Supporting Information for:

Dynamic Antimicrobial Poly(disulfide) Coatings Exfoliate Biofilms On-Demand via Triggered Depolymerization

Yang Lou[a] and Edmund F. Palermo[a,b,*]

(a) Materials Science and Engineering, (b) Biomedical Engineering

Rensselaer Polytechnic Institute, 110 8th St, Troy, NY, 12180, United States. *E-mail: palere@rpi.edu

Table of Contents

1 MATERIALS **2**

2 INSTRUMENTATION **3**

3 EXPERIMENTAL METHODS **4**

3.1 Synthesis of compound **1** 4

3.2 Synthesis of crosslinker **2** 6

3.3 Preparation of the poly(1-co-2) coating 8

3.4 Contact killing assay of poly(1-co-2) coating 9

3.5 MIC and MBC assay 9

3.6 Hemolytic activity assay 11

3.7 Live/Dead Staining Assay 12

3.8 Table of degradation conditions 12

4 SUPPLEMENTARY FIGURES **12**

1 Materials

Materials used in this study were listed in Table S1. All chemical s were used as received unless otherwise specified.

Table S1. Materials information

Item	Company	Product #	Lot #
Dichloromethane (DCM, anhydrous, ⩾ 99.8%)	Sigma	270997	--
Methanol	Fisher Chemical	A412-1	214311
Ethyl acetate (ACS reagent)	J. T. Baker	9280-22	22F0961076
Hexane (anhydrous, 95%)	Sigma-Aldrich	296090-1L	--
Acetonitrile	Sigma-Aldrich	437557-4L	
Chloroform-d (99.8 atom%D)	Sigma-Aldrich	151823-100G	0000180651
Methanol-d4 (99 atom% D)	Sigma	422878-10ML	
Deuterium oxide (99.9 atom% D, contains 1% (w/w) 3-(trimethylsilyl-1-propanesulfonic acid, sodium salt (DSS))	Sigma	343773-25G	1003477465
DL-α -lipoic acid	TCL	L0058	G33QJ
Ethylene glycol	VWR	BDH1125-1LP	0000285985
2-dimetylethanolamino	Sigma-Aldrich	471453-100ML	
1-bromododecane	Sigma-Aldrich	B65551-100G	MKCF2449
lithium phenyl-2,4,6-trimethylbenzoylphosphinate	Sigma-Aldrich	900889-1G	
1,8-Diazabicyclo(5.4.0)undec-7-ene	Sigma-Aldrich	33482-50ML-F	BCBV5145
pentaerythritol tetrakis(3-mercaptopropionate)	Sigma-Aldrich	381462-100ML	MKCJ9289
4-(dimethylamino)pyridine (DMAP)	Sigma	107700	MKBS3287V
N,N’-diisopropylcarbodiimide (DIC)	Sigma	D125407-25G	--
Phosphate buffered saline tablet (pH 7.4)	Sigma	P4417-100TAB	SLBL5997V
Triton X-100	Sigma	T8787	MKBS6557V

Crystal violet, 1% w/v	Fisher	S25275A	5GJ265
Difco Agar Bacteriological, solidifying agent	Becton Dickinson	214530	9233319
BBL Mueller Hinton II Broth, cation adjusted	Becton Dickinson	212322	4293655
Sheep red blood cells, 10% Suspension	MP Biomedicals	55876	S8427
6-well plate	VWR	10861-554	--
96-well plate	CellTreat	229596	190615-076
50 mL centrifuge tube	CellTreat	229421	210425-060
10 mL serological pipet	CellTreat	229240	140822-070
Microscope cover glass, 20 x 20 mm	Globe Scientific Inc	1402-20	--
Safe-Lock tubes 1.5 mL, natural	Eppendorf	022363204	L202759I

2 Instrumentation

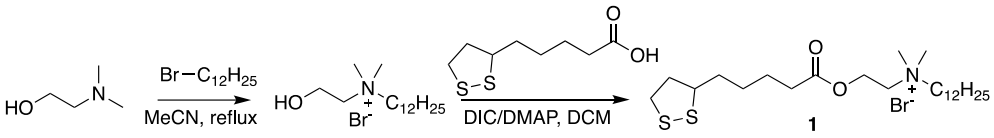
Proton nuclear magnetic resonance (1H NMR) spectra were recorded on a 500 MHz Agilent NMR spectrometer at 25 °C. NMR chemical shifts were reported in parts per million (ppm, δ) and referenced to residual solvent signals ($CDCl_3$ δ = 7.26 ppm, CD_3OD δ = 3.31 ppm, D_2O δ = 4.79 ppm).

Profilometry was performed on a Dektak 6 profilometer. A 18000 µm region was scanned, using a force of 2 mg, resolution of 1.0 µm/sample, measurement range of 2620 kÅ, and profile of Valleys. Leica TCS SP8 STED 3X was used to obtain confocal images of bacteria. SYTO-9 showing live bacteria was excited at 488 nm wavelength. The collected emission was within 505- 545 nm. Propidium iodide (PI) for visualization of dead bacteria was excited at 594 nm. The emission was collected at 605- 655 nm. The acquisition was performed using a 100x objective with oil. 1024×1024-pixel images were recorded from average of eight scans for 2D images, using bidirectional x setting, scan speed at 400 Hz, optical section of 0.896 µm, pixel dwell time 1.04 µs, frame rate 0.194/s. Same settings were applied for 3D images, at z-step size 0.70 µm. Image analysis was reconstructed by Imaris x64 9.5.0 (Copyright Bitplane AG).

UV/vis spectra were performed on an Agilent Cary 60 double beam spectrophotometer. Molecular Devices SpectraMax M2 was warmed up for at least 30 min before use. It was used for both optical density measurement of bacterial suspension, and Hemolytic Activity measurements. Witec Alpha 300R confocal Raman imaging system was used to take Raman spectra. T1: 600 g/mm, BLZ = 500 nm, spectral center: 2099/cm, lens: 100x, integration time: 1.0 s, accumulation: 30.

3 Experimental methods

3.1 Synthesis of compound **1**



Scheme S1 Synthetic scheme of **1**.

N-(2-hydroxyethyl)-N,N-dimethyldodecan-1-aminium (C12 ammonium)

2-dimetylethanolamino (0.03 mol, 2.67 g) and 1-bromododecane (0.033 mol, 7.30 g) were dissolved in 15 mL acetonitrile. The mixture was heated to 85 ˚C and refluxed for 30 min. Heat was turned off and the mixture was cooled to r.t. after 30 min. White solid was obtained by filtration. The white solid was redissolved in ethyl acetate and recrystallized. The obtained solid from recrystallization was filtered, washed with cold ethyl acetate, dried under high vacuum overnight to afford a white salt (94% yield). 1H NMR (500 MHz, CDCl3): δ (ppm) 4.00 (s, 2H), 3.49 (t, 2H), 3.42 (t, 2H), 3.18 (s, 6H), 1.82 (m, 2H), 1.46-1.26 (m, 18H), 0.92 (t, 3H).

