Supporting Information

Direct catechol conjugation of mussel-inspired biomacromolecule coatings to polymeric membranes with antifouling, anticoagulant and cytocompatibility

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Ultrafiltration of creatinine, uric acid and PEGs solutions

In this study, the creatinine solution (0.05 mg/mL, PBS, pH 7.4) was applied to the membranes at the same pressure. Each time, the creatinine solution passed through the filter for about 30 min to get steady state. Then both the permeated solution and bulk solution were collected at the same time. The creatinine concentration was measured by an UV–vis spectrophotometer (UV-1750, Shimadzu, Japan) at 232 nm. The ultrafiltration of uric acid solution (0.07 mg/mL, PBS, pH 7.4) had similar procedure with creatinine and the concentration was measured by the UV–vis spectrophotometer at 292 nm.

Then, for the ultrafiltration of PEGs, three kinds of PEG were used: PEG-2000,
PEG-6000 and PEG-10000. The feed solution was prepared by dissolving PEG in deionized water with a concentration of 100 mg/L. The solution was applied to the membranes at the same pressure. Each time, the PEG solution passed through the filter for about 30 min to get steady state. Then both the permeated solution and bulk solution were collected at the same time.

The concentration of the PEG solution was determined by the UV-vis spectrophotometer after the reaction with barium chloride solution and iodine solution. First, 1 mL of 5.0 wt.% BaCl\textsubscript{2} aqueous solution was added to 1 mL of the PEG solution, then 1 mL of 0.05 mol/L iodine standard solution (13 g I\textsubscript{2} and 36 g KI was dissolved in 1000 mL pure water) was added. After about 10 min, the solution was diluted by deionized water, and the absorbance of PEG-2000 was determined at the wavelength of 562 nm; the absorbance of PEG-6000 and PEG-10000 was determined at the wavelength of 510 nm, then the observed sieving coefficient (SC\textsubscript{o}) was calculated using Eq. (1) as following:

\[
SC_0 = \frac{C_p}{C_b}
\]

where \(C_p\) is the PEG concentration of the permeated solution (mg/L) and \(C_b\) is the concentration of the bulk solution (mg/L).

**Protein adsorption**

BSA and BFG were employed as two model proteins to measure the protein adsorption properties of pristine and modified membranes. Briefly, every 2 mL of BSA (or BFG) phosphate buffer solution with a concentration of 1 mg mL\textsuperscript{-1} was added to a 24-well cell culture plate containing one piece of normal saline pre-equilibrated sample in each well. The plate was kept static for 1 h to reach the adsorption equilibrium. Afterward, the membranes were scrupulously rinsed with PBS solution and DI water, and then put in a new 24-well cell culture plate followed
with the addition of 2 mL of sodium dodecyl sulfate solution (2 wt. %) to each well. The plate was shaken at 37 °C for 2 h to wash down the adsorbed protein on the surface. The protein concentration of the washing solution was determined via Micro BCA™ Protein Assay Reagent Kit.

**Ultrafiltration of BSA solution**

For the ultrafiltration experiments, bovine serum albumin (BSA) was dissolved in isotonic phosphate-buffered saline solution (PBS, pH 7.4) with a concentration of 1.0mg/mL. The flux was calculated by the following equation:

\[
\text{Flux} = \frac{V}{SPt}
\]

where V (mL) is the volume of the permeated solution; S (m²) is the effective membrane area; P (mmHg) is the pressure applied to the membrane and t (h) is the time for collecting permeated solution.

After protein filtration, the membrane was cleaned with deionized water; then the PBS flux of the cleaned membrane was measured again and the flux recovery ratio was calculated using the following equation:

\[
F_{\text{RR}} (%) = \left(\frac{F_2}{F_1}\right) \times 100
\]

where \(F_1\) and \(F_2\) (mL/m²·h·mmHg) are the PBS fluxes before and after protein ultrafiltration, respectively.

**Clotting time**

Platelet-poor plasma (PPP) was obtained via centrifugation of fresh human blood (male, 26 years old, collected using a clinical anticoagulant vacuum tube) at 4000 rpm for 15 min. Activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) tests were performed to evaluate the anticoagulant capability of membranes and solutions. The as-prepared membranes were firstly immersed in
phosphate buffer (pH 7.4) overnight at 4 °C, followed by incubation at 37 °C for 1 h. Two pieces of each kind of membrane (1×1 cm²) were then placed in a 48-well cell culture plate. Afterward, PPP (220 mL) was added and incubated in the well at 37 °C for 0.5 h. For the solutions, LSCS or HSCS was dissolved in normal saline and the concentrations were controlled at 0.1, 0.2, 0.5, 1.0 and 2.0 mg/mL. Then the solution (10 μL) was added to PPP (200 μL) and incubated in the well at 37 °C for 1h. The incubated PPP solutions were then separately collected and analyzed through an automatic coagulation analyzer (sysmex CA-530, Japan). The clotting times were automatically collected and printed by the equipment, and the assays were performed in triplicate for each sample.

**Platelet adhesion**

Platelet adhesion experiments were carried out by using platelet-rich plasma (PRP) which was obtained via centrifugation of fresh human blood at 1000 rpm for 15 min, afterward, 200 μL of the fresh PRP was added to each well containing one piece of normal saline pre-equilibrated sample and then incubated at 37 °C for 2 h. FE-SEM was applied to observe the platelets adhered on the substrates. The average number of the platelets adhered on each sample was calculated via SEM images.

