Supporting Information

Sulfonate-Based Polypeptide toward Infection-Resistant Coatings

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Materials

Triethylamine (99%), acryloyl chloride (99%), allyl alcohol (99%), ethanol (99%)，*n*-butylamine, triphosgene were purchased from Aladdin. Tetrahydrofuran (THF), toluene, ethyl acetate (EA), diethyl ether (Et_2O), and *N,N*-dimethylformamide (DMF) were purchased from Sinopharm Chemical Reagent Co., Ltd. and used as received. Deionized water (DI H_2O) was obtained from LA753 water-purification system (ELGA Lab Water Co. Ltd.). Sodium 2-mercaptoethane sulfonate was purchased from Energy Chemical. Dopamine hydrochloride was purchased from Aladdin Industrial Corporation. Gram-negative *Escherichia coli* (*E. coli*, ATCC 25922) and Gram-positive *Staphylococcus aureus* (*S. aureus*, ATCC 6538) were provided by Nanjing Clinic Biological Technology Co., Ltd. Luria-Bertani (LB) agar, LB broth, soybean casein agar medium and tryptone soybean broth medium were purchased from Suzhou Great Pharmaceutical Technology Co., Ltd. SYTO 9 green fluoresce and propidium iodide (PI) were purchased from Invitech (Shanghai) Trading Co., Ltd. HEK 293T (human embryonic) and NIH 3T3 (mouse embryonic fibroblast) cells were purchased from American type culture collection. All cells were purchased from the American Type Culture Collection (Rockville, MD). HEK 293T and NIH 3T3 cells were cultured in Dulbecco's modified Eagle medium (DMEM) (Gibco, Grand Island, NY) containing 10% fetal bovine serum (FBS) at 37 °C in 5% CO_2 humidified air. Bovine serum albumin (BSA) and fibrinogen (Fg) were obtained from Suzhou Corsain Biotechnology Co., Ltd. BCA protein assay kit was purchased from Thermo Scientific.

Instrumentation and Methods

Fourier transform infrared (FTIR) spectra were collected on a Bruker Hyperion FTIR spectrometer. ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian^{UNITY}INOVA-400 spectrometer at room temperature. Chemical shifts (δ) were reported in the units of ppm and referenced to the protonic impurities. Gel permeation chromatography (GPC) analyses were performed on a system equipped with an isocratic pump (Model 1100, Agilent Technology, Santa Clara, CA, USA), a DAWN HELEOS multi-angle laser light scattering detector (MALLS) detector (Wyatt Technology, Santa Barbara, CA, USA), and an Optilab rEX refractive index detector (Wyatt Technology, Santa Barbara, CA, USA). The detection wavelength of HELEOS was set at 658 nm. Separations were performed using serially connected size exclusion columns (100 Å, 500 Å, 10^3 Å and 10^4 Å Phenogel columns, 5 μm, 300

× 7.8 mm, Phenomenex, Torrance, CA, USA) at 60 °C using DMF containing 0.05 M LiBr as the eluent phase at a flow rate of 1.0 mL·min^{-1}. Circular dichroism (CD) measurements were carried out on a JASCO J-700 CD spectrometer. Polymers were dissolved in DI water at 0.2 mg·mL^{-1} unless otherwise specified. The solution was placed in a quartz cell with a path length of 0.1 cm. The mean residue molar ellipticity of each polypeptide was calculated based on the measured apparent ellipticity according to reported formulas: Ellipticity ([θ] in deg·cm^2·dmol^{-1}) = (millidegrees × mean residue weight)/(path length in millimeters × concentration of polypeptide in mg·mL^{-1}). Dynamic light scattering (DLS) analysis was conducted on a Zeta sizer Nano ZS90 (Malvern Instruments, Ltd., UK) with a He-Ne laser (λ= 633 nm) at a scattering angle of 90° (25 °C). The FEI F20 transmission electron microscope (TEM) was used to observe the morphology of PLC-SO_3Na in aqueous solution. The surface elemental composition was measured by X-ray photoelectron spectroscopy (XPS, Thermo Scientific K-Alpha+, Japan) at 600-100 eV. Fixed angle spectroscopic ellipsometer (Ellipsometry, USA) was used to measure the thickness of the coating. At room temperature, the water contact angle (DataPhysics OCA, Germany) was measured by the fixed drop method using 5 μL water droplets, and the samples were measured at least 3 times from different locations. Bacterial was observed with confocal laser scanning microscope (CLSM, Zeiss 800, Germany) and field emission scanning electron microscope (SEM, Zeiss G500, Germany).

