Supporting Information

Immobilization of Amphiphilic Polycations by Catechol Functionality for Antimicrobial Coatings

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S. 1. DMA synthesis (Lee et al. [1])

$NaHCO_3$ (1.6g, 19.0 mmol) and $Na_2B_4O_7$ (4g, 10.5 mmol) were dissolved in H_2O (50 mL) under N_2. Dopamine-HCl (2g, 10.5 mmol) was added into aqueous solution, and then methacrylate anhydride (1.9 mL, 11.6 mmol) in THF (10 mL) was then added dropwise. NaOH aq (1M) was added so that the pH was kept above 8, and the mixture was stirred overnight with N_2 bubbling. The pH of the aqueous solution was adjusted to 2 and extracted with ethyl acetate (100 mL) three times. The combined organic layer was dried over $MgSO_4$. After removing the solvent under reduced pressure, the solution was added into hexane (100 mL) and then stored at 4 °C overnight. The gray precipitate was filtered and washed with hexane. (1.6 g, 68.9% yield). ^{1}H NMR (Inova, 400MHz, DMSO-D_6) δ 6.64 (d, 1H, $C_6H_2H(OH)_2$-), 6.61 (s, 1H, $C_6H_2H(OH)_2$), 6.42 (d, 1H, $C_6H_2H(OH)_2$-), 5.60 (s, 1H, -C(=O)-C(-CH_3)=CH_2), 5.29 (s, 1H, -C(=O)-C(-CH_3)=CH_2), 3.22 (q, 2H, $C_6H_3(OH)_2$-CH_2-CH_2(NH)-C(=O)-), 2.58(t, 2H, $C_6H_3(OH)_2$-CH_2-CH_2(NH)-C(=O)-), 1.83 (s, 3H, -C(=O)-C(-CH_3)=CH_2).

S. 2. Synthesis of cationic monomer DMAEMAC$_{12}$ (Ravikumar et al. [2])

2-(Dimethylamino)ethyl methacrylate DMAEMA (10.8 mL, 62.7 mmol) was added to 1-bromododecane $C_{12}H_{25}Br$ (17.5 mL, 70.7 mmol) in a mixture of acetonitrile (50 mL) and chloroform (25 mL). The mixture was stirred at 40 °C overnight under N_2. The solvent was removed under reduced pressure. Diethyl ether was added to the remaining oily product, giving precipitate. This precipitate was collected by filtration and recrystallized from a mixture of diethyl ether and acetonitrile. (20.8 g, 81.5% yield). ^{1}H NMR (400MHz, CD_3OD) δ 6.12 (s, 1H, -C(-CH_3)=CH_2), 5.62 (s, 1H, -C(-CH_3)=CH_2), 4.58 (m,

2H, -N-CH_2-CH_2-C(=O)-), 3.66 (m, 2H, -N-CH_2-CH_2-C(=O)-), 3.38 (m, 2H, $CH_3(CH_2)_{10}$-CH_2-$(CH_3)_2$-N^+-CH_2-CH_2-C(=O)-), 3.15 (s, 6H, $CH_3(CH_2)_{10}$-CH_2-(CH_3)$_2$-N^+), 1.90 (s, 3H, -C(-CH_3)=CH_2), 1.76 (m, 2H, $CH_3(CH_2)_9$-CH_2-CH_2 -$(CH_3)_2$-N^+), 1.29 (br, 18H, CH_3(CH_2)$_9$-CH_2-CH_2-$(CH_3)_2$-N^+), 0.80 (t, 3H, C$H_3(CH_2)_{11}$-$(CH_3)_2$-N^+).