**Hemolysis test**

5 mL of whole blood was added to 10 mL of calcium- and magnesium-free PBS solution, and then red blood cells (RBCs) were isolated from plasma by centrifuging at 500 rpm for 10 min for 5 repeated times. The obtained RBCs were diluted in PBS to a volume of 100 mL for further use. For the hemolysis test, 0.2 mL of the diluted RBCs suspension (around $5 \times 10^8$ cells per mL) was added to 0.8 mL of normal saline which contained one piece of membrane. The RBCs solution dispersed in normal saline was selected as a negative control and RBCs solution dispersed in DI water was
used as a positive control. All the suspensions were centrifuged at 1000 rpm for 3 min after being incubated in a rocking shaker at 37 °C for 3 h. The absorbance of the released hemoglobin was measured at 540 nm by an UV-vis spectrometer (UV-1750, Shimadzu Co., Ltd, Japan), and then the hemolysis ratio was calculated by the following formula:

\[
\text{Hemolysis ratio (\%) = } \left( \frac{\text{Solution}_{\text{abs}} - \text{Negative control}_{\text{abs}}}{\text{Positive control}_{\text{abs}} - \text{Negative control}_{\text{abs}}} \right) \times 100
\]

Contact activation of the coagulation system and complement activation in human blood system

Platelet activation and coagulation activation were evaluated by platelet factor 4 (PF4) and thrombin-antithrombin III complex (TAT), respectively, through commercial enzyme-linked immunosorbent assays (ELISA, CUSABIO BIOTECH CO., Ltd, China). The same as the PF4 and TAT detections, complement activation (C3a and C5a) evaluation for the samples was also carried out using ELISA method. Briefly, whole blood was incubated with the as-prepared samples for 2 h and then centrifuged for 15 min to obtain plasma. The centrifugal rotational speed for PF4, TAT, C3a and C5a were 2500, 3000, 1000 and 1000 rpm, respectively. Afterwards, evaluations were performed according to the respective instructions from the manufacturer.

Endothelial cell compatibility

Human umbilical vein endothelial cells (HUVECs) were grown in R1640 medium. 100 μL of the cells was seeded in a 24-well tissue culture polystyrene plate (TCPS) at a density of 2.5 × 10^4 cells per cm². After cell culture for 4 h, 400 μL of the culture media was further added. MTT assay was used for examining the cell proliferation.
After culturing for 48 h, 50 μL of MTT solution (1 mg/mL in PBS) was added to each well and incubated at 37 °C for 4 h, and then the MTT solution was replaced with 400 μL/well ethanol, followed by 30 min shaking. The optical density (OD) of each well was determined with a Microplate reader (model 550, Bio-Rad) at 492 nm. In the present study, the cells cultured for 2, 4 and 6 days were used for the MTT assay.

To observe viable cell morphology, the culture polystyrene plate after being cultured for 6 d was washed three times with PBS solution, then 400 μL FDA (10 μg/mL PBS) was added to each well, and the cell culture plate was placed in an incubator (37 °C, 5% CO₂). After incubating for 5 min, 200 μL PI (20 μg/mL PBS) was added to each well, and then the cell culture plate was washed three times with PBS solution and observed by fluorescence microscope. Confocal laser scanning microscopy (CLSM) was also used for the endothelia cells adhesion and morphology.

**Ultrafiltration of pure water and NaCl solution**

The water flux of the membrane was determined by collecting the solution after getting steady, and was calculated by using the eq. (2).

For the ultrafiltration of NaCl aqueous solutions, salts were dissolved in deionized water with the ionic strengths varied from 0.0 M to 1.0 M. For each salt solution, the flux was determined by collecting the solution after getting steady and calculated by Eq. (2). To measure the reversibility of the membrane, pure water and salt solution (ionic strength=1.0 M) were alternately applied to the membrane and the fluxes were then calculated.

**Preparation of smooth PES film**

The smooth PES film was prepared by vacuum evaporation. The concentration of PES was controlled at 10 wt. % in DMAc. The temperature and time were controlled at 60 °C and 12 h, respectively.
Figure S1. Solubility of LSCS in acid, neutral and alkaline solution (0.01 g LSCS was added into the 1 mL solution).

Figure S2. XPS C 1s core-level spectra for PES, LSCS-H0 and HSCS-H0 membranes.

Figure S3. The images of smooth PES film and PES UF membrane.
Figure S4. Pore size distributions for the LSCS-Hx membranes (A) and HSCS-Hx membranes.

Figure S5. APTT, TT and PT for the LSCS and HSCS solutions, and the concentration of LSCS and HSCS in PPP were controlled at 0.5, 1.0 2.5, 5.0 and 10.0 μg/100 mL PPP.

Figure S6. Clotting times for the LSCS coatings and HSCS coatings that deposited on PTFE, PVDF and PSF membranes.
Figure S7. MTT assay was performed to quantify the cell proliferation.

Figure S8. The fluorescence images of viable HUVECs cultured with the media contained HSCS. The concentrations of HSCS in the culture media were 0, 0.05, 0.10, 0.25, 0.50 and 1.00 mg/mL.

Figure S9. The changes of catechol moieties under acidic and base conditions.
Figure S10. (A) UV-vis absorption spectra for the reaction between LSCS and catechol under air atmosphere; (B) Absorbance of the solution monitored at 294 nm with the time increasing.

Figure S11. Color changes of HCl, NaOH and SDS solutions after immersing membranes.
### Table S1. The peak ratios of S 2p spectra for membranes

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<th>167.5 eV (%)</th>
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<th>169.7 eV (%)</th>
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<tr>
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<td>HSCS-H2</td>
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### Table S2. The increase of the absorbance at 254 nm after immersing membranes

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<th>LSCS-H2</th>
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<td>HCl</td>
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<td>&lt;0.01</td>
<td>&lt;0.01</td>
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