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Safe and efficient membrane permeabilizing polymers based on PLLA for antibacterial applications

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1. Materials

2-Bromoethanol (95%), Sodium azide (NaN_3, >99.5%), 2-Bromopropionyl bromide (97%), poly(ethylene glycol) methyl ether (MPEG, M_n = 550), propargyl bromide solution (80 wt. % in toluene), Sodium hydride (NaH, 60% dispersion in mineral oil), α-Bromoisobutyryl bromide (98%), triethylamine (>99%), 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA, 99%), N,N,N′,N″,N″-Pentamethyldiethylenetriamine (PMDETA, 99%), copper(I) bromide(CuBr, 99%), stannous octoate [$Sn(Oct)_2$] (95%), anhydrous 1,4-Dioxane (99.8%), anhydrous toluene (99.8%), and anhydrous N,N-dimethylformamide (99.8%) were obtained from Sigma-Aldrich. 2-(Dimethylamino)ethyl methacrylate (DMAEMA) stabilized with hydroquinone monomethyl ether was obtained from Merck and used as received. Ethylene glycol (99.8%, Sigma-Aldrich) was distilled over CaH_2 before use. L-lactide (L-LA) (Purac Biochem, The Netherlands) were used without further purification. Alkyne-ended MPEG was prepared according to the previous procedures.[1]

2. Synthesis of PLLA-diBr Macroinitiators

PLLA-diBr macroinitiators were synthesized using ring opening polymerization (ROP), and followed by terminal groups modification. The process was based on the previously described method with some modification (Scheme S1).[2] Typically, L-LA monomers were weighed into a dry and nitrogen purged flask together with ethylene glycol as initiator. The molar ratio of initiator and monomer was fixed at 1:25 for both reactions. The polymerization was performed in concentrated solution (ca. 3.0 M in anhydrous toluene) at 130°C for 24 h in the presence of $Sn(Oct)_2$ as catalyst. Purified PLLA-diol was obtained by precipitation of the reaction mixture into excess cold methanol twice, followed by overnight vacuum drying at 80 °C. Subsequently, PLLA-diol were modified into ATRP macroinitiator by esterification of the hydroxyl end groups with α-bromoisobutyryl bromide in anhydrous THF. A 20 times

excess of α-bromoisobutyryl bromide with respect to -OH end groups was added and triethylamine was used to trap hydrobromic acid generated during the reaction. Insoluble ammonium salt produced was first removed by centrifugation and the reaction mixture was further purified by passing through a short Al_2O_3 column using THF as eluent. The dilute solution was concentrated and precipitated into excess cold methanol twice. PLLA-diBr macroinitiators were obtained after drying under vacuum at 50°C overnight.

3. Synthesis of PDMAEMA-*b*-PLLA-*b*-PDMAEMA (D-PLLA-D) Triblock Copolymers

PDMAEMA-*b*-PLLA-*b*-PDMAEMA triblock copolymers were prepared by controlled ATRP. Molar feed ratio of [PLLA-diBr] : [DMAEMA] : [CuBr] : [HMTETA] = 1 : 1000 : 1 : 2 was applied for all polymer synthesis. As a typical example, PLLA-diBr was first introduced into a nitrogen filled round bottom flask (RBF) followed by successive addition of 1,4-dioxane and DMAEMA monomer through syringe injection. Afterwards, the RBF was purged and refilled with nitrogen using *vacuum-nitrogen-cycling* system three times. HMTETA and CuBr were added quickly under nitrogen atmosphere. Polymerization was allowed to proceed under continuous stirring at 60 °C for a desired reaction time. The molecular weight was monitored by gel permeation chromatography (GPC) analysis. After polymerization, the reaction was stopped by diluting the reaction mixture with THF and exposing it to ambient atmosphere for 1 h. Catalyst complex was removed by passing the reaction mixture through a short neutral Al_2O_3 column. After concentrating the filtrates, the solutions were precipitated into excess ether and the final product PDMAEMA-*b*-PLLA-*b*-PDMAEMA (D-PLLA-D) was obtained through centrifugation.

4. Synthesis of 2-Azidoethyl-2-bromopropanoate (AEBP)

AEBP was prepared from the synthesis of 2-Azidoethanol, followed by esterification with 2-bromopropionyl bromide. First, 2-Azidoethanol was prepared according to a reported procedure.[3] In a typical reaction, 2-bromoethanol (8.8 g, 0.07 mol) and sodium azide (8.7 g, 0.14 mol) were placed into the reaction flask together with 100 mL of water. The mixture was stirred at 50 °C for 24 h and then cooled to room temperature. The solution was extracted with 100 mL ether and repeated for three cycles, followed by drying with magnesium sulphate overnight and filtered. Purified 2-azidoethanol was obtained as a colorless liquid after vacuum dry. For the synthesis of AEBP, 2-Azidoethanol (6.0 g, 0.07 mol) and anhydrous Et_3N (10.6 mL, 0.08 mol) were dissolved in 50 mL anhydrous THF in a 150 mL RBF. A 1.1 times excess of 2-bromopropionyl bromide (7.9 mL, 0.08 mol) with respect to the hydroxyl groups was added dropwise into the flask at 4 °C over a period of 1 h. After the addition was completed, the reaction mixture was continually stirred for another 24 h at room temperature. The undissolved solid was removed by centrifuge and the concentrated solution was further purified by a silica gel column chromatograph using THF/hexane (1:4 v/v) as an eluent. The solvent was removed by vacuum drying and purified AEBP was obtained as a colourless oil.[4]

5. Synthesis of Alkyne-terminated PEG

Alkyne-terminated PEG was synthesized according to previous method.[1] Typically, NaH (60% w/w in mineral oil, 0.77 g, 32.01mmol, 1.1 equiv.) was added into 80 mL of anhydrous THF solution dissolved with 16 g of MPEG (29.1mmol). Traces of water in PEG were removed by azeotropic distillation with toluene. The reaction was kept at 0 °C for 15 min with frequent venting. Then, propargyl bromide (80% in toluene, 4.74 mL, 32.01mmol, 1.1 equiv) was added dropwise through the dropping funnel, after which the mixture was stirred at 25 °C for

24 h. For purification, the solution was concentrated and extracted with 400 mL $H_2O/CHCl_3$ (1/3, v/v) and repeated for three cycles, followed by drying with magnesium sulphate and filtered. The concentrated solution was precipitatedin excess hexane and vacuum dried to obtain the final purified product which is colourless liquid (yield, 87.5%).

6. Synthesis of PEG Conjugated D-PLLA-D (D-PLLA-D@PEG) Copolymers

D-PLLA-D@PEG was synthesized through one-pot approach using AEBP as coupling agent. In a typical procedure, D-PLLA-D (1.0 g) prepared as described above was dissolved in DMF (10 mL) and AEBP (0.072 mL) was then introduced into the mixture. The [AEBP]/[DMAEMA] molar ratio was fixed at 1 : 10, targeting at a 10% quaternization degree of D-PLLA-D@N_3. After stirring at 50 °C for 48 h, the mixture was cooled to room temperature. Next, 0.55 g of propargyl-terminated PEG and 0.09 g of PMDETA were added and the flask was purged with N_2 for 30 min. CuBr (0.07 g) were added quickly under nitrogen atmosphere. After stirring for 24 h at ambient temperature, the solution was dialyzed against deionized water using a dialysis membrane (Spectrum dialysis membrane, MWCO 1000) for 48 h to remove the excess PEG, and the final product (D-PLLA-D@PEG) was subsequently collected through lyophilization.

