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Biodegradable Broad-Spectrum Antimicrobial Polycarbonates: Investigating the Role of Chemical Structure on Activity and Selectivity

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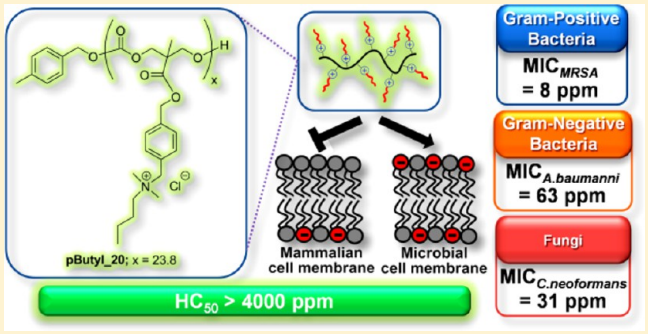
Supporting Information

ABSTRACT: A series of biodegradable polycarbonate polymers was designed and synthesized via organocatalytic ring-opening polymerization of functional cyclic carbonate monomer (MTC−OCH_2BnCl). By adopting a facile post-polymerization functionalization strategy, the polycarbonates were quaternized to yield cationic polymers with quaternary ammonium groups of various pendant structures (e.g., alkyl, aromatic, imidazolinium). The biological properties of these polymers were investigated by microbial growth inhibition assays against clinically relevant Gram-positive and Gram-negative bacteria, fungus as well as hemolysis assays using rat red blood cells. A judicious choice in the structure of the cationic appendages elucidated that the amphiphilic balance of the polymers is a pertinent determinant to render substantial antimicrobial potency and low hemolysis, consequently affording the polymer pButyl_20 (degree of polymerization, 20; quaternary ammonium group, *N*,*N*-dimethylbutylammonium) as a highly efficacious and nonhemolytic antimicrobial agent with a remarkable selectivity of more than 1026. To ameliorate the selectivity against a wider spectrum of microbes including the difficult-to-kill *Pseudomonas aeruginosa*, it was shown that polymers containing *N*,*N*-dimethylbutylammonium and *N*,*N*-dimethylbenzylammonium groups in 1:1 molar ratio exerted considerable antimicrobial potency while remaining relatively nonhemolytic. Biophysical studies encompassing the determination of water-octanol partition coefficients (log *P*) and dye leakage studies from model liposomes provided useful insights which delineate the pivotal role of cationic group structure in the antimicrobial activity and mechanism of these polymers. Through field emission scanning electron microscopy (FE-SEM), a physical lysis of microbial cell membranes was deemed operative in the antimicrobial action of these macromolecular agents, which would consequently reduce the propensity toward resistance development. These polymers are envisaged to be promising antimicrobial agents for the prevention and treatment of multidrug-resistant pathogenic infections.



1. INTRODUCTION

The ongoing prevalence of drug-resistant bacterial infections represents a challenging and eminent health issue facing society today.[1] Exacerbated by the diminishing number of novel and effective antibiotics,[2] this precarious situation has since prompted unremitting efforts for developing alternative compounds that not only show high efficacy toward pathogenic microbes, but also exhibit reduced propensity toward resistance development. To that end, an unconventional class of antimicrobial agents known as natural antimicrobial peptides (AMPs) has emerged in the past decade as effective macromolecules that are capable of exerting their antimicrobial activity by disrupting the microbial cell membranes.[3] Owing to the physical nature of its mode of activity, it is postulated to be less susceptible than metabolic targeting of conventional antibiotics to the development of resistance. Despite being highly efficacious, their applicability is still beset with a number of limitations including: high cytotoxicity (e.g., hemolysis),

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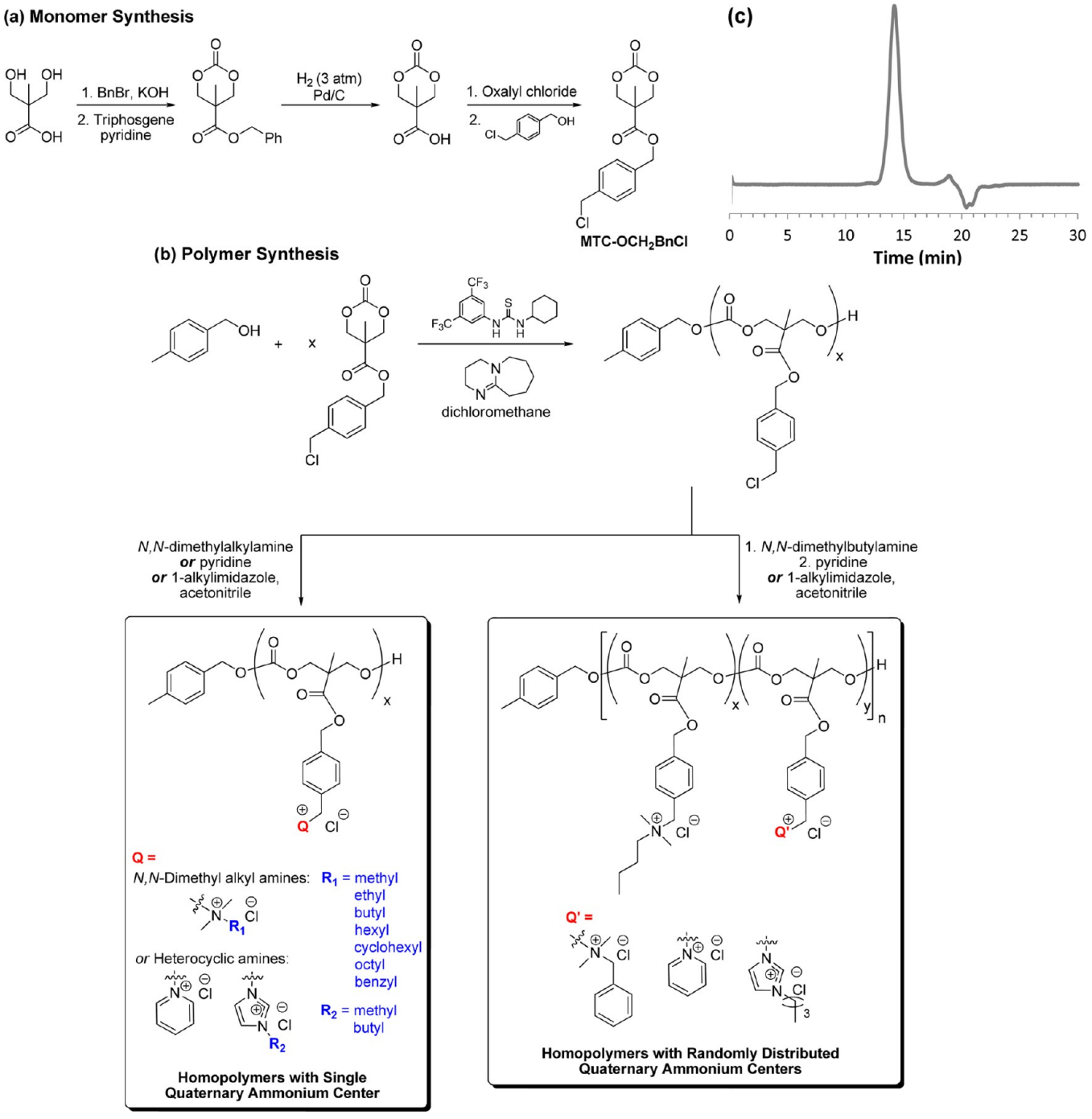
Scheme 1. Synthetic Scheme and Structure of (a) MTC−OCH_2BnCl Monomer and (b) Amphiphilic Cationic Polycarbonates by Varying the Cationic Group Structures and (c) Gel-Permeation Chromatograph of Precursor Polymer Prior to Post-Polymerization Quaternization



poor proteolytic stability and pharmacokinetics (i.e., short half-life *in vivo*), and high manufacturing cost.[4]

The design principles of most AMPs have hitherto been governed by two common characteristics, which are a facially amphiphilic nature and cationic charge.[5] Such attributes allow the AMPs to electrostatically adhere to the negatively charged microbial cell surface while the hydrophobic moieties disrupt the membrane by insertion into the bilayer core. By drawing "molecular inspiration" from AMPs so as to mimic their cationic and amphiphilic structure, synthetic antimicrobial polymers[6,7] (e.g., polymethacrylates[8] and polynorbornenes[9]) have also recently demonstrated their ability to target pathogens via a membrane disruption mechanism. Notwithstanding the numerous synthetic efforts in developing potent macromolecules for targeting a wide spectrum of microbes, the relatively low selectivity of many polymers resulting in cytotoxicity to mammalian cells (in particular, hemolysis) remains an unsolved issue.[9] Furthermore, the nonbiodegradable polymeric backbones observed in the majority of these synthetic materials would present significant problems during *in vivo* administration, thus hampering their potential for clinical applications.

