Supporting Information

Antimicrobial Peptide-Conjugated Hierarchical Antifouling Polymer Brushes for Functionalized Catheter Surfaces

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1. Experimental Section

1.1 Materials.

Polyurethane (PU) films were purchased by Shanghai Qinggen Industrial Co. (China). *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (PMDETA) and Triton X-100 were purchased from Sigma-Aldrich (USA). Methylene blue, ethanol and tetrahydrofuran were purchased from Beijing Chemical Works (China). Sodium methacrylate (MAA), 3-dimethyl(methacryloyloxyethyl) ammonium propane sulfonate (DMAPS), 3-aminopropyl triethoxysilane(APTES), anhydrous dichloromethane triethylamine (TEA) , 2-(*N*-morpholino)ethanesulfonic acid monohydrate (MES), 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methyl morpholinium chloride (DMTMM) and copper (I) bromide (CuBr) were purchased from Energy Chemical (China). 2-Bromoisobutyryl bromide (BIBB) was purchased from TCI Chemical Industry Co. (China). Tryptone and yeast extract were purchased from Oxoid (UK)

1.2 Synthesis of 3-(2-Bromoisobutyramido) Propyl(Triethoxy) Silane (APTES-Br).

In a 50 mL flask, 3-aminopropyl triethoxysilane (4.68 mL, 26.74 mmol) and triethylamine (3.35 mL, 24.07 mmol) were added into 30 mL of anhydrous tetrahydrofuran (THF), and then 2-bromoisobutyryl bromide (2.72 mL, 21.82 mmol) was added dropwise under ice cooling. The mixture was slowly warmed to room temperature and magnetically stirred for 5 h. Next, triethylammonium bromide was separated by centrifugation (3500 r/min for 3 min), and THF was removed by rotary evaporation for 0.5 h. Residual triethylammonium bromide that precipitated upon evaporation was further removed by centrifugation (3500 r/min for 3 min). The liquid product of APTES-Br with light yellow color was stored at 4 °C under seal, which was the initiator of surface-initiated atom transfer radical polymerization (SI-ATRP).[S1]

1.3 Preparation of SI-ATRP Initiator-Modified PU (PU-Br).

PU films were cut into small pieces (1 cm × 1 cm), sonicated alternately in ethanol and deionized water for 20 min, and dried in a drying oven. Then, the PU samples were treated with oxygen plasma (90 W, 5 min). Subsequently, the samples were immersed into APTES-Br and kept for three days at room temperature. To remove any organic residues from the surfaces, the samples were rinsed alternately with deionized water and ethanol for three times by a vortex oscillator. Finally, the washed samples were cured in a vacuum oven at 85° C for 12 h. The initiator-functionalized PU (PU-Br) were stored at room temperature under seal.

1.4 Preparation of PDMAPS-modified PU (PU-D).

Poly(3-[dimethyl-[2-(2-methylprop-2-enoyloxy)ethyl]azaniumyl]propane-1-sulfonate) (PDMAPS) brushes were grafted from the PU surface under the typical condition of SI-ATRP. Briefly, DMAPS (1.12 g, 4.00 mmol) and PMDETA (0.08 mL, 0.40 mmol) were added into a 10 mL round flask containing deionized water (3 mL). The mixture was degassed by bubbling with nitrogen for 5 min, and then CuBr (57.36 mg, 0.4 mmol) was added into the tube. The mixture was degassed for another 5 min. Then, PU-Br samples were placed into the flask, and the flask was sealed for ATRP reaction. After being magnetically stirred at 60 ℃ for 3 h, the samples were removed from the reaction solution and alternately washed with deionized water and ethanol for three times, then dried in a blast drying oven to obtain PU-DMAPS (PU-D).

1.5 Preparation of P(DMAPS-*b*-MAA)-Modified PU (PU-DM).

MAA (1.30g, 1.20mmol) and PMDETA (0.11 mL, 0.24 mmol) were added into a 10 mL round flask containing water (3 mL). The mixture was degassed by bubbling with nitrogen for 5 min, and then CuBr (68.80 mg, 0.50 mmol) was added into the flask. The mixture was degassed for another 5 min. PU-D samples were added into the flask,

and the flask was sealed for ATRP reaction. After magnetically stirred at 60 ℃ for 2.5 h, the P(DMAPS-*b*-PMAA)-modified PU samples (PU-DM) were removed from the reaction solution and alternately washed with deionized water and ethanol for three times, followed by drying in a drying oven.[S2]

1.6 Preparation of HHC36-Functionalized PU (PU-DMH).

The PU-DM samples were immersed in 3 mL of MES buffer with 100 mg DMTMM and magnetically stirred at room temperature for 1 h. The DMTMM-activated PU-DM samples were removed and immersed into the solution of HHC36 in 3 mL of PBS at the concentration of 3.33 mg/mL for 12 h in room temperature. The HHC36-immobilized PU-DM (PU-DMH) were washed with deionized water for three times, and naturally dried.