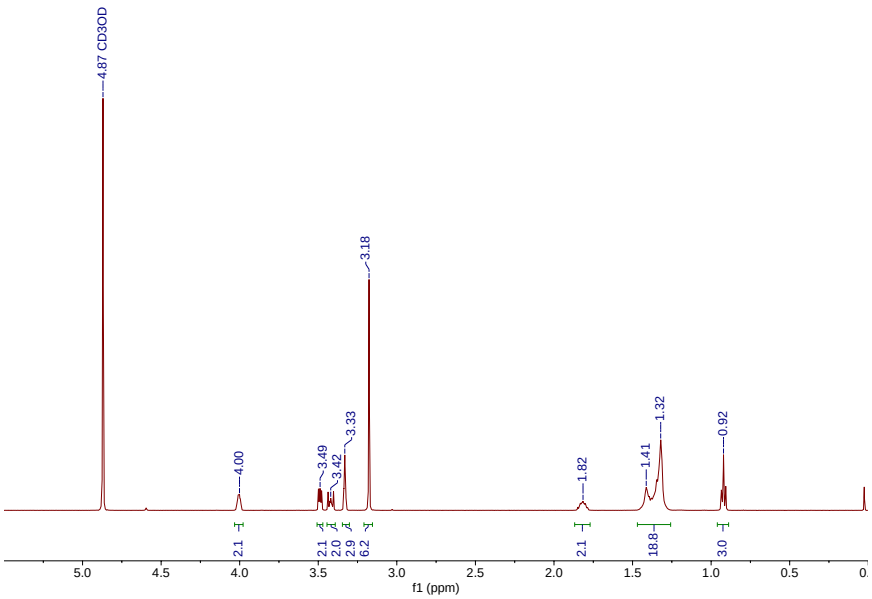


Figure S1 1H NMR spectrum of N-(2-hydroxyethyl)-N,N-dimethyldodecan-1-aminium in methanol-d4.

N-(2-((5-(1,2-dithiolan-3-yl)pentanoyl)oxy)ethyl)-N,N-dimethyldodecan-1-aminium (1)

DL-α-lipoic acid (5.5 mmol, 1.14 g) and C12 ammonium (5 mmol, 1.47 g) were dissolved in 10 mL anhydrous dichloromethane (DCM) in a flask and cooled with ice water bath. In a separate container, 4-(dimethylamino)pyridine (DMAP) (0.5 mmol, 0.06 g) and N,N'-diisopropylcarbodiimide (DIC) (15 mmol, 1.90 g) were dissolved in 10 mL anhydrous DCM. The solution of DIC and DMAP was added dropwise into the solution of DL-α-lipoic acid and C12 ammonium. The solution was stirred at r.t. for 24 h. Column chromatography was used to purify the product and afford a yellow solid. 1H NMR (500 MHz, CD3OD): δ (ppm) 4.56 (t, 2H), 3.74 (t, 2H), 3.61 (m, 1H), 3.19 (s, 6H), 3.24-3.10 (m, 2H), 2.49 (m, 1H), 2.45 (m, 2H), 1.93 (m, 1H), 1.83 (m, 2H), 1.78-1.62 (m, 4H), 1.52 (m, 2H), 1.47-1.27 (m, 18H), 0.92 (t, 3H). Same procedure was conducted to synthesize N-(2-((5-(1,2-dithiolan-3-yl)pentanoyl)oxy)ethyl)-N,N-dimethylalkan-1-aminium with different length of alkyl chains, which are named as LA-C2, LA-C4, LA-C6, LA-C8, LA-C10.

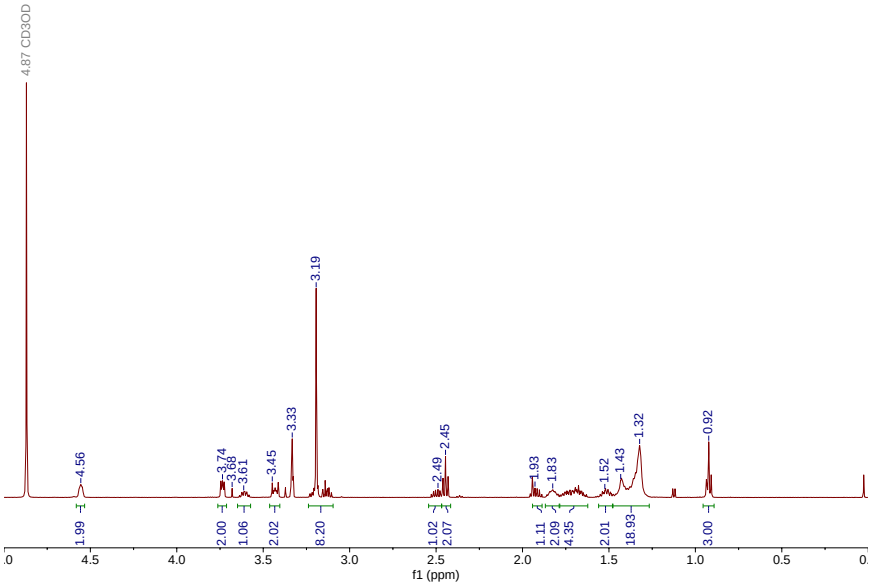
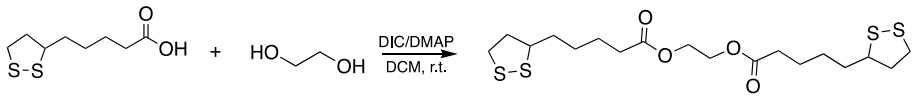


Figure S2 1H NMR spectrum of **1** in methanol-d4.

3.2 Synthesis of crosslinker **2**



Scheme S2 Synthetic scheme of **2**.

DL-α-lipoic acid (5.0 mmol, 1.03 g) and ethylene glycol (2.5 mmol, 0.16 g) were dissolved in 7.5 mL anhydrous dichloromethane (DCM) in a flask and cooled with ice water bath. In a separate container, DMAP (0.5 mmol, 0.06 g) and DIC (7.5 mmol, 0.95 g) were dissolved in 7.5 mL anhydrous DCM. The solution of DIC and DMAP was added dropwise into the solution of DL-α-lipoic acid and ethylene glycol. The solution was stirred at r.t. for 24 h. Column chromatography was used to purify the product and afford yellow liquid. 1H NMR (500 MHz, CD3OD): δ (ppm) 4.31 (s, 4H), 3.60 (m, 2H), 3.24-3.10 (m, 4H), 2.49 (m, 2H), 2.38 (t, 4H), 1.92 (m, 2H), 1.78-1.62 (m, 8H), 1.50 (m, 4H).