Study on the bacteria morphologies

The polypeptide coated PDMS slides (1.5 cm × 1.5 cm) were placed in a 12-well plate. Bacterial solution (500 μL, 1 × 10^6 CFU·mL^{-1}) was added to each well to ensure that the samples were just submersed. After incubation at 37 °C for 24 h, the samples were gently washed with PBS buffer for three times to remove loosely adherent bacteria. Then, the samples were transferred to another 24-well plate and immersing in glutaraldehyde (2.5 wt%, 1 mL) for 2 h, followed by washing with PBS three times. After that, the samples were washed with a mixed solvent of ethanol and water (20%, 30%, 50%, 70%, 99% (v/v)) for 15 minutes each time. At last, the morphologies of bacteria attached to the surfaces were observed by SEM.

LIVE/DEAD staining assay

After incubating with bacteria for 24 h, the polypeptide coated PDMS slides were washed three times with PBS buffer and H_2O to remove loosely adherent bacteria. Then the samples were transferred to another 24-well plate and stained by SYTO 9

(10 μg·mL^{-1}) and Propidium Iodide (PI, 10 μg·mL^{-1}) for 20 min in the dark. The samples were then observed by CLSM.

Agar plate colony counting assay

The antibacterial activity of the PLC-SO_3H coating was quantitatively evaluated by agar plate colony counting assay according to JIS Z2801 standard. All samples with the size of 1.5 cm × 1.5 cm were placed in a 12-well plate and 25 μL of bacterial suspension (1 × 10^6 CFU·mL^{-1}) was added at the center region of the PDMS slide. Then the samples were covered with a pristine PE membrane with the size of 1.0 cm × 1.0 cm. After that, a small amount of H_2O was added into the wells around the samples, and placed the 12-well plate in a 37 °C incubator to ensure the appropriate temperature and humidity required for normal growth of bacteria. After cultivated for 24 h, 2 mL of PBS buffer was added to each well directly to make sure the samples were entirely immersed. Then the plate was ultrasonicated for 5 min to release the bacteria adherent on the PDMS slide. After that, PBS buffer containing bacteria was serially diluted and plated for colony counts. The number of the colony-forming units (CFUs) was counted after incubation at 37 °C for 24 h. Each sample was carried out at least in triplicate.

Hemolysis experiment

For the *in vitro* hemolysis test, the eyeballs of mice were removed to obtain fresh blood. The blood was centrifuged at 1000 rpm for 10 min. The red blood cells were collected at the bottom and washed with PBS twice. Then, the red blood cells were diluted to 5% (volume) with PBS. After that, the diluted red blood cell solution and PBS solution containing the sample were mixed at a volume ratio of 1:1 (both 300 μL) at 37 °C for 1 hour. The release of hemoglobin was measure by a microplate reader (λ = 450 nm). In this experiment, red blood cells treated with PBS were used as negative control while red blood cells treated with Triton X-100 were used as positive control. The absorbance of erythrocytes treated with Triton X-100 was 100% hemolysis. T;he following formula was used to calculate the percentage of hemolysis:

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

Methyltetrazolium (MTT) assay

The toxicity of the coated samples against 3T3 fibroblasts and 293T epithelial cells was tested by MTT assay. The cells were cultured in DMEM medium containing 10% fetal bovine serum (FBS). The cells were seeded into a 24-well plate at a concentration of 5 × 10^4 per well, and incubated overnight at 37 °C in a humidified

atmosphere of 5% CO_2. The cells were gently covered by the sample and incubated for 24 hours. Then add 200 μL medium and MTT reagent (40 μL) and incubate at 37 °C for 3 hour. Then remove the MTT reagent and dissolve the purple crystals with 100% dimethyl sulfoxide (DMSO, 500 μL), and measure the absorbance of the sample at 570 nm.

