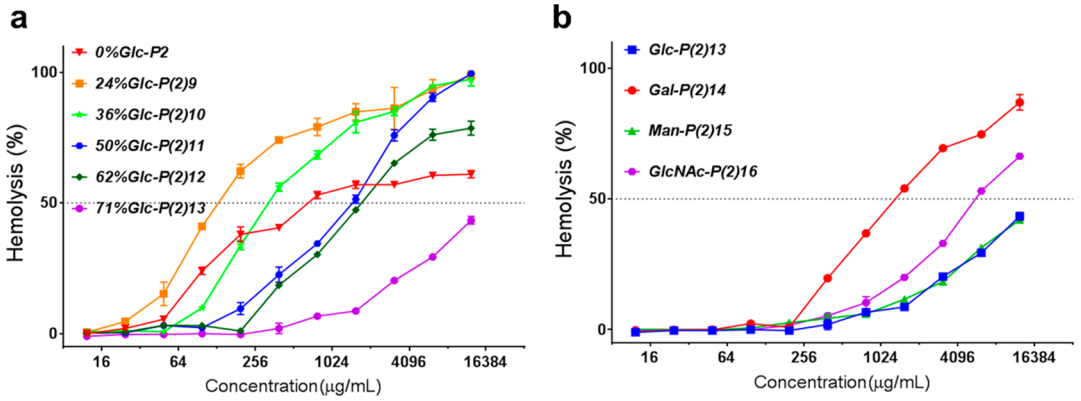

Figure 3. Dose-dependent hemolytic activity of resultant PCLs. (a) PCLs bearing different ratios of glucose. (b) PCLs bearing different carbohydrates. Glc = glucose, Gal = galactose, Man = mannose, GlcNAc = glucosamine.