1.7 SI-ATRP inside PU catheters

PU catheters with internal diameter of 5.0 mm and external diameter of 8.0 mm were washed with water/ethanol, then sonicated and dried before use. According to the above-described procedures of SI-ATRP, the PMAA was grafted in the internal surface the PU catheters (PU tube-M). The grafted PMAA was visualized by immersing the samples in methylene blue solution for 30 min and washing the samples with deionized water.

1.8 Methylene Blue Staining.

The samples were immersed for 30 min in a methylene blue solution in PBS with the concentration of 2×10^{-3} mol/L at room temperature. Then the samples were removed and washed with deionized water for three times and dried at room temperature.[S3]

1.9 Physical Characterization.

The chemical structures of APTES-Br was characterized by 400 MHz ^{1}H NMR spectrum. The morphologies of the samples were investigated by scanning electron

microscopy (SEM, JSM-7500F, JEOL, Japan) and atomic force microscopy (AFM, Bruker Dimension Icon, Bruker, USA). Surface chemical compositions of the samples were investigated by X-ray photoelectron spectroscopy (XPS, Kratos AXIS-His, Shimadzu, Japan) equipped with Al Kα X-ray source. Attenuated total reflection Fourier transformed infrared spectroscopy (ATR-FTIR, Nicolet IS 10, Thermo Scientific, USA) was conducted to determine the functional groups of the samples. The static water contact angles (WCA) of samples were tested with Dataphysics OCA system (Dataphysics, Germany) at room temperature by injecting 3 μL of deionized water on the surface. At least three measurements were conducted on each sample to calculate the average WCA values.

1.10 Measurement of Thickness of the Polymer Brushes.

Surface functionalization was performed on glass slices with the same process of PU samples. Then the scratches were made by a steel needle to penetrate the polymer layers without affecting the substrate according to the differences in mechanical properties between polymer brushes and glass substrates. Atomic force microscopic scanning was used to obtain the morphological images of the scratched areas, and then the thicknesses of polymer layers were measured based on the AFM images.

1.11 Protein Adsorption Assay

Bovine serum albumin (BSA) was used as representative protein for protein adsorption assay. After being equilibrated with normal saline for 2 h, the samples were immersed in BSA solution (2 mg/mL) for 4 h and then rinsed with saline. The adsorbed proteins were detached from the sample surfaces by sonication for 10 minutes in 2% sodium dodecyl sulfonate (SDS). The amounts of absorbed protein were determined using a BCA Protein Assay Kit (Pierce®, Thermo Fisher, USA) following the instruction of the kit.

1.12 Bacterial Adhesion and Bactericidal Assay.

PU, PU-D, PU-DM, and PU-DMH samples were incubated in phosphate buffer saline (PBS, 200 μL) containing 1.0×10^8 CFU/mL of bacteria (*E. coli* or *S. aureus*). After the incubation in shaker at 37 °C for 9 h, the surfaces were gently rinsed with normal saline solution for three times. Then, the samples were soaked into a staining solution containing SYTO 9 (6 μmol/L) and propidium iodide (PI, 30 μmol/L) from a live/dead staining kit (L7012, Thermo Fisher Scientific, USA) in the dark. After 10 min, the stained samples were observed by confocal laser scanning microscope (CLSM, Leica SP8, Germany) using oil immersed 63× objective lens. After observation, each sample was placed into a 1.5 mL centrifuge tube. In each tube, 1 mL of normal saline solution was added. The samples were shaken vigorously with a vortex shaker for 3 s and then observed by CLSM again.

Antibacterial properties were also evaluated in LB media. After being equilibrated with normal saline for 2 h, the samples were incubated in LB media containing 1.0×10^5 CFU/mL of *S. aureus* at 37 °C for 12 h. Then, the samples were removed and gently washed with saline. The bacteria on the samples were stained by Live/Dead Staining Kit (L7012, Thermo Fisher, USA) for 15 minutes in the dark. The stained bacterial were observed by CLSM (SP8, Leica, Germany).

1.13 Platelet and Whole Blood Adhesion.

Fresh blood was extracted from a healthy rabbit. All of the animal experiments were performed in compliance with the guidelines issued by the Ethical Committee of the Chinese Academy of Sciences (CAS), and approved by the Ethical Committee of CAS. Platelet rich plasma (PRP) was separated from the whole blood by centrifuging at 1500 rpm for 10 min. Then 200 μL of PRP or whole blood was dropped respectively onto the samples, and the samples were incubated for 2 h at 37 °C. Residual platelets

or whole blood on the samples were washed with normal saline for three times. The samples were fixed with 2.5 % glutaraldehyde in phosphate buffer for 12 h at 4 ° C. The fixed samples were sequentially dehydrated in aqueous solutions with increasing concentrations of ethanol (25 % and 50 % for 1.5 min, 75 %, 87.5 %, 100% and 100% for 2 min, respectively). The samples were naturally dried and subjected to SEM observation.

