Catechol Chemistry Inspired Approach to Construct Self-Crosslinked Polymer Nanolayers as Versatile Biointerfaces

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Supporting Information

Materials

Sodium 4-vinylbenzenesulfonate (SS, 90%), poly(ethylene glycol) methyl ether methacrylate (EGMA, average molecular weight 475 Da), 1-vinyl-2-pyrrolidone (VP,

99%), (2-(methacryloyloxy)ethyl) trimethylammonium chloride solution (MTAC, 75 wt. % in H_2O), dioxane (99%), acrylic acid (AA, >99%), and *N*,*N*-dimethylacetamide (DMAc, 99.0%) were purchased from Aladdin Reagent Co., China. VP and AA were purified by vacuum distillation to remove inhibitors before use. (2-(methacryloyloxy)ethyl) dimethyl-(3-sulfopropyl) ammonium hydroxide (SBMA, 97%) was purchased from Sigma Aldrich. *S,S'*-bis(α, α'-dimethyl-α''-acetic acid)-trithiocarbonate (CTA) was synthesized according to a previous report.[1] 4,4'-azobis(4-cyanovaleric acid) (ACVA, ≥98.0%), 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), and 3, 4-dihydroxyphenethylamine-hydrochloride (dopamine·HCl, 99%) were acquired from Alfa Aesar, USA. Sodium phosphate dibasic and sodium phosphate monobasic were used to prepare phosphate-buffered saline (PBS) solution at certain pH value.

Polyethersulfone (PES, Ultrason E6020P, BASF, Germany) was used as the model polymeric substrate for the surface coating studies. PES solution (16 wt. % in DMAc) was spin-casted onto glass slides and followed with a liquid-liquid phase separation method to get the PES polymeric substrates.[2] Bovine serum albumin (BSA, fraction V, 95%), bovine serum fibrinogen (BFG) and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) were obtained from Sigma Aldrich. 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI, D8200-10) and Rhodamine Phalloidin (PHDR1) were from Solarbio, Canada and Cytoskeleton, USA, respectively. Micro BCA™ Protein Assay Reagent kits were the products of PIERCE.

APTT and TT reagent kits were purchased from Siemens. All other reagents were commercially obtained and used without purification. All aqueous solutions were prepared with de-ionized water (DI water), except that ultrapure water (UP water) was used for synthesis. Dialysis membranes (MWCO = 3500 Da and 7000 Da) were obtained from Solarbio, Canada.

Gel Permeation Chromatography (GPC)

Gel permeation chromatography (GPC) was performed on a Waters-1515 instrument (Waters, USA) against poly(ethylene oxide) (PEO) standards with water as an eluent. The sample concentration was 2-3 mg/mL, while the flow rate was 1.0 mL/min.

Contact Angle Measurement

Goniometric measurement is a facile way to characterize the hydrophilic polymer modified substrates. With a contact angle goniometer (Dataphysics OCA20, Germany), static water contact angle measurements were performed for polymeric substrates before and after the coating. One droplet of DI water (3μL) was dropped on the surface of the substrate with an automatic piston syringe and photographed by a video capture.

Plasma Collection

Healthy fresh human blood (male, 23 years old) was collected in vacuum tubes, containing sodium citrate as the anticoagulant (anticoagulant/blood, 1:9 v/v). The

blood was centrifuged at 1500 rpm and 4000 rpm for 15 min to get platelet-rich plasma (PRP) and platelet-poor plasma (PPP) respectively.

Protein Adsorption

Bovine serum albumin (BSA) and bovine serum fibrinogen (BFG) were employed in protein adsorption experiments, for which the protein was dissolved in isotonic PBS (pH 7.4, 1 mg/mL). The substrates (1 cm × 1 cm) were immersed in PBS, and incubated at 37 °C for 1 h, subsequently immersed in the aforementioned protein solution (2 mL per piece) for 1h at 37 °C under static state. During the desorption process, the substrates were gently rinsed by PBS solution and UP water, and placed in sodium dodecyl sulfate (SDS, 2 wt. %) solution for another hour at 37 °C under constant agitation (200 rpm). The vast majority (>95%) of the adsorbed protein could be extracted into the final solution so that the amount of adsorbed protein was calculated. The protein concentration in SDS eluant was determined by Micro BCA protein assay reagent kits employing a UV-Vis spectrometer (UV-1750, Shimadzu, Japan).

