Supporting Information

Antibacterial Activity of Cationic Polymers: Side-chain or Main-chain Type?

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Experimental Section

Materials. Imidazole, triethylenediamine, 1-bromohexane, 1,6-dibromohexane, dimethylamine, p-xylylene dibromide, 1,4-bis(chloromethyl)benzene, vinylbenzyl chloride, N, N-dimethylformamide, acetonitrile, ether, methanol, ethanol, acetone, dichloromethane, 2,2-azobisisobutyronitrile (AIBN), anhydrous sodium sulfate, sodium hydroxide, potassium hydroxide, potassium carbonate were purchased from Shanghai Chemical Reagents Co. (Shanghai, China). Hexylimidazolium (HIM),

4-hexyl-1,4-diazoniabicyclo[2.2.2]octane-1-ium bromide [HDABCO][Br] and hexyl dimethylamine [HDMA] were synthesized as previously described (**Scheme S2**).[1] d_6-Dimethylsulfoxide (d_6-DMSO), deuterium oxide (D_2O), chloroform ($CDCl_3$), phosphate buffered saline (PBS, pH=7.4), Luria-Bertani (LB) medium, and poly(ethylene terephthalate) (PET) membranes were used as purchased. Deionized water was used throughout the experiments. AIBN was purified by recrystallization in ethanol. 4-Vinylbenzyl chloride liquid was passed through a column filled with neutral alumina powder to remove the inhibitor. The chain transfer agent (CTA) (S)-2-(ethyl propionate)-(o-ethyl xanthate) was synthesized according to a method described in the literature.[2] Strains of *S. aureus* (ATCC 6538) and *E. coli* (8099) were kindly provided by Dr. Shengwen Shao (Huzhou University School of Medicine, China).

Characterization

^{1}H NMR spectra of the synthetic chemicals were recorded on a Varian 400 MHz spectrometer at 400 MHz using D_2O, d_6-DMSO, or $CDCl_3$ as the solvents. ^{13}C NMR spectra were recorded on a Bruker Avance 300 at 75 MHz. Fourier transform infrared (FT-IR) spectra were recorded on a Specode 75 model spectrometer in the range of 4000-400 cm^{-1}. A scanning electron microscope (Hitachi Model S-4700) was used to record the morphology of the bacteria fixed on the PET membranes. The optical density (OD) values were obtained with a microplate reader (Thermo Scientific Multiskan Go 1510). Gel permeation chromatography (GPC, TOSOH HLC-8320)

equipped with refractive-index detectors using two TSKgel SuperMultiporeHM-M (6.0×150 mm, 3.0 μm beads size) columns was used to measure the number-average molecular weight (Mn) and molecular weight distribution (Mw/Mn) of the synthesized cationic polymers (after the anion-exchanged with PF_6^-). Dimethyl formamide containing 0.01M lithium bromide was used as the eluent at a flow rate of 0.6 mL/min at 40 ℃. Data acquisition was performed using EcoSEC software, and calculated with polystyrene (PS) standards.

Synthesis of small molecule cationic compounds

Cationic compounds, including 1-vinylbenzyl-3-hexylimidazolium chloride [Im][Cl], vinylbenzyl dimethylhexylammonium chloride [Qa][Cl], and 1-vinylbenzyl-4-hexyl-1,4-diazoniabicyclo[2.2.2]octane-1,4-diium chloride bromide [DABCO][Cl][Br] were synthesized by mixing vinylbenzyl chloride and hexylimidazolium (or hexyl dimethylamine, or 4-hexyl-1,4-diazoniabicyclo[2.2.2]-octane-1-ium bromide) in ethyl acetate at room temperature for 72 h, respectively. Afterwards, the product was washed with diethyl ether more than three times. The remaining viscous liquid was collected after the evaporation of solvent and dried under vacuum at room temperature. All the obtained products were characterized by ^{1}H NMR and ^{13}C NMR.