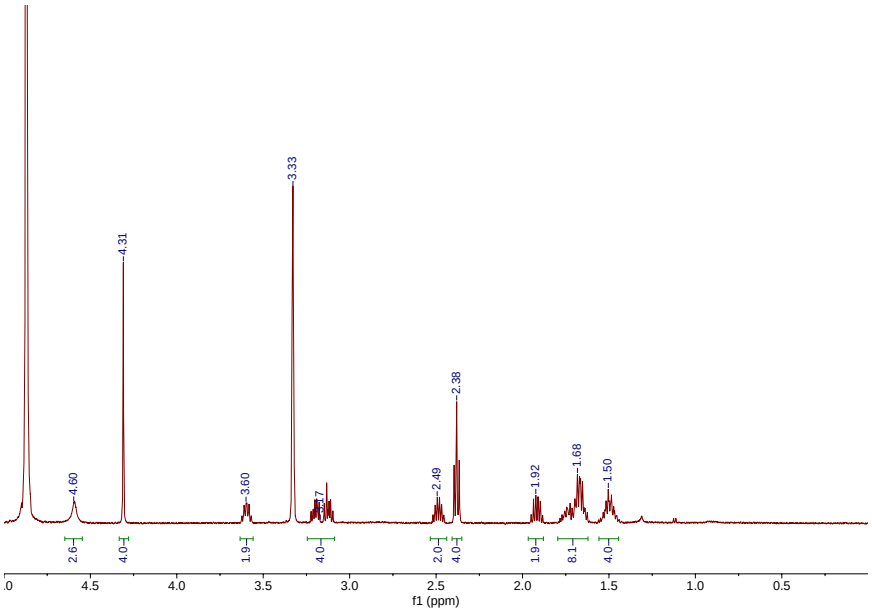
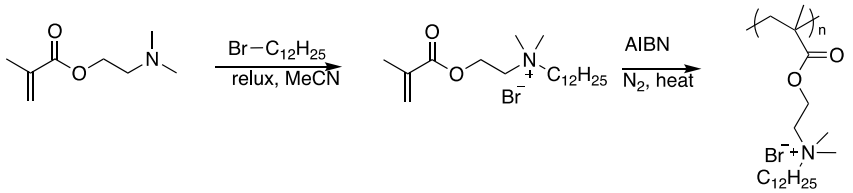


Figure S3 1H NMR spectrum of **2** in methanol-d4.

Synthesis of poly(N-(2-(methacryloyloxy)ethyl)-N,N-dimethyldodecan-1-aminium) (PMA-C12)



Scheme S3 Synthetic scheme of **PMA-C12**.

2-(dimethylamino)ethyl methacrylate (1 eq., 10 mmol, 1.5721 g) and 1-bromododecane (1.1 eq., 11 mmol, 2.433 g) were dissolved in 7 mL acetonitrile. The solution was refluxed for 18 h. After reflux, the solution was allowed to cool to r.t. then transferred into fridge. The white solid precipitated from the solution, was filtered, and washed with cold ethyl acetate (yield=52.7 %). 1H NMR (500 MHz, CD3OD): δ (ppm) 6.17 (s, 1H), 5.74(s, 1H), 4.63 (t, 2H), 3.78 (t, 2H), 3.44 (t, 2H), 3.20 (s, 6H), 1.99 (s, 3H), 1.82 (m, 2H), 1.44-1.24 (m, 18H), 0.92 (t, 3H).

N-(2-(methacryloyloxy)ethyl)-N,N-dimethyldodecan-1-aminium (MA-C12) (1 eq., 2 mmol, 756 mg) and AIBN (0.01 eq., 20 μmol, 3.28 mg) were dissolved in 4 mL DMF. The solution was bubbled with nitrogen for 15 min, then stirred under 80 ˚C for 18 h. The obtained solution was cooled. Then the product was precipitated from hexane and dried under high vacuum overnight (yield = 82.69 %).

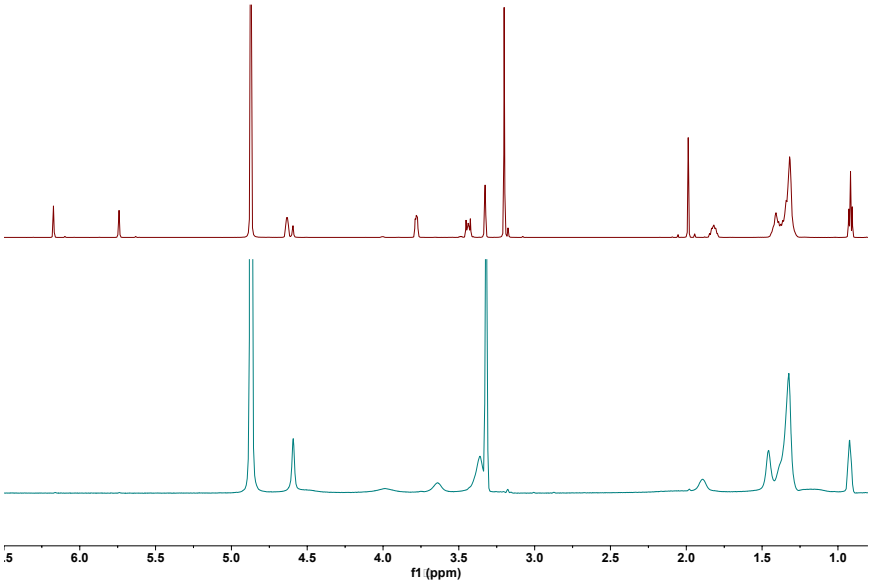


Figure S4 1H NMR in methanol-d4 of MA-C12 (red) and PMA-C12 (green).

3.3 Preparation of the poly(1-co-2) coating

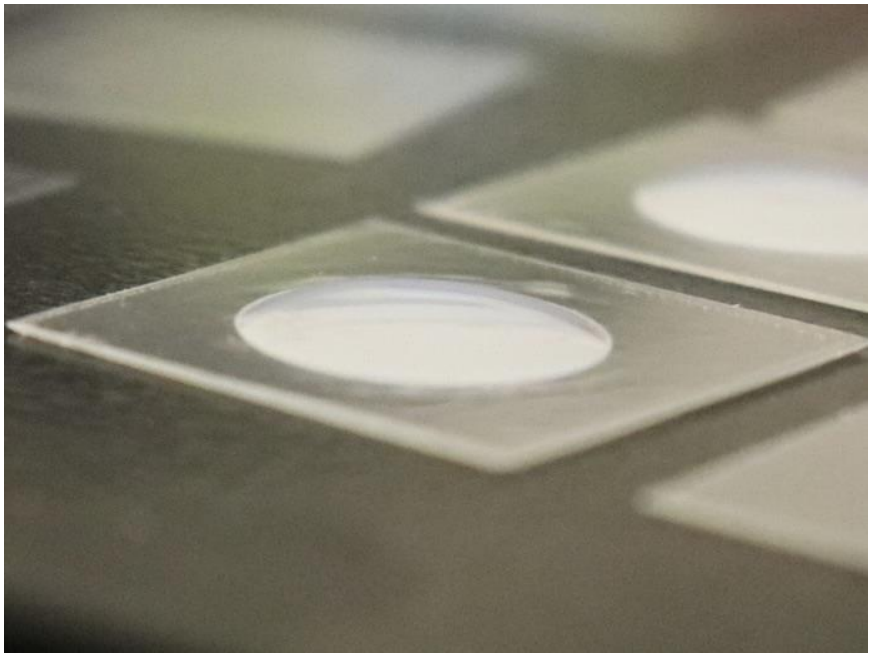


Figure S5 A photo of poly(1-co-2) preparation during air drying.