Platelet Adhesion

Platelet-rich-plasma (PRP) was adopted in platelet adhesion testing to rule out the interference of other blood components. After equilibrium in PBS solution at 37 °C for 1 h, the substrates (1 cm × 1 cm) were incubated in 1 mL of fresh PRP at 37 °C for 2 h. Then, the samples were slightly rinsed by PBS, and fixed with glutaraldehyde solution (2.5 wt. % in PBS) for 24 h, which were afterward underwent a drying

process by immersing them in a series of graded alcohol-PBS solutions (25%, 50%, 70%, 75%, 90%, 95% and 100%, 15 min for each). Surface platelet adhesion was observed by a FE-SEM (JSM-7500F, JEOL, Japan). Counting of platelets adhered was obtained from five SEM images at 500 × magnification from different sites on the same substrate.

APTT and TT Tests

Activated partial thromboplastin time (APTT) and thrombin time (TT) were two indicators to evaluate the antithrombogenicity of substrates before and after coating. The two tests were carried out on a semi-automated blood coagulation analyzer CA-50 (Sysmex Corporation, Japan), using fresh platelet-poor plasma (PPP). With regard to APTT test, 4 pieces of substrate (0.5 cm × 0.5 cm) were incubated in 200 μL of isotonic PBS (pH 7.4) for 1 h at 37 °C. Then the PBS was removed and 100 μL of PPP was added. After contacting with the substrates at 37 °C under shaking (200 rpm) for 30 min, 50 μL of the incubated PPP was introduced into the test cup, and then 50 μL of APTT agent and 50 μL of $CaCl_2$ solution (50 mM) were successively added. APTT was ultimately determined. TT was measured in a similar procedure. 100 μL of TT agent was added into the test cup containing 50 μL of the incubated PPP, and then TT was measured.

Cell Culture and Seeding

Human umbilical vein endothelial cells (HUVECs) were incubated in an R1640 medium consisted of 10% fetal bovine serum (FBS, Hyclone, USA) and 2 mM L-glutamine supplemented with 1 v/v% antibiotics mixture (10000 U penicillin and

10 mg streptomycin) in a humidified atmosphere containing 5% CO_2 at 37 °C (Queue Incubator, France). Confluent cells were segregated from the culture flask by using sterile PBS and 0.05% trypsin/EDTA solutions, and culture medium was changed daily. After cell culture, the substrates (1 cm × 1 cm), suitable for the size of 24-well cell culture polystyrene plates, were immersed in 75% ethanol aqueous solution for 3 h. Afterward, the samples were rinsed with PBS and sterilized by UV radiation. HUVECs were seeded onto the substrates at a density of ~2.5 x 10^4 cells/cm^2.

MTT Assay

The viability of the HUVECs was evaluated via MTT assay after the cells were cultured for 2, 4 and 6 days. Meanwhile, the cells cultivated in the full culture media without samples were acted as the controls. After fixed time intervals, 45 μL of MTT solution (1 mg/mL in the test medium) was added to each well, which was subsequently incubated for 4 h at 37 °C. Mitochondrial dehydrogenases from the viable cells selectively cleaved to the tetrazolium ring, resulting in the formation of blue/purple formazan crystals. After removing of the solution, 400 μL of ethanol was then added to dissolve the formazan crystals. Thus, the level of the cell metabolism could be evaluated by the quantity of the formazan crystals dissolved in ethanol. After being jogged by a shaker for approx. 15 min, a homogeneous solution was obtained, which was subsequently aspirated on a microliter plate and then the optical density of the formazan solution was calculated via a Microplate reader (Model 550, Bio-Rad, USA) at a wavelength of 492 nm. In order to minimize the test error, eight samples for each type substrate were tested. The results were expressed as means ± SD. The

statistical significance was evaluated by Student's t -test, and the level of significance was chosen as $P < 0.05$.

Fluorescence Staining

Fluorescein diacetate (FDA) and propidium iodide (PI) are popular nuclear counterstains used in multicolor fluorescent techniques, and chosen to stain the cells in the present work. Four days after seeding, the culture polystyrene plate was washed twice by PBS solution, then 400 μL of FDA solution (10 μg/mL in PBS) was added to each well, and the cell culture plate was placed into an incubator (37 °C, 5% CO_2). After incubation for 5 min, 400 μL of PI solution (10 μg/mL in PBS) was added and kept for another 5 min at 37 °C. Then the cell culture plate was rinsed three times by PBS solution. The whole experimentation was manipulated free from light. A fluorescence microscope (IX53, Olympus, Japan) was used to observe the fluorescence images of the samples.