1-Vinylbenzyl-3-hexylimidazolium chloride [Im][Cl]. ^{1}H NMR (400 MHz, D_2O): δ 8.81 (s, 1H), 7.53 (d, 2H), 7.48 (s, 2H), 7.36 (d, 2H), 6.77 (dd,1H), 5.86 (d, 1H), 5.34 (d,3H), 4.16 (t, 2H), 1.88-1.74 (m, 2H), 1.19 (d, 6H), 0.80 (t, 3H). ^{13}C NMR (75 MHz,

D_2O): δ 140.45, 138.32, 137.68, 136.31, 131.33, 129.35, 125.21, 117.41, 54.99, 52.19, 32.77, 31.58, 27.45, 24.35, 15.79.

Vinylbenzyl dimethylhexylammonium chloride [Qa][Cl]. ^{1}H NMR (400 MHz, D_2O): δ 7.60 (s, 2H), 7.50 (s, 2H), 6.95-6.73 (m, 1H), 5.94 (d, 1H), 5.42 (d, 1H), 4.44 (s, 2H), 3.24 (s, 2H), 3.00 (s, 6H), 1.85 (s, 2H), 1.34 (s, 6H), 0.88 (t, 3H). ^{13}C NMR (75 MHz, D_2O): δ 139.59, 135.68, 133.10, 126.61, 116.30, 67.15, 63.98, 49.67, 30.42, 25.16, 21.82, 13.23.

1-Vinylbenzyl-4-hexyl-1,4-diazoniabicyclo[2.2.2]octane-1,4-diium chloride bromide [DABCO][Cl][Br]. ^{1}H NMR (400 MHz, D2O): δ 7.66 (d, 2H) 7.53 (d, 2H), 6.83 (m, 1H), 5.97 (d, 1H), 5.44 (d, 1H), 3.99 (d, 12H), 3.63-3.47 (m, 2H), 3.28-3.11 (m, 2H), 1.78 (d, 2H), 1.34 (d, 6H), 0.86 (t, 3H). ^{13}C NMR (75 MHz, D_2O): δ 140.45, 135.62, 133.29, 127.20, 124.09, 116.78, 68.51, 65.42, 50.94, 44.20, 30.30, 24.86, 21.52, 13.20.

Synthesis of side-chain cationic polymers

Briefly, the side-chain cationic polymers were synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization. The synthesis of polymers were performed by mixing cationic monomer, (S)-2-(ethyl propionate)-(o-ethyl xanthate) (1 mol%, as RAFT agent) and AIBN (0.1 mol%, as initiator) in DMF, The reaction was stirred at 65 ℃ for 3 h and 14 h under nitrogen atmosphere, respectively. The resultant raw product was purified by precipitating into cold acetone three times to remove unreacted raw materials and other impurities,

followed by drying in a vacuum oven at 50 ℃ for 24 h to obtain raw polymers. The prepared polymeric aqueous solution was dialyzed against distilled water for 72 h using a dialysis membrane (MWCO 3500). The aqueous phase was collected and freeze-dried before characterization by ^{1}H NMR and FTIR.

Synthesis of main-chain cationic polymers

1. Synthesis of M-P[Im][Br]

Imidazole (5.1 g, 75.0 mmol) and potassium hydroxide (4.2 g, 75.0 mmol) were dissolved in 25.0 mL DMSO to obtain a homogeneous solution at room temperature. Afterwards, 1,6-dibromohexane (8.3 g, 34.1 mmol) was slowly added to the prepared mixture, which was constantly stirred for another 12 h. The reaction mixture was poured into ice water and followed by extraction with dichloromethane multiple times. The obtained organic layer was washed with water and dried with anhydrous sodium sulfate and filtered before being concentrated under reduced pressure to give a colorless viscous liquid. The obtained 1,6-bis(imidazol-1-yl) hexane (**Scheme S3**) was analyzed by ^{1}H NMR and ^{13}C NMR. ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.59 (s, 2H), 7.15 (s, 2H), 6.87 (s, 2H), 3.92 (t, 4H), 2.55 (s, 1H), 1.67 (d, 4H), 1.21 (s, 4H). ^{13}C NMR (75 MHz, d_6-DMSO): δ 137.00, 124.01, 122.73, 49.00, 29.54, 25.21.