S.3. ^{1}H NMR characterization of polymers

^{1}H NMR (400MHz, CD_3OD) for the copolymer P0 (11% DMA, 89% MEA, 69% yield): δ 6.7- 6.8 (br, 2H, C_6H_2H$(OH)_2$-), 6.58 (d, 1H, $C_6H_2H(OH)_2$-), 4.21 (br, 2H, CH_3-O-CH_2-CH_2-O-C(=O)-), 3.56 (br, 2H, CH_3-O-CH_2-CH_2-O-C(=O)-), 3.35 (s, 2H, CH_3-O-CH_2-CH_2-O-C(=O)-), 2.69 (s, 2H, $C_6H_3(OH)_2$-CH_2-CH_2(NH)-C(=O)-), 2.39 (s, 1H, -O-C(=O)-CH(-CH_2)-CH_2-), 2.22(br, 2H, $C_6H_3(OH)_2$-CH_2-CH_2(NH)-C(=O)-), 1.90 (s, 3H, -C(=O)-C(-CH_3)-CH_2-), 1.68 (m, 2H, -O-C(=O)-CH(-CH_2)-CH_2-), 0.98 (m, 2H, -C(=O)-C(-CH_3)-CH_2-).

^{1}H NMR (400MHz, CD_3OD) for the copolymer P1 (12% DMA, 72% MEA, 16% $DMAEMAC_{12}$, 70% yield): δ 6.7- 6.8 (br, 2H, C_6H_2H$(OH)_2$-; 1H, $C_6H_2H(OH)_2$-), 4.40 (br, 2H, -N-CH_2-CH_2-C(=O)-, 2H, -N-CH_2-CH_2-C(=O)-), 3.80 (br, 2H, $CH_3(CH_2)_{10}$-CH_2-$(CH_3)_2$-N^+-CH_2-CH_2-C(=O)-), 4.20-3.80 (br, 2H, CH_3-O-CH_2-CH_2-O-C(=O)-; 2H, CH_3-O-CH_2-CH_2-O-C(=O)-), 3.60 (s, 6H, $CH_3(CH_2)_{10}$-CH_2-(CH_3)$_2$-N^+), 3.42 (s, 2H, CH_3-O-CH_2-CH_2-O-C(=O)-), 2.60 (s, 2H, $C_6H_3(OH)_2$-CH_2-CH_2(NH)-C(=O)-), 2.40 (s, 1H, -O-C(=O)-CH(-CH_2)-CH_2-), 2.20(br, 2H, $C_6H_3(OH)_2$-CH_2-CH_2(NH)-C(=O)-), 1.90 (br, -C(=O)-C(-CH_3)-CH_2-), 1.68 (br, -O-C(=O)-CH(-CH_2)-CH_2-), 1.10-1.45 (br, 20H, CH_3(CH_2)$_{10}$-CH_2-$(CH_3)_2$-N^+), 0.80 (br, -C(=O)-C(-CH_3)-CH_2-).

^{1}H NMR (400MHz, CD_3OD) for the copolymer P3 (13% DMA, 31% MEA, 56% $DMAEMAC_{12}$, 72% yield): δ 6.6- 6.8 (br, 2H, $C_6H_2H(OH)_2$-; 1H, $C_6H_2H(OH)_2$-), 4.50 (br, 2H, -N-CH_2-CH_2-C(=O)-, 2H, -N-CH_2-CH_2-C(=O)-), 3.85 (br, 2H, $CH_3(CH_2)_{10}$-CH_2-$(CH_3)_2$-N^+-CH_2-CH_2-C(=O)-), 4.20-3.80 (br, 2H, CH_3-O-CH_2-CH_2-O-C(=O)-; 2H, CH_3-O-CH_2-CH_2-O-C(=O)-), 3.60 (s, 6H, $CH_3(CH_2)_{10}$-CH_2-$(CH_3)_2$-N^+), 3.30 (s, 2H, CH_3-O-CH_2-CH_2-O-C(=O)-), 2.70 (s, 2H, $C_6H_3(OH)_2$-CH_2-CH_2(NH)-C(=O)-), 2.10 (br, 1H, -O-C(=O)-CH(-CH_2)-CH_2-), 2.20(br, 2H, $C_6H_3(OH)_2$-CH_2-CH_2(NH)-C(=O)-), 1.80-1.90 (br, -C(=O)-C(-CH_3)-CH_2-); -O-C(=O)-CH(-CH_2)-CH_2-), 1.20-1.45 (br, 20H, $CH_3(CH_2)_{10}$-CH_2-$(CH_3)_2$-N^+), 0.90 (br, -C(=O)-C(-CH_3)-CH_2-).