Scanning Electron Microscopy

For SEM observation, six days after cell seeding, the HUVECs seeded substrates were firstly washed with PBS and then fixed with glutaraldehyde (2.5 wt. % in PBS) at 4 °C for 12 h. Before cell morphology observation under a FE-SEM (JSM-7500F, JEOL, Japan), the fixed samples were subjected to a drying procedure as follows: successively passing them through a series of graded alcohol-PBS solutions (30, 50, 70, 80, 90, 95 and 100%, 15 min for each) and isoamyl acetate-alcohol solutions (30, 50, 70, 80, 90, 95 and 100%, 15min for each). The specimens were further subjected

to a liquid CO_2 critical point drying process, which were then sputter-coated with gold layers.

Confocal Laser Scanning Microscopy

Six days later after cell seeding, the substrates were scrupulously washed with PBS for three times, and then fixed with 1mL of paraformaldehyde (3.7 wt. % in PBS) at 4 °C overnight. Afterward, the substrates were washed with PBS for 0.5 min and then 1 mL of Triton X-100 solution (0.5 % in PBS) was added to penetrate the cell substrate for 5min. Subsequently, the substrates were stained with 200 μL of Rhodamine phalloidin solution (100 nM in PBS) for 30 min, and then 1 mL of DAPI solution (5 μg/mL in PBS) for 10 min. Finally, the substrates were immersed in PBS solution and observed by a confocal laser scanning microscope (CLSM, Leica, Switzerland). The whole procedure should be manipulated free from light.

Antibacterial Activity

Escherichia coli (*E. coli,* gram-negative) and *Staphylococcus aureus* (*S. aureus,* gram-positive) bacteria were selected as typical bacteria to evaluate the antibacterial ability of the substrates. Before seeding, all the substrates were sterilized by ultraviolet irradiation for 30 min. After resuscitation and purification, 1×10^6 colony forming units (CFU) of *E. coli* and *S. aureu* in 2 mL of suspension were separately seeded onto substrates (1 cm × 1cm), and kept under constant agitation at 37 °C.

Quantification of bacterial adhesion and viability on the pristine and coated polymeric substrates was carried out by the spread plate method. After 4 h, the substrates were gently washed three times with sterile PBS and then cleaned in 2 mL

of sterile PBS solution under mild ultrasonic for 5-7 min. The bacterial suspension was mixed in a vortex mixer for 15 s, followed by 10-fold serial dilution. 100 μL aliquots of the serially diluted suspension were spread onto the triplicate solid agar. After incubation of the plates at 37 °C for 24 h, the number of the viable bacteria colonies was counted manually, and the results were expressed as the relative viability, defined as the percentage of viable bacteria on the coated substrates relative to those on the pristine polymeric substrate.

On the other hand, after incubation for 24 h, the adhered bacteria on the substrates were observed under a FE-SEM (Inspect F, FEI, USA) after being fixed with 2.5 % glutaraldehyde and dehydration by a series of graded alcohol, which is similar to the procedure for platelets adhered observation as mentioned above.

Statistical Analysis

All experiments were repeated at least three times. Statistical analysis was carried out utilizing SPSS 17.0 software. Results were expressed as a mean ± standard deviation (SD). Student's *t*-test at 95% confidence levels ($P < 0.05$) was conducted to assess statistical significance within the data.

Calculation of Grafting Degree

The conjugation degree of the dopamine for polymer-Cat was determined by using ^{1}H NMR spectroscopy, and all the substitutions ranged from 19% to 27%. For PSS-Cat, the integral ratio of the aromatic protons (6.3-7.8 ppm) to the methylene protons in catechol (2.8-3.2 ppm) was used, and the conjugation degree was then obtained. For other grafted polymers, the integral ratios of the aromatic protons in

catechol (6.5-7.5 ppm) to any characteristic protons in the major monomer units were used, for instance, the methyl protons in the pendant groups of SBMA (see Figure S1 B-2, i signal). The degrees of the catechol substitution were further calculated. All the measurements were repeated three times to get reliable values.