26.4 mg **1** (0.05 µmol, 1 eq.) was dissolved in 910 µL DI water. In a separate vial, a stock solution of 15 mg/mL crosslinker **2** was prepared. In another vial, a stock solution of 9.5 µL pentaerythritol tetrakis(3-mercaptopropionate) (thiol) and 16.4 µL 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in 10 mL methanol was prepared. 735 µL of **2** stock solution (0.025 mmol, 0.5 eq.) and 50 µL of

thiol/DBU stock solution (thiol: 0.125 μmol, 0.0025 eq., DBU: 0.55 μmol, 0.011 eq.) were added to **1** solution. The solution was agitated thoroughly. 100 μL of the mixture was added on a 20 x 20 mm glass coverslip with a spacer attached to confine the solution. The mixture was dried at room temperature overnight to obtain a transparent cross-linked polymer coating.

3.4 Contact killing assay of poly(1-co-2) coating

A single colony of *Staphylococcus aureus* (*S. aureus*) ATCC 25926 was inoculated in MH broth at 37 ˚C in shaking incubator overnight. The *S. aureus* suspension was diluted to OD_{600} = 0.1, regrown for 120 min at 37 ˚C to midlogarithmic phase in MH broth. The *S. aureus* suspension was diluted with PBS to OD_{600}=0.1. 10 μL *S. aureus* suspension was added on the polymer coating. The drop of *S. aureus* suspension was covered with a sterile glass coverslip to make sure good contact and maintain moisture. After 1 h incubation at r.t., The polymer coating and the coverslip was rinsed with 990 μL PBS, followed with 10x dilution and another 10x dilution. 10 μL solution from the solution for rinsing and the dilutions was streaked on clean and sterile agar plates respectively. All agar plates were incubated at 37 ˚C overnight. Average number of colonies was calculated from plates. All the experiments were conducted in triplicate. After each 1-h bactericidal challenge, the coating and coverslips were rinsed thoroughly with sterile PBS.

3.5 MIC and MBC assay

Minimum Inhibitory Concentration (MIC)

A single colony of *Escherichia coli* ATCC 25922 was inoculated in Muller-Hinton broth at 37 ˚C in shaking incubator overnight. The *E. coli* suspension was diluted to OD_{600} = 0.1 (absorbance measured at 600 nm by Molecular Devices SpectraMax M2), regrown for 90 min to midlogarithmic phase in MH broth. The *E. coli* suspension was diluted with MH broth to OD_{600} = 0.1 then diluted 10x with MH broth for twice until a *E. coli* suspension of OD_{600}=0.001 was obtained. In a round-bottom 96-well plate, 10 μL LA-C12 solution of serial concentrations and 90 μL *E. coli* suspension was added to each well. 100 μL sterile MH broth was added to several cells as negative control. 10 μL *E. coli* suspension and 90 μL MH broth were added in several wells as positive control. All experiments were done in triplicate. The plate was incubated at 37 ˚C overnight. MIC was calculated by averaging the minimum concentration where the solution was completely clear.

Minimum Bactericidal Concentration (MBC)

A single colony of *Escherichia coli* (*E. coli*) ATCC 25922 was inoculated in Muller-Hinton broth at 37 ˚C in shaking incubator overnight. The *E. coli* suspension was diluted to OD_{600} = 0.1, regrown for 90 min to midlogarithmic phase in MH broth. The *E.coli* suspension was diluted with PBS to OD_{600}=0.1 then diluted 10x with PBS for twice until a *E. coli* suspension of OD_{600} = 0.001 was obtained. In a round-bottom 96-well plate, 10 µL lipoic acid derivative solution of serial concentrations and 90 µL *E. coli* suspensions were added to each well. 100 µL PBS was added to several cells as negative control. 10 µL PBS and 90 µL *E. coli* suspensions were added in several wells as positive control. The plate was covered with lid and incubated for 2 hrs at 37 ˚C. All experiments were done in triplicate. In a clean and sterile 96-well plate, 90 µL MH broth was added to each well. 10 µL of solution of the first plate was transferred to the second plate at the same position. The second plate was incubated at 37 ˚C overnight. MBC was calculated by averaging the minimum concentration where the solution was completely clear.

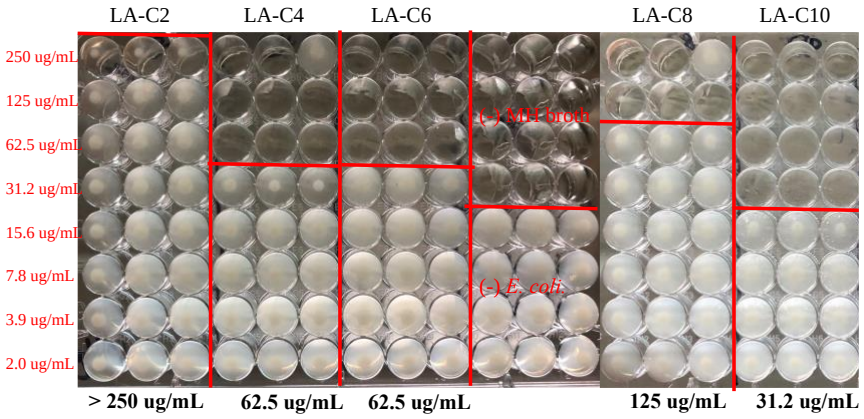


Figure S6 The photo of MIC results of lipoic acid derivatives with different alkyl chains.