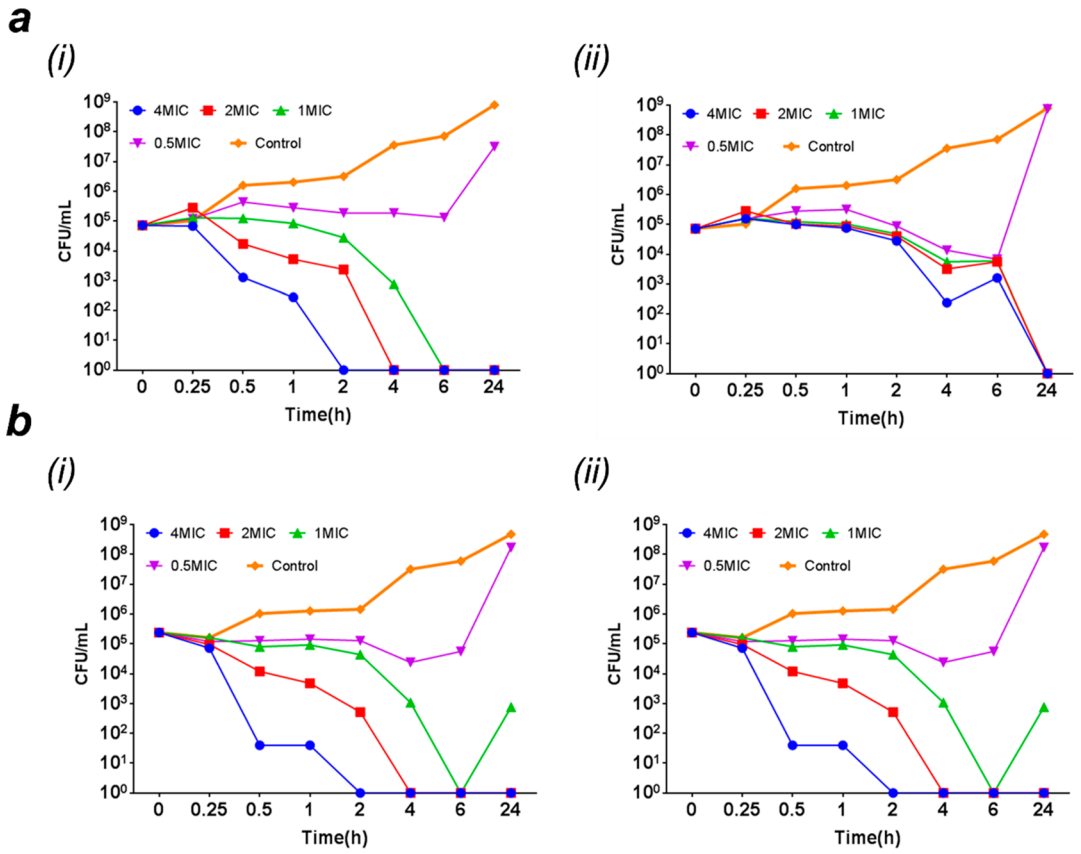

Figure 4. Killing kinetics against (a) MRSA BAA40: (i) P(2)15, (ii) vancomycin. (b) MRSA USA300: (i) P(2)15, (ii) vancomycin.

possibly because the PCL backbone is relatively hydrophobic. Also, with the decreasing ratios of imidazolium in the intermediate range (Table 1, P5–P7, and P9–P12), the MIC values increased moderately for Gram-positive bacteria but dramatically for Gram-negative bacteria. Overall, the hemolysis of the PCL-Im derivatives are generally quite good (with selectivity ratios of at least 40 even for P3).

Furthermore, to study the effects of different carbohydrates, various sugars (glucose, galactose, mannose, glucosamine) were attached in comparable molar ratios. No significant difference was observed in MIC values (Table 1, P13–P16). However, it is noteworthy that PCLs with C6-Im bearing glucose and mannose are relatively less hemolytic probably attributed to their difference in polarity (Table 1 and Figure 3b), and C6-Im with glucose, mannose and glucosamine had markedly improved 3T3 compatibility.

With regard to selectivity against bacteria over red blood cells, P13 and P15 showed the best results (>195), followed by P2 (>172) and P16 (>90). However, considering both the hemolytic selectivity and cytotoxicity, P15 ($P(C6Im)_{0.35}CL$-*co*-$P(Man)_{0.65}CL$) was postulated to have the most satisfactory balance in this polymer system.

With this promising compound in hand, the killing kinetics of the compound was studied and compared against vancomycin, which is the last resort antibiotics against MRSA infection. By exposing MRSA BBA40 to a series of concentrations of synthesized polymer and vancomycin, rapid bactericidal effects were observed at 1 × MIC concentration of

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our polymer whereas vancomycin could not kill the bacteria within 6 h even at 4 × MIC concentration (Figure 4a). Another MRSA strain tested (USA300) has revealed the same trend although some regrowth could be observed at lower concentration probably due to slight degradation of antibacterial agents (Figure 4b). Besides, to examine the degradability of our polymers, a model study was carried out with P15 in physiological pH condition at 37 °C. The preliminary results indicated that the polymers are hydrolytically degradable. In addition, no noticeable degradation occurred before inhibiting bacteria and significant loss of activity after degradation was observed (Figure S5). This feature endows the polymers with great advantages over enzymatically degradable polymers, rendering such polymers more promising in further applications.

Compared with bacteriostatic antibiotics, cationic polymers are preferred in many situations against resistant bacteria, biofilm bacteria or persistent bacteria where clinical studies have proven the therapeutic efficacy of bactericidal agents.[61] Moreover, cationic polymers could minimize the emergence of resistance in early clinical usage, due to minimal bacteria survival and mutation.[62] This could be an advantage of our polymer series due to its true sterilization effect within a short period of time at a relatively low concentration.[62] Thus, the PCL bearing mannose and imidazolium could be a potentially effective antibacterial agent in clinical applications.