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Recently, we reported biodegradable antimicrobial polycarbonates, which were designed via the "segregated monomer" approach, wherein a relatively hydrophobic monomer is randomly copolymerized with a cationic monomer to afford either statistical or block copolymers.[10,11] It is widely established that the hydrophobic/hydrophilic balance (i.e., amphiphilicity) of antimicrobial polymers represents a pivotal determinant in influencing how they interact with cellular membranes, and as a consequence imparts selectivity toward bacteria over mammalian cells.[12] The amphiphilic balance of polycarbonates could thus be fine-tuned by varying the monomeric composition across the polymer chain. These materials demonstrated antimicrobial activities toward a wide spectrum of microbes with good hemolytic profiles; however, the observed minimum inhibitory concentrations (MICs) were still considerably high when compared to most AMPs, which consequently limits their usage in biomedical applications.

As highlighted in our recent review,[13] many classes of antimicrobial polymers have primarily focused on adopting the "segregated monomer" and "facially amphiphilic monomer" (i.e., each repeat unit is comprised of a separate hydrophobic and cationic section) approaches for tuning the hydrophobic/hydrophilic balance. In contrast, relatively fewer studies have been dedicated toward polymers employing the "same centered" approach for developing macromolecular antimicrobial agents. In the "same centered" approach, a hydrophobic moiety (usually an alkyl chain) is directly conjugated to a cationic center, allowing the facile optimization of its amphiphilicity by varying various structural parameters pertaining to the hydrophobic component. A notable work described by Sens and co-workers[14] have shown that polymethacrylates with cationic pyridinium and hydrophobic tails on the "same center" led to improved selectivities while a spatial separation of the cationic and hydrophobic components (i.e., "segregated monomer") rendered the polymers to become significantly biocidal (hemolytic and antimicrobial). Taking into account the findings gathered from these studies, it therefore remains desirable to design and synthesize biodegradable antimicrobial polycarbonates via the less explored "same centered" approach which we postulate to be able to lead to a pronounced membrane-lytic activity toward microbes while retaining or even augmenting their previously observed biocompatibility, consequently giving rise to promising materials with potent antimicrobial activity and high selectivity.

The study presented herein describes a series of amphiphilic polycarbonates with the "same centered" structure, which was synthesized via metal-free organocatalytic ring-opening polymerization of functional cyclic carbonate monomer to investigate the structure−activity relationship. By adopting a facile and efficient postpolymerization quaternization reaction, the cationic group structure and their respective compositions were systematically modulated to study their effect on antimicrobial activity against clinically relevant microbes and toxicity against mammalian erythrocytes. Results revealed that by carefully controlling the hydrophobic/hydrophilic balance of the polymers, it is possible to dramatically enhance the selectivity through subtle structural modifications in the cationic groups, giving rise to biodegradable polymers with remarkably high and unprecedented selectivity toward a broad range of pathogenic microbes over mammalian cells. To elucidate the role of the cationic groups in the observed biological activity, a series of biophysical studies such as water-octanol partition coefficients and polymer-induced dye leakage from model liposomes was carried out. In addition, the antimicrobial mechanism of action was explored by visualizing the morphological changes incited by the polymers on the bacteria cell surfaces.

2. EXPERIMENTAL SECTION

2.1. Materials. 2,2-Bis(hydroxymethyl)propionic acid (bis-MPA) and *N*-(3,5-trifluoromethyl) phenyl-*N*′-cyclohexylthiourea (TU) were prepared according to our previous protocol.[15,16] TU was dissolved in dry tetrahydrofuran (THF), stirred with CaH_2, filtered, and freed of solvent *in vacuo*. Prior to use, 1,8-diazabicyclo [5,4,0]undec-7-ene (DBU) were stirred over CaH_2 and vacuum distilled before being transferred to a glovebox. All other chemical reagents were purchased from Sigma-Aldrich and used as received unless specified. Ultra pure (HPLC grade) water was obtained from J.T. Baker (U.S.A.). Phosphate-buffered saline (PBS) at 10× concentration was purchased from 1st BASE (Singapore) and diluted to the intended concentrations before use. Tryptic soy broth (TSB) powder was bought from BD Diagnostics (Singapore) and used to prepare the microbial broths according to the manufacturer's instructions. Methanol, ethanol, formalin solution (10% neutral buffered) and calcein were purchased from Sigma-Aldrich (Singapore) and used as received. The phospholipids 1,2-dioleoyl-snglycero-3-phospho-(10-*rac*-glycerol) (DOPG), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) were obtained as dry powder from Avanti Polar Lipids, Inc. *Staphylococcus aureus* (ATCC No. 6538), *Escherichia coli* (ATCC No. 25922) and *Pseudomonas aeruginosa* (ATCC No. 9027) were obtained from ATCC (U.S.A) and reconstituted according to the suggested protocols. Methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), carbapenem-resistant *Acinetobacter baumannii* and fluconazole-resistant *Cryptococcus neoformans* were extracted from patients' blood (MRSA, VRE and carbapenem-resistant *A. baumannii*) and cerebrospinal fluid (fluconazole-resistant *C. neoformans*) samples, and kindly provided by Z. Q. Wei, Department of Infectious Diseases, The First Affiliated Hospital, College of Medicine, Zhejiang University, P. R. China.

2.2. Synthesis of MTC−OCH_2BnCl Monomer (Scheme 1a). MTC−OCH_2BnCl was synthesized with reference to the protocol reported in the previous work.[15] Briefly, in a dry three-neck round-bottom flask equipped with a stir bar, MTC−OH (3.08 g, 19.3 mmol) was dissolved in dry THF (50 mL) with a 3−4 drops of dimethylformamide (DMF). A solution of oxalyl chloride (2.45 mL, 28.5 mmol) in THF (50 mL) was subsequently added from a dropping funnel. Under an inert atmosphere, the solution was stirred for 1 h, after which volatiles were removed under vacuum, yielding an off-white solid (i.e., 5-chlorocarboxy-5-methyl-1,3-dioxan-2-one intermediate). The solid was heated to 60 °C for a brief 2−3 min to remove any residual solvent, and then redissolved in dry CH_2Cl_2 (50 mL) and cooled down to 0 °C via an ice bath. A mixture of *p*-chloromethyl benzyl alcohol (2.79 g, 17.8 mmol) and pyridine (1.55 mL, 19.3 mmol) dissolved in dry CH_2Cl_2 (50 mL) was then added dropwise over a duration of 30 min, and allowed to stir at 0 °C for an additional 30 min before leaving it at ambient temperature for further stirring overnight. After removal of solvent, the crude product was subjected to purification by flash column chromatography using silica gel and a hexane-ethyl acetate solvent system as the eluent (gradient elution up to 80% vol. ethyl acetate) to yield MTC−OCH_2BnCl as a white solid (4.1 g, 13.6 mmol, 70% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 22 °C): δ 7.36 (dd, J = 30.3, 8.0 Hz, 4H, Ph-*H*), 5.20 (s, 2H, OCH_2), 4.69 (d, J = 10.8 Hz, 2H, CH_aH_b), 4.58 (s, 2H, CH_2Cl), 4.20 (d, J = 10.8 Hz, 2H, CH_aH_b), 1.31 (s, 3H, CCH_3).

2.3. Synthesis of Representative Cationic Polymers (Scheme 1b). The detailed procedures for the ring-opening polymerization (ROP) of MTC−OCH_2BnCl with 4-methyl benzyl alcohol as initiator are given as a representative example. Using a glovebox, MTC−OCH_2BnCl (448 mg, 1.2 mmol) was added to a reaction vial containing 1-(3,5-bis(trifluoromethyl)-phenyl)-3-cyclohexyl-2-thiourea (TU) (22.2 mg, 0.05 mmol) dissolved in dry DCM (2 mL). The

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mixture was subsequently charged with 4-methyl benzyl alcohol (7.32 mg, 0.06 mmol), before adding 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (8.96 μL, 0.05 mmol) and left to stir at room temperature for approximately 30 min. At the end of the reaction, an excess of benzoic acid (10 mg, 0.08 mmol) was added to quench the catalyst. The crude polymer was then precipitated twice into cold methanol and the supernatant decanted to obtain a white solid (91% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 22 °C): δ 7.41−7.26 (m, 89H, Ph-*H*), 5.18−5.06 (m, 48H, −OCOCH_2-), 4.58−4.52 (m, 45H, −CH_2Cl), 4.34−4.21 (m, 83H, −OCOOCH_2- and −OCH_2CCH$_3$−), 2.34 (s, 3H, initiator CH_3), 1.28−1.15 (m, 69H, −CH_3).