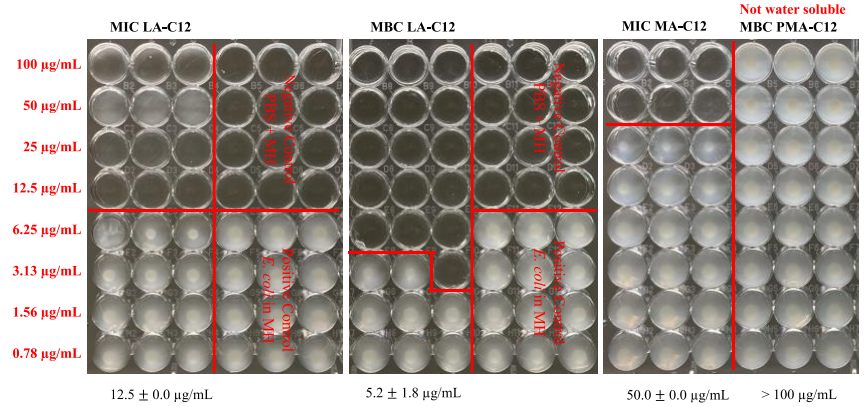


Figure S7 The photo of MIC and MBC results of **1**, MA-C12, and PMA-C12

3.6 Hemolytic activity assay

Hemolytic activity of polymers was determined by hemoglobin release assay using 2 mg/mL lipoic acid derivatives stock solution in methanol. 1 ml of 10% (v/v) sheep RBCs was centrifuged at 1000 rpm for 10 min and washed with PBS. The supernatant was carefully removed by pipetting. The RBCs were washed with PBS two more times. The resulting stock was diluted 10-fold in PBS to provide 1.11% (v/v) RBC assay stock. In a sterile 96-well round-bottom polypropylene microplate, 90 µl of 1.11% (v/v) RBC assay stock was mixed with 10 µl of each of the polymer dilution. As negative controls, PBS, and as positive control 0.1% (v/v) Triton X-100 were used. Microplate was put in orbital shaker at 37 ˚C and incubated at 180 rpm for 60 min. The microplate was centrifuged at 1000 rpm for 10 min. In another sterile flat-bottom microplate, 10 µl of supernatant was diluted with 90 µl PBS. The absorbance at 415 nm was recorded by microplate reader. Hemolysis was plotted as a function of polymer concentration. The HC_{50} that is described as the polymer concentration causing 50% hemolysis relative to the positive control. This value was estimated by the fitting the experimental data to the function

$$H = \frac{1}{1+(\frac{HC_{50}}{[P]})^n} \qquad \textbf{(Equation S1)}$$

where H is the hemolysis fraction $H = \frac{OD_{415}(polymer)-OD_{415}(buffer)}{OD_{415}(Triton)-OD_{415}(buffer)}$, P is the polymer concentration, n and HC_{50} are variable parameters. All experiments were done in triplicate. The absorbance values from each trial were averaged. The HC_{50} was calculated by fitting the data with **Equation S1**.

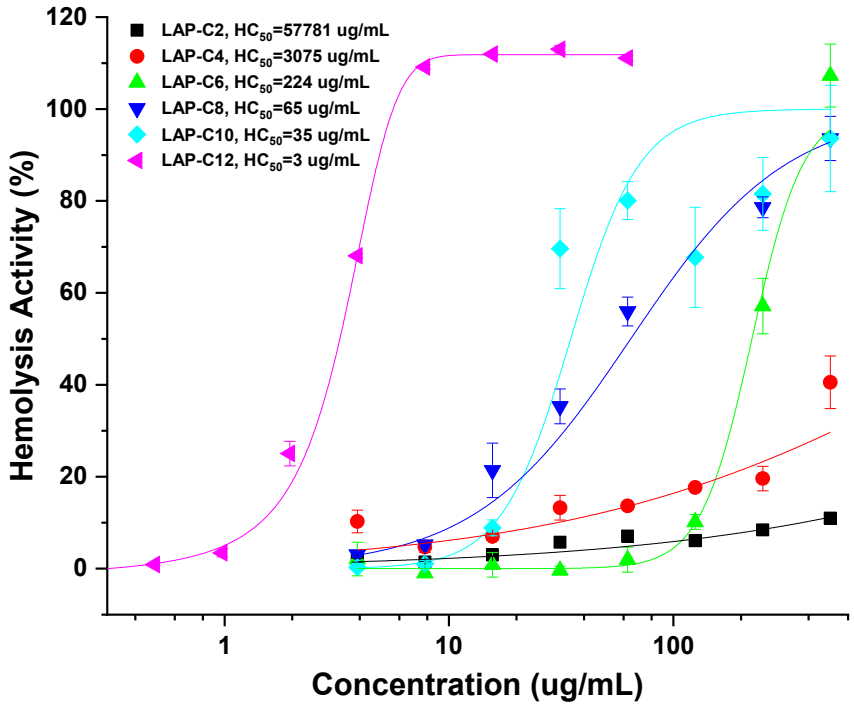


Figure S8 Hemolysis activity over concentrations of compound **1** analogues with alkyl chains of different length

3.7 Live/Dead Staining Assay

The stock solution of the LIVE/DEAD BacLight staining reagent was obtained by dissolving the contents of one Component A pipet (containing yellow-orange SYTO 9) and one Component B pipet (containing red propidium iodide) in 5 mL PBS. The final concentration of each dye will be 6 µM SYTO 9 stain and 30 µM propidium iodide. Prior to imaging, 10 µL of stock solution was added on the sample, incubated at r.t. for 15 min in dark, and rinsed with PBS. Confocal images were taken with Laser scanning confocal microscopy.

3.8 Table of degradation conditions

Poly(1-co-2)	+	+	+	+	+	+	+	+	+	+	+	+
Biofilms	+	+	+	+	+	+	+	+	-	-	-	-
LAP	+	-	+	-	+	-	+	-	+	-	+	-
UV 5 min	+	-	-	+	+	-	-	+	+	-	-	+
Crystal violet	+	+	+	+	-	-	-	-	-	-	-	-
Degraded or Not	+	-	-	-	+	-	-	-	+	-	-	-

4 Supplementary figures

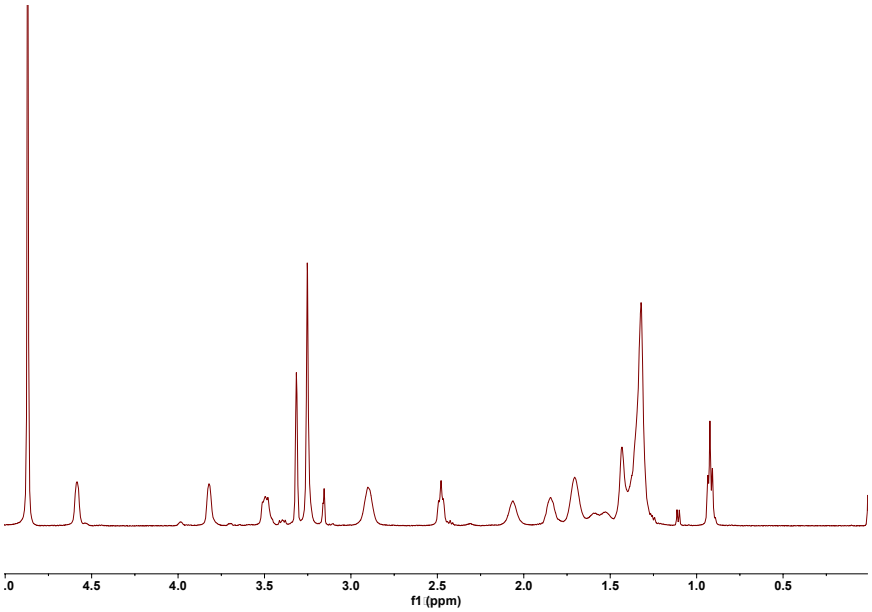


Figure S9 1H NMR in methanol-d4 of **1** dried from water and washed with DCM.