^{1}H NMR (400MHz, CD_3OD) for the copolymer P4 (12% DMA, 88% $DMAEMAC_{12}$, 80% yield): δ 6.5- 6.8 (br, 2H, $C_6H_2H(OH)_2$-; 1H, $C_6H_2H(OH)_2$-), 4.40 (br, 2H, -N-CH_2-CH_2-C(=O)-, 2H, -N-CH_2-CH_2-C(=O)-), 3.85 (br, 2H, $CH_3(CH_2)_{10}$-CH_2-$(CH_3)_2$-N^+-CH_2-CH_2-C(=O)-), 3.55 (s, 6H, $CH_3(CH_2)_{10}$-CH_2-$(CH_3)_2$-N^+), 2.70 (br, 2H, $C_6H_3(OH)_2$-CH_2-CH_2(NH)-C(=O)-), 2.20(br, 2H, $C_6H_3(OH)_2$-CH_2-CH_2(NH)-C(=O)-), 1.80-1.90 (br, -C(=O)-C(-CH_3)-CH_2-); -O-C(=O)-CH(-CH_2)-CH_2-), 1.20-1.45 (br, 20H, $CH_3(CH_2)_{10}$-CH_2-$(CH_3)_2$-N^+), 0.90 (br, -C(=O)-C(-CH_3)-CH_2-).

^{1}H NMR (400MHz, CD_3OD) for the copolymer P5 (73% MEA, 27% $DMAEMAC_{12}$, 79% yield): δ4.20 (br, 2H, -N-CH_2-CH_2-C(=O)-), 3.78 (br, 2H, -N-CH_2-CH_2-C(=O)-), 3.58 (br, 2H, $CH_3(CH_2)_{10}$-CH_2-$(CH_3)_2$-N^+-CH_2-CH_2-C(=O)-), 3.30 (s, 6H, $CH_3(CH_2)_{10}$-CH_2-$(CH_3)_2$-N^+), 2.30-2.50 (br, 1H, -O-C(=O)-CH(-CH_2)-CH_2-), 1.80 (br, 2H, -C(=O)-C(-CH_3)-CH_2-), 1.20-1.30 (br, 18H, $CH_3(CH_2)_{10}$-CH_2-$(CH_3)_2$-N^+), 0.85 (br, 3H, -C(=O)-C(-CH_3)-CH_2-).

^{1}H NMR (400MHz, CD_3OD) for the copolymer P7 (26% MEA, 74% $DMAEMAC_{12}$, 77% yield): δ4.40 (br, 2H, -N-CH_2-CH_2-C(=O)-), 3.70 (br, 2H, -N-CH_2-CH_2-C(=O)-), 3.52 (br, 2H, $CH_3(CH_2)_{10}$-CH_2-$(CH_3)_2$-N^+-CH_2-CH_2-C(=O)-), 3.30 (s, 6H, $CH_3(CH_2)_{10}$-CH_2-(CH_3)$_2$-N^+), 2.20 (br, 1H, -O-C(=O)-CH(-CH_2)-CH_2-), 1.80 (br, 2H, -C(=O)-C(-CH_3)-CH_2-), 1.20-1.30 (br, 18H, CH_3(CH_2)$_{10}$-CH_2-$(CH_3)_2$-N^+), 0.85 (br, 3H, -C(=O)-C(-CH_3)-CH_2-).