7. Molecular Characterization

Nuclear Magnetic Resonance (^{1}H-NMR). ^{1}H-NMR spectra were recorded on a Bruker AV-400 NMR spectrometer at room temperature. Chemical peaks are reported in ppm with reference to solvent peaks (DMF:δ 8.03, 2.92 and 2.75 ppm; $CHCl_3$: δ 7.3 ppm; and H_2O:

δ4.8 ppm). Chemical compositions of the copolymers were evaluated from the proton integral regions as assigned in Figure 1.

Gel Permeation Chromatography (GPC). The molecular weight and polydispersity of the as-synthesized copolymers were determined by GPC (Shimadzu SCL-10A and LC-8A system) equipped with two Phenogel columns (10^3 and 10^5Å) (size: 300 × 7.80 mm) in series and a Shimadzu RID-10A refractive index detector. DMF (0.1M LiBr) was used as the eluent at a flow rate of 1.0 mL/min at 40°C. Monodispersed poly(methylmethacrylate) (PMMA) standards were used to obtain a calibration curve. The PEG conjugation percentage was calculated from the molecular weight difference before and after the conjugated reaction.

8. Cell viability assay

The cytotoxicity of the polymers was evaluated using the MTT assay in HEK 293T and Hela cell lines. They were cultured in DMEM, supplemented with 10% FBS, 100 units/mL of penicillin/streptomycin at 37 °C under 5% CO_2, and 95% relative humidity atmosphere. The cells were seeded in a 96-well plate at a density of $1x10^4$ cells/well and incubated in 100 μL of DMEM/well for 24 h. The culture media were replaced with fresh culture media containing serial dilution of polymers (15. 6 to 500 μg/mL), and the cells were incubated for 24 h. Then, 10 μL of sterile-filtered MTT stock solution in PBS (5 mg/mL) was added to each well. After 4 h, the unreacted dye was removed by aspiration and the produced formazan crystals were dissolved in 100 μL of DMSO/well. The absorbance was measured using a microplate reader (Infinite M200, Tecan) at wavelength of 570 nm. The cell viability (%) of polymers was the absorbance of polymer treated cells divided by the absorbance of control cells.

9. Determination of miminum inhibitory concentration (MIC)

Stock solutions were prepared by dissolving the polymers in deionized water and subsequently diluted to 256 μg/mL in Mueller Hinton broth (MHB). 100 μL of the polymer solution was added to two wells of the 96 well plates for duplicates and another 200 μL was added to one well for zero reference. Serial dilution was performed to obtain the following concentrations: 256μg/ml, 128μg/ml, 64μg/ml, 32μg/ml, 16μg/ml, 8μg/ml, 4μg/ml and 2μg/ml. The inoculum suspension was prepared by adding a few colonies extracted from the Tryptic Soy Agar (TSA) plate into the MHB. The concentration of the inoculum suspension was adjusted according to the 0.5 McFarland standard and the final concentration of each well was ~10^5 Colony Forming Units (CFU)/mL. Upon the addition of 100 μL of the inoculum suspension into the polymers solutions, the plates were incubated at 37 ˚C for 24 hours. Absorbance was then read at 600nm. The positive and the negative controls used in the experiment was inoculum with MHB and MHB only. Vancomycin solution was also used as a control drug.

10. Hemolysis experiments

Hemolysis was performed using fresh rabbit red blood cells which were isolated from the whole blood of the New Zealand white rabbits. The retrieval process follows the standards of the Association for Research in Vision and Ophthalmology and approval was obtained from the IACUC of SingHealth. The whole blood was centrifuged at 3000 rpm at 4 ˚C for 10 min. The supernatant was discarded while the red blood cells were subjected to two times of washing with 2 ml sterile PBS. The purified red blood cells were then diluted with 10 mM sterile PBS (pH 7) to make 8 % (v/v) red blood cell stock solution. Polymer solutions were prepared and mixed with the red blood cells solutions to obtain the desired concentrations with 4 % (v/v) red blood cells. Negative and positive controls used in the experiment were

PBS with red blood cells and Triton X with red blood cells respectively. Following the addition of the red blood cells to the polymer solutions, the solutions were incubated at 37 ˚C for 1 h. After the incubation, the solutions were centrifuged at 3000 rpm for 3 min and 100 μL of the supernatant was transferred to a 96 well plate. The absorbance of the supernatants were measured using TECAN infinite 200 microplate reader at 576nm and the values were subsequently input into the following equation to calculate the % hemolysis.

% hemolysis = ([Abs] - [Neg]) / ([Pos] - [Neg]) x 100 %

where [Abs] is the absorbance reading of the supernatant, [Neg] is the absorbance reading of the negative control and [Pos] is the absorbance reading of the positive control.

11. Cytoplasmic membrane depolarization assay

The effect of the polymers on the membrane potential of Pseudomonas Aeruginosa (ATCC 9027) was probed by membrane sensitive DiSC3-5 fluorescent assay. Briefly, Pseudomonas Aeruginosa (ATCC 9027) was harvested at an early exponential growth phase and washed with buffer solution (5 mM HEPES at pH 7) and resuspended in the same buffer until an optical density of 0.09 at 620 nm [OD_{620}] was obtained. The cell suspension was incubated with 0.4 μM DiSC3-5 (Invitrogen) and 0.1 M potassium chloride (KCl) solution at 37 °C until DiSC3-5 uptake was maximal (when the reduction of fluorescence intensity was stable due to self-quenching of DiSC3-5 in the untreated bacteria). The desired concentration of

polymer was added into a stirred cuvette. The fluorescence reading was monitored for 500 s with a Photon Technology International Model 814 fluorescence spectrophotometer, at an excitation wavelength of 660 nm and an emission wavelength of 675 nm. DMF alone had no

effect on depolarization. Experiments were repeated at least three times and were reproducible.