For postpolymerization quaternization, the aforementioned polymer (0.35 g, 0.05 mmol) was initially added to a reaction vial and dissolved in acetonitrile (10 mL). The quaternizing agent was added in excess (5 equiv of benzyl chloride groups) and the reaction mixture left to stir overnight at ambient temperature. Quaternization reactions involving trimethylamine (TMA) were carried out in a pressure safe Schlenk tube owing to the gaseous nature of TMA. The addition of TMA gas was carried out by initially cooling the reaction mixture to −78 °C using dry ice and the condensed TMA gas (4 mL, 42.6 mmol) was subsequently added and sealed before leaving it to stir overnight at ambient temperature. For the quaternization of polymers with pyridine, the reaction was carried out at 40 °C. The synthesis of polymers containing randomly distributed quaternary ammonium centers was carried out in two consecutive steps, whereby the precursor polymer was first quaternized with *N*,*N*-dimethylbutylamine to the desired molar composition, as verified by *in situ* ^{1}H NMR. After that, the partially quaternized polymer was reacted with an excess of the second quaternizing agent.

Following quaternization, the crude product was then subjected to purification via dialysis against a 1:1 acetonitrile-isopropanol solvent system. After removal of solvent, the resultant polymer was freeze-dried to yield a white solid. The yields and analytical data for all quaternized polymers are provided in the Supporting Information.

2.4. MIC Measurements. All bacteria strains obtained from ATCC were reconstituted from its lyophilized form according to the manufacturer's protocol. Bacteria from ATCC and clinical samples were cultured in tryptic soy broth (TSB) and Mueller−Hinton broth (MHB) solutions, respectively, at 37 °C under constant shaking of 100 rpm. The MICs of the polymers were measured using the broth microdilution method. Briefly, 100 μL of TSB or MHB solution containing the polymer (with a fixed deionized (DI) water concentration of 20% v/v) at various concentrations (0−500 μg mL^{-1}) was placed into each well of a 96-well microplate. An equal volume of microbial suspension (3×10^5 CFU mL^{-1}) was added into each well. Prior to mixing, the microbial sample was first inoculated overnight to enter its log growth phase. The concentration of bacterial solution was adjusted to give an initial optical density (OD) reading of approximately 0.07 at 600 nm wavelength on a microplate reader (TECAN, Switzerland), which corresponds to the concentration of McFarland 1 solution (3×10^8 CFU mL^{-1}). The bacterial solution was then further diluted 1000-fold to achieve an initial inoculum of 3×10^5 CFU mL^{-1}. The 96-well plate was kept in an incubator at 37 °C under constant shaking of 100 rpm for 18 h. The MIC was taken as the concentration of the antimicrobial polymer at which no microbial growth was observed with unaided eyes and the microplate reader at the end of 18 h incubation. Broth containing microbial cells alone was used as the negative control, and each test was carried out in 6 replicates.

2.5. Time-Kill and Killing Efficiency Tests. The bacteria were inoculated and prepared according to the same procedure in the MIC measurement described above. The samples were treated with the polymer at various concentrations (0, 1/2 × MIC, MIC, and 2 × MIC), and were incubated at 37 °C under constant shaking of 100 rpm. At regular time intervals (0, 0.5, 1, 2, 4, 6, 8, and 18 h), the bacteria samples were taken out from each well for a series of 10-fold dilutions. Twenty μL of the diluted bacterial solution was streaked onto an agar plate (LB Agar from 1st Base). The plate was incubated for 24 h at 37 °C and counted for colony-forming units (CFU). For killing-efficiency studies, the samples were taken after 18 h incubation and plated using the same protocol for viable counts.

2.6. Hemolysis Assay. The toxicity of the polymers against mammalian erythrocytes was tested using fresh rat red blood cells (rRBCs). Briefly, rRBCs were diluted 25-fold in PBS to achieve 4% v/v of blood content. The polymers were dissolved in PBS at concentrations ranging from 0 to 4000 μg mL^{-1} by serial dilutions. Equal volumes of polymer solutions (100 μL) were then mixed with the diluted blood suspension (100 μL). The mixtures were then incubated at 37 °C for 1 h to allow for the interactions between rRBC and the polymers to take place. After that, the mixture was subjected to centrifugation (1000 g for 5 min, 4 °C), and 100 μL aliquots of the supernatant was pipetted into a 96-well microplate. The hemoglobin release was measured spectrophotometrically by measuring the absorbance of the samples at 576 nm using the microplate reader (TECAN, Switzerland). Two control groups were employed for this assay: untreated rRBC suspension (negative control), and rRBC suspension treated with 0.1% Triton-X (positive control). Each assay was performed in 4 replicates. The percentage of hemolysis was defined as follows:

Hemolysis (%) = [($OD_{576\ nm}$ of the treated sample − $OD_{576\ nm}$ of the negative control)/($OD_{576\ nm}$ of positive control − $OD_{576\ nm}$ of negative control)] × 100%.

2.7. FE-SEM. Bacteria cells grown in TSB with or without polymer treatment were performed using a similar protocol as MIC measurements but with a 2 h incubation time. All the samples were collected into a microfuge tube and pelleted at 4000 rpm for 5 min, and then washed twice with PBS buffer. Subsequently, fixing the samples with formalin solution (10% neutral buffered) for 60 min was conducted, followed by washing with DI water twice. Dehydration of the samples was performed by using a series of ethanol/water solution (35%, 50%, 75%, 90%, 95%, and 100%). The dehydrated samples were dried at room temperature for 2 days before being mounted on carbon tape and coated with platinum for imaging using a JEOL JSM-7400F (Japan) field emission scanning electron microscope.

2.8. Water−Octanol Partition Coefficient (log *P*). Solutions containing representative dansyl-conjugated polymers (500 μL, 63 μg mL^{-1}) were prepared in PBS buffer in a microfuge tube and octanol (500 μL) was added. The tube was vortexed for 5 min and then allowed to sit overnight in the dark. After 5 min of centrifugation at 3000 rpm, an aliquot of each phase was diluted 10-fold into methanol and the fluorescence spectra were recorded. The concentration of polymer in each phase was determined with reference to calibration curves obtained by measuring the fluorescence spectra of the polymers at various concentrations in methanol. The partition coefficient was defined as log P = log ($[P]_{oct}/[P]_{aq}$) where $[P]_{oct}$ and $[P]_{aq}$ are the concentration of the polymer in the octanol and aqueous phases, respectively. Measurements were performed in triplicates.

2.9. Liposome Dye-Leakage Assay. To prepare the liposomes, the following buffers were prepared: buffer 1 consisted of 10 mM Na_2HPO_4 in H_2O at pH = 7.0, buffer 2 consisted of 10 mM Na_2HPO_4 and 90 mM NaCl. Calcein dye was dissolved in buffer 1 to achieve a concentration of 40 mM. To a clean round-bottom flask, appropriate volumes of lipid stocks (25 mg/mL $CHCl_3$) were added to make up 1 mL of $CHCl_3$ (For 4:1 PE/PG vesicles, 238 μL of PE and 63.6 μL of PG were used; For PC vesicles, 314.4 μL of PC was used). The solvent was removed by a stream of nitrogen gas to obtain a thin lipid film, which was then hydrated by 1 mL of calcein solution. The mixture was left to stir for 1 h, after which it was subjected to 10 freeze−thaw cycles (using dry ice/acetone to freeze and warm water to thaw). The suspension was extruded 20 times through a polycarbonate membrane with 400 nm pore diameter. The excess dye was removed using Sephadex G-50 column and buffer 2 as the eluent. The dye-filled vesicle fractions were diluted 2000 times with buffer 2 (final lipid concentration: ∼5.0 mM). This suspension (90 μL) was subsequently mixed with polymer stock solutions (10 μL) on a 96-well black microplate (Greiner, flat bottom). Buffer 2 (10 μL) and Triton-X (0.1% v/v, 10 μL) were employed as the negative and positive controls, respectively. After 1 h, the fluorescence intensity in each well was recorded using the microplate reader (TECAN, Switzerland) with