Protein resistance assay

Protein adsorption (Fg and BSA) on the PDMS and coating surfaces was determined using the micro-BCA assay. Briefly, all surfaces were balanced in PBS of pH 7.4 for 12 h and then separately submersed in 2 mL of protein solutions with a concentration of 5 mg·mL^{-1} and incubated at 37 °C for 24 h. All surfaces were then cleaned softly with deionized water several times to ensure complete removal of non-adsorbed protein. All samples were separately placed in a 24-well plate, as well as 1 mL aliquot of 2.0 wt % aqueous sodium dodecyl sulfate (SDS) solution subsequently and separately. Then, the plate was shaken for 2 h and ultrasonically processed for 1 h in an ice bath to detach the surface-absorbed protein. The protein solution (100 μL), which was sampled from every well, was placed into a new 96-well plate, supplementing with 100 μL of BCA reagent, and then incubated at 60 °C for 1 h. The protein concentration was measured based on the absorbance at 560 nm by using a microplate reader. The adsorbed protein was determined by the following equation:

$$Adsorbed\ protein\ (\%) = \frac{OD\ of\ Coating - OD\ of\ blank}{OD\ of\ PDMS - OD\ of\ blank} \times 100\%$$

Bacteria adhesion assay

Bacterial suspension (10^8 CFU·mL^{-1}) was placed on uncoated and coated surfaces and incubated at 37 °C for 120 min. Then, the surfaces were rinsed with PBS, fixed with glutaraldehyde. After that, the samples were washed with a mixed solvent of ethanol and water (20%, 30%, 50%, 70%, 99%, volume percentage of ethanol) for 15 minutes each time, followed by SEM measurements. The amount of bacteria adhered to the surfaces were calculated by ImageJ (software) according to SEM images.

***In vivo* experiments**

Twelve female BALB/c mice (8 weeks, 19−22 g) were assigned to one of the three groups randomly, anesthetized, and shaved clearly. For *in vivo* biocompatibility assay, pristine and coating samples (1 × 5 mm^2) were subcutaneously implanted in the same animal for comparing. After 3 and 7 days implantation, the samples were visually scored. The entire implant and associated tissues were surgically removed to conduct histological analysis. In brief, the fresh tissue was immobilized, dehydrated, stained,

and visualized.

The implant-related bacterial infection experiment is demonstrated as follows. The implant sample films were first soaked in a *S. aureus* suspension solution (10^8 CFU·mL^{-1}) at 37 °C for 360 min, then soak in sodium bicarbonate solution (0.1 M) 10min, and rinse gently with PBS three times. Pristine and coating samples (1 × 5 mm^2) were subcutaneously implanted in the same animal for comparing. After the implant samples were implanted for 3 days, the implants with surrounding tissues were surgically removed. To quantify the bacterial number, the implanted samples were immersed and homogenized in a buffer aqueous solution. The resulting *S. aureus* suspension was coated on a plate as a bacterial count.

Statistical analysis

All data were presented as mean ± standard deviation (SD). The statistical significance was assessed by analysis of variance (ANOVA), *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$).