12. Nitrocefin assay

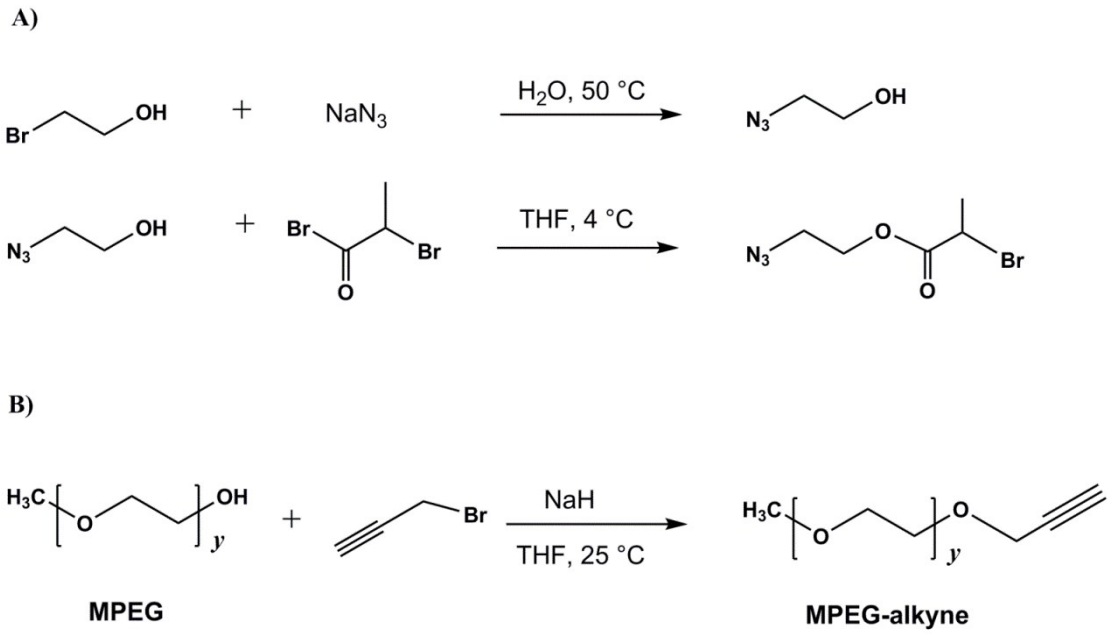
Nitrocefin was used as the probe to characterize the outer membrane permeabilization. 10 mg/mL stock of nitrocefin was prepared with DMSO as the solvent. This stock solution was then diluted to 2 mg/mL using sterile PBS. A stock solution of bacteria and nitrocefin was prepared in PBS. Colonies of bacteria were added to a 100 mM PBS solution to achieve an optical density of 0.4. Nitrocefin was then added to the prepared solution in the ratio of 1μL of nitrocefin to 200 μL of solution. 200 μL of the stock solution was added to each well in a 96 well plate. Reading was taken for 5 min which accounts for the baseline. Stock solutions of the various polymers were then added to attain final concentrations of: 1 μg/mL, 2 μg/mL, 4 μg/mL, 8 μg/mL, 16 μg/mL, 32 μg/mL, 64 μg/mL, 128 μg/mL, 256 μg/mL, 512 μg/mL and 1024 μg/mL. Absorbance of the wells was recorded for 40 min using PerkinElmer Multimode plate reader at an excitation wavelength of 500nm while a reading was collected every minute.

The typical method employed in determining the PC_{50} values of the polymers with regards to their potential in outer membrane permeabilization involved determining the difference in absorbance between the baseline and the final plateau of the curve. The average of the first five values read before polymer addition was subtracted from the average of the final five values after the addition of the polymer. The same analysis was also run on positive control PMB, and the computed results from the polymers were taken as percentage values of the obtained value from PMB. The percentage values of each concentration per polymer were determined, and plotted against the polymer concentration. This allowed for the generation of

a curve that could be fitted to the one phase association model. Through the equation of the fitted model, interpolation could be conducted to determine the PC_{50} values of the polymers.

13. Antibacterial activity in chemically defined media

Bacteria were diluted to ~5 x 10^5 CFU.mL^{-1} in M9 minimal media (as per ATCC Medium: 2511 M9 Minimal Broth containing inorganic salts, glucose and thiamine, pH = 7.4) for E. coli and minimum essential medium (MEM) for S. aureus. 50 μL of the polymers were added to a 96 well plate containing 150 μL bacterial solutions. The plate was then incubated at 37 C for a period of 18-24 h. 20 μL of bacterial suspension was spot-plated on agar plates. The viable colonies (<100) were counted after 48 h incubation at 37 ^{o}C.



Scheme S1. Synthesis of (A) 2-Azidoethyl-2-bromopropanoate (AEBP) and (B) MPEG-alkyne.

Bacteria strains/MIC_{50}	D-PLLA-D	D-PLLA-D@Q 1	D-PLLA-D@Q 2	D-PLLA-D@Q-PEG-1	D-PLLA-D@-Q-PEG-2
MRSA 57964	68 ± 3	176 ± 8	87 ± 4	201 ± 14	290 ± 15
MRSA 42412	11 ± 1	29 ± 2	30 ± 2	11 ± 0.5	32 ± 2
MRSA 21595	13 ± 1	33 ± 2	72 ± 3	22 ± 1	97 ± 5
MRSA 9808	10 ± 1	36 ± 2	47 ± 3	10 ± 0.5	2 ± 0.1
MRSA 6506	18 ± 2	25 ± 2	114 ± 5	20 ± 1	76 ± 4

Table S1. MIC_{50} of antibacterial polymers against methicillin-resistant Staphylococcus aureus (MRSA).

Table S2 Minimum inhibitory concentration of copolymers against a panel of Gram-negative pathogens. The source of clinical isolates is indicated in parentheses. The antimicrobial activity of the polymers against tobramycin-resistant strains is highlighted in bold.

Gram-negative bacteria	Minimum Inhibitory Concentration (µg/mL)		
	D-PLLA-D@Q-PEG-1	D-PLLA-D@-Q-PEG-2	Tobramycin
Pseudomonas aeruginosa ATCC 15442	64	64	< 0.5
Pseudomonas aeruginosa ATCC 27853	64	64	< 0.5
Pseudomonas aeruginosa ATCC 9027	64	64	< 0.5
Pseudomonas aeruginosa 07DM023376 (eye)	64	64	< 0.5
Pseudomonas aeruginosa PAE230DR4877/07 (sputum)	**64**	**64**	**> 64**
Pseudomonas aeruginosa 412DR18531 (unknown)	**32**	**32**	**> 64**
Pseudomonas aeruginosa DM023257 (eye)	64	64	< 0.5
Pseudomonas aeruginosa DU14476/07 (urine)	**64**	**64**	**> 64**
Pseudomonas aeruginosa DM023155 (eye)	64	64	< 0.5
Pseudomonas aeruginosa DR5790 (wound)	**64**	**64**	**> 64**
Pseudomonas aeruginosa DM0004150R (PsD) (eye)	64	64	< 0.5
Escherichia coli ATCC 10536	32	64	1
Escherichia coli ATCC 8739	32	64	2
Escherichia coli ATCC 25922	32	64	2

Table S3 Minimum inhibitory concentration of copolymers against a panel of Gram-positive pathogens. The source of clinical isolates is indicated in parentheses. The antimicrobial activity of the polymers against tobramycin-resistant strains is highlighted in bold.