An equimolar amount of 1,6-dibromohexane was slowly added dropwise into 1,6-bis(imidazol-1-yl) hexane solution with DMF as the solvent at 60 ℃. Afterwards, the mixture was refluxed at 110 ℃ for 96 h. Subsequently, the resultant polymer was precipitated in acetone and further washed with excess acetone. The yellowish

precipitate was filtered and dried in a vacuum oven at 40 ℃ overnight. The raw polymer was dissolved with deionized water and dialyzed with a dialysis membrane (MWCO 3500) for 72 h. Then, the aqueous phase was collected and freeze-dried to obtain M-P[Im][Br], a white solid that was characterized by ^{1}H NMR. ^{1}H NMR (400 MHz, D_2O): δ 8.76 (1H, -N-CH-N-, a), 7.45 (2H, -N-CH-CH-N-, b), 4.15 (4H, -N-CH2-, c), 1.83 (4H, -CH2-, d), 1.31 (4H, -CH2-, e).

2. Synthesis of M-P[Qa][Br]

A mixture containing 40% dimethylamine (1.5 mL), K_2CO_3 (1.0 g), and p-xylylene dichloride (0.5 g, 2.8 mmol) in 25 mL acetonitrile was stirred for 1 h at room temperature. The reaction was quenched by 25% NaOH aqueous solution after refluxing for another 10 h. The obtained solution was extracted with diethyl ether three times to collect the organic layer. The obtained solvent was dried in a vacuum oven to obtain (p-phenylenedimethylene)bis(dimethylammonium) (**Scheme S4**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (s, 4H), 3.42 (s, 4H), 2.24 (s, 12H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.54, 128.98, 64.07, 45.29.

M-P[Qa][Br] was synthesized follow the procedure described for M-P[Im][Br], using (p-phenylenedimethylene)bis(dimethylammonium) and 1,6-dibromohexane as raw materials. The final target product was a white solid, which was characterized by ^{1}H NMR. ^{1}H NMR (400 MHz, D_2O): δ 7.69 (4H, Ph, a), 4.57 (4H, Ph-CH_2-N-, b), 3.38 (4H, -N-CH_2-, d), 3.05 (12H, -N-CH_3, c), 1.94 (4H, -CH_2-, e), 1.48 (4H, -CH_2-, f).

3. Synthesis of M-P[DABCO][Br]

M-P[DABCO][Br] was synthesized follow the procedure described for M-P[Im][Br], and a white solid was obtained, which was characterized by ^{1}H NMR. ^{1}H NMR (400 MHz, D_2O): δ 4.02 (12H, -N-CH_2-CH_2-N-, a), 3.61 (4H, -CH_2-, b), 1.88 (4H, -CH_2-, c), 1.48 (4H, -CH_2-, d).

Minimum inhibitory concentration (MIC) test

E. coli and *S. aureus* were cultured in LB medium at 37 ℃ on a shaker bed at 150 rpm for 24 h. Then the bacteria were diluted with LB medium to a certain concentration, which was confirmed by measuring the optical density at a wavelength of 600 nm (OD_{600}). According to Clinical & Laboratory Standards Institute guidelines, minimum inhibitory concentration (MIC) values were used to investigate the antibacterial activities of the synthesized cationic compounds and polymers through the broth microdilution format.[3] Samples were prepared in LB medium at an initial concentration (for example, 2048 μg/mL), which was then serially diluted by 2-fold dilutions in a 96-well plate using LB broth. A standard 0.5 bacterial suspension (1-2 × 10^8 CFU/mL) was prepared by direct colony suspension in LB broth. This suspension was further diluted to achieve a final test concentration of OD=0.1 in the wells of the microtiter plate. Then 100 μL of LB medium containing of each bacterium was dispensed to the wells of a 96-well plate. After that, another 100 μL of LB broth containing synthesized compounds was added into the bacterium solution to achieve a final concentration (for example, from 1 to 1024 μg mL^{-1}). The microtiter plates were

then incubated at 37 ^{o}C for 24 h under moistened conditions, the optical density (OD) was tested with a microplate reader (Thermo Scientific Multiskan Go 1510). The inhibition efficiency was determined from the following formula:

$$\text{Inhibited Efficiency} = \frac{OD_1 - OD_2}{OD_0 - OD_3} \times 100\%$$

where OD_0 is the absorbance of the bacterial culture in LB medium, OD_1 indicates the absorbance of the bacterial culture containing the synthesized compounds, and OD_2 and OD_3 are the absorbance of LB medium with or without the synthesized compounds, respectively. Similarly, the MIC was taken as the concentration of the antimicrobial compounds at which 90% microbial growth was observed with the microplate reader. The assay was performed in three replicates, and the experiments were repeated at least three times.