CONCLUSIONS

We have successfully synthesized a new biodegradable series of polymers: poly(ε-caprolactone)-*graft*-alkylimidazolium-*graft*-carbohydrates via ring-opening polymerization followed by clicking with alkyne-imidazoliums and alkyne-carbohydrates. The actual ratio of alkyne-Im to alkyne-carbohydrates clicked on PCL was achieved through careful control of reagent ratios. The synthesized PCLs have good antibacterial efficacy toward Gram-positive bacteria. However, the hemolytic and 3T3 toxicities of these PCL derivatives were generally high for the PCL graft alkyl-Im polymers. These toxicities can be improved upon addition of carbohydrates, at the expense of some bacteria activity. Through our screening efforts, we have successfully identified a candidate polymer, P15, P-$(C6Im)_{0.35}$CL-*co*-P$(Man)_{0.65}$CL, that demonstrated good MICs with very low toxicities against red blood cells and 3T3 cells. This candidate compound was demonstrated to have faster killing kinetics when compared against vancomycin. In conclusion, we have developed a new class of clinically relevant antibacterial polymers that is biodegradable, potent and biocompatible, paving the way for clinical applications of antibacterial polymers in the future.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biomac.8b01577.

Experimental details, supplementary figures and tables, NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Zasloff, M. Antimicrobial Peptides of Multicellular Organisms. *Nature* **2002**, *415*, 389.
(2) Kenawy, E.-R.; Worley, S. D.; Broughton, R. The Chemistry and Applications of Antimicrobial Polymers: A State-of-the-Art Review. *Biomacromolecules* **2007**, *8*, 1359.
(3) Muñoz-Bonilla, A.; Fernández-García, M. Polymeric Materials with Antimicrobial Activity. *Prog. Polym. Sci.* **2012**, *37*, 281.
(4) Xue, Y.; Xiao, H.; Zhang, Y. Antimicrobial Polymeric Materials with Quaternary Ammonium and Phosphonium Salts. *Int. J. Mol. Sci.* **2015**, *16*, 3626.
(5) Brogden, K. A. Antimicrobial Peptides: Pore Formers or Metabolic Inhibitors in Bacteria? *Nat. Rev. Microbiol.* **2005**, *3*, 238.
(6) Tew, G. N.; Scott, R. W.; Klein, M. L.; DeGrado, W. F. De Novo Design of Antimicrobial Polymers, Foldamers, and Small Molecules: From Discovery to Practical Applications. *Acc. Chem. Res.* **2010**, *43*, 30.
(7) Tew, G. N.; Liu, D.; Chen, B.; Doerksen, R. J.; Kaplan, J.; Carroll, P. J.; Klein, M. L.; DeGrado, W. F. De Novo Design of Biomimetic Antimicrobial Polymers. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 5110.
(8) Liu, D.; Choi, S.; Chen, B.; Doerksen, R. J.; Clements, D. J.; Winkler, J. D.; Klein, M. L.; DeGrado, W. F. Nontoxic Membrane-Active Antimicrobial Arylamide Oligomers. *Angew. Chem., Int. Ed.* **2004**, *43*, 1158.
(9) Al-Badri, Z. M.; Som, A.; Lyon, S.; Nelson, C. F.; Nüsslein, K.; Tew, G. N. Investigating the Effect of Increasing Charge Density on the Hemolytic Activity of Synthetic Antimicrobial Polymers. *Biomacromolecules* **2008**, *9*, 2805.
(10) Ilker, M. F.; Nüsslein, K.; Tew, G. N.; Coughlin, E. B. Tuning the Hemolytic and Antibacterial Activities of Amphiphilic Polynorbornene Derivatives. *J. Am. Chem. Soc.* **2004**, *126*, 15870.
(11) Gabriel, G. J.; Madkour, A. E.; Dabkowski, J. M.; Nelson, C. F.; Nüsslein, K.; Tew, G. N. Synthetic Mimic of Antimicrobial Peptide with Nonmembrane-Disrupting Antibacterial Properties. *Biomacromolecules* **2008**, *9*, 2980.
(12) Lienkamp, K.; Madkour, A. E.; Musante, A.; Nelson, C. F.; Nüsslein, K.; Tew, G. N. Antimicrobial Polymers Prepared by ROMP with Unprecedented Selectivity: A Molecular Construction Kit Approach. *J. Am. Chem. Soc.* **2008**, *130*, 9836.
(13) Nederberg, F.; Zhang, Y.; Tan, J. P. K.; Xu, K.; Wang, H.; Yang, C.; Gao, S.; Guo, X. D.; Fukushima, K.; Li, L.; Hedrick, J. L.; Yang, Y.-Y. Biodegradable Nanostructures with Selective Lysis of Microbial Membranes. *Nat. Chem.* **2011**, *3*, 409.
(14) Voo, Z. X.; Khan, M.; Narayanan, K.; Seah, D.; Hedrick, J. L.; Yang, Y. Y. Antimicrobial/Antifouling Polycarbonate Coatings: Role of Block Copolymer Architecture. *Macromolecules* **2015**, *48*, 1055.
(15) Ng, V. W. L.; Tan, J. P. K.; Leong, J.; Voo, Z. X.; Hedrick, J. L.; Yang, Y. Y. Antimicrobial Polycarbonates: Investigating the Impact of Nitrogen-Containing Heterocycles as Quaternizing Agents. *Macromolecules* **2014**, *47*, 1285.