Gram-positive bacteria	Minimum Inhibitory Concentration (µg/mL)		
	D-PLLA-D@Q-PEG-1	D-PLLA-D@-Q-PEG-2	Tobramycin
Bacillus cereus ATCC 11778	16	32	2
Bacillus subtilis ATCC 6633	64	64	< 0.5
Enterococcus faecalis ATCC 29212	64	> 128	> 16
Enterococcus faecalis ATCC 700802	128	> 128	> 16
Enterococcus hirae ATCC 9790	32	> 128	> 16
MRSA ATCC 43300	**32**	**32**	**> 64**
MRSA ATCC 700699	**16**	**32**	**> 64**
MRSA ATCC BAA-38	> 128	128	< 0.5
Staphylococcus aureus ATCC 29213	16	32	1
Staphylococcus aureus ATCC 6538	16	32	1
Staphylococcus aureus ATCC 29737	16	32	1
Staphylococcus epidermidis ATCC 12228	4	16	0.5
MRSA DB21455 (eye)	**16**	**32**	**> 64**
MRSA DB68004 (blood)	**32**	**32**	**> 64**
Staphylococcus aureus DM0004001R (eye)	16	16	1
Staphylococcus aureus DM4299 (eye)	16	16	< 0.5
Staphylococcus aureus DM0004583R (eye)	16	16	1
Staphylococcus aureus DM0004400R (cornea)	16	32	< 0.5

Table S4 Minimum inhibitory concentration of copolymers against yeasts pathogens. The source of clinical isolates is indicated in parentheses.

Fungal/Yeasts strains	Minimum Inhibitory Concentration (µg/mL)	
	D-PLLA-D@Q-PEG-1	D-PLLA-D@-Q-PEG-2
Candida albicans ATCC 2091	16	16
Candida abicans ATCC 10231	16	16
Candida albicans ATCC 24433	16	64
Candida albicans DF0001976R (colon)	16	32
Candida albicans DF0002672R (urine)	16	16

Table S5. Minimum inhibitory concentration of copolymers against *E. coli* in chemically defined media.

Gram-negative bacteria	Minimum Inhibitory Concentration (µg/mL)		
	D-PLLA-D@Q-PEG-1	D-PLLA-D@-Q-PEG-2	D-PLLA-D
Escherichia coli ATCC 10536	32	64	256
Escherichia coli ATCC 8739	32	64	256
Escherichia coli ATCC 25922	32	64	256

Table S6. Minimum inhibitory concentration of copolymers against *S. aureus* in chemically defined media. The source of clinical isolates is indicated in parentheses.

Gram-positive bacteria	Minimum Inhibitory Concentration (μg/mL)		
	D-PLLA-D@Q-PEG-1	D-PLLA-D@-Q-PEG-2	D-PLLA-D
Staphylococcus aureus ATCC 29213	32	32	64
Staphylococcus aureus ATCC 6538	32	32	128
Staphylococcus aureus ATCC 29737	32	32	128
Staphylococcus epidermidis ATCC 12228	16	32	64
Staphylococcus aureus DM0004001R (eye)	32	32	64
Staphylococcus aureus DM4299 (eye)	32	32	64
Staphylococcus aureus DM0004583R (eye)	32	16	64
Staphylococcus aureus DM0004400R (cornea)	32	32	64

Table S7. Minimum inhibitory concentration of copolymers against *E. coli* after incubation in medium for 24h.

Gram-negative bacteria	Minimum Inhibitory Concentration (μg/mL)		
	D-PLLA-D@Q-PEG-1	D-PLLA-D@-Q-PEG-2	D-PLLA-D
Escherichia coli ATCC 10536	64	64	>512
Escherichia coli ATCC 8739	64	64	>512
Escherichia coli ATCC 25922	64	64	>512

Table S8. Minimum inhibitory concentration of copolymers against *S. aureus* after incubation in medium for 24h. The source of clinical isolates is indicated in parentheses.

Gram-positive bacteria	Minimum Inhibitory Concentration (μg/mL)		
	D-PLLA-D@Q-PEG-1	D-PLLA-D@-Q-PEG-2	D-PLLA-D
Staphylococcus aureus ATCC 29213	32	32	>512
Staphylococcus aureus ATCC 6538	32	32	>512
Staphylococcus aureus ATCC 29737	32	32	>512
Staphylococcus epidermidis ATCC 12228	16	32	>512
Staphylococcus aureus DM0004001R (eye)	32	32	>512
Staphylococcus aureus DM4299 (eye)	32	32	>512
Staphylococcus aureus DM0004583R (eye)	32	16	>512
Staphylococcus aureus DM0004400R (cornea)	32	32	>512