^{1}H NMR (400MHz, CD_3OD) for the copolymer P8 (100% $DMAEMAC_{12}$, 80% yield): δ4.50 (br, 2H, -N-CH_2-CH_2-C(=O)-), 3.90 (br, 2H, -N-CH_2-CH_2-C(=O)-), 3.60 (br, 2H, $CH_3(CH_2)_{10}$-CH_2-$(CH_3)_2$-N^+-CH_2-CH_2-C(=O)-), 3.39 (s, 6H, $CH_3(CH_2)_{10}$-CH_2-(CH_3)$_2$-N^+), 1.80 (br, 2H, -C(=O)-C(-CH_3)-CH_2-), 1.20-1.40 (br, 18H, CH_3(CH_2)$_{10}$-CH_2-$(CH_3)_2$-N^+), 0.85 (br, 3H, -C(=O)-C(-CH_3)-CH_2-)

S. 4. Molecular weight determination of polymers

Acid hydrolysis of P1-P4. A portion of the polymer (10 mg) was dispersed in acetic acid (2.0 mL) containing hydrochloric acid (1 M; 1.0 mL) and heated for 1 day at 90 °C. After cooling to room temperature, the sample was concentrated under reduced pressure.

Alkaline hydrolysis of P5-P8. A portion of the polymer (100 mg) was dispersed in deionized water (1.0 mL) containing NaOH (100 mg) and heated for 2 days at 90 °C. After cooling to room temperature, the solution was acidified by addition of hydrochloric acid. The sample was concentrated under reduced pressure.

GPC. The crude polymer after hydrolysis was dissolved in THF and filtered through a 0.45 micron syringe filter. Gel permeation chromatography (GPC) was carried out using a Waters 440 GPC, a Wyatt Optilab Refractive Index detector, and Waters' Millennium software for GPC data acquisition and processing with an HT-4, HT-3, and HT-2 3

columns placed in series. The molecular weights were determined relative to narrow molecular weight polystyrene standard.

Table S1. Molecular weight of polymers

Polymer	Molecular weight of hydrolyzed polymer M_n ($\times 10^3$)	M_w ($\times 10^3$)	PDI	Hydrolysis condition
w/catechol				
P1	39	77	2.0	acid
P2	30	89	2.9	acid
P3	28	76	2.7	acid
P4	30	81	2.7	acid
w/o catechol				
P5	30	97	3.3	base
P6	20	69	3.4	base
P7	28	90	3.3	base
P8	33	89	2.7	base