DOI: 10.1021/acs.biomac.8b01577
Biomacromolecules XXXX, XXX, XXX−XXX

(16) Chin, W.; Zhong, G.; Pu, Q.; Yang, C.; Lou, W.; De Sessions, P. F.; Periaswamy, B.; Lee, A.; Liang, Z. C.; Ding, X.; Gao, S.; Chu, C. W.; Bianco, S.; Bao, C.; Tong, Y. W.; Fan, W.; Wu, M.; Hedrick, J. L.; Yang, Y. Y. A Macromolecular Approach to Eradicate Multidrug Resistant Bacterial Infections while Mitigating Drug Resistance Onset. *Nat. Commun.* **2018**, *9*, 917.
(17) Nimmagadda, A.; Liu, X.; Teng, P.; Su, M.; Li, Y.; Qiao, Q.; Khadka, N. K.; Sun, X.; Pan, J.; Xu, H.; Li, Q.; Cai, J. Polycarbonates with Potent and Selective Antimicrobial Activity toward Gram-Positive Bacteria. *Biomacromolecules* **2017**, *18*, 87.
(18) Mowery, B. P.; Lee, S. E.; Kissounko, D. A.; Epand, R. F.; Epand, R. M.; Weisblum, B.; Stahl, S. S.; Gellman, S. H. Mimicry of Antimicrobial Host-Defense Peptides by Random Copolymers. *J. Am. Chem. Soc.* **2007**, *129*, 15474.
(19) Liu, R.; Chen, X.; Falk, S. P.; Mowery, B. P.; Karlsson, A. J.; Weisblum, B.; Palecek, S. P.; Masters, K. S.; Gellman, S. H. Structure−Activity Relationships among Antifungal Nylon-3 Polymers: Identification of Materials Active against Drug-Resistant Strains of Candida albicans. *J. Am. Chem. Soc.* **2014**, *136*, 4333.
(20) Liu, R.; Chen, X.; Falk, S. P.; Masters, K. S.; Weisblum, B.; Gellman, S. H. Nylon-3 Polymers Active against Drug-Resistant Candida albicans Biofilms. *J. Am. Chem. Soc.* **2015**, *137*, 2183.
(21) Zhou, C.; Qi, X.; Li, P.; Chen, W. N.; Mouad, L.; Chang, M. W.; Leong, S. S. J.; Chan-Park, M. B. High Potency and Broad-Spectrum Antimicrobial Peptides Synthesized via Ring-Opening Polymerization of α-Aminoacid-N-carboxyanhydrides. *Biomacromolecules* **2010**, *11*, 60.
(22) Xiong, M.; Lee, M. W.; Mansbach, R. A.; Song, Z.; Bao, Y.; Peek, R. M.; Yao, C.; Chen, L.-F.; Ferguson, A. L.; Wong, G. C. L.; Cheng, J. Helical Antimicrobial Polypeptides with Radial Amphiphilicity. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, 13155.
(23) Kuroda, K.; DeGrado, W. F. Amphiphilic Polymethacrylate Derivatives as Antimicrobial Agents. *J. Am. Chem. Soc.* **2005**, *127*, 4128.
(24) Ivanov, I.; Vemparala, S.; Pophristic, V.; Kuroda, K.; DeGrado, W. F.; McCammon, J. A.; Klein, M. L. Characterization of Nonbiological Antimicrobial Polymers in Aqueous Solution and at Water−Lipid Interfaces from All-Atom Molecular Dynamics. *J. Am. Chem. Soc.* **2006**, *128*, 1778.
(25) Sambhy, V.; Peterson, B. R.; 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.* **2008**, *47*, 1250.
(26) 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. Robust Antimicrobial Compounds and Polymers Derived from Natural Resin Acids. *Chem. Commun.* **2012**, *48*, 916.