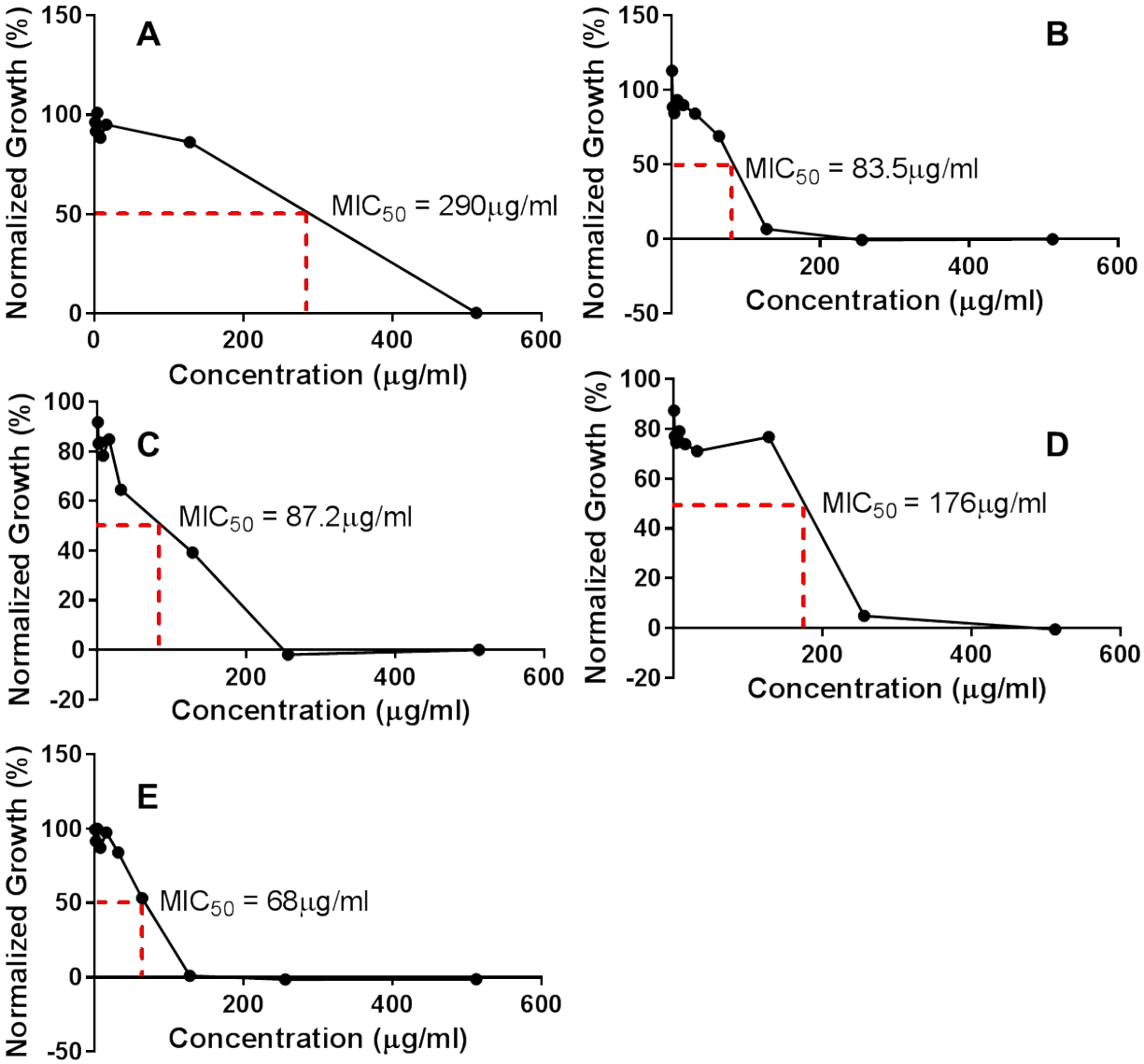


Figure S1. Growth of MRSA57964 in the presence of different concentrations of polymers (A) D-PLLA-D@-Q-PEG-2 (B) D-PLLA-D@-Q-PEG-1 (C) D-PLLA-D@Q 2 (D) D-PLLA-D@Q 1 (E) D-PLLA-D

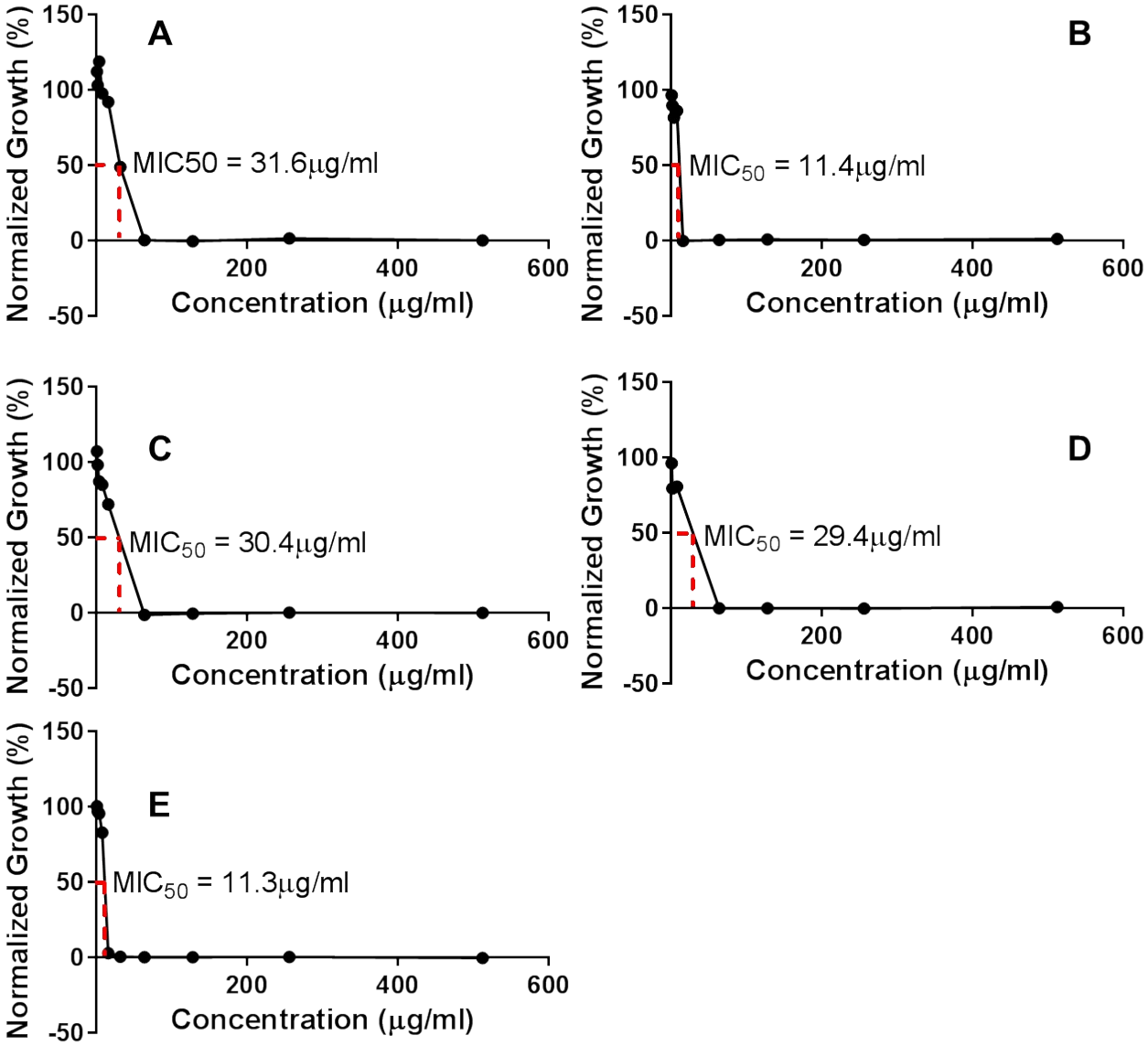


Figure S2. Growth of MRSA42412 in the presence of different concentrations of polymers (A) D-PLLA-D@-Q-PEG-2 (B) D-PLLA-D@-Q-PEG-1 (C) D-PLLA-D@Q 2 (D) D-PLLA-D@Q 1 (E) D-PLLA-D