1.14 Hemolysis Assay.

Fresh blood was extracted from a healthy rabbit. Fresh blood (1 mL) was washed with 25 mL of normal saline by centrifugation (2000 rpm, 15 min) for three times. Then the red blood cells after centrifugation were diluted to 4% with normal saline. The diluted suspension of red blood cells (0.5 mL) and normal saline solution (0.5 mL) were added onto the samples. The samples were kept at 37 °C for 3 h and then centrifuged at 2000 rpm for 10 min. The supernatants were transferred to a 96-well plate, and the absorbance was measured at 545 nm using a microplate reader (Biotek, USA). The mean values of three measurements were calculated. 1% Triton X-100 was taken as the positive control, and normal saline as the negative control. Hemolysis ratio was calculated according to the following formula:

$$\text{Hemolysis ratio}\ (\%) = \frac{OD_{test} - OD_{neg}}{OD_{pos} - OD_{neg}} \times 100\%$$

where OD_{test}, OD_{pos} and OD_{neg} represented the absorbance values of the tested sample, the positive control (Triton X-100) and the negative control (normal saline), respectively.

1.15 Cytotoxicity Assay.

The samples were sterilized in 75% ethanol solution overnight. Then the samples of each group were rinsed by PBS for three time and placed into a 15 mL centrifuge

tube. In each tube, DMEM liquid medium containing 10% FBS (1 mL of media to 1 cm^2 of samples) was added and incubated for 24 h at 37 °C to prepare the extracts. The cryopreserved L929 cells were resuscitated and cultured. After growing with 80% confluence of the bottom of the culture flask, the cells were digested by 2.5% trypsin (Sigma, USA) and separated by centrifugation. The cell density was adjusted by culture media to 5.0×10^5 cells/mL. After culture for 24 h, the media in the plate were replaced with the extracts (100 μL per well) and the plate was incubated for another 24 h. The activity of the cells was studied using the conventional MTT method. MTT solution (5 mg/mL) was mixed with culture media at a volume ratio of 1:10 to obtain MTT-containing media. The culture media in the wells were replaced with 0.20 mL of culture media containing MTT, and incubated at 37 °C for 1.5 h. Then, 0.1 mL of the culture solution was taken from each well and placed in a new 96-well plate, and the OD 450 was measured with a microplate reader.

1.16 Antibacterial Effect of the Modified Sheets under Flow Conditions.

All the device were sterilized at 121 °C and 103.4 kPa for 20 min, and dried by a drying oven. The silicone tubes for circulation were approximately 60 cm in length and 6.4 mm in inner diameter. The samples of pristine PU and PU-DMH were placed into the outlets of silicone tubes. Suspension of *S. aureus* (1.0×10^8 CFU/mL in 200 mL 1× PBS) was stirred continuously to avoid sedimentation of bacteria. The silicone tubes were mounted onto a peristaltic pump (BT301L-YT15, Baoding Lead Fluid Technology Co., Ltd., China) that maintained the flow rate of 5 mL/min for 48 h and replaced fresh bacterial suspension every 24 h. After a 48-hour incubation under flow conditions, each sample of pristine PU and PU-DMH was harvested. The samples were washed by normal saline for three times to remove unbound bacteria, stained by live/dead staining kit and observed using CLSM.

The antibacterial properties were also tested in LB media. Suspension of *S. aureus* (1.0×10^8 CFU/mL in 200 mL of 1× PBS and 1.0×10^5 CFU/mL in 200 mL of LB) was stirred continuously to avoid sedimentation of bacteria. The silicone tubes were mounted onto a peristaltic pump (BT301L-YT15, Baoding Lead Fluid Technology Co., Ltd., China) that maintained the flow rate of 5 mL/min for 48 h and replaced fresh bacterial suspension every 24 h. After a 48-hour incubation under flow conditions, each sample of pristine PU and PU-DMH was harvested. The samples were observed as described above.

In order to accurately simulate the velocity and shear stress distributions in actual medical catheters, rheological characterizations were conducted with the samples for *in vitro* circulation. We assumed that there were steady uniform velocity profile at flow entrance, and no slip condition existed at the walls of tube. This allows us to calculate shear rate by:

$$\dot{r} = \frac{\mathrm{d}u}{\mathrm{d}r} = 4\,\frac{Q}{\pi r^3}$$

where u is the velocity of the fluid in m/s and r is the radius of the tube. Q is volumetric flow rate in m^3/s.[S4] Reynolds number is a dimensionless number that can be used to characterize fluid flow, expressed as *Re*,

$$Re = \frac{\rho v d}{\mu}$$

where v, ρ and η were the flow velocity, density and viscosity coefficient of the fluid, respectively, while d is the diameter of the tube.

1.17 *In Vivo* Anti-Infection Assay.