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Table 1. Antimicrobial (MIC, μg mL^{-1}) and Haemolytic (HC_{50}, μg mL^{-1}) Activities of Homopolymers with Varying Cationic Group Structures

			MIC (μg mL^{-1})				selectivity (HC_{50}/MIC)		
polymer	R_1 or R_2	DP	*E. coli*	*S. aureus*	*P. aeruginosa*	HC_{50} (μg mL^{-1})	*E. coli*	*S. aureus*	*P. aeruginosa*
pMethyl_20	methyl	22.5	125	62.5	>500	>4000	>32	>64	<8
pEthyl_20	ethyl	23.6	125	31.3	>500	>4000	>32	>128	<8
pButyl_20	butyl	23.8	15.6	3.9	>500	>4000	>256	>1026	<8
pHexyl_20	hexyl	23.5	7.8	3.9	62.5	15.6	2	4	0.25
pCyhexyl_20	cyclohexyl	23.9	15.6	7.8	>500	250	16	32	<0.5
pOctyl_20	octyl	20.7	62.5[a]	31.3[a]	125[a]	7.8	0.12	0.25	0.06
pBenzyl_20	benzyl	22.7	15.6	7.8	125	250	16	32	2
pPyr_20	pyridine	21.2	15.6	7.8	125	1000	64	128	8
pMeImi_20	1-methyl imidazole	20.1	15.6	3.9	>500	>1000	>64	>256	<2
pBuImi_20	1-butyl imidazole	23.4	7.8	3.9	62.5	250	32	64	4

[a]Poorly soluble in nutrient broth; measured as finely ultrasonic-distributed suspensions.

excitation and emission wavelengths of 490 and 515 nm, respectively. The percentage of leaked calcein dye in each well was determined as follows: leakage (%) = $[(F - F_0)/(F_{TX} - F_0)] \times 100\%$ where F is the fluorescence intensity recorded in the well, F_0 is the intensity in the negative control well, and F_{TX} is the intensity in the positive control well.

3. RESULTS AND DISCUSSION

3.1. Monomer and Polymer Synthesis. In light of designing antimicrobial polymers with hydrophobic pendent groups bearing synthetically accessible and reactive moieties toward various tertiary, cyclic as well as heterocyclic amines for facile postpolymerization quaternization, a carbonate monomer containing a benzyl chloride functional group was synthesized using a previously established route,[15] as shown in Scheme 1. Metal-free organocatalytic ring-opening polymerization (ROP) of the functional carbonate was subsequently employed to yield homopolymers with varying degrees of polymerization (20−60). Owing to the living nature and highly controlled ability of organocatalytic ROP, the homopolymers were obtained with respective lengths that were dictated by initial monomer:-initiator feed ratios, as ascertained by comparing the integrated intensities of the relevant ^{1}H NMR resonances from the terminal initiator (4-methyl benzyl alcohol) relative to the side chains (Figure S1 in the Supporting Information). All polymers exhibited narrow molecular weight distribution with a polydispersity index ranging between 1.2 and 1.3, which was determined by gel permeation chromatography prior to postpolymerization quaternization. The metal-free nature of the catalysts and low catalyst loadings (5 mol %) are also anticipated to circumvent potential cytotoxic issues typically associated with catalyst residues.

As amphiphilic balance has been shown to play a pivotal role in the antimicrobial and hemolytic properties, a series of polymers was synthesized by reacting the precursor polymer with a variety of quaternizing agents in a simple and facile quaternization reaction performed under ambient conditions (Scheme 1). An exception was made for the reaction with pyridine as the quaternizing agent most likely owing to the slightly reduced nucleophilicity of the nitrogen center, which thus led to the need for slight heating (40 °C) during quaternization. It was anticipated that the amphiphilicity of the resultant polymers could be tuned by simple modifications of the cationic group structures either by varying the length of the alkyl chains of homologous tertiary (i.e., *N,N*-dimethylalkylamines) and heterocyclic amines (i.e., 1-alkylimidazoles) or by virtue of its chemical structure (e.g., pyridine). As a consequence of the reactive benzylic center, the reaction was found to be remarkably efficient in all the cases where near-quantitative (>99%) degree of quaternization was typically achieved, as determined by ^{1}H NMR spectroscopy (Figure S1). Such high extent of quaternization would indubitably be an attractive synthetic approach for the facile tuning of the polymers' composition and amphiphilicity, so as to effectively investigate their influence on antimicrobial and hemolytic activities.

As a means to characterize the hydrophobic/hydrophilic balance of polycarbonates, water-octanol partition coefficients were investigated. Representative polymers conjugated with a highly fluorescent dansyl group at the chain end were prepared in a similar fashion to that described above using a modified hydroxyl-terminated dansyl as the initiator (Scheme S1). The wavelengths of maximum absorbance and fluorescence emission of the polymers in methanol ranged from 332 to 336 nm and 519−520 nm respectively (Table S1), and are comparable to those of free dansyl and dansyl-conjugated proteins reported in literature.[17,18] These data indicated that the conjugation to these polymers did not affect or diminish the absorbance and emission properties of the dansyl fluorophore.

For clarity, the polymers are labeled according to the structure of their respective quaternary ammonium centers and their respective composition, as well as the degree of polymerization. As a representative example, pButyl_20 denotes a homopolymer containing 20 repeating units quaternized with *N,N*-dimethylbutylamine, while pBu-tyl$_{0.5}$Benzyl$_{0.5}$_20 denotes a polymer of similar chain length containing two dissimilar quaternary ammonium centers (i.e., *N,N*-dimethylbutylammonium and *N,N*-dimethylbenzylammo-nium) randomly distributed along the polymeric backbone in a final molar ratio of 1:1.

3.2. Antimicrobial and Hemolytic Activities of Homopolymers. The antimicrobial activity of all polymers in this study was probed against three representative clinically relevant bacterial strains, namely *S. aureus* (Gram-positive), *E. coli* and *P. aeruginosa* (Gram-negative). By employing a broth microdilution assay, minimal inhibitory concentrations (MICs) were taken at the lowest polymer concentrations required for the inhibition of bacterial growth after 18 h of incubation with an initial inoculum size of 3×10^5 CFU/mL. Relevant polymer toxicity against erythrocytes was also evaluated via hemolysis assays using rat red blood cells to determine the HC_{50}, i.e.,

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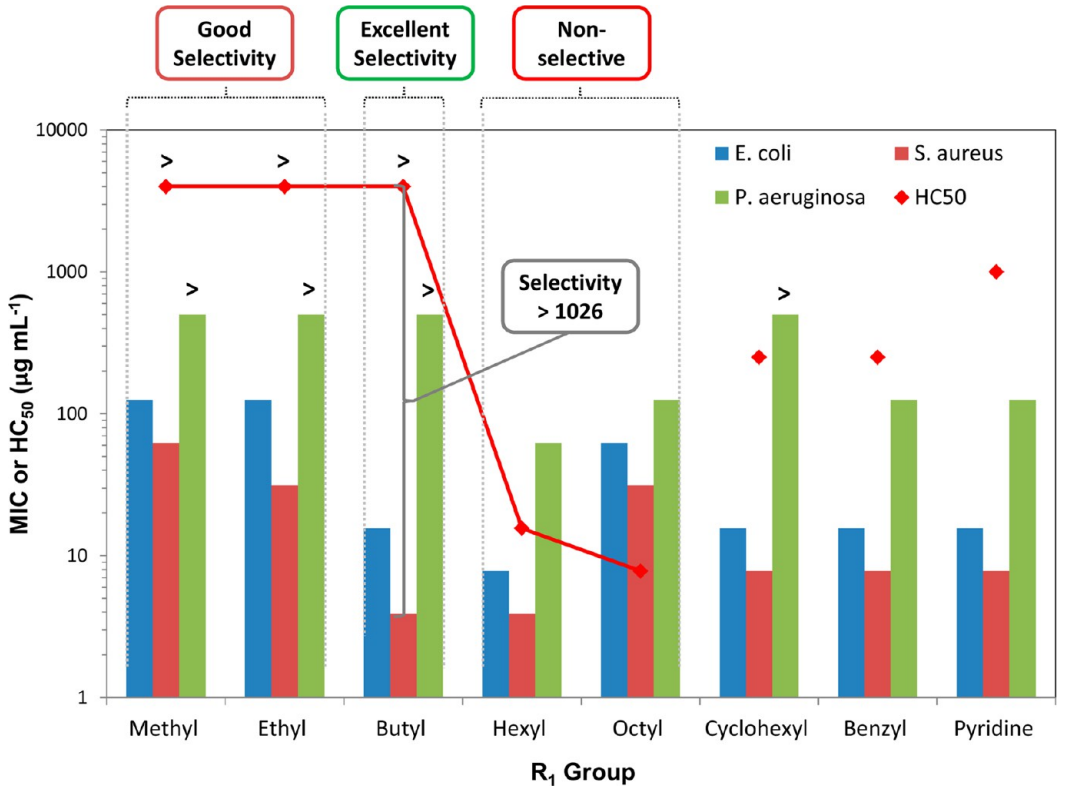