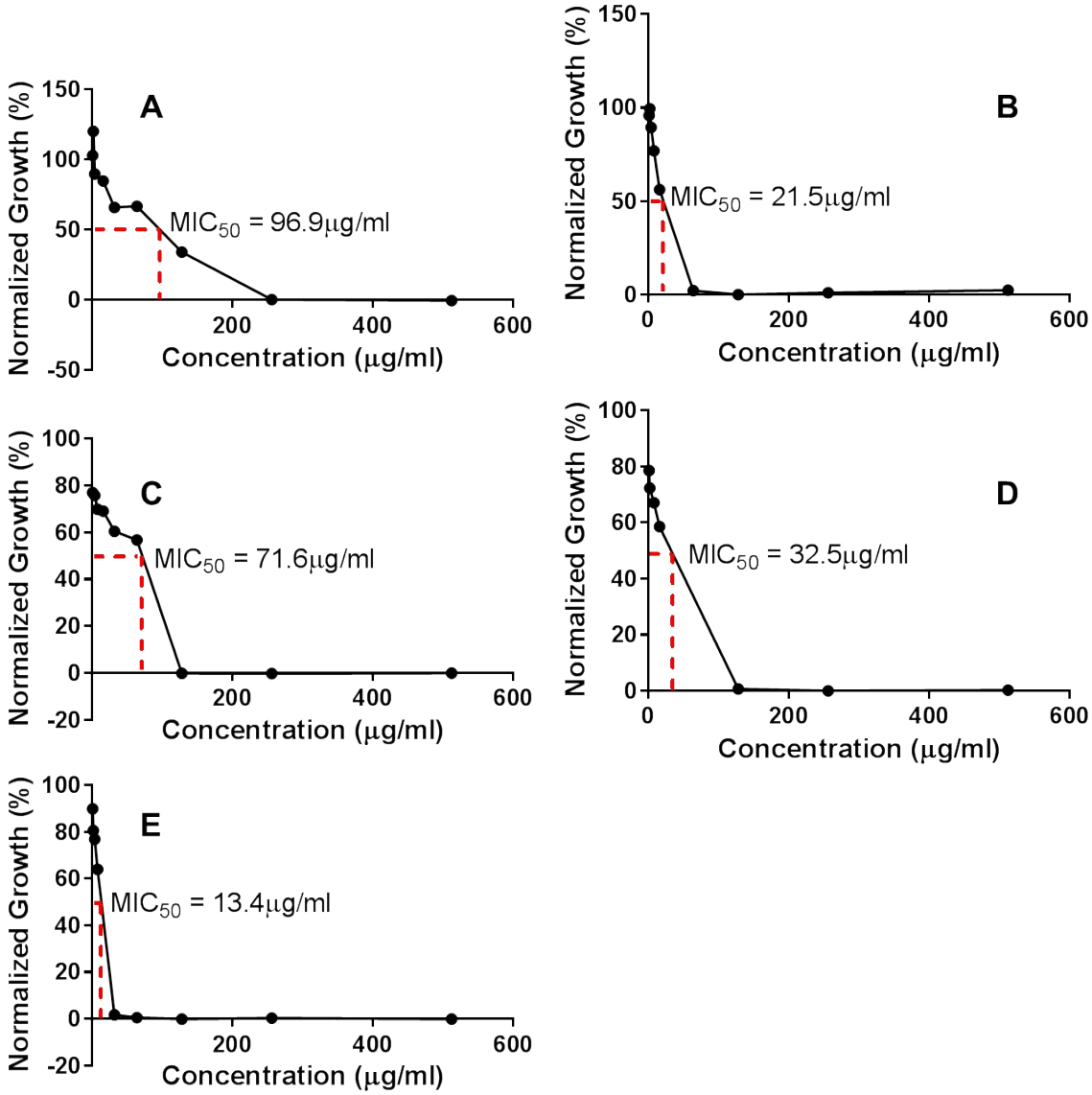


Figure S3. Growth of MRSA21595 in the presence of different concentrations of polymers (A) D-PLLA-D@-Q-PEG-2 (B) D-PLLA-D@-Q-PEG-1 (C) D-PLLA-D@Q 2 (D) D-PLLA-D@Q 1 (E) D-PLLA-D

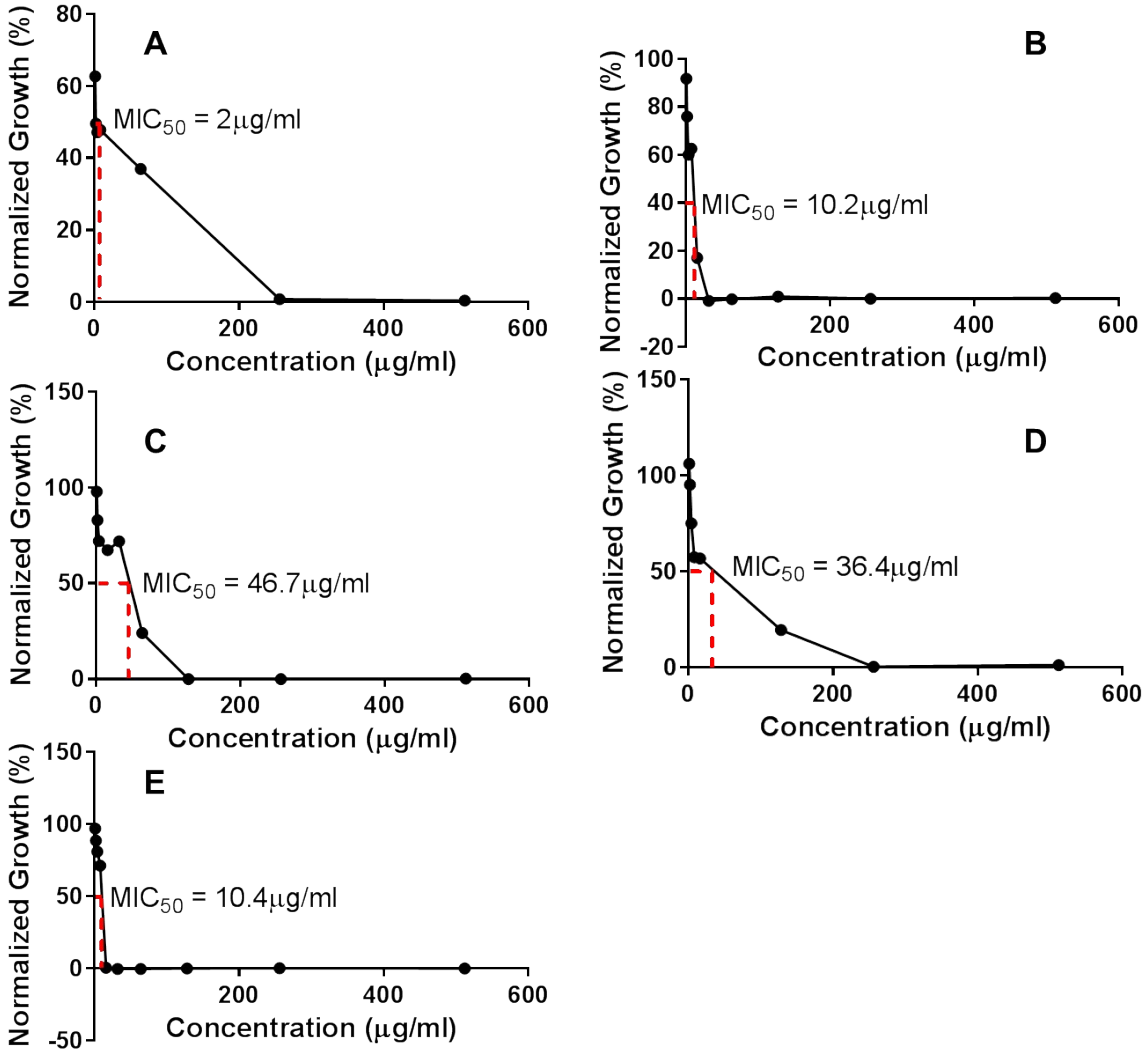


Figure S4. Growth of MRSA9808 in the presence of different concentrations of polymers (A) D-PLLA-D@-Q-PEG-2 (B) D-PLLA-D@-Q-PEG-1 (C) D-PLLA-D@Q 2 (D) D-PLLA-D@Q 1 (E) D-PLLA-D