S. 5. SFG spectra of P1-4 coatings wet by water and after removal of water

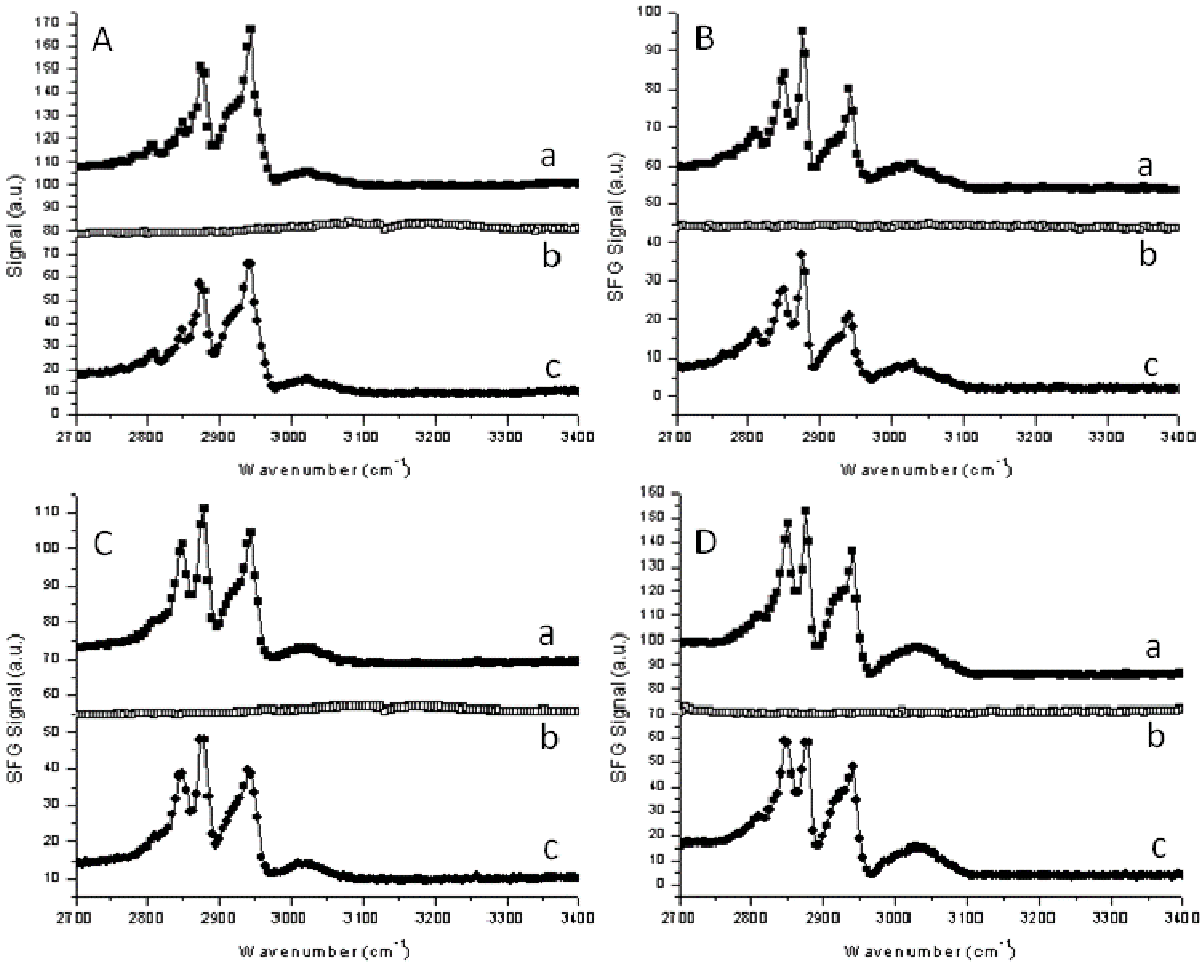

Figure S1. SFG spectra of P1 (A), P2 (B), P3 (C), and P4 (D). a) in air; b) in contact with water; and c) in air, after removal from water contact.

S. 6. Antimicrobial activity of polymers in solution: MIC determination

Antimicrobial activity of polymer coatings was determined by a modified standard protocol: *ASTM E2149-01 Standard Test Method for Determining the Antimicrobial Activity of Immobilized Agents Under Dynamic Contact Conditions.*[3, 4] The *E. coli* ATCC 25922 or *S. aureus* ATCC 25923 was grown in Mueller-Hinton (MH) broth (5 mL, pH 7.4) at 37°C overnight. The cell culture was diluted with MH broth to give OD_{600} = 0.1, which was incubated at 37°C with orbital rotation at 200 rpm for 90 minutes for *E. coli* or 140 minutes for *S. aureus*. The bacterial culture in the mid-logarithmic phase (OD_{600} =

0.5-0.6) was diluted to OD_{600} = 0.1 by MH broth, and further diluted by Phosphate Buffer Saline (PBS, pH 7.4, without calcium and magnesium) serially (OD_{600} = ~0.001, corresponding to ~2×10^5 CFU/mL). The polymer-coated glass slides were completely immersed into this bacterial suspension (5mL) in a sterile 15 mL conical tube at 37°C for 1 hr. A non-coated glass slide was used as a control. An aliquot of the bacterial sample solution (100μL) was diluted serially, plated on the agar plates, and incubated at 37°C overnight to count the number of bacterial colonies. Three samples for the polymer coating or control glass slide were tested, each in triplicate, and three independent experiments were performed. The average numbers of viable cells (colony-forming unit, cfu) in solution and standard deviations were calculated from the results of all experiments.