Female Balb/c mice aged eight weeks with body weight around 20 g were used for *in vivo* anti-infection assay. The surgical operations were performed after one-week adaption. The mice were anesthetized by isoflurane and the hair on the operating sites

was shaved. Then, two incisions with the length of 1.0 cm were cut parallel to the spine on each side of the back. Pristine PU and PU-DMH samples were sterilized. Subsequently, *S. aureus* (1.0×10^9 CFU in 1 μL of PBS) were inoculated on the samples. Then, the samples with bacteria were implanted into the incisions of mice, with pristine PU on the left side and PU-DMH on the right side of the mice. The incisions were closed with 4−0 sutures. On 1 d and 3 d after surgery, the mice were humanely euthanized, and the implanted samples were harvested. The samples were stained by live/dead staining kit and analyzed by CLSM. Soft tissues around the samples were collected and fixed in 4% paraformaldehyde, dehydrated, and embedded in paraffin. The tissues were cut into small slices and stained with hematoxylin-eosin (H&E) according to the standard protocols.

1.18 Statistical Analysis.

At least three samples were tested in each group. The results were presented as mean ± standard deviation. When two groups were compared, the differences were assessed by *t*-test. A significance level of $p = 0.05$ were performed using Origin 7.1 software.

2. Supporting figures

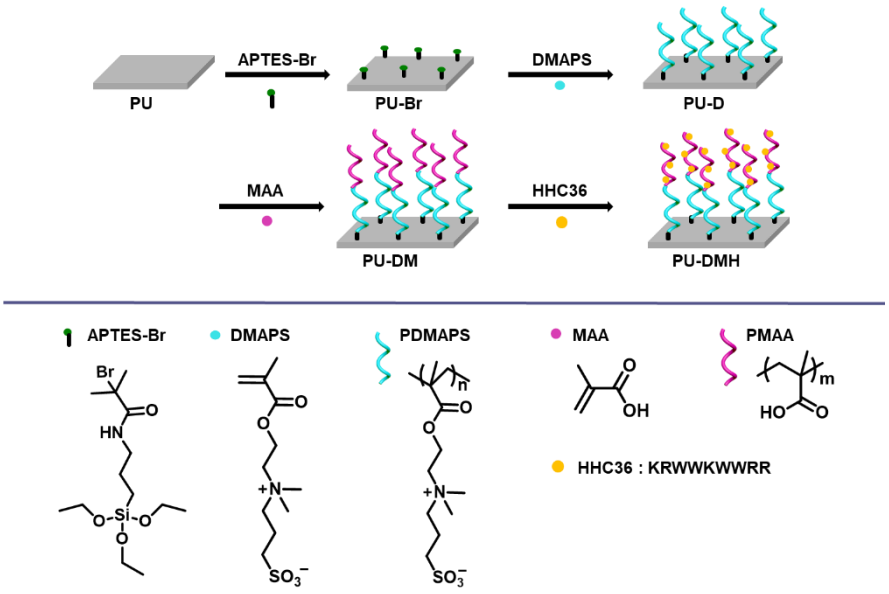

Figure S1. Schematic functionalization process of PU-DMH.

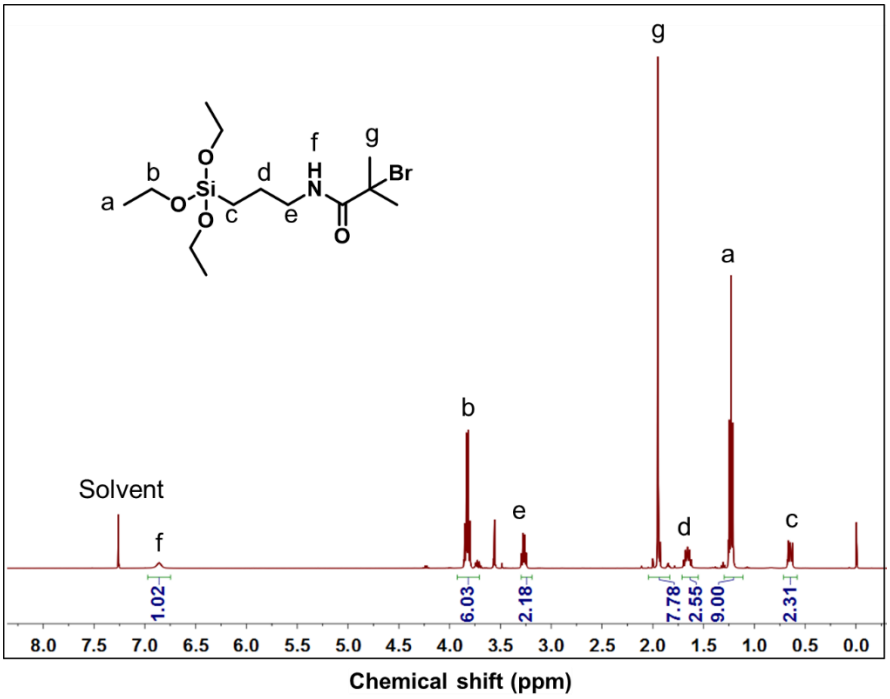

Figure S2. ^{1}H NMR spectrum of APTES-Br. The characteristic peaks of APTES-Br are as follows: δ = 1.2 ppm (a, -C<u>H</u>$_3$), 3.8 ppm (b, CH$_3$-C<u>H</u>$_2$-O), 0.6 ppm (c, Si-C<u>H</u>$_2$-CH$_2$), 1.6 ppm (d, CH$_2$-C<u>H</u>$_2$-CH$_2$), 3.2 ppm (e, CH$_2$-C<u>H</u>$_2$-NH), 6.8 ppm (f, CH$_2$-N<u>H</u>-CO) and 1.9 ppm (g, C<u>H</u>$_3$-C).