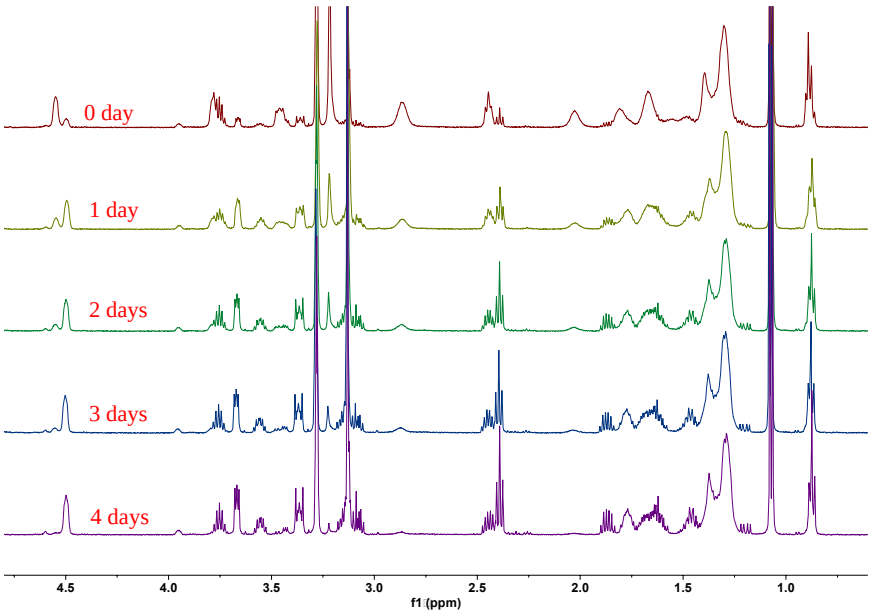


Figure S10 1H NMR spectra in methanol-d4 showing time-dependent degradation of 10 mg/mL **1**in solution.

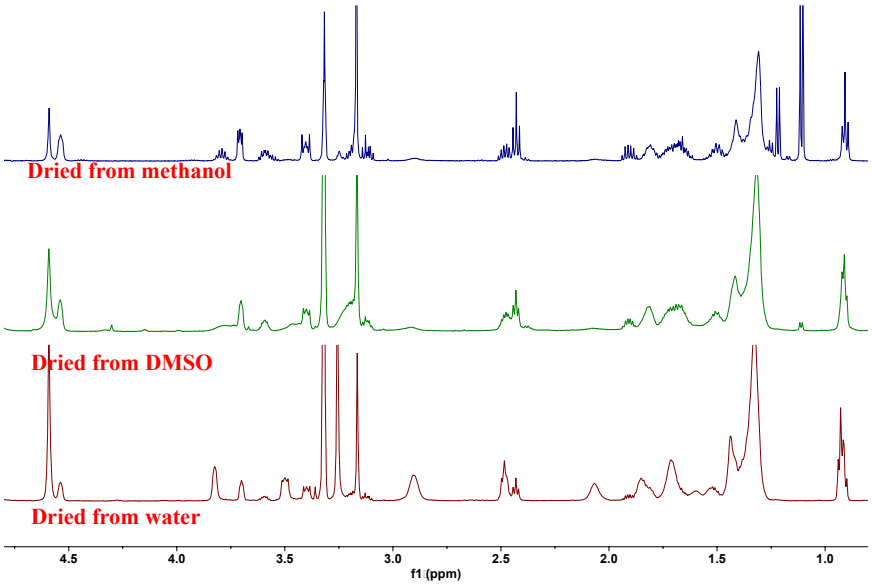


Figure S11 1H NMR in methanol-d4 of **1** dried from methanol (blue), DMSO (green), and water (red).

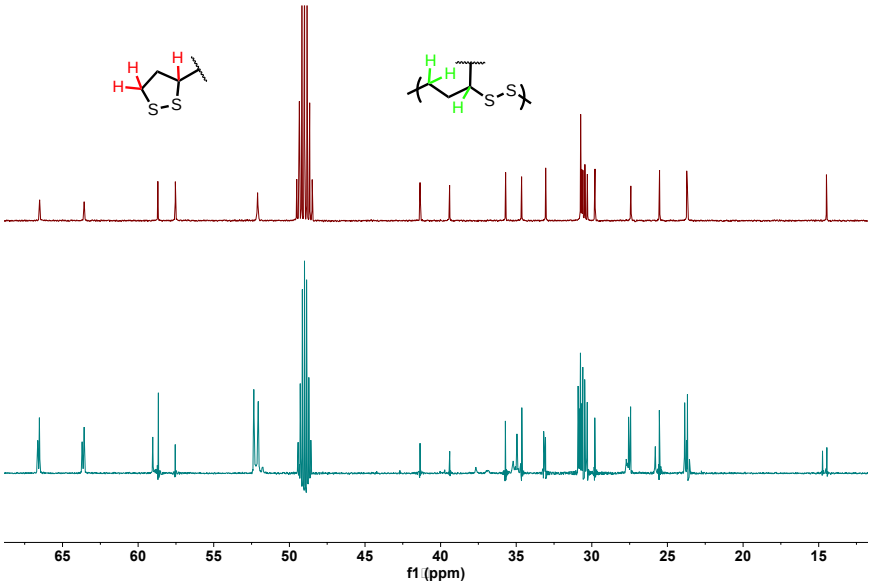


Figure S12 13C NMR in methanol-d4 of **1** (red) and **1** dried from water (green).

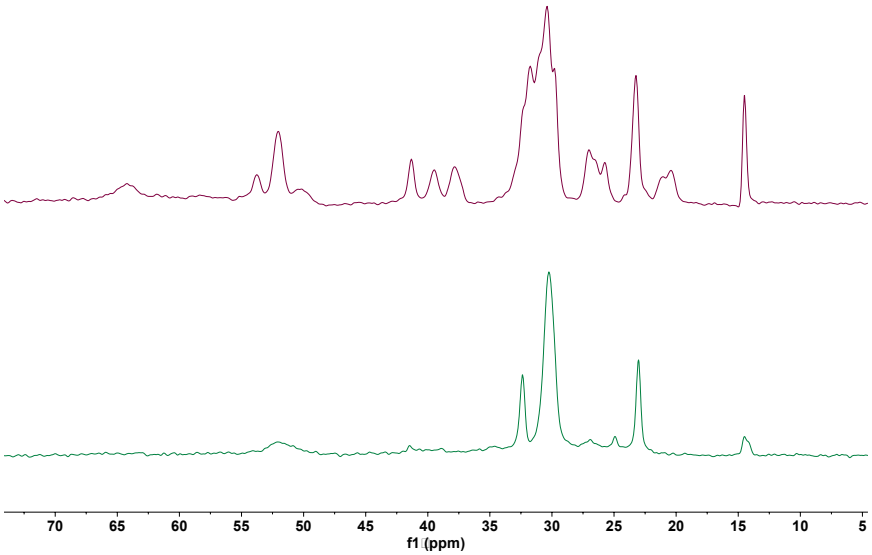


Figure S13 Solid state 13C NMR of **1** (red) and **1** dried from water (green).