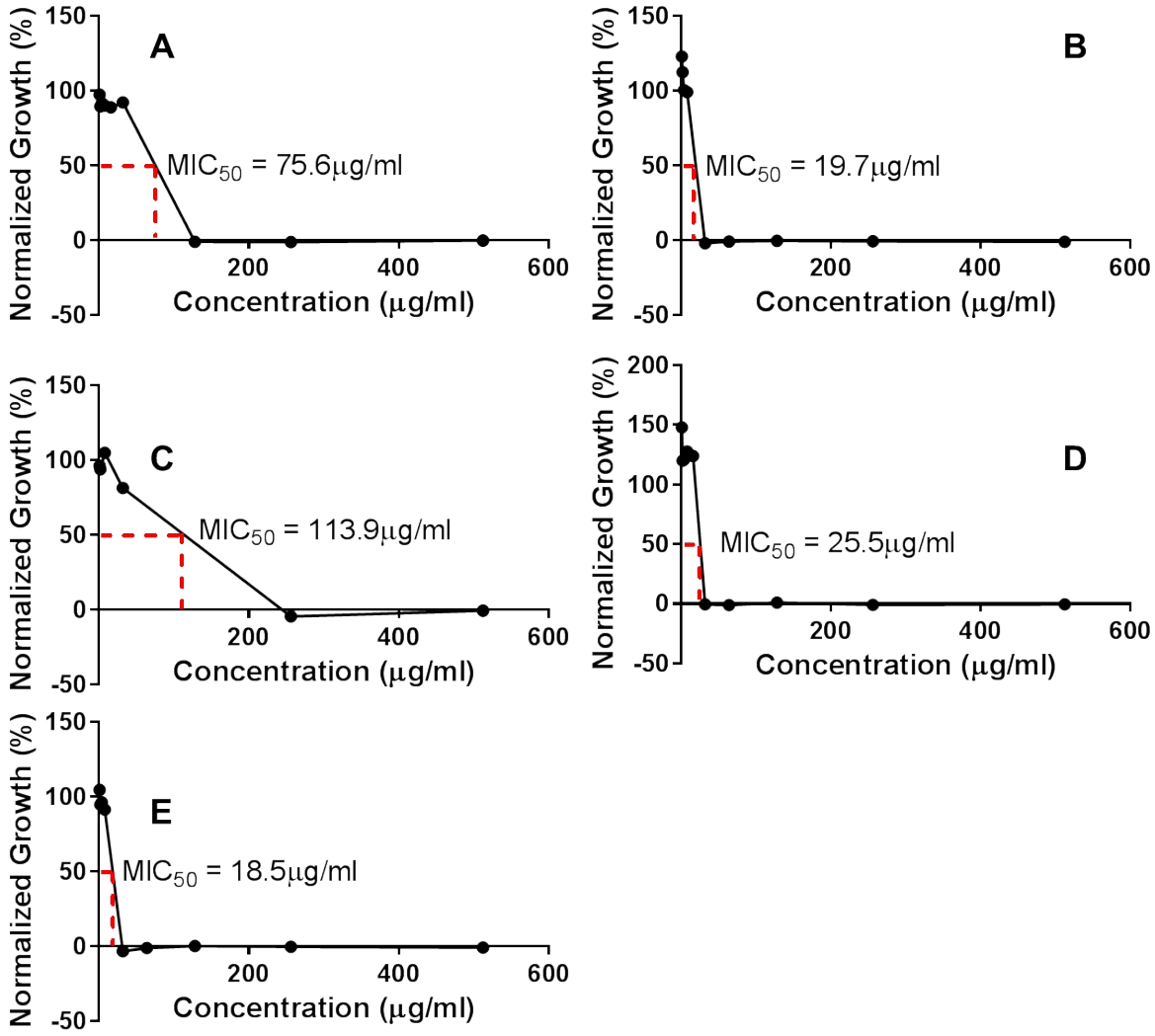


Figure S5. Growth of MRSA6506 in the presence of different concentrations of polymers (A) D-PLLA-D@-Q-PEG-2 (B) D-PLLA-D@-Q-PEG-1 (C) D-PLLA-D@Q 2 (D) D-PLLA-D@Q 1 (E) D-PLLA-D

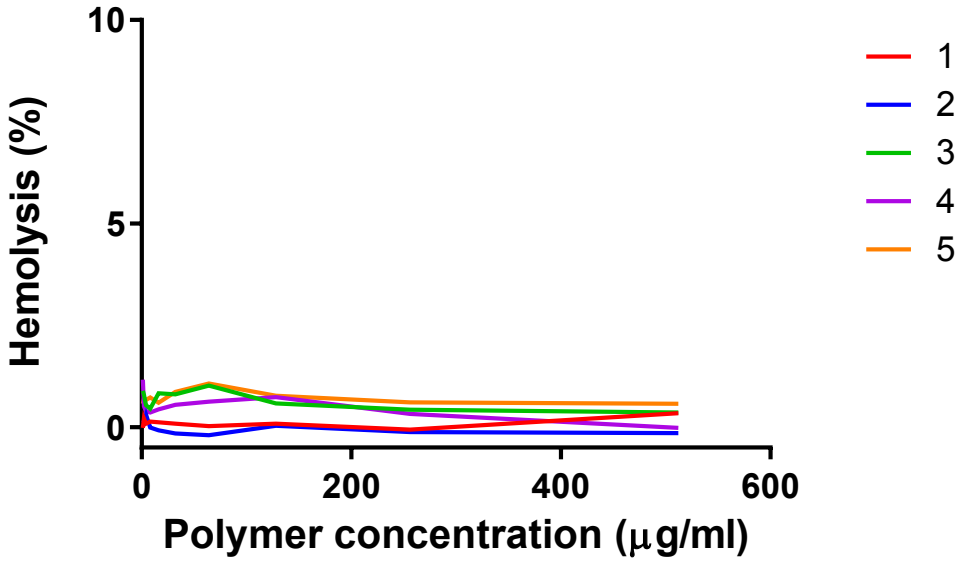


Figure S6. Hemolysis profile of D-PLLA-D@-Q-PEG-2 at different polymer concentrations. (1) D-PLLA-D@-Q-PEG-2 (2) D-PLLA-D@-Q-PEG-1 (3) D-PLLA-D@Q 2 (4) D-PLLA-D@Q 1 (5) D-PLLA-D