(27) Li, Y.; Fukushima, K.; Coady, D. J.; Engler, A. C.; Liu, S.; Huang, Y.; Cho, J. S.; Guo, Y.; Miller, L. S.; Tan, J. P. K.; Ee, P. L. R.; Fan, W.; Yang, Y. Y.; Hedrick, J. L. Broad-Spectrum Antimicrobial and Biofilm-Disrupting Hydrogels: Stereocomplex-Driven Supramolecular Assemblies. *Angew. Chem., Int. Ed.* **2013**, *52*, 674.
(28) Jung, J. H.; Ree, M.; Kim, H. Acid- and base-catalyzed hydrolyses of aliphatic polycarbonates and polyesters. *Catal. Today* **2006**, *115*, 283.
(29) Sanchez, J. G.; Tsuchii, A.; Tokiwa, Y. Degradation of Polycaprolactone at 50 °C by a Thermotolerant *Aspergillus* sp. *Biotechnol. Lett.* **2000**, *22*, 849.
(30) Pranamuda, H.; Chollakup, R.; Tokiwa, Y. Degradation of Polycarbonate by a Polyester-Degrading Strain, *Amycolatopsis* sp. Strain HT-6. *Appl. Environ. Microb.* **1999**, *65*, 4220.
(31) Mizutani, M.; Palermo, E. F.; Thoma, L. M.; 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*, 1554.
(32) Ergene, C.; Palermo, E. F. Cationic Poly(benzyl ether)s as Self-Immolative Antimicrobial Polymers. *Biomacromolecules* **2017**, *18*, 3400.
(33) Chamsaz, E. A.; Mankoci, S.; Barton, H. A.; Joy, A. Nontoxic Cationic Coumarin Polyester Coatings Prevent Pseudomonas aeruginosa Biofilm Formation. *ACS Appl. Mater. Interfaces* **2017**, *9*, 6704.
(34) Mankoci, S.; Kaiser, R. L.; Sahai, N.; Barton, H. A.; Joy, A. Bactericidal Peptidomimetic Polyurethanes with Remarkable Selectivity against Escherichia coli. *ACS Biomater. Sci. Eng.* **2017**, *3*, 2588.
(35) Lenoir, S.; Riva, R.; Lou, X.; Detrembleur, C.; Jérôme, R.; Lecomte, P. Ring-Opening Polymerization of α-Chloro-ε-caprolactone and Chemical Modification of Poly(α-chloro-ε-caprolactone) by Atom Transfer Radical Processes. *Macromolecules* **2004**, *37*, 4055.
(36) Bexis, P.; Thomas, A. W.; Bell, C. A.; Dove, A. P. Synthesis of Degradable Poly(ε-caprolactone)-based Graft Copolymers via a "grafting-from" Approach. *Polym. Chem.* **2016**, *7*, 7126.
(37) Riva, R.; Schmeits, S.; Jérôme, C.; Jérôme, R.; Lecomte, P. Combination of Ring-Opening Polymerization and "Click Chemistry": Toward Functionalization and Grafting of Poly(ε-caprolactone). *Macromolecules* **2007**, *40*, 796.
(38) Xu, N.; Wang, R.; Du, F.-S.; Li, Z.-C. Synthesis of Amphiphilic Biodegradable Glycocopolymers Based on Poly(ε-caprolactone) by Ring-opening Polymerization and Click Chemistry. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 3583.