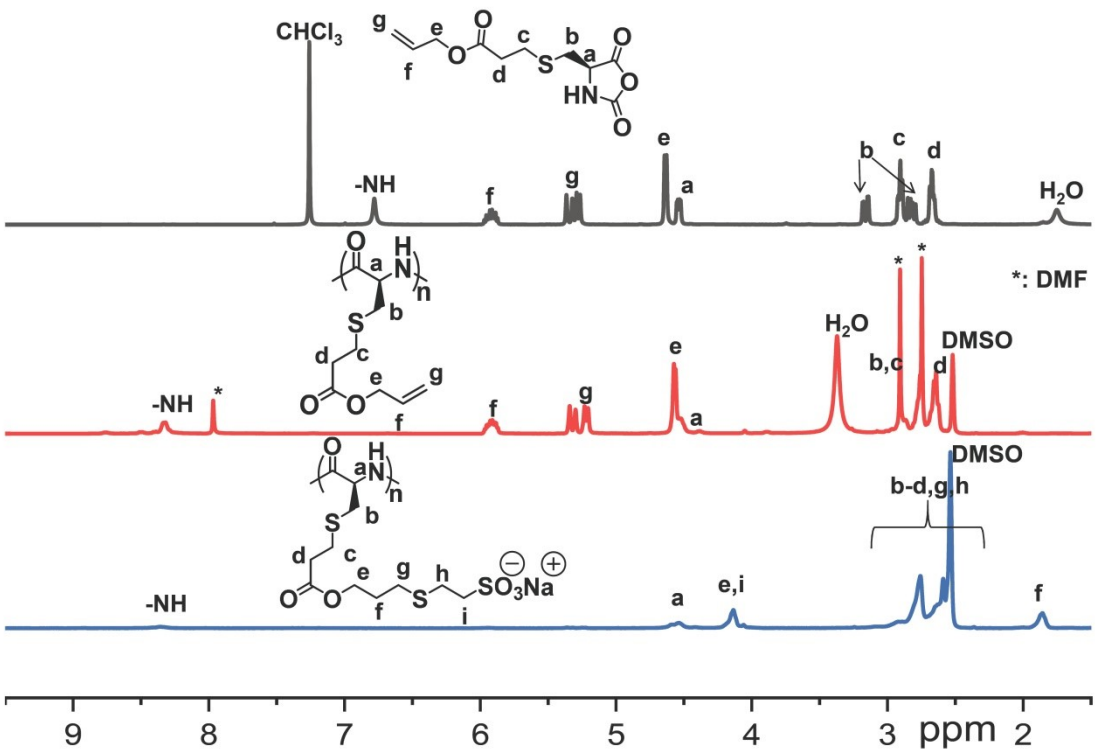

Figure S1. ^{1}H NMR spectra of ALC-NCA in $CDCl_3$ (top curve), PALC (middle curve) and PLC-SO_3Na in DMSO-d_6 (bottom curve).

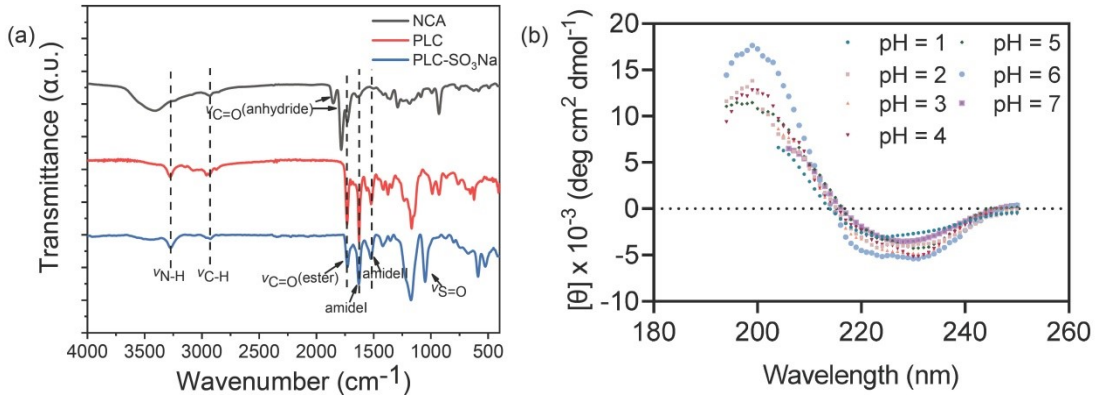

Figure S2. (a) FTIR spectra of the ALC-NCA, PALC, and PLC-SO_3Na. (b) CD spectra of PLC-SO_3Na aqueous solutions in the pH range of 1-7 at room temperature (0.2 mg·mL^{-1}).