Figure 1. Antimicrobial and hemolytic activities for polymer series containing homologous linear alkyl chain (pMethyl_20 to pOctyl_20), pCyhexyl_20, pBenzyl_20, and pPyr_20. Bars labeled with a ">" symbol indicate that the MIC or HC_{50} was greater than the highest polymer concentration tested (500 $\mu g\ mL^{-1}$ for MIC and 4000 $\mu g\ mL^{-1}$ for HC_{50}). Through an extensive structure–activity study, a highly efficacious and nonhemolytic polymer, pButyl_20, was achieved. Remarkably, it exhibited a high selectivity ratio of an unprecedented value (>1026 against *S. aureus*) for biodegradable synthetic polymers.

concentration at which 50% of red blood cells are lysed upon exposure to the polymer.

3.2.1. Effect of Molecular Weight. As a preliminary study, the influence of molecular weight on antimicrobial and hemolytic activities was evaluated with a series of polycarbonates bearing a common quaternary ammonium center where R_1 = methyl (i.e., pMethyl_20, pMethyl_30, pMethyl_40, and pMethyl_60) (Table S2). Changes in molecular weight over the investigated range did not result in significant changes in hemolytic activities (HC_{50} > 1000 mg/L for all polymers), whereas they did influence antimicrobial activities depending upon the type of bacteria strains. While the polymers were seen to be relatively inactive (nonbiocidal and nonhemolytic) against *P. aeruginosa* across the range of molecular weights tested, a modest extent of antimicrobial activity and selectivity was seen for the polymers against *E. coli* and *S. aureus*. Interestingly, the polymers demonstrated an apparent correlation between molecular weight and antimicrobial activity against the Gram-positive *S. aureus*, wherein the antimicrobial activity was observed to increase as the molecular weight decreased from 23 800 g mol^{-1} (pMethyl_60) to 8170 g mol^{-1} (pMethyl_20). This may be ascribed to the "sieving effect" as previously reported by Lienkamp et al.,[5] which accounts for such a loss in antimicrobial activity as higher molecular weight polymers are trapped by the dense outermost peptidoglycan layer of *S. aureus*.

3.2.2. Effect of Cationic Group Structure. Having established the optimal polymer chain length, the role of the cationic group structure on the antimicrobial and hemolytic activities was subsequently evaluated using a series of homopolymers bearing a variety of quaternary ammonium centers (Table 1) and at a fixed uniform chain length of about 20 repeating units. By comparing the MICs and HC_{50} for different polymers, it appeared evident that the length of the alkyl substituent in homologous quaternary centers had a significant bearing on the antimicrobial and hemolytic activities. For the series of polymers quaternized with *N,N*-dimethyl alkylamines of varying alkyl chain lengths, the potency of the polymers ameliorated with increasing alkyl chain length while maintaining its nonhemolytic properties, until it reached a threshold in terms of hydrophobicity imparted by the alkyl chain wherein the polymers started to lose its initial selectivity and became gradually biocidal (i.e., nonselective) in nature. To elaborate the observed trend, the findings are collectively summarized in Figure 1. As the number of carbon atoms in the alkyl substituent increased from pMethyl_20 to pButyl_20, the antimicrobial activities of the polymers, particularly toward *E. coli* and *S. aureus*, increased as much as 8-fold (125 $\mu g\ mL^{-1}$ to 15.6 $\mu g\ mL^{-1}$) and 16-fold (62.5 $\mu g\ mL^{-1}$ to 3.9 $\mu g\ mL^{-1}$) respectively, while concomitantly retaining its nonhemolytic properties (HC_{50} > 4000 mg/L). This optimum in MIC and HC_{50} values consequently gave rise to a remarkably efficacious antimicrobial polymer with a measured selectivity (HC_{50}/MIC) of more than 256 against *E. coli* and 1026 against *S. aureus* respectively. To the best of our knowledge, these are the highest selectivity values reported in the literature for biodegradable synthetic polymers. Furthermore, the MIC values for *E. coli* and *S. aureus* are comparable to and in some cases lower than many highly effective naturally occurring antimicrobial peptides (e.g., cecropin A and B, magainin 1 and

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2, and defensing,[3,19−22]) which also typically exhibit pronounced hemolytic activities. As the alkyl substituent length further increased to six carbons (pHexyl_20), the MIC against *E. coli* and *S. aureus* remained relatively constant while for *P. aeruginosa,* it decreased significantly to 62.5 μg mL^{-1}. Notwithstanding the lowest MIC values observed with the hexyl substituent, the dramatic increase in its hemolytic activity (HC_{50} = 15.6 μg mL^{-1}) and resultant biocidal properties precluded its usefulness for therapeutic applications. A similar extent of nonselective biocidal activity was also seen for the octyl-substituted polymer pOctyl_20, albeit its poor solubility in the nutrient broth.

From Figure 1, additional intriguing findings pertaining to the influence of cationic group structure on the polymers' antimicrobial and hemolytic activities are revealed. While the polymers pHexyl_20 and pCyhexyl_20 contain the same number of pendent carbon atoms in their respective alkyl substituent, the cyclic homologue was apparently much less hemolytic (HC_{50} = 250 μg mL^{-1}) relative to pHexyl_20 despite having a comparably similar extent of potent antimicrobial activity, particularly toward *E. coli* and *S. aureus*. Interestingly, polymers containing aromatic substituents in their quaternary ammonium centers exhibited an analogous trend as well. The polymer pBenzyl_20 showed similar extent of antimicrobial and hemolytic activities relative to pCyhexyl_20, with the notable exception of its stronger activity against *P. aeruginosa* (MIC = 125 μg mL^{-1} vs >500 μg mL^{-1}). Similarly, pPyr_20 exerted considerable antimicrobial potency against the microbes tested while being less hemolytic than linear alkyl chains of comparable number of carbons (pHexyl_20). These results thus led us to posit that the properties of fine structures and conformations (aliphatic cyclic and aromatic vs linear alkyl) does have a profound influence on the observed biological activities. Such observations could be putatively attributed to the cyclohexyl, benzyl and pyridinium groups being fixed in an extended conformation, consequently limiting the insertion of the respective substituents into the hydrophobic lipid region of the cellular membrane, relative to the polymers bearing flexible and linear alkyl groups which therefore render them more hemolytic in nature.

To shed further light on investigating the role of cationic group structure, two different polymers containing homologous 1-alkylimidazolinium moieties were evaluated for their corresponding antimicrobial and hemolytic activities. Interestingly, the polymer pMeImi_20 which contains 1-methylimidazolinium as the cationic group, was found to exhibit high selectivity toward *E. coli* and *S. aureus* (Table 1). As the alkyl substituent chain length increased to four carbon atoms, the polymer became more potent toward the pathogens in general, even against the elusive *P. aeruginosa.* However, this apparent improvement in antimicrobial activity was concomitantly accompanied by an undesirable increase in its hemolytic activity as well, thus causing pBuImi_20 to exhibit a weak extent of selectivity against the microbes.

The results from these studies have effectively demonstrated the cogent influence of nuanced structural changes on the biological activities of these amphiphilic polymers. The observed increase in activity with increasing alkyl substituent chain length suggested that hydrophobicity is a pivotal determinant in modulating the antimicrobial potency of the polymers. Increasing the hydrophobicity augments the propensity of the polymers to bind onto the lipid membrane, which in turn leads to pronounced membrane disruption and ultimately causes cell death. For similar reasons in each polymer series, the HC_{50} decreased accordingly with increasing hydrophobicity imparted by the alkyl chain length. Much akin to native antimicrobial peptides,[3,23] a general comparison of polymers across each series in Table 1 highlights the delicate balance of hydrophobicity and hydrophilicity required to develop effective macromolecular antimicrobials with favorable biocompatibility toward mammalian cells.