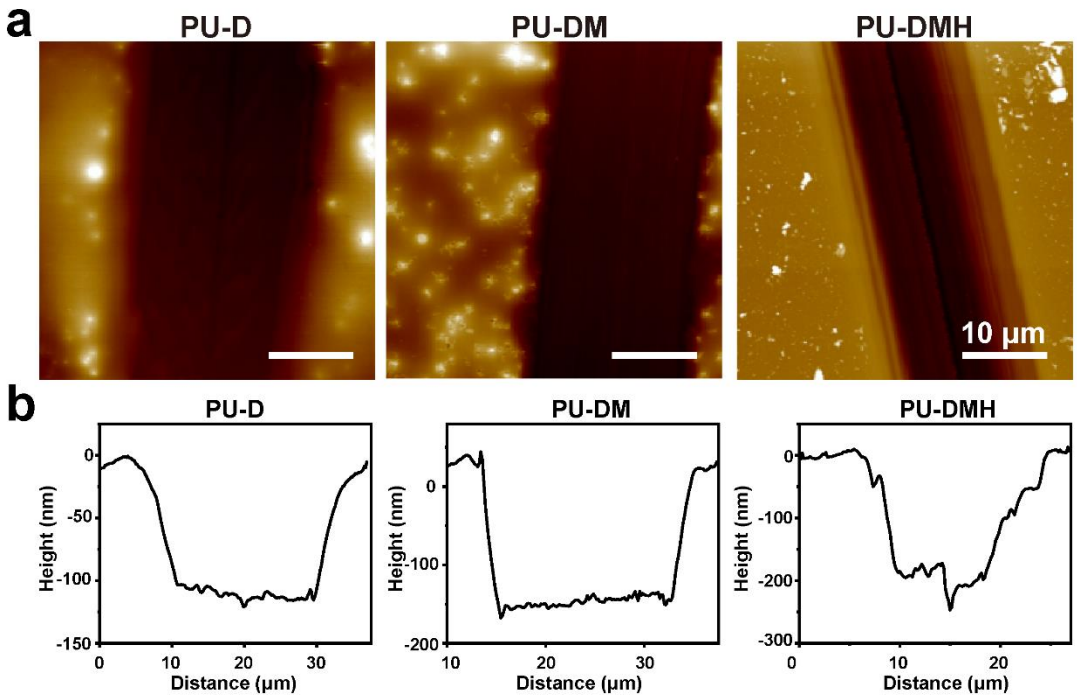

Figure S3. (a) AFM images of the scratches and (b) thickness profiles of polymer brushes of PU-D, PU-DM and PU-DMH.

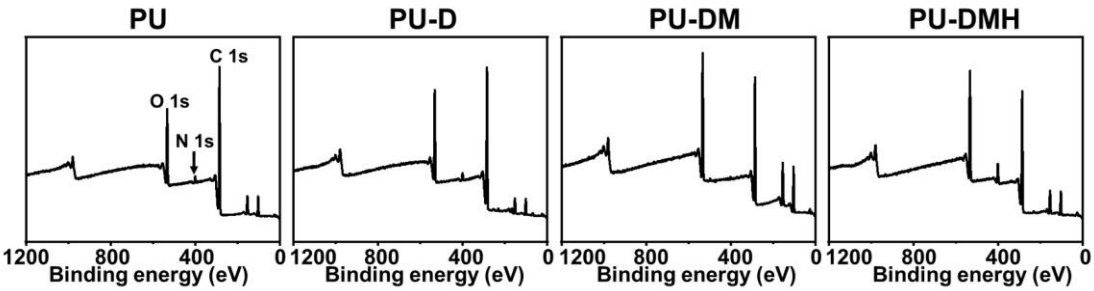

Figure S4. XPS wide-scan spectra of PU, PU-D, PU-DM and PU-DMH.

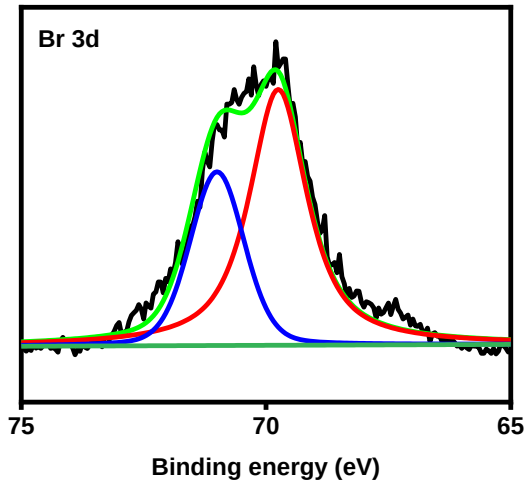

Figure S5. XPS Br 3d spectra of PU-Br.