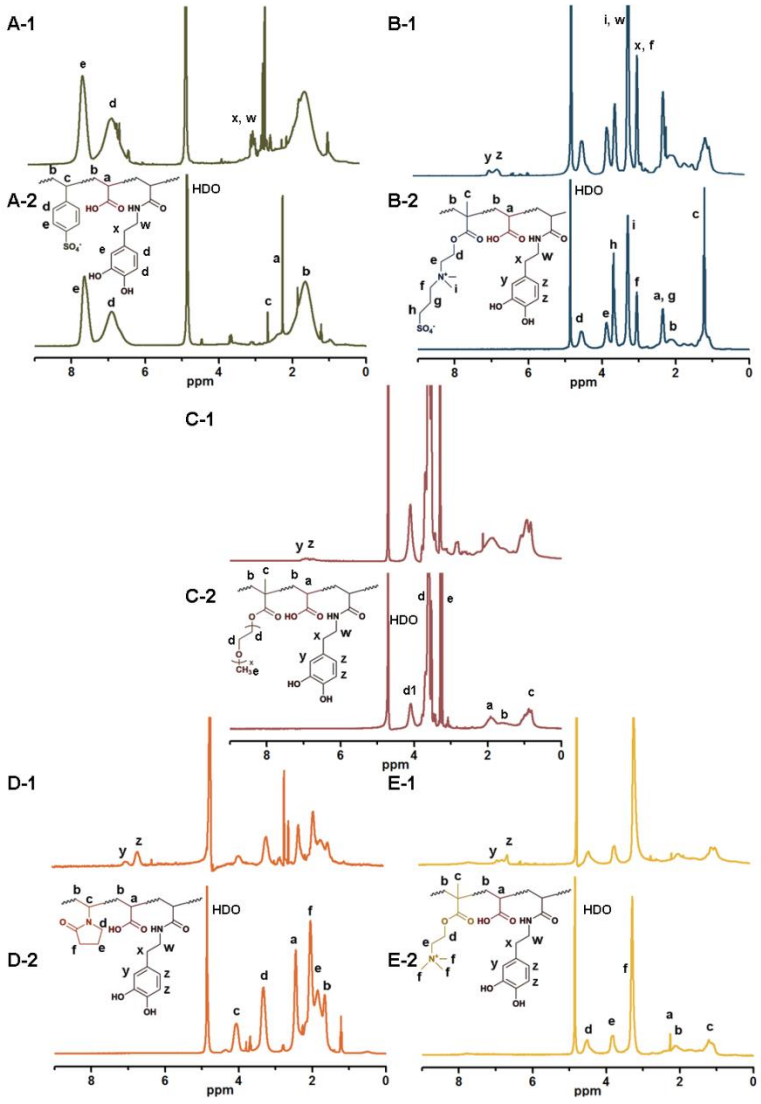

Figure S1. ^{1}H NMR spectra of PSS-Cat (A-1), P(SS-*co*-AA) (A-2), PSBMA-Cat (B-1), P(SBMA-*co*-AA) (B-2), PEGMA-Cat (C-1), P(EGMA-*co*-AA) (C-2), PVP-Cat (D-1), P(VP-*co*-AA) (D-2), PMTAC-Cat (E-1), and P(MTAC-*co*-AA) (E-2).

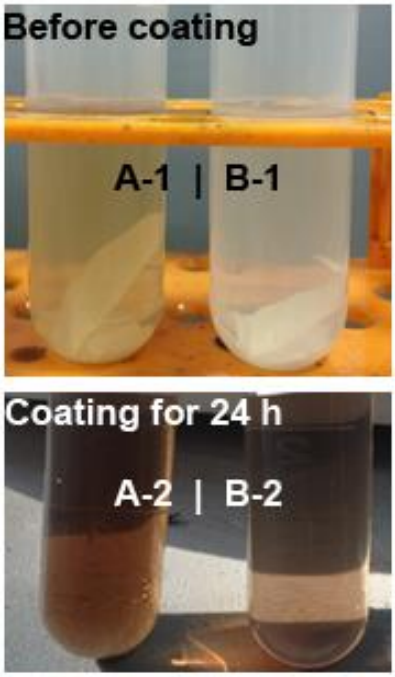

Figure S2. Typical pictures for PSS-Cat coating solution before coating (A-1), after coating for 24 h (A-2); PSBMA-Cat coating solution before coating (B-1), after coating for 24 h (B-2).

As shown in Figure S2, during the coating process, the color of the catecholic polymer solution will experience a gradual change from colorless to dark brown. However, no visible or insoluble polymer particles are observed in the PBS buffer. That is different from the pH-induced polymerization of dopamine, which may generate a lot of visible polydopamine particles.[3] For the catechol conjugated polymers, the grafting degrees of dopamine on polymers are relatively low, so it will not lead to any insoluble particle.