Table S2. Antimicrobial activity of polymers in solution

Polymer	MIC (μg/mL)[a)]	
	E. coli	*S. aureus*
P1	< 7.8	500
P2	< 7.8	125
P3	< 7.8	250
P4	< 7.8	500
P5	< 7.8	62.3
P6	< 7.8	62.3
P7	< 7.8	250
P8	< 7.8	500

a) the assay media contained 5%DMSO.

S.7. Antimicrobial activity of polymers leached from coatings

Polymer-coated glass slides were incubated with 5 mL of PBS (pH 7.4, without calcium and magnesium) in a sterile 15 mL Falcon tube at 37°C with orbital rotation at 200 rpm for 1 hour. An untreated glass slide was used as a control. The PBS solution (4.5 mL) was removed from each Falcon tube and added to another tube containing *E. coli* fresh culture (0.5mL, OD_{600} = ~0.01, corresponding to ~2×10^6 cfu/mL), then incubated at 37°C for 1 hour. The bacterial suspension was diluted serially, plated on Mueller-Hinton (MH) agar plates, and incubated at 37°C overnight.

Table S3. Antimicrobial effects of leachables from coatings

Sample solution[a)]	viable *E. coli* cells ($x10^5$ cfu)
Control[b)]	3.4±0.6
w/catechol	
P1	3.8±0.6
P2	4.1±0.3
P3	3.6±0.3
P4	3.8±0.3
w/o catechol	
P5	0[c)]
P6	0[c)]
P7	0[c)]
P8	0[c)]

a) The PBS solution was incubated with an untreated (control) and polymer-coated glass slides; b) Untreated glass slide; c) No viable cell in solution was detected in the assay condition.

S.8. ^{1}H NMR characterization of leached polymers

Experimental procedure. The polymer-coated glass slides were incubated with 1 mL of D_2O in a sterile 15 mL Falcon tube at 37°C with orbital rotation at 200 rpm for 1 hour. The D_2O solution was collected for ^{1}H NMR measurement.

Results. To identify the leached polymers, we examined ^{1}H NMR spectra of the polymers. The coating was incubated in deuterium oxide (D_2O), and ^{1}H NMR spectra of leached compounds in D_2O solutions were measured. The P5 and P6 polymers, which contain high % of MEA, showed small broad peaks (Figs. S1 and S2), and no detectable peaks were found in the spectra for the coatings of P7 and P8 containing high $DMAEMAC_{12}$ contents (Fig. S2). However, the residues after removing D_2O was dissolved in methanol-d4 and displayed distinctive peaks in the NMR spectra (Figs. S1 and S3). These results indicate that the polymers P5-8 without DMA are released from the surfaces and are likely to aggregate in water. The NMR spectra also indicate that no or much fewer amounts of polymers were released from the coatings P1-4 as compared to the polymers P5-P8.