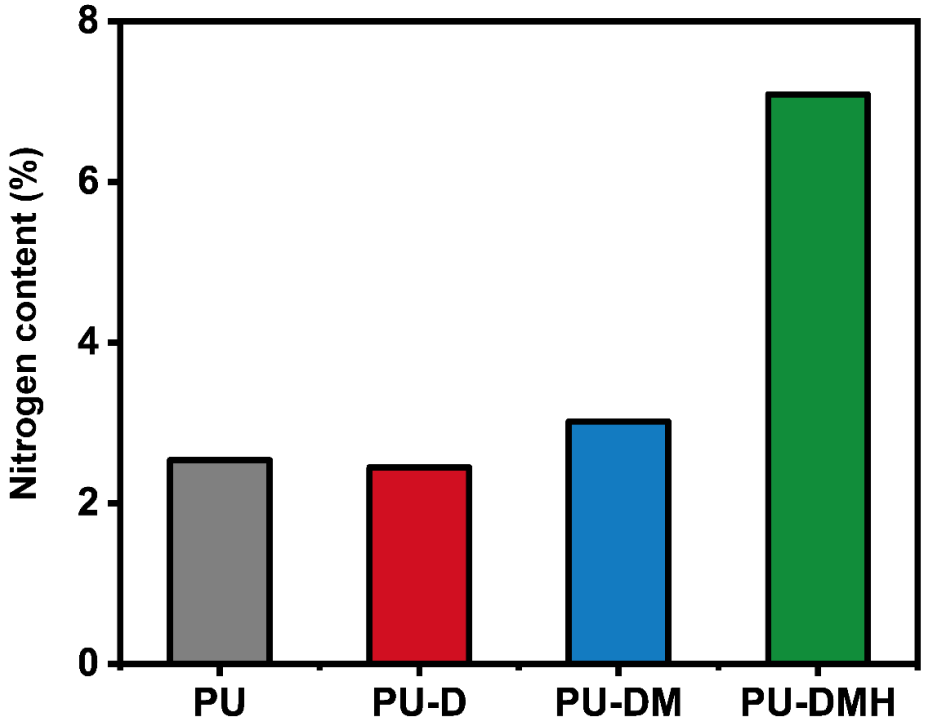

Figure S6. Element ratios of nitrogen of PU, PU-D, PU-DM and PU-DMH.

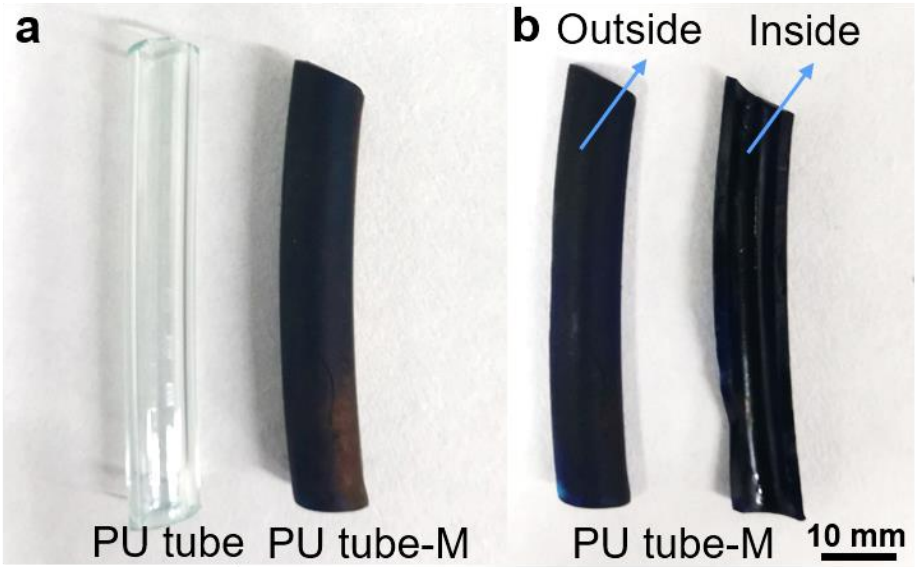

Figure S7. Images of methylene blue staining of PU tube, and the external and internal surfaces of PU tube-M.