(39) Roychoudhury, S.; Zielinski, N. A.; Ninfa, A. J.; Allen, N. E.; Jungheim, L. N.; Nicas, T. I.; Chakrabarty, A. M. Inhibitors of Two-Component Sgnal Transduction Systems: Inhibition of Aginate Gene Activation in *Pseudomonas Aeruginosa*. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 965.
(40) Pernak, J.; Sobaszkiewicz, K.; Mirska, I. Anti-microbial Activities of Ionic Liquids. *Green Chem.* **2003**, *5*, 52.
(41) Riduan, S. N.; Zhang, Y. Imidazolium Salts and Their Polymeric Materials for Biological Applications. *Chem. Soc. Rev.* **2013**, *42*, 9055.
(42) Kuroda, K.; Caputo, G. A.; DeGrado, W. F. The Role of Hydrophobicity in the Antimicrobial and Hemolytic Activities of Polymethacrylate Derivatives. *Chem. - Eur. J.* **2009**, *15*, 1123.
(43) Lienkamp, K.; Madkour, A. E.; Kumar, K.-N.; Nüsslein, K.; Tew, G. N. Antimicrobial Polymers Prepared by Ring-Opening Metathesis Polymerization: Manipulating Antimicrobial Properties by Organic Counterion and Charge Density Variation. *Chem. - Eur. J.* **2009**, *15*, 11715.
(44) Qiao, Y.; Yang, C.; Coady, D. J.; Ong, Z. Y.; Hedrick, J. L.; Yang, Y.-Y. Highly Dynamic Biodegradable Micelles Capable of Lysing Gram-Positive and Gram-Negative Bacterial Membrane. *Biomaterials* **2012**, *33*, 1146.
(45) Engler, A. C.; Shukla, A.; Puranam, S.; Buss, H. G.; Jreige, N.; Hammond, P. T. Effects of Side Group Functionality and Molecular Weight on the Activity of Synthetic Antimicrobial Polypeptides. *Biomacromolecules* **2011**, *12*, 1666.
(46) Palermo, E. F.; Kuroda, K. Chemical Structure of Cationic Groups in Amphiphilic Polymethacrylates Modulates the Antimicrobial and Hemolytic Activities. *Biomacromolecules* **2009**, *10*, 1416.
(47) Eren, T.; Som, A.; Rennie, J. R.; Nelson, C. F.; Urgina, Y.; Nüsslein, K.; Coughlin, E. B.; Tew, G. N. Antibacterial and Hemolytic Activities of Quaternary Pyridinium Functionalized Polynorbornenes. *Macromol. Chem. Phys.* **2008**, *209*, 516.
(48) Venkataraman, S.; Zhang, Y.; Liu, L.; Yang, Y.-Y. Design, Syntheses and Evaluation of Hemocompatible Pegylated-Antimicrobial Polymers with Well-Controlled Molecular Structures. *Biomaterials* **2010**, *31*, 1751.
(49) Lopez, A. I.; Reins, R. Y.; McDermott, A. M.; Trautner, B. W.; Cai, C. Antibacterial Activity and Cytotoxicity of PEGylated Poly(amidoamine) Dendrimers. *Mol. BioSyst.* **2009**, *5*, 1148.
(50) Colak, S.; Nelson, C. F.; Nüsslein, K.; Tew, G. N. Hydrophilic Modifications of an Amphiphilic Polynorbornene and the Effects on its Hemolytic and Antibacterial Activity. *Biomacromolecules* **2009**, *10*, 353.
(51) Yang, C.; Krishnamurthy, S.; Liu, J.; Liu, S.; Lu, X.; Coady, D. J.; Cheng, W.; De Libero, G.; Singhal, A.; Hedrick, J. L.; Yang, Y. Y. Broad-Spectrum Antimicrobial Star Polycarbonates Functionalized