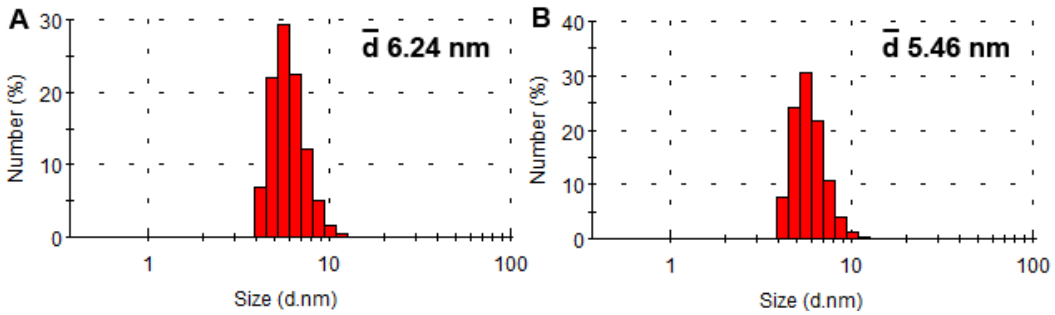

Figure S3. The size distribution for the developed nanogels in PSS-Cat (A) and PSBMA-Cat (B) coating solutions (coating time = 24 h).

Although there is no visible large particle, dynamic light scattering (DLS, Zetasizer ZS90, Malvern) demonstrated that smaller nanogels formed in the polymer-Cat solutions after coating, the nanogel diameters are smaller than 10 nm. As seen in Figure S3, the mean diameters are 6.24 nm and 5.46 nm for the PSS-Cat and PSBMA-Cat coating solutions, respectively, which also provide the evidence that the nanogel sizes are too small, and no insoluble particle can be observed.

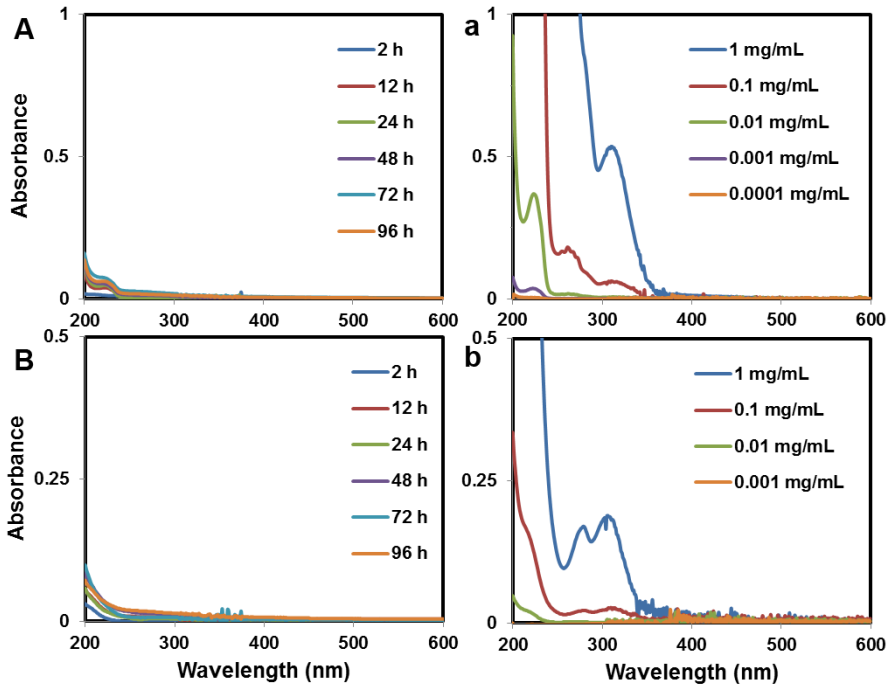

Figure S4. The UV-vis spectra for the solutions where PSS-Cat (A) and PSBMA-Cat (B) coated substrates were immersed (coating time = 24 h); and the original UV-vis spectra for the PSS-Cat (a) and PSBMA-Cat (b) polymer solutions at different concentrations.

Further experiment was conducted to investigate the long/short term behavior of the polymer-Cat coating layer using a UV-vis spectrophotometer. Typically, the PSS-Cat and PSBMA-Cat were used as the model coating polymer to illustrate the stability. The as-prepared polymer-Cat substrates (1 cm × 1 cm for each) were firstly immersed into 3 mL of DI water, followed by the collection of the spectra from 600 nm to 200 nm at 2 h, 12 h, 24 h, 48 h, 72 h, and 96 h. As exhibited in Figure S4, both the PSS-Cat and PSBMA-Cat coated surfaces showed excellent stability in the long term (> 96 h), when kept in the water.