3.3. Antimicrobial and Hemolytic Activities of Homopolymers with Two Different Quaternary Ammonium Centers. To broaden the extent of the polymers' antimicrobial activity toward the highly opportunistic and difficult-to-kill Gram-negative *P. aeruginosa* without compromising much of its nonhemolytic properties, a series of homopolymers bearing a mixture of two different quaternary ammonium centers randomly distributed along the polymer chain was designed and synthesized as shown in Scheme 1. It was envisaged that the low hemolytic activity of pButyl_20 and potent antimicrobial activities of pBenzyl_20, pPyr_20 and pBuImi_20 across the range of microbes tested, would allow for the concomitant installation of dissimilar quaternary ammonium centers on a single polymeric chain as a facile synthetic strategy for optimizing activity and selectivity. As the precursor polymer remains essentially the same as that employed for the homopolymerization studies, such a synthetic route rendered the various polymeric compositions to be easily explored without compromising narrow polydispersities, in contrast to statistical copolymerization and polycondensation approaches.[24] From Figure 2, Table 1 and Table S3, it was shown that the polymer pButyl$_{0.5}$Benzyl$_{0.5}$_20 with *N,N*-dimethylbutylammonium and *N,N*-dimethylbenzylammonium groups randomly distributed in a final molar ratio of 1:1 effectively alleviated the hemolytic activity previously observed in the comparatively more hydrophobic pBenzyl_20, con-

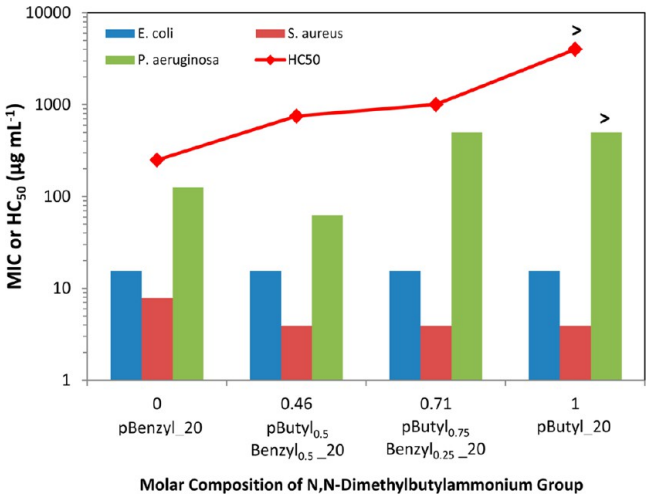

Figure 2. Effect of cationic group composition on the antimicrobial and hemolytic activities of polymers with varying molar compositions of *N,N*-dimethylbutylammonium and *N,N*-dimethylbenzylammonium groups. Bars labeled with a ">" symbol indicate that the MIC or HC_{50} was greater than the highest polymer concentration tested (500 μg mL^{-1} for MIC and 4000 μg mL^{-1} for HC_{50}). The results revealed that 50% of *N,N*-dimethylbutylammonium group content was sufficient to alleviate hemolytic activity by 3 times, consequently enhancing its potency against a wide spectrum of microbes including the highly opportunistic and elusively hard-to-kill Gram-negative *P. aeruginosa*.

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Table 2. Antimicrobial (MIC, μg mL^{-1}) Activity and Selectivity of Polymers against Clinically Isolated Nosocomial Microbes

	MIC (μg mL^{-1})					selectivity (HC_{50}/MIC)			
polymer	MRSA	VRE	*A. baumannii*	*C. neoformans*	HC_{50} (μg mL^{-1})	MRSA	VRE	*A. baumannii*	*C. neoformans*
pMethyl_20	250	31.3	500	31.3	>4000	>16	>128	>8	>128
pButyl_20	7.8	3.9	62.5	31.3	>4000	>513	>1026	>64	>128
pBenzyl_20	7.8	1.95	15.6	7.8	250	32	128	16	32
$pButyl_{0.5}$ $Benzyl_{0.5}$_20	3.9	3.9	31.3	7.8	750	192	192	24	96

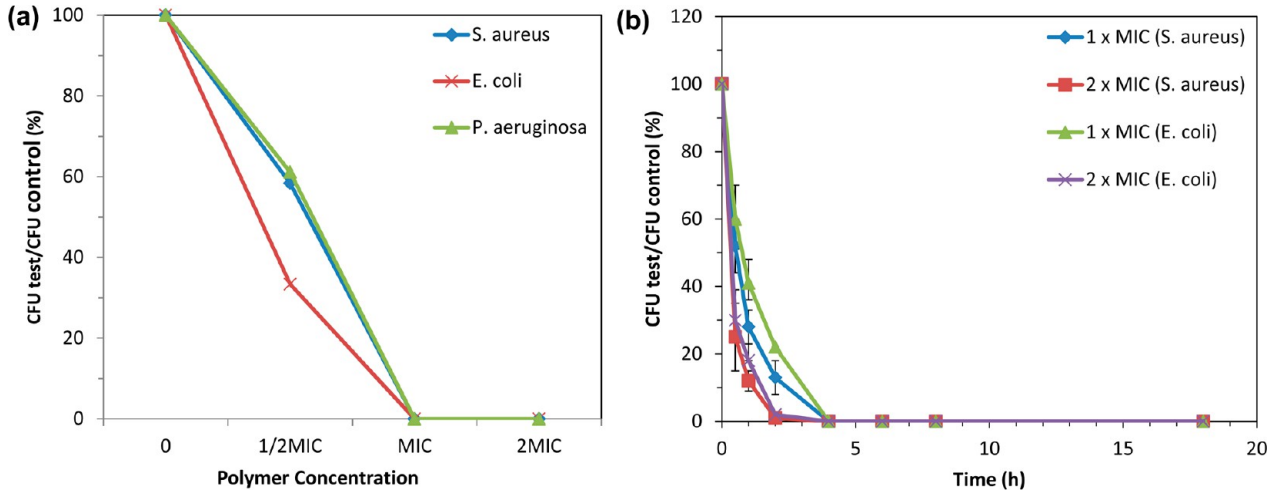

Figure 3. Killing efficiency and bactericidal kinetics of polymers. (a) Fractional cell survival of *S. aureus*, *E. coli* and *P. aeruginosa* after 18h incubation with pButyl_20 and $pButyl_{0.5}Benzyl_{0.5}$_20 at various concentrations (0, 1/2 × MIC, MIC and 2 × MIC) (b) Bactericidal kinetics of *S. aureus* and *E. coli* after 0.5, 1, 2, 4, 6, 8, and 18 h incubation with pButyl_20. Taken together, the results evidently demonstrate that the mode of antimicrobial activity incited by the polymers is bactericidal, rather than just bacteriostatic.

sequently augmenting the selectivity against *E. coli*, *S. aureus*, and, most importantly, *P. aeruginosa*. Notably, 50% of *N,N*-dimethylbutylammonium group content was sufficient to increase the HC_{50} by 3 times, which consequently afforded a broad-spectrum antimicrobial polymer with favorable antimicrobial potency and selectivity (Table 1). Notwithstanding the successful attempt to ameliorate its biocompatibility (HC_{50} > 1000 μg mL^{-1}), a further increase in the molar ratio of *N,N*-dimethylbutylammonium group content for $pButyl_{0.75}Benzyl_{0.25}$_20 however resulted in an apparent loss of activity toward *P. aeruginosa*. From Table S3 and Table 1 a similar observation was made for the polymers $pButyl_{0.5}Pyr_{0.5}$_20 and $pButyl_{0.75}Pyr_{0.25}$_20, wherein the introduction of *N,N*-dimethylbutylammonium groups significantly improved their hemolytic activities but was not adequate to exhibit observable potency toward *P. aeruginosa*. In contrast, while the polymers bearing *N,N*-dimethylbutylammonium and 1-butylimidazolium groups ($pButyl_{0.5}BuImi_{0.5}$_20 and $pButyl_{0.75}BuImi_{0.25}$_20) retained their broad-spectrum and potent activities against the tested microbes, they were still generally hemolytic in nature, with minimal differences relative to pBuImi_20. The polymers from this study are excellent examples for demonstrating the dramatic effect of fine-tuning the hydrophibic/hydrophilic balance (i.e., amphiphilicity) via a synthetically facile method, which in turn allows for the development of efficacious antimicrobial polymers ($pButyl_{0.5}Benzyl_{0.5}$_20) with broad-spectrum activity and favorable selectivity.