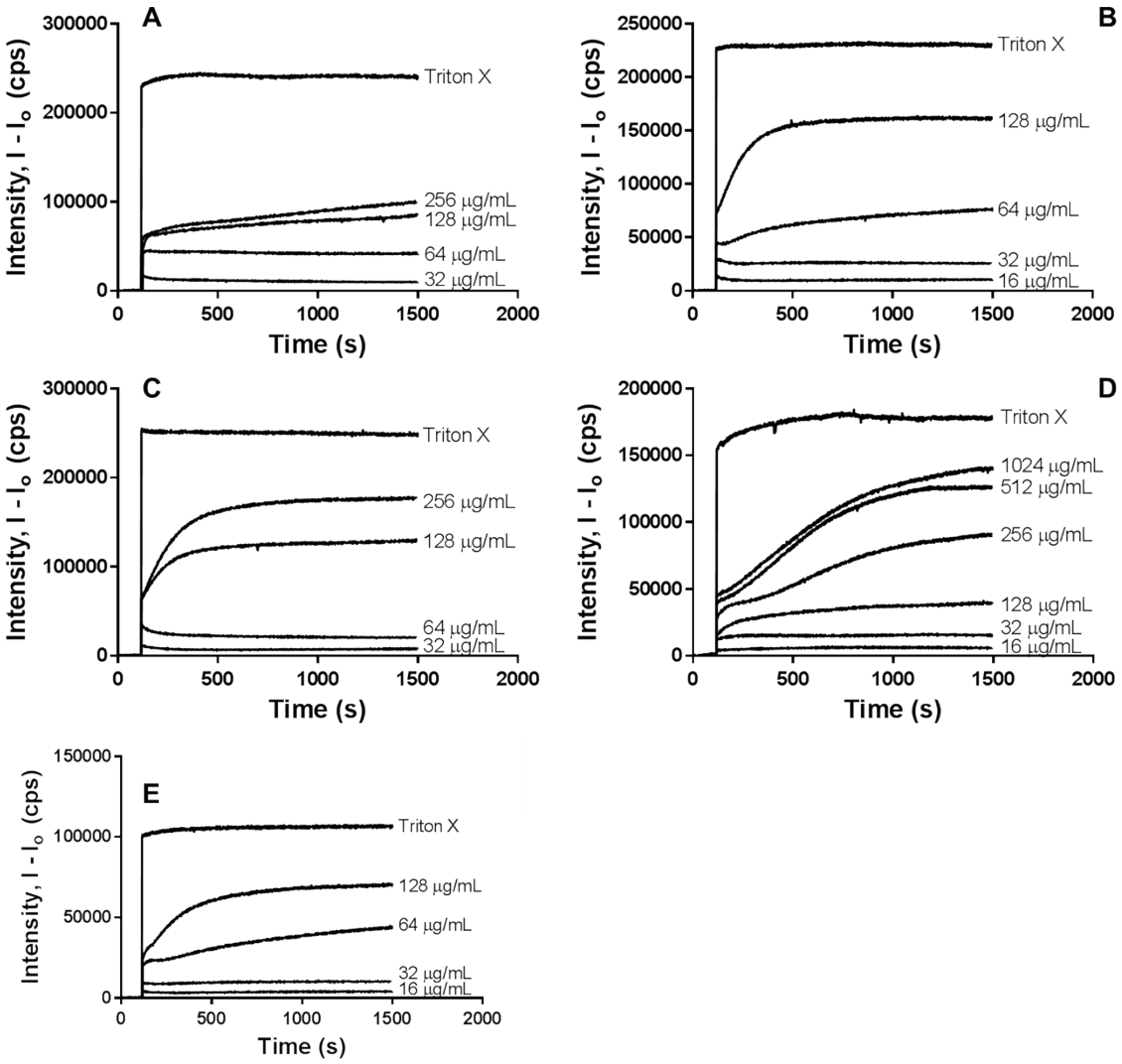


Figure S7. Effects of different concentrations of polymers on the depolarization of inner membrane monitored by a membrane-sensitive di$SC_3$5 assay, incubated with Pseudomonas Aeruginosa (ATCC 9027) (A) D-PLLA-D@-Q-PEG-2 (B) D-PLLA-D@-Q-PEG-1 (C) D-PLLA-D@Q 2 (D) D-PLLA-D@Q 1 (E) D-PLLA-D

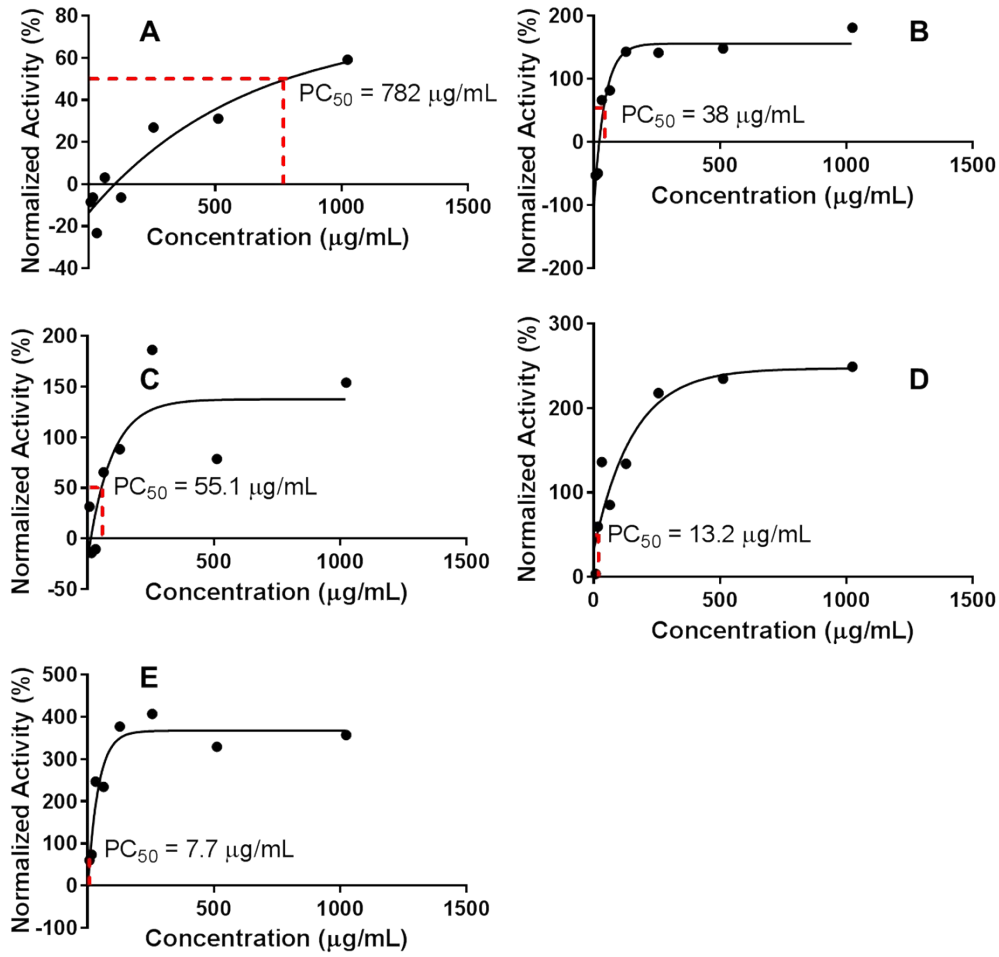


Figure S8. Effects of different concentrations of polymers on the outer membrane permeabilization probed by nitrocefin assay, incubated with Pseudomonas Aeruginosa (ATCC 9027) (A) D-PLLA-D@-Q-PEG-2 (B) D-PLLA-D@-Q-PEG-1 (C) D-PLLA-D@Q 2 (D) D-PLLA-D@Q 1 (E) D-PLLA-D

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