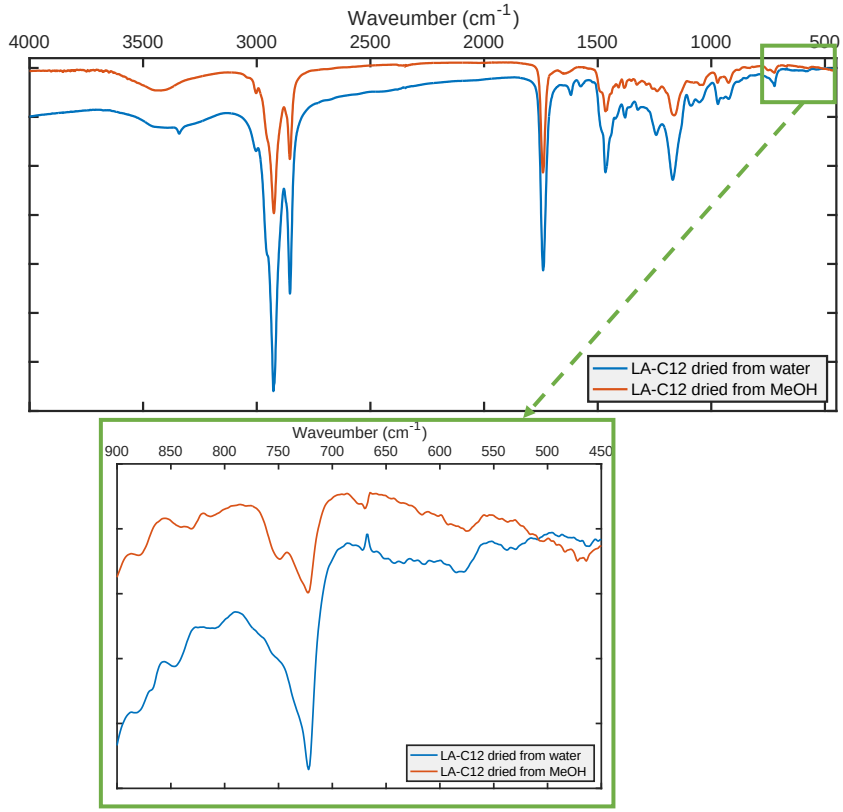


Figure S14 FT-IR spectra of **1** dried from methanol (red) and **1** dried from water (green).

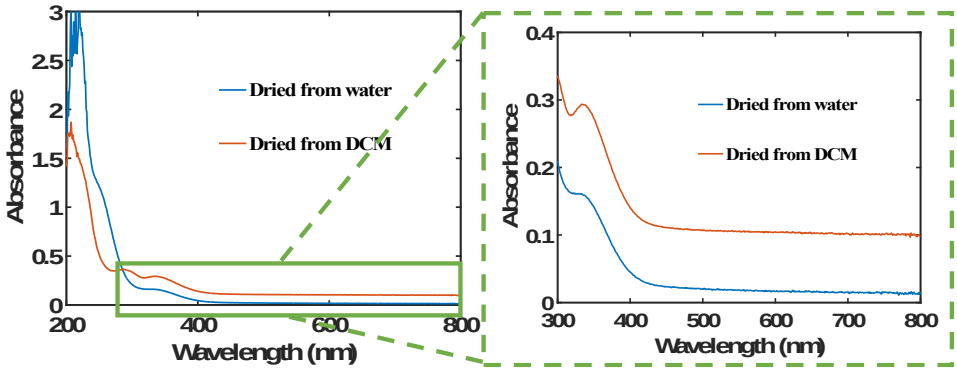


Figure S15 UV-vis spectra of drop coated **1** on glass with water (blue curve), and DCM (red curve) as solvent.

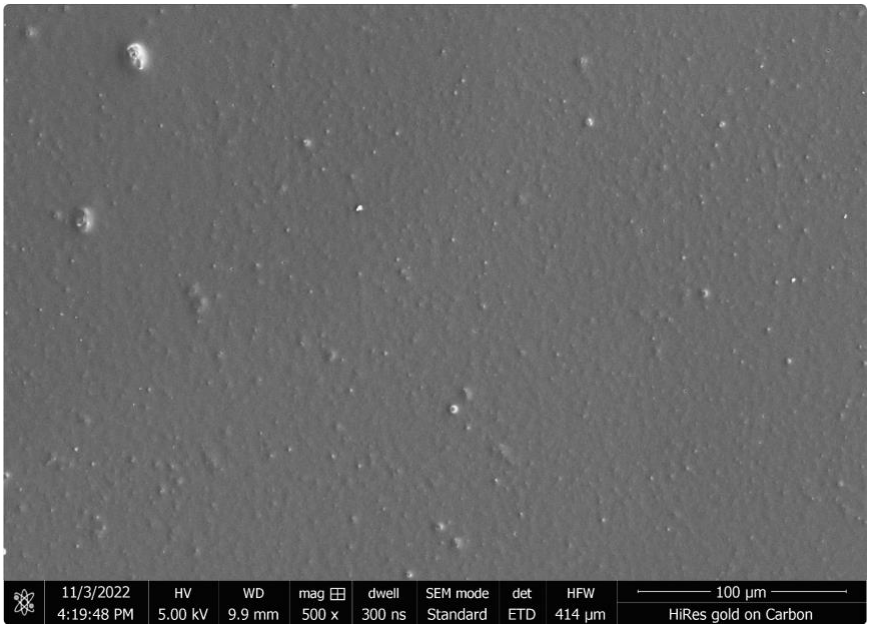


Figure S16 SEM image of poly(1-co-2) coating

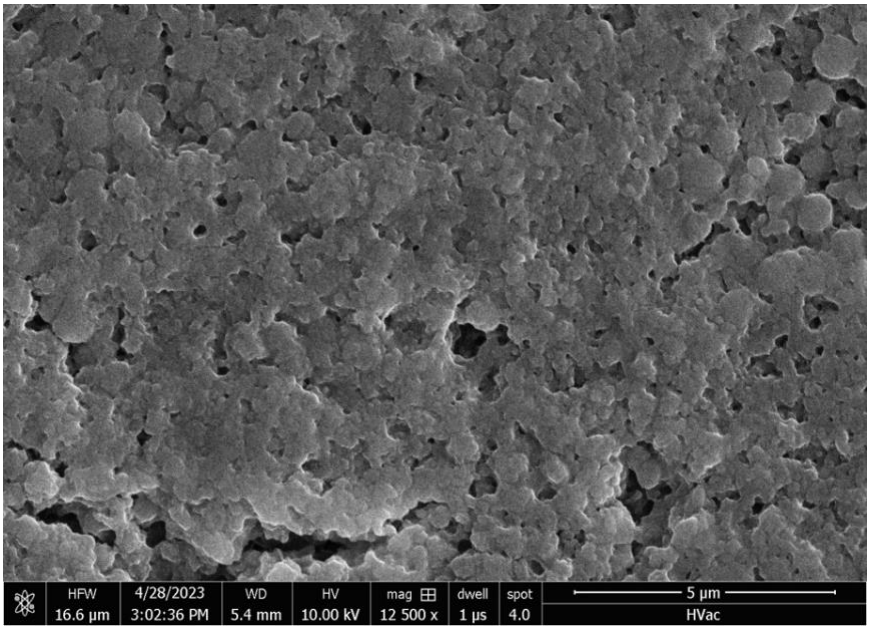


Figure S17 SEM image of *S. aureus* biofilms after 5-day incubation at room temperature.