Colony assay for the antibacterial activity

The concentrations of *S. aureus* (ATCC 6538) and *E. coli* (8099) were diluted to 1×10^6 CFU/mL before use. The bacteria were treated with the synthesized compounds at the concentration of MIC$\times 10^{-3}$. All the samples were taken out of each well after 4 h. For plating, 10 μL of the diluted sample was spread on growth medium agar plates, and colonies were counted after overnight incubation at 37 ^{o}C. All the experiments were performed in duplicates and repeated three times.

Morphological changes of the bacteria

Scanning electron microscopy (SEM) images were obtained to observe the

morphological changes of the bacteria cultured with the synthesized cationic compounds and polymers under MIC with PET as the substrate materials. Typically, the diluted bacterial suspension (OD_{600}=0.1) of *E. coli* or *S. aureus* and the synthetic samples (under MIC) were dropped onto the aseptic PET membrane surfaces and incubated at 37 ℃ for 6 h. PET membranes without samples were used as controls. Afterwards, the PET membranes were immersed in 2.5 wt% glutaraldehyde solution for 2 h, followed by gradient dehydration with ethanol solution (10 vol%, 30 vol%, 50 vol%, 70 vol%, 80 vol%, 90 vol%, and 100 vol%) for 10 min at each step.

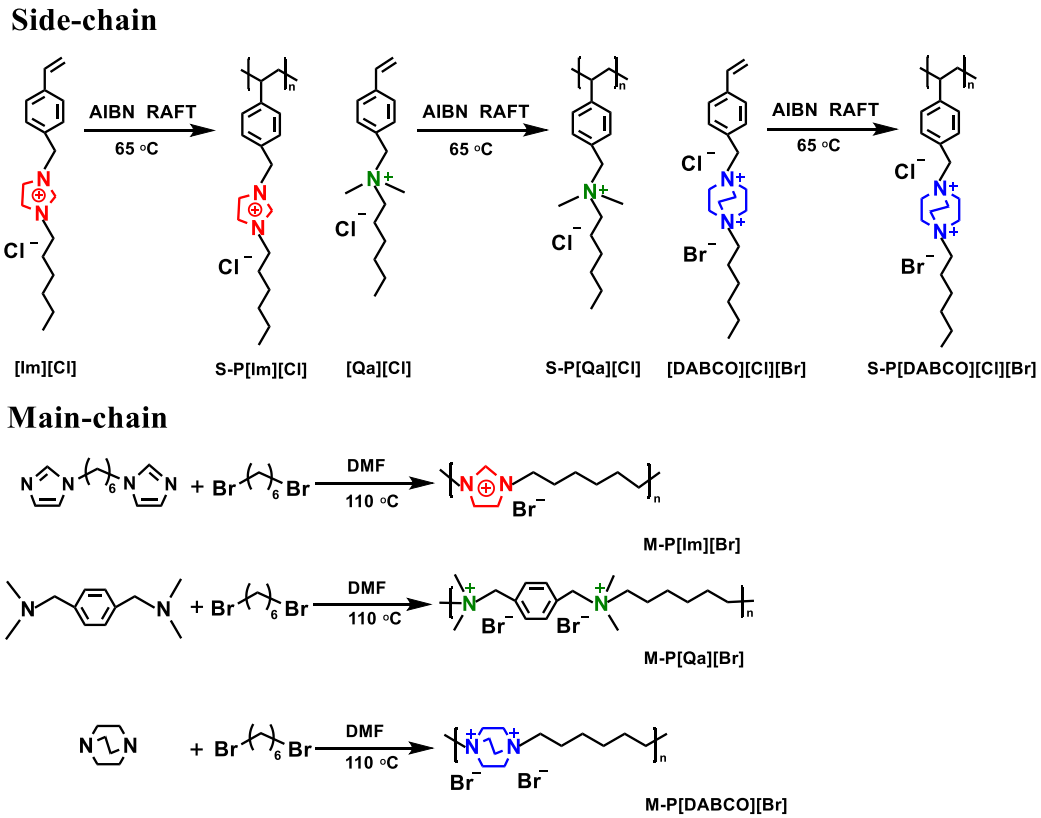
Hemolytic assay

Fresh adult whole blood was collected in anticoagulation tubes, and then diluted with physiological saline (4:5 v/v). The synthesized compounds were diluted stepwise with physiological saline to the MIC (the side-chain polymers with Mn in the range of 16200-17700 were chose as the model side-chain polymers). Afterwards, 200 μL of diluted samples were dropped into a standard tube containing 10 mL physiological saline and 200 μL of diluted blood and incubated at 37 °C. After 1 h, 100 μL of the supernatant