DOI: 10.1021/acs.biomac.8b01577
Biomacromolecules XXXX, XXX, XXX−XXX

with Mannose for Targeting Bacteria Residing inside Immune Cells. *Adv. Healthcare Mater.* **2016**, *5*, 1272.
(52) Wong, E. H. H.; Khin, M. M.; Ravikumar, V.; Si, Z.; Rice, S. A.; Chan-Park, M. B. Modulating Antimicrobial Activity and Mammalian Cell Biocompatibility with Glucosamine-Functionalized Star Polymers. *Biomacromolecules* **2016**, *17*, 1170.
(53) Álvarez-Paino, M.; Muñoz-Bonilla, A.; López-Fabal, F.; Gómez-Garcés, J. L.; Heuts, J. P. A.; Fernández-García, M. Effect of Glycounits on the Antimicrobial Properties and Toxicity Behavior of Polymers Based on Quaternized DMAEMA. *Biomacromolecules* **2015**, *16*, 295.
(54) Ma, G.; Li, D.; Wang, J.; Zhang, X.; Tang, H. Methoxy-Poly(ethylene glycol)-block-Poly(ϵ-caprolactone) Bearing Pendant Aldehyde Groups as pH-Responsive Drug Delivery Carrier. *Aust. J. Chem.* **2013**, *66*, 1576.
(55) Wiegand, I.; Hilpert, K.; Hancock, R. E. W. Agar and Broth Dilution Methods to Determine the Minimal Inhibitory Concentration (MIC) of Antimicrobial Substances. *Nat. Protoc.* **2008**, *3*, 163.
(56) Gabriel, G. J.; Maegerlein, J. A.; Nelson, C. F.; Dabkowski, J. M.; Eren, T.; Nüsslein, K.; Tew, G. N. Comparison of Facially Amphiphilic versus Segregated Monomers in the Design of Antibacterial Copolymers. *Chem. - Eur. J.* **2009**, *15*, 433.
(57) Huang, R. T. W.; Peng, K. C.; Shih, H. N.; Lin, G. H.; Chang, T. F.; Hsu, S. J.; Hsu, T. S. T.; Lin, I. J. B. Antimicrobial Properties of Ethoxyether-Functionalized Imidazolium Salts. *Soft Matter* **2011**, *7*, 8392.
(58) Docherty, K. M.; Kulpa, J. C. F., Jr. Toxicity and Antimicrobial Activity of Imidazolium and Pyridinium Ionic Liquids. *Green Chem.* **2005**, *7*, 185.
(59) Richter, M. F.; Drown, B. S.; Riley, A. P.; Garcia, A.; Shirai, T.; Svec, R. L.; Hergenrother, P. J. Predictive Compound Accumulation Rules Yield a Broad-Spectrum Antibiotic. *Nature* **2017**, *545*, 299.
(60) Delcour, A. H. Outer Membrane Permeability and Antibiotic Resistance. *Biochim. Biophys. Acta, Proteins Proteomics* **2009**, *1794*, 808.
(61) Pankey, G. A.; Sabath, L. D. Clinical Relevance of Bacteriostatic versus Bactericidal Mechanisms of Action in the Treatment of Gram-Positive Bacterial Infections. *Clin. Infect. Dis.* **2004**, *38*, 864.
(62) Stratton, C. W. Dead Bugs Don't Mutate: Susceptibility Issues in the Emergence of Bacterial Resistance. *Emerging Infect. Dis.* **2003**, *9*, 10.

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