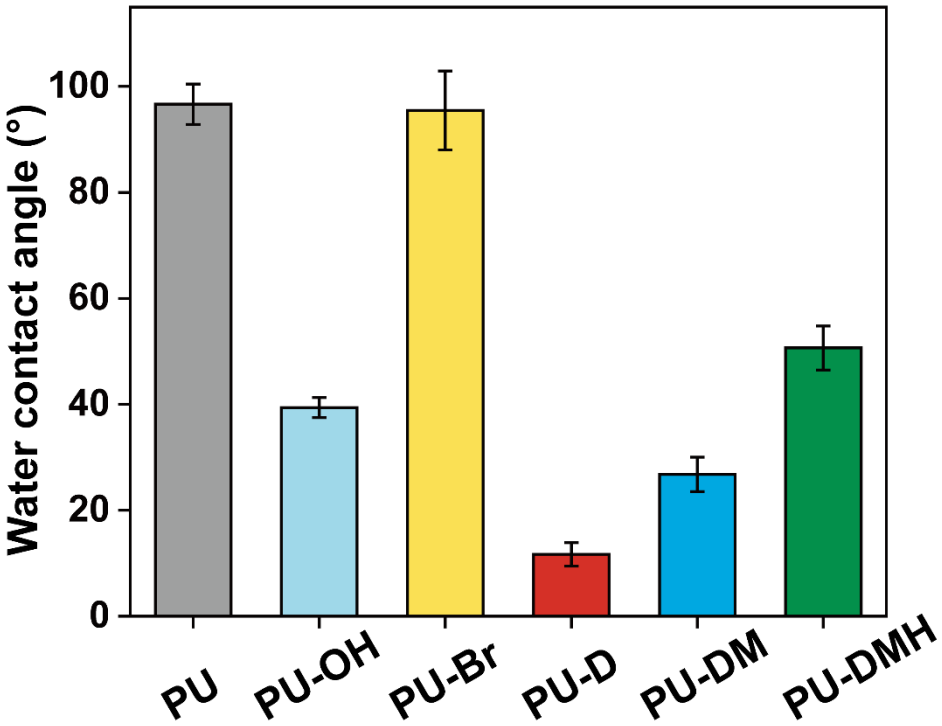

Figure S8. Water contact angles of PU, PU-OH, PU-Br, PU-D, PU-DM and PU-DMH.

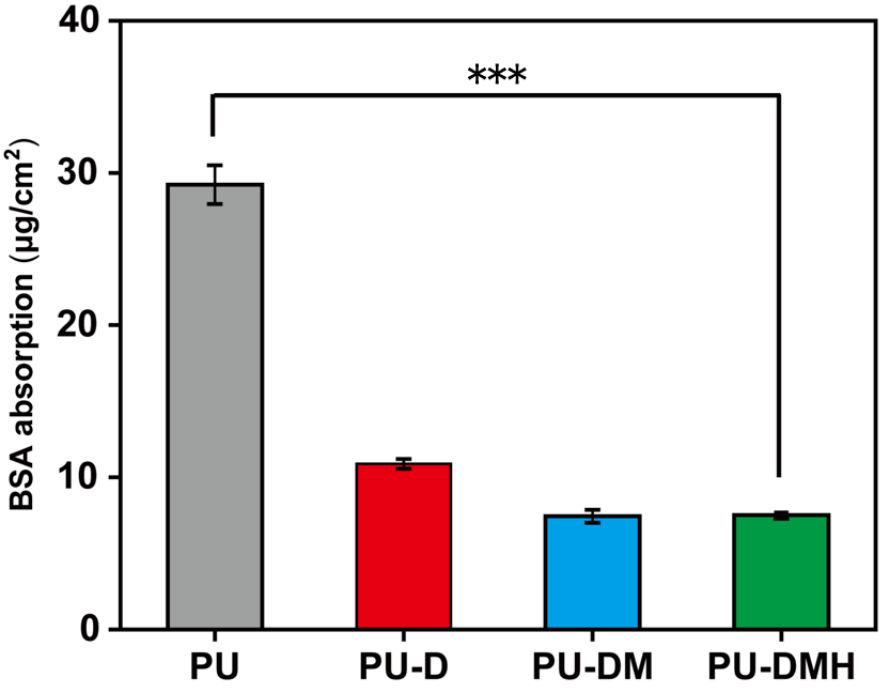

Figure S9. Protein adsorption on the surfaces of PU, PU-D, PU-DM and PU-DMH. Statistical significance (***, p<0.005) was evaluated by Student's *t*-test.

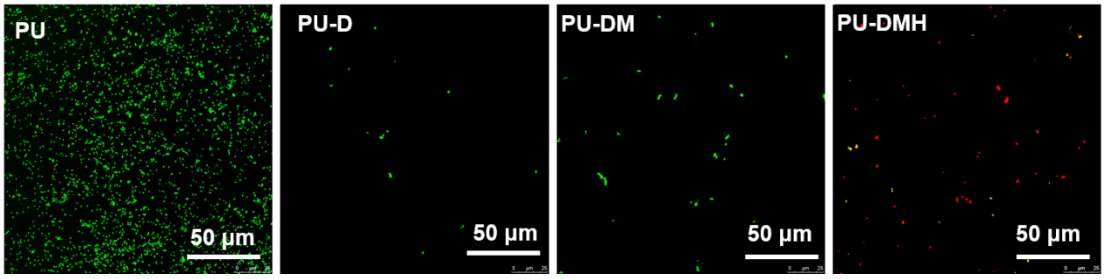

Figure S10. CLSM images of *S. aureus* on surfaces of PU, PU-D, PU-DM and PU-DMH in LB media.