obtained by centrifugation of the mixed solution at 1500 rpm for 10 min was transferred into a 96-well plate. An Eon microplate spectrophotometer (Bio Tek Instruments, Inc.) was used to evaluate the hemoglobin release by recording the absorbance of the supernatant at 545 nm. A mixture containing 200 μL diluted blood and 10 mL deionized water was used as a positive control (producing 100% hemolysis), while a mixture of 200 μL diluted blood and 10 mL physiological saline

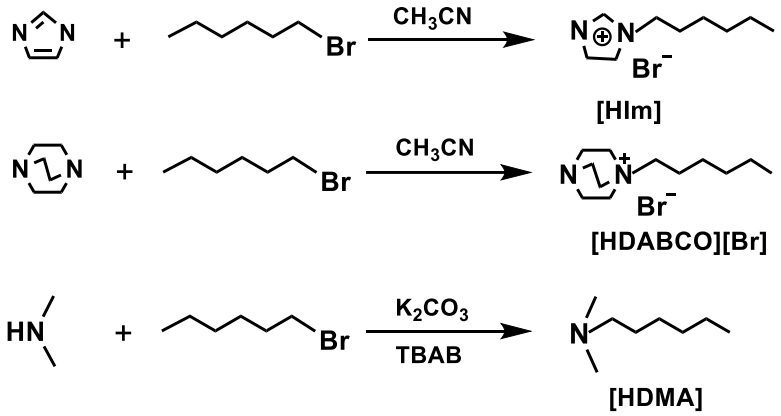
was used as a negative control (without hemolysis). All the experiments were performed three times. The degree of hemolysis was calculated according to the following formula:[4]

$$\text{Hemolysis}(\%) = \frac{OD_{\text{sample}} - OD_{\text{negative control}}}{OD_{\text{negative control}} - OD_{\text{positive control}}} \times 100\%$$

Scheme S1. General synthetic route of side-chain and main-chain cationic polymers.



Scheme S2. General synthetic route of [HIM], [HDABCO][Br] and [HDMA].



Scheme S3. General synthetic route of 1,6-bis(imidazol-1-yl) hexane.

Scheme S4. General synthetic route of (p-phenylenedimethylene) bis(dimethylammonium).

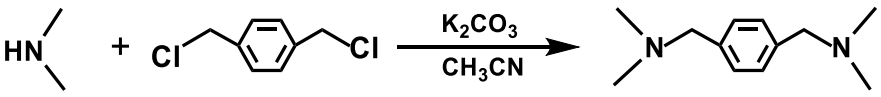


Table S1. The GPC results of side-chain/main-chain cationic polymers.

Samples	M_n	M_w	M_p	M_z	M_w/M_n
S-P[Im][Cl]-L[#]	16200	25900	23100	44400	1.60
S-P[Im][Cl]	56900	93900	85800	140800	1.65
S-P[Qa][Cl]-L[#]	15900	24100	22300	42900	1.52
S-P[Qa][Cl]	56400	92300	90600	136300	1.64
S-P[DABCO][Cl][Br]-L[#]	17700	27900	25100	52500	1.58
S-P[DABCO][Cl][Br]	53600	90700	88400	136900	1.69
M-P[Im][Br]	16800	29500	24300	55700	1.76
M-P[Qa][Br]	22300	39600	31800	64200	1.78
M-P[DABCO][Br]	17800	31800	24700	52800	1.79

[#]S-P[Im][Cl]-L, S-P[Qa][Cl]-L, and S-P[DABCO][Cl][Br]-L denote the side-chain polymer with relatively lower molecular weight.