3.4. Antimicrobial Activity against Clinically Isolated Microbes. To assess the potential of these polymers toward clinical applications as broad-spectrum therapeutics, the antimicrobial activity was investigated against a series of clinically isolated nosocomial microbes (Table 2). Similar to the *in vitro* results against *E. coli*, *S. aureus*, and *P. aeruginosa*, pButyl_20 showed superior extent of selectivities against a wide spectrum of pathogens in comparison to pMethyl_20 despite both polymers being nonhemolytic. Notably, pButyl_20 exhibited excellent antimicrobial potency and selectivity toward methicillin-resistant *S. aureus* (MRSA, Gram-positive, HC_{50}/MIC > 513), vancomycin-resistant *Enterococcus* (VRE, Gram-positive, HC_{50}/MIC > 1026) as well as carbapenem-resistant *Acinetobacter baumannii* (Gram-negative, HC_{50}/MIC > 64). Furthermore, the polymers were also shown to possess efficacious antifungal activities against fluconazole-resistant *Cryptococcus neoformans*, which are generally comparable to their antibacterial activity. A further survey of the results also revealed that $pButyl_{0.5}Benzyl_{0.5}$_20 retained most of its antimicrobial activity (relative to pBenzyl_20) toward the range of nosocomial microbes tested while having its hemolytic activity ameliorated by about 2-fold. This further substantiates the synthetic feasibility of tuning the amphiphilic balance so as to allow the preparation of potent and selective antimicrobial polymers. These promising results using clinical isolates of drug-resistant microbes thus suggest excellent potential of pButyl_20 and $pButyl_{0.5}Benzyl_{0.5}$_20 for use as antimicrobial agents in future clinical applications.

3.5. Mechanistic Studies. *3.5.1. Bactericidal Mechanism and Kinetics.* To study the antimicrobial mechanism of the polymers, colony counting assays via a traditional surface plating method were performed. Each bacterial strain was treated using the most potential candidates, which were identified as the most potent and selective (i.e., pButyl_20 for *E. coli* and *S. aureus*, $pButyl_{0.5}Benzyl_{0.5}$_20 for *P. aeruginosa*). As shown in Figure 3a, at both the MIC and 2xMIC

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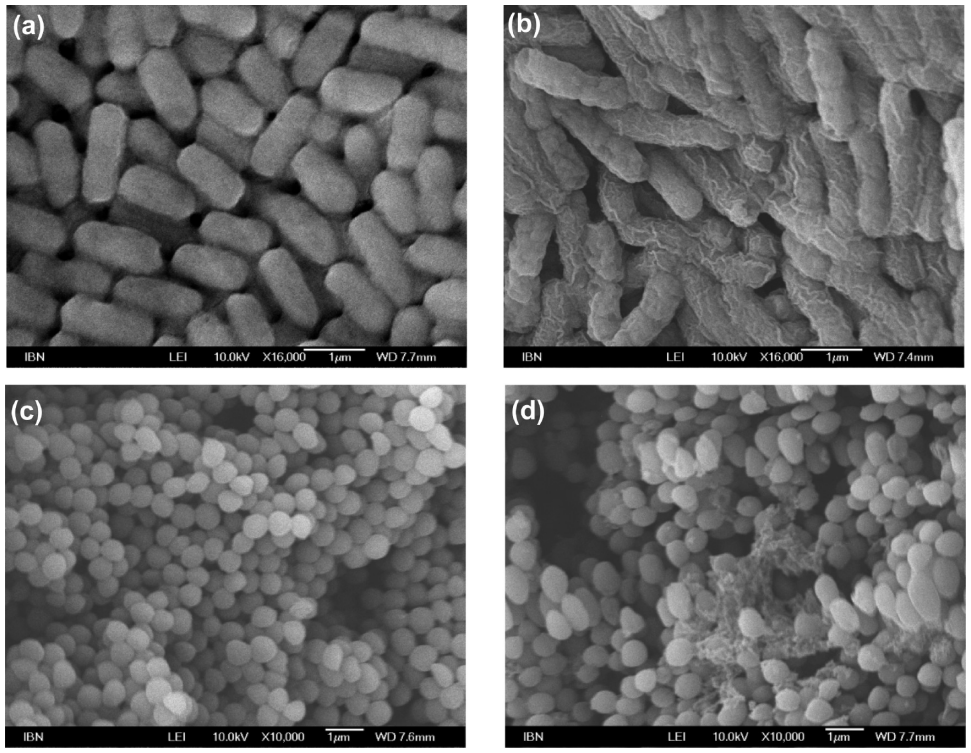

Figure 4. Field emission scanning electron microscopy (FE-SEM) images of *E. coli* (a, b) and *S. aureus* (c, d) before (a, c) and after (b, d) 2 h treatment with pButyl_20 at 2 × MIC. Compared with the intact controls, the treated *E. coli* cells exhibited distorted and corrugated surfaces. Significant fusion of bacterial membrane and debris were observed for *S. aureus* cells.

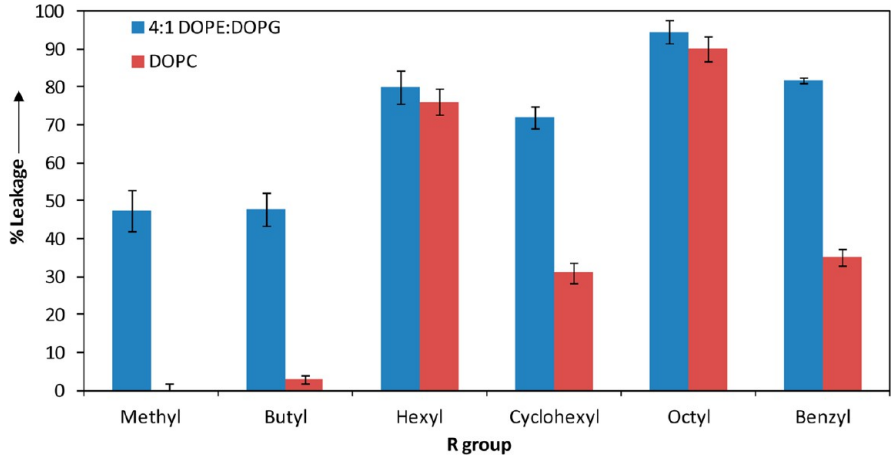

Figure 5. Extent of calcein efflux in neutral vesicles (DOPC) and negatively charged vesicles (4:1 DOPE/DOPG) after treatment with representative polymers for 1 h. In general, a good correlation between leakage and selectivity was observed, wherein nonhemolytic polymers such as pMethyl_20 and pButyl_20 induced little or negligible extent of dye leakage for neutral vesicles while relatively more hydrophobic polymers such as pHexyl_20 and pOctyl_20 demonstrated a nonselective behavior toward both type of vesicles.

concentrations of the respective polymers, more than 99.9% killing efficiency (i.e., 3-log reduction of the initial inoculum) was achieved, strongly suggesting its definitive bactericidal mechanism. Further substantiation of this antimicrobial mechanism was evidently shown from time-kill experiments using pButyl_20 as a model polymer against *E. coli* and *S. aureus* over an 18-h period. From Figure 3b, pButyl_20 displayed expeditious bactericidal effect against the pathogens: more than 3-log reduction in terms of viable colonies was observed within 4 h of treatment, at the respective lethal concentrations for each bacterial strain. Taken together, these results conferred definitive evidence for indicating the polymers to be bactericidal, rather than being merely bacteriostatic (i.e., growth inhibiting) in nature.