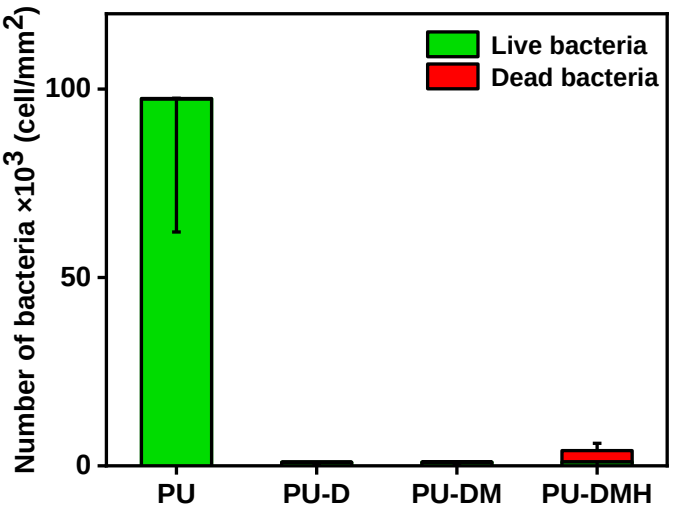

Figure S11. Bacterial densities on the surfaces of PU, PU-D, PU-DM and PU-DMH in LB media.

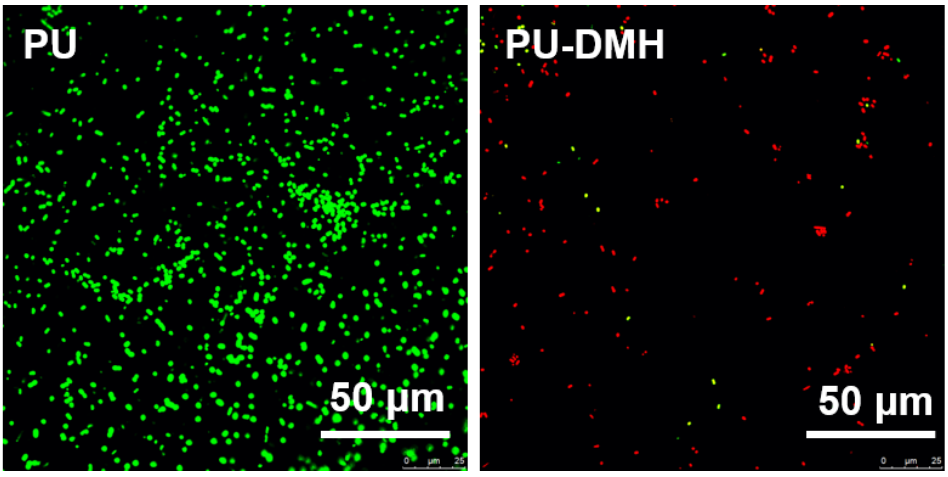

Figure S12. CLSM images of *S. aureus* on the surfaces of PU and PU-DMH in flow conditions.

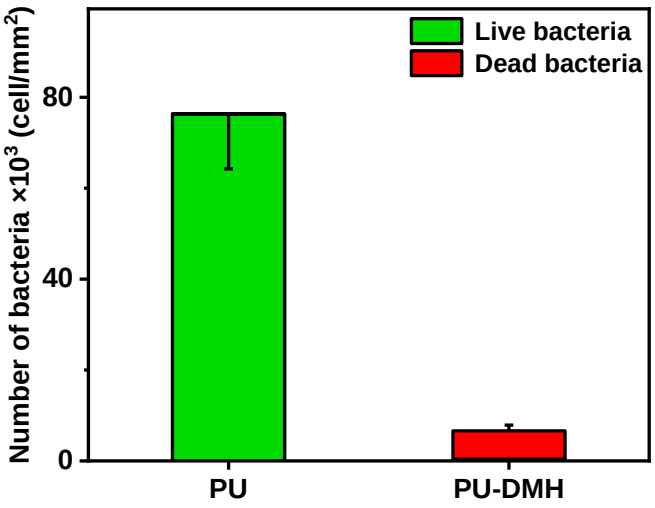

Figure S13. Bacterial densities on the surfaces of PU and PU-DMH in flow conditions.

Table S1. The roughness of samples in air and saline

Samples	**PU**	**PU-D**	**PU-DM**	**PU-DMH**
R_q in air (nm)	6.63	21.30	40.40	18.57
R_q in saline (nm)	7.17	65.70	65.57	27.10

Table S2. Atomic composition (At%) of PU and PU-Br based on XPS.

Element / **Sample**	**C**	**N**	**O**	**S**	**Br**
PU	73.16	7.17	18.60	1.07	0
PU-Br	75.50	7.00	13.62	1.23	2.65

3. References

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(S2) Wu, Y.; Wang, A.; Ding, X.; Xu, F. J. Versatile Functionalization of Poly(Methacrylic Acid) Brushes with Series of Proteolytically Cleavable Peptides for Highly Sensitive Protease Assay. *ACS Appl. Mater. Interfaces* **2017,** *9*, 127-135.

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