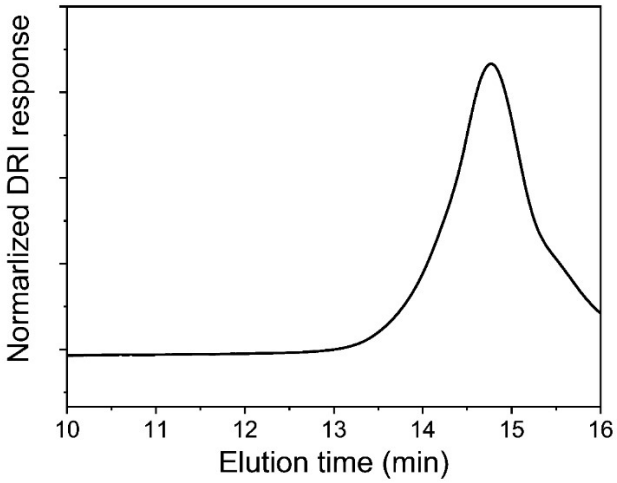

Figure S3. GPC curve of PALC.

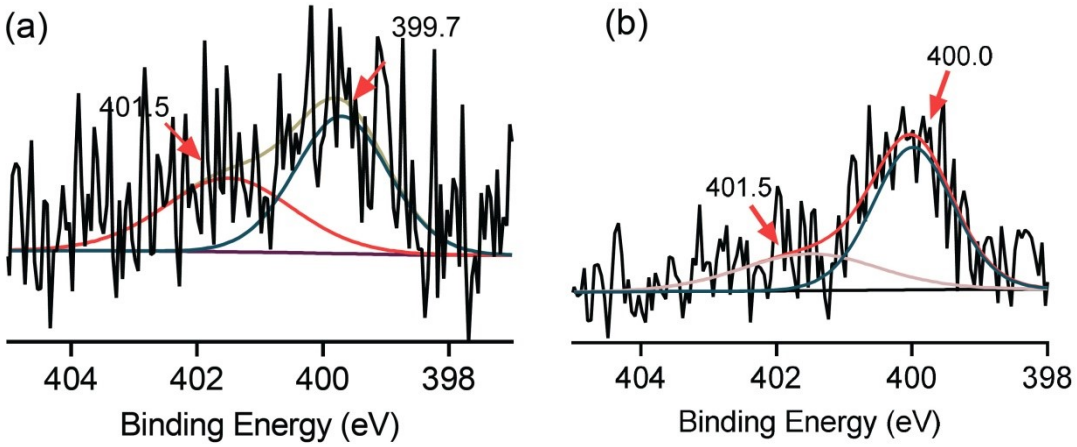

Figure S4. XPS spectra of (a) PDA and (b) PLC-SO_3Na coated PDMS.