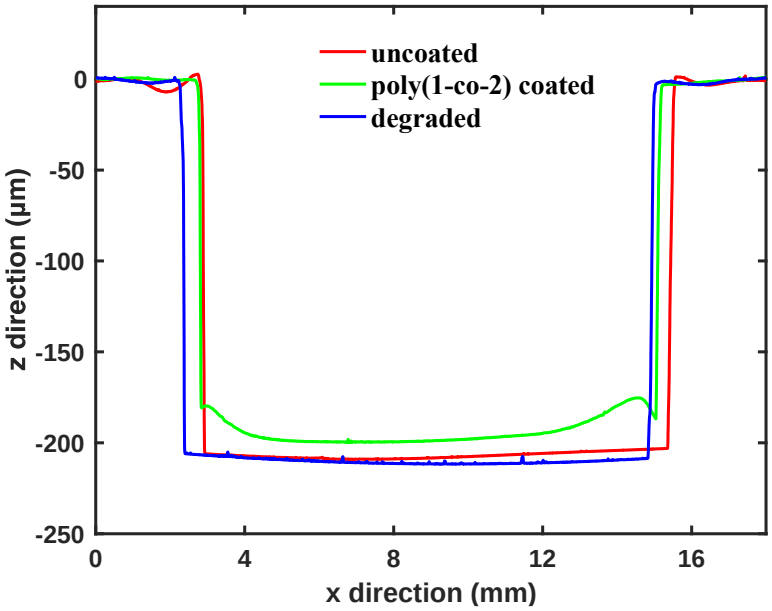


Figure S18 Profilometer curve of the surface of uncoated glass slide, coated slide, and the coated slide after degradation.

Figure S19 A photo of poly(1-co-2) coated glass slide after 15 min incubation in 0.1% crystal violet aqueous solution.

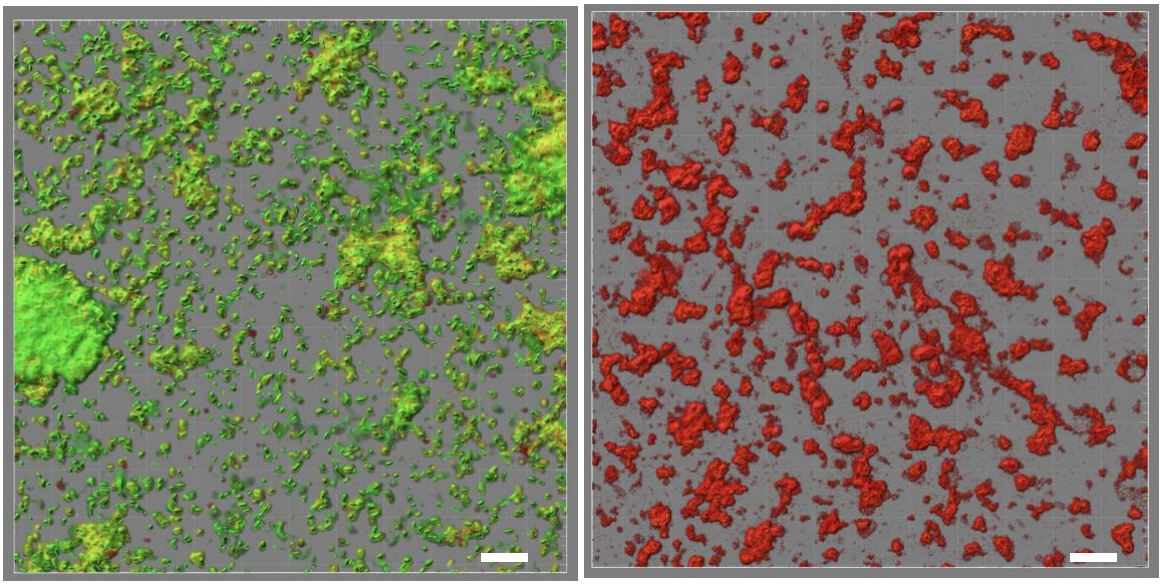


Figure S20 Confocal images of bacteria on glass surface (left), and poly(1-co-2) coated surface (right), after 1-day incubation. Scale bar represents 10 μm.

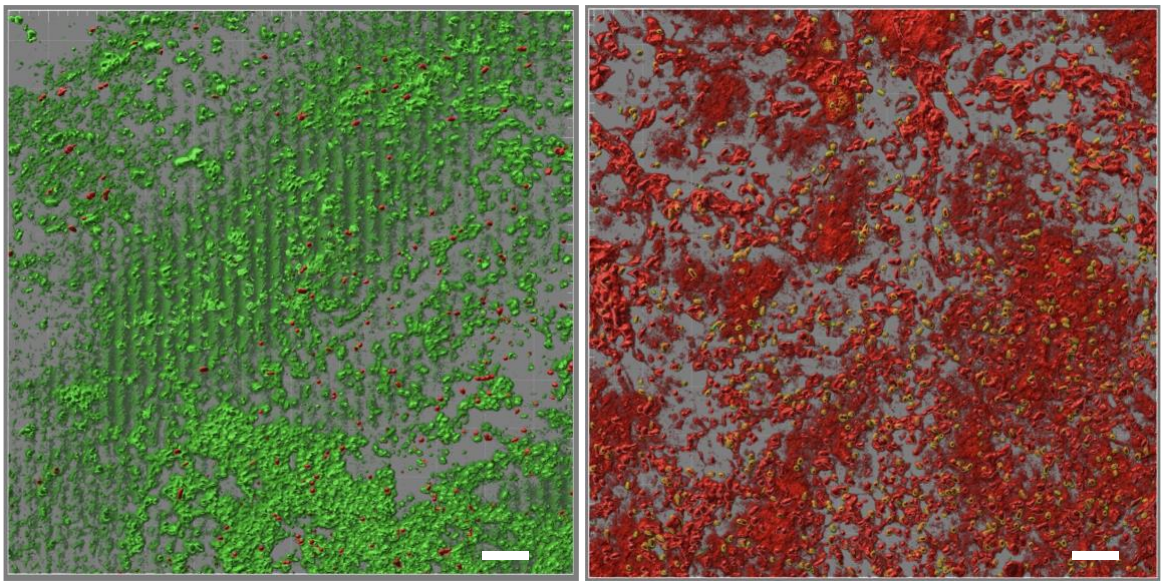


Figure S21 Confocal images of bacteria on glass surface (left), and poly(1-co-2) coated surface (right), after 3-day incubation. Scale bar represents 10 μm.