3.5.2. Membrane-Lytic Studies with FE-SEM. Using field emission scanning electron microscopy (FE-SEM), the antimicrobial mechanism of the polymers was further probed through observation of the pathogen cell morphologies upon polymer treatment. With reference to Figure 4, the microbial cells (*E. coli* and *S. aureus*) treated with pButyl_20 as a model polymer (at a lethal dose well above its MIC) exhibited distinct and stark changes in their morphology when compared to their corresponding intact controls. In contrast to the smooth surfaces of the control cells, distorted and wrinkled cell walls

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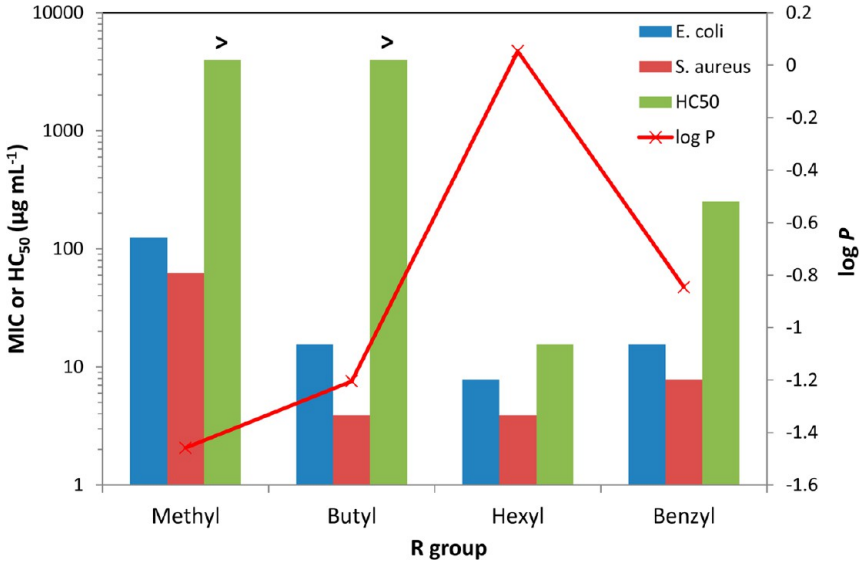

Figure 6. Correlation studies between water-octanol partition coefficient (log *P*) of representative polymers with their respective antimicrobial (MIC) and hemolytic (HC_{50}) activities. Bars labeled with a ">" symbol indicate that the HC_{50} was greater than the highest polymer concentration tested (i.e., 4000 μg mL^{-1}).

were clearly observed upon polymer treatment, particularly for *E. coli* cells (Figure 4a,b). Some *S. aureus* cells were also seen to be damaged to such an extent that the cell structure wholly collapsed and consequently had their cell contents leaked out and membranes fused together to form distinctive groups of cell debris (Figure 4c,d). These results gathered from FE-SEM images collectively indicate that the polymer caused severe cell wall and membrane lysis via physical means, analogous to antimicrobial peptides.[3,23]

3.5.3. Membrane-Lytic Mechanism Studied Through Dye Leakage Assay. In order to assess the ability of the polymers to disrupt the integrity of model membranes, we monitored the leakage of an encapsulated fluorescent dye (calcein) from within negatively charged (4:1 DOPE/DOPG) and neutral (DOPC) large unilamellar vesicles, which mimic Gram-negative bacteria and mammalian cell membranes respectively, upon polymer treatment. Although these assays are known to underestimate certain factors such as cell walls and lipopolysaccharides in bacterial cell membranes, they are nonetheless deemed to be useful in providing biophysical insights underlying the membrane disrupting abilities of macromolecular antimicrobial materials.[19,24] These assays were therefore employed to investigate the overall membrane disruption activities of polymers.

As shown in Figure 5, pMethyl_20 and pButyl_20 were relatively inactive or showed little disruption against neutral vesicles while exhibiting observable activity against negatively charged vesicles, demonstrating a good correlation between leakage and selectivity. A strong agreement between leakage and in vitro results was further seen for the nonselective and biocidal polymers pHexyl_20 and pOctyl_20, which was found to induce almost similar extent of leakage toward negatively charged and neutral vesicles. Similarly, the extent of dye leakage demonstrated by pCyhexyl_20 and pBenzyl_20 was higher from the negatively charged vesicles as compared to the neutral vesicles, which is in agreement with the good selectivity determined from in vitro studies. The general trend gathered from these dye leakage assays provides further substantiation to our postulation wherein amphiphilic balance as well as nuanced structural factors (e.g., alkyl chain length and cationic group structure) are equally important determinants of membranolytic potency and selectivity.

3.5.4. Water–Octanol Partition Coefficients (log P). It has been widely established that the hydrophobic/hydrophilic balance serves as a pivotal structural determinant for the design of membrane active and biocompatible polymers. Despite its significance, a majority of studies concerning the overall hydrophobicity of amphiphilic antimicrobial polymers has been typically based on qualitative determinants, such as molar ratio of monomers in copolymers and length of alkyl side chains. To gain a better understanding of how the overall hydrophobicity of these polymers affect their biological activity, we sought to determine the water-octanol partition coefficient (log *P*), where *P* is defined as the ratio of polymer concentrations in the octanol and water phases at equilibrium. Polymer concentration was measured based on the fluorescence properties of the dansyl-conjugated polymers. When log *P* is lower than 0, a polymer is considered to be relatively hydrophilic, while the polymer is hydrophobic if log *P* is above 0. While log *P* values are conventionally useful in structure–activity relationship studies for small molecules and drugs,[25] it is expected that it would provide an equally germane approach as a quantitative measure of the polymers' overall hydrophobicity or amphiphilic balance.

From Figure 6, the log *P* values of the representative dansyl-labeled polymers appeared to correlate well with its corresponding biological activity. In general, it was shown that as the alkyl substituent of the homopolymers increased in chain length (i.e., from methyl to hexyl groups), their respective log *P* gradually increased from −1.46 to +0.05 as the polymers became concomitantly more potent toward the microbes. It was interesting to note that the log *P* of pButyl_20 (−1.20) increased only slightly from that of pMethyl_20 (−1.46), suggesting the predominantly hydrophilic nature of the polymers albeit the relatively significant increase in the alkyl chain length. As the alkyl chain increased by two carbon atoms to pHexyl_20, the log *P* dramatically increased 24-fold to 0.05. This similar behavior in hemolytic activity as observed earlier

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for the homopolymers in Table 1 led us to ascribe the pronounced toxicity of pHexyl_20 as a highly plausible consequence of the increase in its overall hydrophobicity as reflected in its log *P* value. Another intriguing finding stems from the polymers pBenzyl_20, wherein the log *P* value of −0.85 is seen to be relatively hydrophilic in comparison to pHexyl_20, which might in turn attribute to its lower hemolytic activity.

The observed log *P* values have effectively demonstrated that the overall hydrophobic/hydrophilic balance is not a simple function of the individual monomers' alkyl substituent chain length or the cationic group structure, but also depends on the polymeric structure as a whole ("global amphiphilicity"). This is in accord with our previous hypothesis as evidenced through the dramatic effects these subtle yet pertinent determinants have on the biological activities of the polymers.

4. CONCLUSION

A series of biodegradable and amphiphilic antimicrobial polycarbonates was synthesized via metal-free organocatalytic ring-opening polymerization. By means of a facile and efficient post-polymerization quaternization reaction, the hydrophobic/hydrophilic balance was easily controlled by varying the cationic group structure as well as their respective compositions along the polymeric chain. Nuanced modifications to the hydrophobic character of the cationic amphiphilic polymers were demonstrated to dramatically influence the antimicrobial and hemolytic activities, which consequently afforded the polymer pButyl_20 as a highly efficacious (MICs ranging from 3.9 to 62.5 μg mL^{-1} against clinically isolated drug-resistant Gram-positive and Gram-negative bacteria as well as fungi) and nonhemolytic (HC_{50} > 4000 μg mL^{-1}) antimicrobial polymer. In an effort to ameliorate its antimicrobial activity while retaining its nonhemolytic character, polymers bearing a mixture of two dissimilar quaternary ammonium centers were systematically prepared so as to fine-tune their amphiphilic balance. It was successfully demonstrated that 50% of *N*,*N*-dimethylbutylammonium group content was sufficient to alleviate hemolytic activity by 3 times, consequently enhancing its potency against a wide spectrum of microbes including the highly opportunistic and elusively hard-to-kill Gram-negative *P. aeruginosa*. Through a series of mechanistic studies, the polymers are demonstrated to be expeditiously bactericidal in nature, with a membrane-lytic mechanism deemed to be operative. It is envisaged that these excellent broad-spectrum and highly selective antimicrobial polymers hold great potential for use as promising antimicrobial agents in combating many challenging pathogenic infection.

■ ASSOCIATED CONTENT

Supporting Information

General sysnthesis scheme for dansyl-conjugated polycarbonates, ^{1}H NMR spectrum and data of representative polymers, characterization data of dansyl-conjugated polycarbonates, and additional data on biological activity of polycarbonates. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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