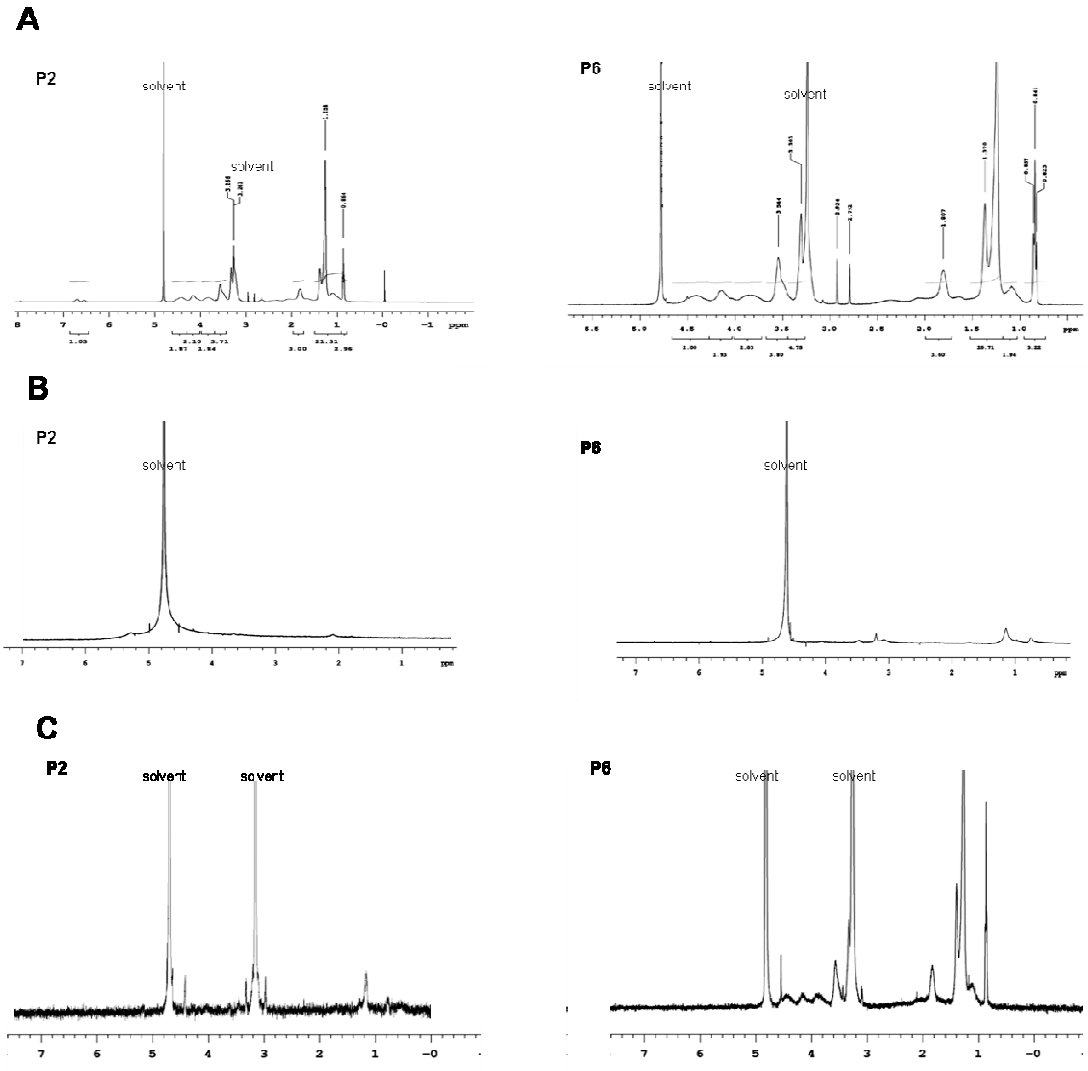

Figure S2. ^{1}H NMR spectra of polymers P2 and P6. (A) Polymers before coatings (CD_3OD), (B) D_2O solutions incubated with coatings of P2 and P6, and (C) residues in CD_3OD after removing D_2O solutions.