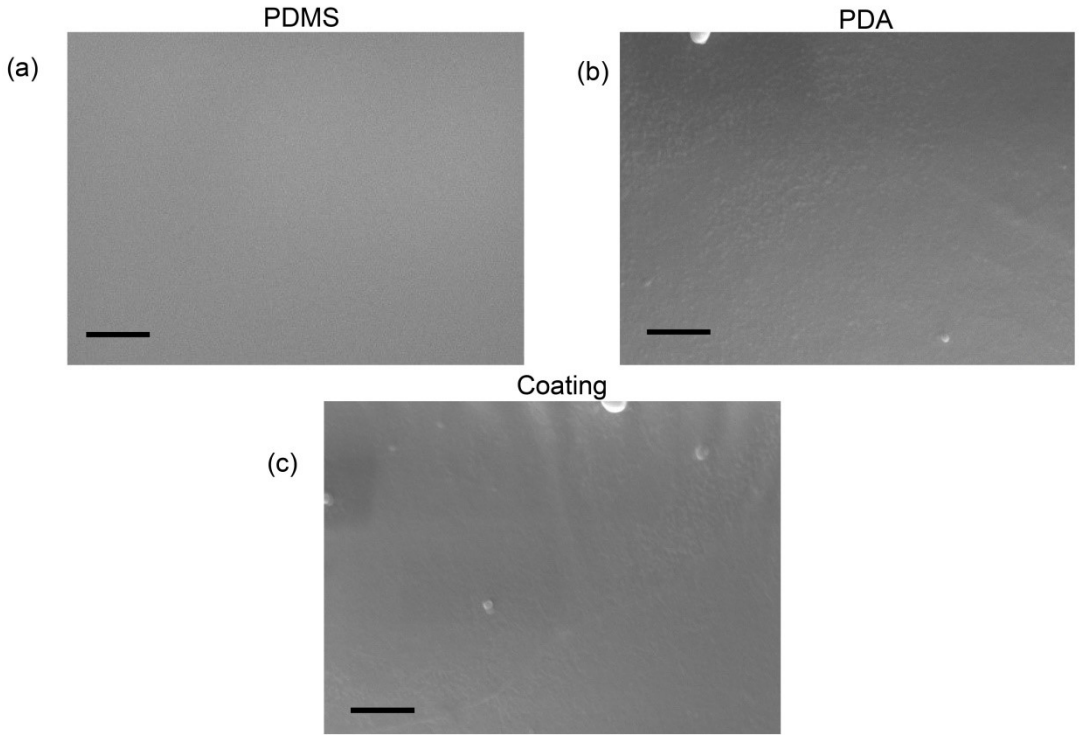

Figure S5. SEM images of (a) uncoated, (b) PDA, and (c) PLC-SO_3Na coated PDMS. Scale bar = 400 nm.

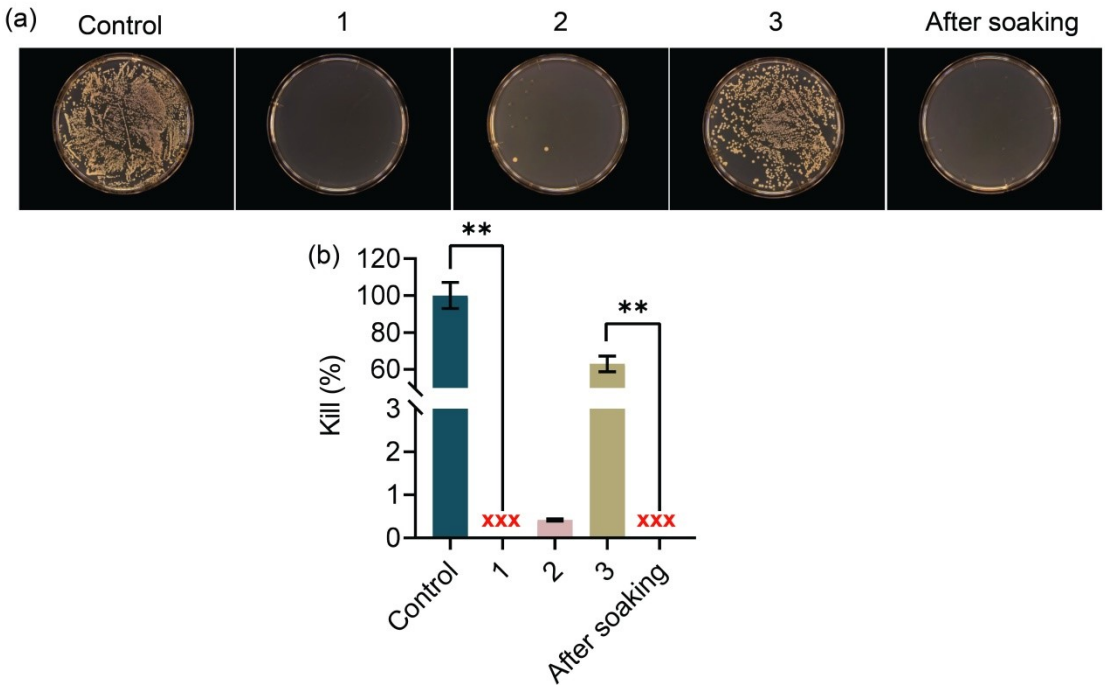

Figure S6. Antibacterial activities against *E. coli in vitro*. (a) The LB agar plates treated with uncoated (control) and PLC-SO_3H coated surfaces which were used for 1-3 times or activated by soaking in HCl solution (0.1 mol·L^{-1}, 10 min) after using for 3 times. (b) Killing efficacy of uncoated and PLC-SO_3H coated surfaces. ** $p < 0.01$.