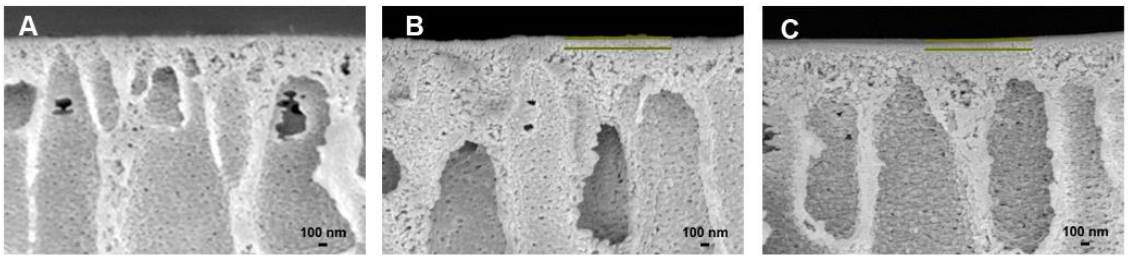

Figure S5. SEM cross-section view for the pristine PES (A), PSS-Cat (B, before washing), and PSS-Cat (C, washed in DI water for 96 h) (coating time = 24 h).

The changes of the thickness on coatings were checked via cross-section SEM observation. Typically, the cross-section images of the PSS-Cat before and after washing with DI water were shown. As exhibited in Figure S5, the thickness for the PSS-Cat before washing with DI water was 112 nm, while it was 108 nm after washing with DI water. No obvious change took place.

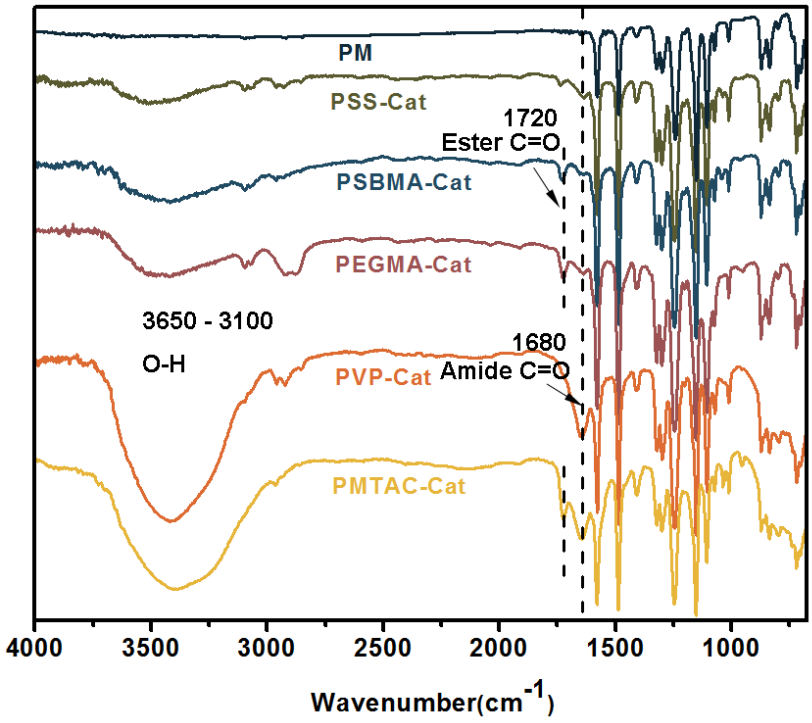

Figure S6. ATR-FTIR spectra for the near surface of the pristine PES substrate and the catecholic polymer coated PES substrates (coating time = 24 h).