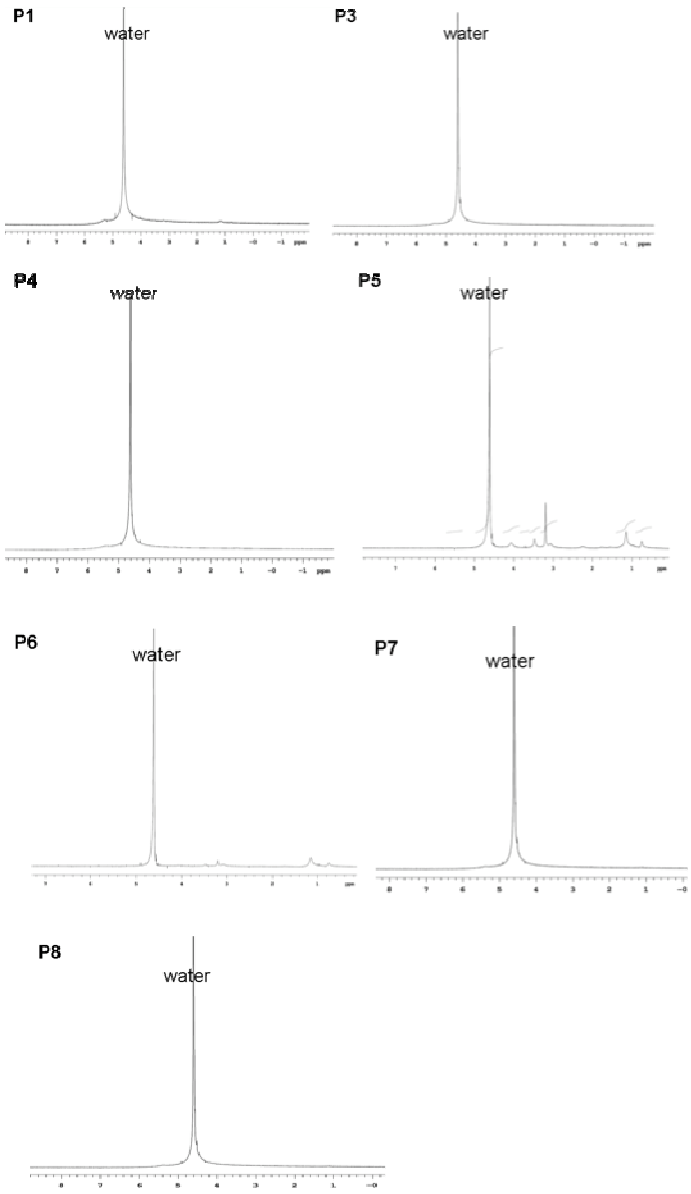

Figure S3. ^{1}H NMR spectra of D_2O solutions incubated with coatings.

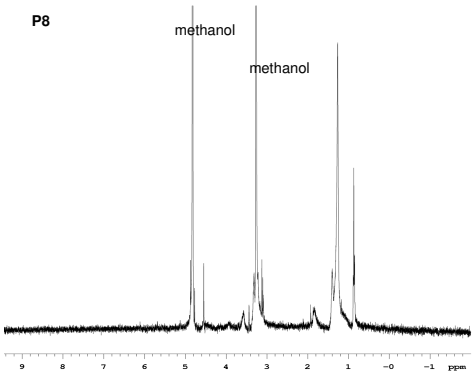

Figure S4. ^{1}H NMR spectra of residues in CD_3OD after removing D_2O solutions incubated with P8 coating.

S. 9. Surface killing ability

To determine the maximum antimicrobial capacity of the P2 and P3 coatings in terms of the number of bacterial cells, we further tested the surfaces with the increased numbers of *E. coli* cells. The P2 and P3 coatings showed complete killing of *E. coli* cells up to 1.2×10^8 and 1.2×10^7 cfu/cm^2, respectively (Table S4). Above these bacterial concentrations, these coatings reduced only fraction of viable cells. This might be due to that, above these bacterial concentrations, the adhesion of a large number of *E. coli* cells likely saturated the surfaces, effectively shielding the antimicrobial activity of polymer chains.

Table S4. Surface killing ability of polymers P2 and P3

Polymer coatings	Number of viable *E. coli* cells (cfu/mL)	
	Control	Coatings
P2	3.7×10^6	0 [a)]
P2	1.2×10^7	0 [a)]
P2	1.2×10^8	0 [a)]
P2	1.0×10^9	8.0×10^8
P3	3.7×10^6	0 [a)]
P3	1.2×10^7	0 [a)]
P3	1.2×10^8	4.7×10^8

a) No viable cell was detected in the assay condition.

S.9. Determination of zone of inhibition

The coated slides were placed on the top of agar plates, which were pre-spread with 100 µl of overnight grown culture of *E.coli* or *S. aureus*. The agar plates were incubated at 37°C overnight to determine the size of zones of inhibition of bacterial growth caused by any leahcables from the coatings.

References

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