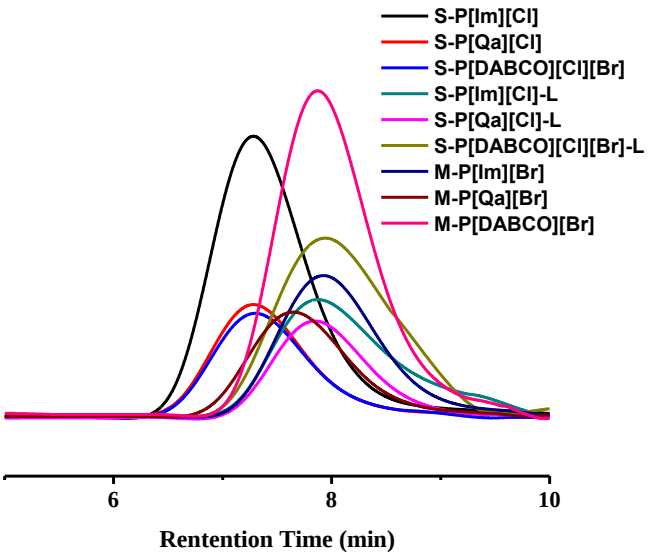


Figure S1. Evolution of the GPC traces of the side-chain/main-chain cationic polymers.

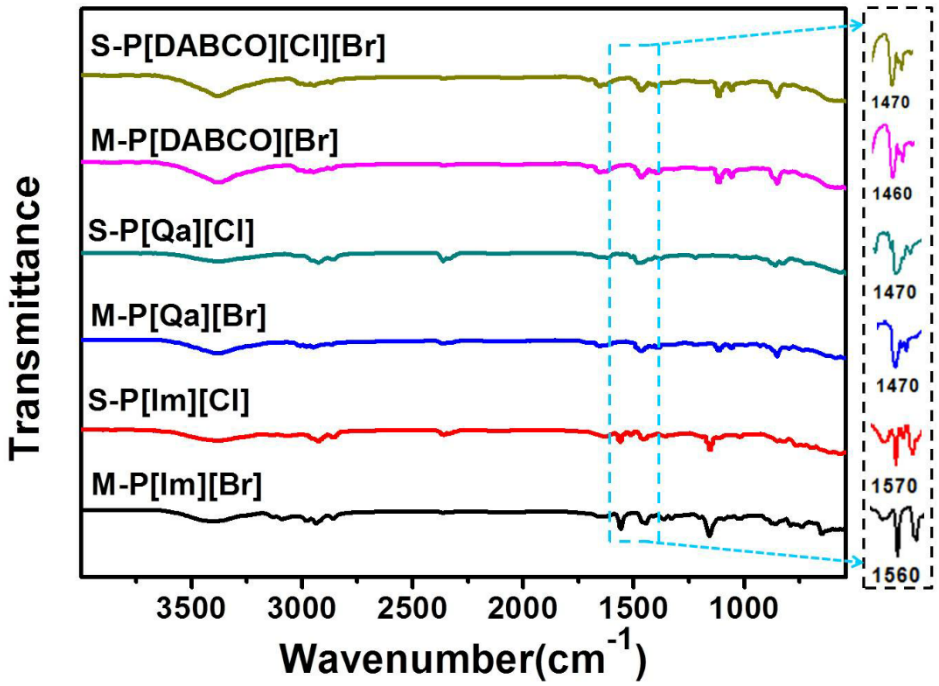


Figure S2. FTIR spectra of the side-chain/main-chain cationic polymers.