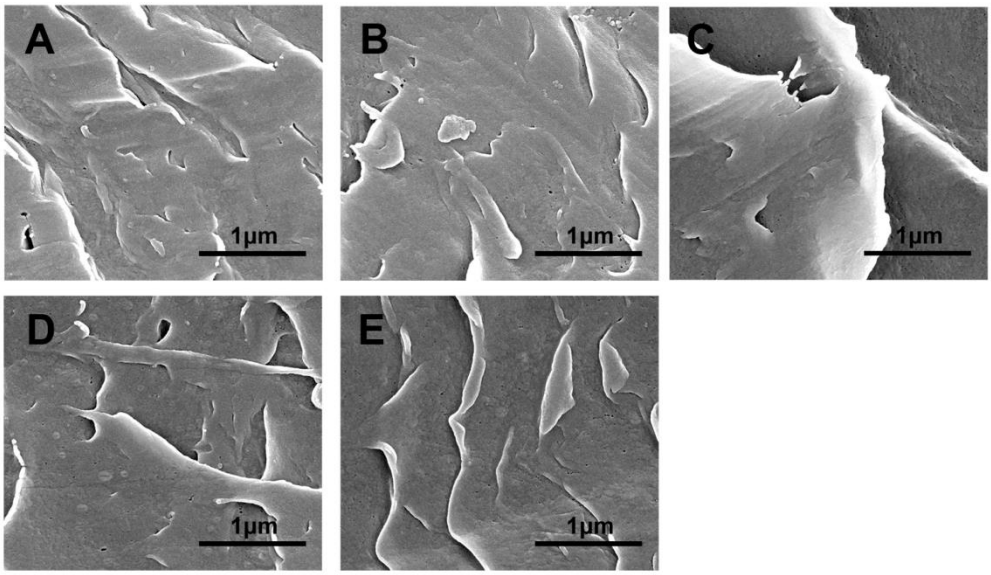

Figure S7. Typical SEM images of the surface morphologies for the PSS-Cat (A, 24 h), PSBMA-Cat (B, 24 h), PEGMA-Cat (C, 24 h), PVP-Cat (D, 24 h) and PMTAC-Cat (E, 24 h) substrates.

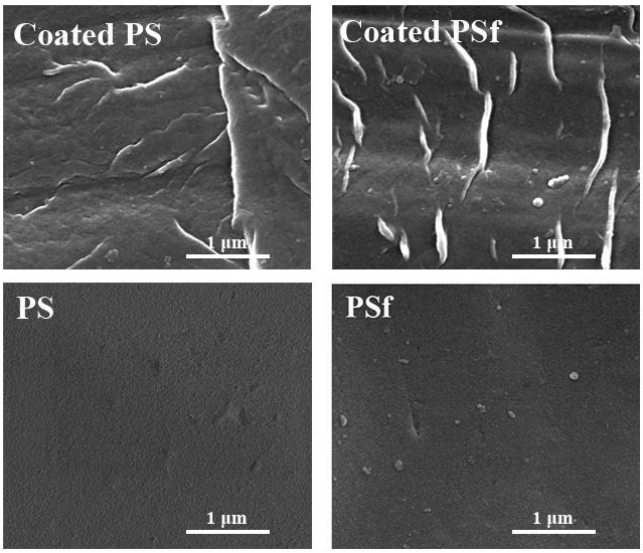

Figure S8. Typical SEM images of the surface morphologies for PSS-Cat coated PS, PSS-Cat coated PSf, the pristine PS, and the pristine PSf (coating time = 48 h).

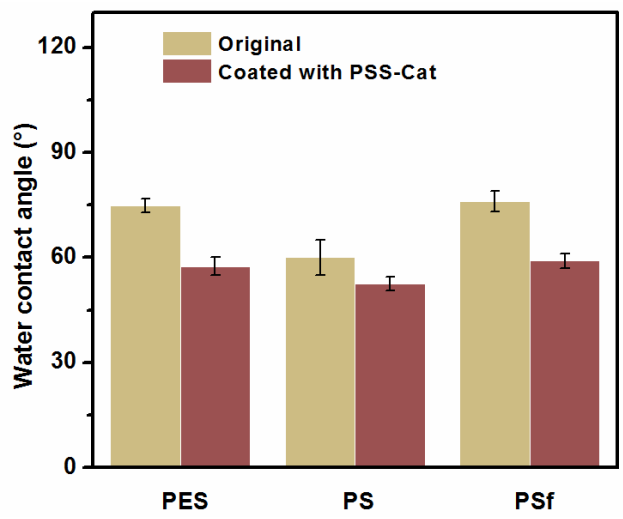

Figure S9. Measurements of water contact angle on different types of substrates (coating time = 48 h).