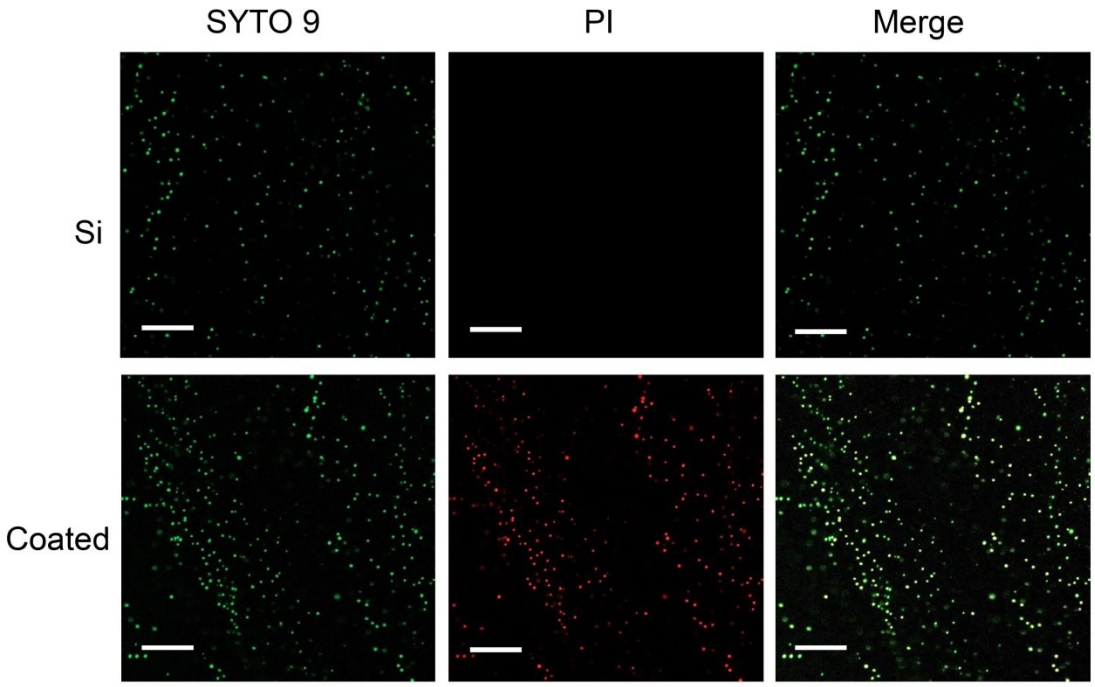

Figure S7. LSCM images of uncoated and PLC-SO_3H coated surfaces against *S. aureus* for 24 h. The bacteria were stained with a mixture of SYTO 9 (green: live and dead) and PI (red: dead) solution (in dark, 20 min). Scale bar = 100 μm

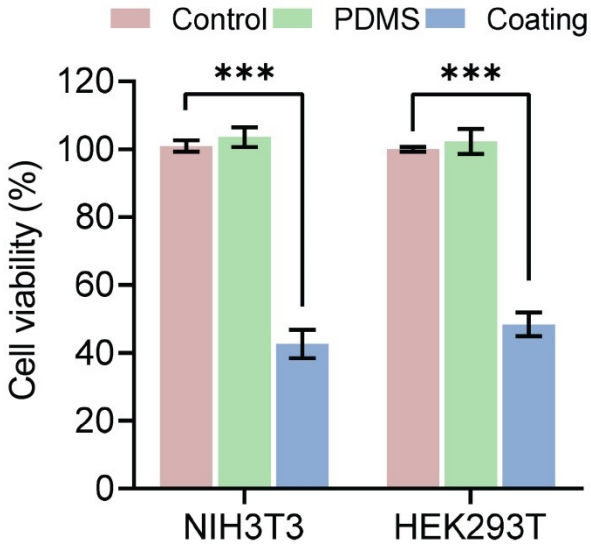

Figure S8. Cell viability of HEK 293T renal epithelial cells and NIH 3T3 fibroblast cells treated by uncoated and PLC-SO_3H coated surfaces. *** $p < 0.001$.