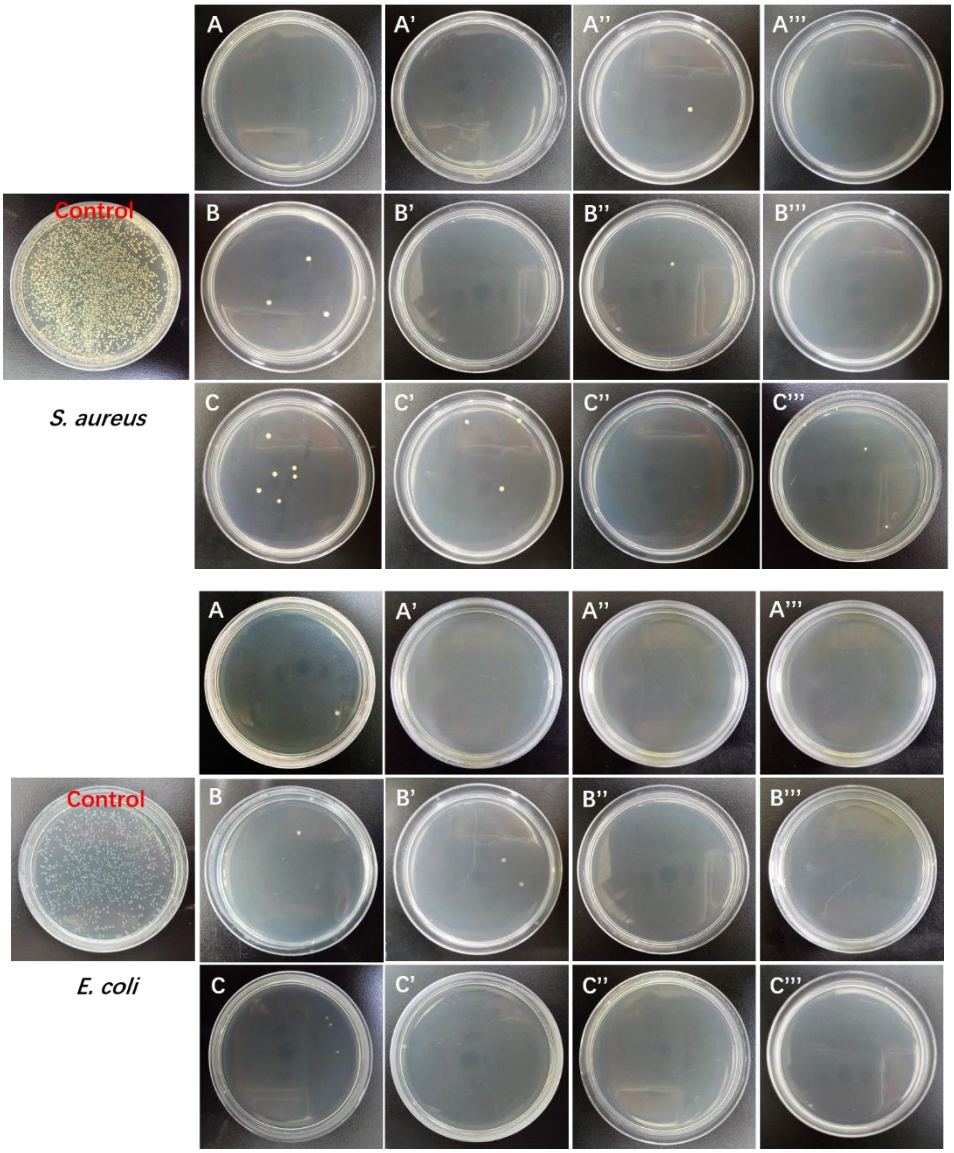


Figure S3. Antibacterial activities of the control and the synthesized small molecules (A-C), side-chain polymers with relatively lower molecular weight (A′-C′)，side-chain polymers with relatively higher molecular weight (A″-C″) and main-chain polymers (A‴-C‴) against S. aureus and E. coli at the concentration of MICx10-3. A) [Im][Cl], B) [Qa][Cl], C) [DABCO][Cl][Br]; A′) and A″) S-P[Im][Cl], B′) and B″) S-P[Qa][Cl], C′) and C″) S-P[DABCO][Cl][Br]; A‴) M-P[Im][Br], B‴) M-P[Qa][Br], C‴) M-P[DABCO][Br]. The bacterial strains were incubated with the synthesized compounds at 37°C for 4h and smeared onto a LB agar plate evenly.

References

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