Figure S8 and S9 indicated that the PSS-Cat have been successfully coated onto different types of substrates, which illustrated that our method is a universal protocol for interface functionalization. Meanwhile, many researchers have confirmed that the dopamine, catechol derivatives, and also the catechol conjugated polymers could be

coated on various substrates, including glass, TiO_2, SiO_2, Si, PU, PTFE, PC, PVDF, and etc..[3-5]

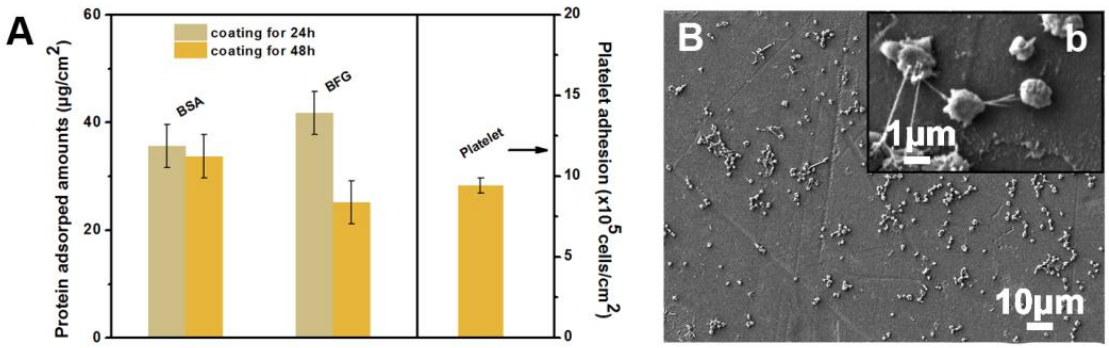

Figure S10. (A) Adsorption amounts of plasma proteins, BSA and BFG, on the PMTAC-Cat coated substrate; average numbers of the adhered platelets onto the PMTAC-Cat coated substrates. (B, b) SEM images of platelets adhered to PMTAC-Cat coated substrates.

Table S2. Comparison of the antifouling properties of different catechol conjugated polymers.

Polymer grafted with catechol	**Catechol position**	**Protein species**	**Testing methods**	**Typical protein adsorption amounts**
polypeptides[6-7]	end	fibrinogen (Fg), lysozyme (Lyz), serum	optical waveguide lightmode spectroscopy	7 ng/cm^2(Fg adsorption to TiO_2 surfaces coated with polypeptoid)
PEG[8]	side	serum	ellipsometry	2.5 nm thickness increase (serum protein adsorption on Si surfaces coated with PEG)

PEG[9]	end	rinary protein	-	10.4 mg/cm^2(for the PEG-Cat coated ureter)
hPG[10]	branched end	BSA, Fg	quartz crystal microbalance	34 ng/cm^2(BSA adsorption to TiO_2 surfaces coated with hPG-Cat10)
pSBMA[11]	end	Fg, Lyz, serum	surface plasmon resonance	2.1 ng/cm^2 (Lyz adsorption to NH_2-SAM surfaces coated with pSBMA)
polyampholytes[12]	end /middle	Fg, Lyz, BSA	surface plasmon resonance	1.7 ng/cm^2 (Fg adsorption to gold surfaces coated with one type of polyampholyte)
P(SBMA-*co*-AA)	side	BSA, BFG	UV-vis spectroscopy	1.29 μg/cm^2 (BFG adsorption to polymeric substrates coated with PSBMA-Cat)
P(EGMA-*co*-AA)	side	BSA, BFG	UV-vis spectroscopy	1.84 μg/cm^2 (BFG adsorption to polymeric substrates coated with PEGMA-Cat)

As shown in Table S2, all the earlier reports demonstrated that the cathchol conjugated antifouling polymers could endow the modified substrates with excellent antifouling ability. However, it is difficult to compare the surface antifouling properties by the protein adsorption amounts in different papers, since the protein adsorption amounts depend on various parameters, including coating layers (surface morphology, wettability, thickness and chemical composition), testing methods (the quartz crystal microbalance and surface plasmon resonance usually exhibit high sensitivity than other methods, resulting in low testing values of protein adsorption amounts), protein species, coating substrates and other factors. Nevertheless, in all of

these papers, the catecholic polymer coated substrates exhibit increased antifouling property than the pristine substrates or other modified substrates within one standard measurement. For example, Cheng et al. designed different surfaces, including pSBMA or pOEGMA grafted surfaces and SAMs with different terminated groups, and further demonstrated both pSBMA and pOEGMA brushes dramatically reduced bacterial adhesion and protein adsorption.[12] Our work also presented a versatile protocol to prepare the anti- protein/bacteria fouling substrates via a facile self-coating process by using different polymer-Cat.

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