Layer by Layer assembly of sulfonic poly(ether sulfone) as heparin-mimicking coatings: scalable fabrication of super-hemocompatible and antibacterial membranes

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Supporting Information

Synthesis of Water-soluble Heparin-mimicking Poly(ether sulfone)

WHP was prepared according to our earlier literature with minor modification.[1] The detail information of the WHP preparation is shown as follows: 3,3'-disulfanted-4,4'-difluorophenyl sulfone, (disodium salt) (1.834 g, 4.0 mmol), 4,4'-difluorobiphenyl sulfone (0.254 g, 1.0 mmol), 4,4'-sulfonyldiphenol (0.25 g, 1.0 mmol), 3,3'-diamino-4,4'-dihydroxydiphenyl sulfone (1.120 g, 4.0 mmol), DMAc (50 mL), toluene (40 mL), and potassium carbonate (K_2CO_3) were introduced to a three-neck round bottom flask equipped with a Dean-Stark device. After vacuuming and back-filling nitrogen for several times, the flask was transferred to an oil bath preheated to 155 °C, and lasted for 5 h at nitrogen atmosphere. Then, the reaction

system was heated to 180 °C and kept for 12 h. K_2CO_3 was used as proton scavenger, and the formed water during the reaction was removed as an azeotrope with toluene by the Dean-Stark device. The reaction mixture was allowed to transfer to an oil bath (preheated to 100 °C) and kept for 18 h, followed by the addition of excess maleic anhydride. After the reaction, the resulted product was cooled to room temperature and precipitated into de-ionized water (D.I. water). The residual monomers, K_2CO_3, and maleic anhydride in the product were removed by dispersing the polymer into D.I. water, followed by centrifugation; and the purification procedure was repeated for several times.

Synthesis of Quaternized Chitosan

QC was prepared according to a reported literature with a minor modification.[2] The detail information of the QC preparation is shown as follows: Step one: 2 g CS, 4.8 g NaI, 11 ml 15 wt. % aqueous sodium hydroxide (NaOH) solution, 11.5 ml methyl iodide, and 80 ml 1-methyl-2-pyrrolidinone, were introduced to a round bottom flask equipped with a Liebig condenser. Then, the flask was transferred to a water bath (preheated to 60 °C), and lasted for 1 h. The product was precipitated and washed using ethanol, and subsequently isolated by centrifugation. Step two: After volatilization of the ethanol, the product of the first step was dissolved in 80 ml 1-methyl-2-pyrrolidinone and heated to 60 °C. Subsequently, 4.8 g NaI, 11 ml 15 wt. % NaOH solution, and 7 ml of methyl iodide, were added with rapid stirring and the mixture was kept at 60 °C for 30 min. Then, an additional 2 ml methyl

iodide and 0.6 g NaOH pellets were added and then stirred for another 1 h. Then, the product of the second step was dissolved in 40 ml 10 wt. % NaCl aqueous solution to exchange the iodide. At last, the solution was centrifuged and washed with ethanol, and subsequently with ether to yield a white, water-soluble powder, which was then dried in a vacuum at 40 °C.

Preparation of PES Membranes

PES was dissolved in DMAc by vigorous stirring until a clear homogeneous solution was obtained. The total concentration of PES in the casting solution was 16 wt. %. After vacuum degassing, the casting solution was dropped on a piece of glass by spin coating till the solution was deposited uniformly on the surface of the glass. Then the glass was immersed into D.I. water; here water was used as the nonsolvent for membrane formation. After the completion of the exchange between water and the solvent, PES substrate membrane was prepared.

Hydrophilicity Tests

The hydrophilicity of the membrane surface was characterized by contact angle measurement using a contact angle goniometer (OCA20, Dataphysics, Germany) equipped with video capture. A piece of 2 × 2 cm^2 membrane was attached on a glass slide. For the dynamic changes of water contact angle measurement, a total of 3 μL double distilled water was dropped on the airside surface of the membrane, and the contact angle was measured every two seconds. The measurement error was ±3°.

Protein Adsorption

Protein adsorption experiments were carried out with BSA and BFG solutions under static condition, respectively. Firstly, the membrane with an area of 1 × 1 cm^2 was immersed in phosphatic buffer solution (PBS, pH = 7.4), containing BSA or BFG with the concentration of 1 mg/mL; and incubated at 37 °C for 1 h; subsequently rinsed slightly with PBS solution and double distilled water. Then the membrane was placed in a washing solution (2 % sodium dodecyl sulfate (SDS) at 37 °C), and shaken for 2 h to remove the adsorbed protein. The adsorption and desorption times were carefully determined in preliminary experiments. The protein concentration in the washing solution was determined by using the Micro BCA™ Protein Assay Reagent Kit (PIERCE), and then the adsorbed protein amount was calculated.

Cell Culture

Human umbilical vein endothelial cells (HUVECs) were grown in R1640 medium supplemented with 10% fetal bovine serum (FBS, Hyclone, USA), 2 mM L-glutamine and 1% (*V/V*) antibiotics mixture (10000 U penicillin and 10 mg streptomycin). Cultures were maintained in humidified atmosphere of 5 % CO_2 at 37 °C (Queue Incubator, Paris, France). Confluent cells were detached from the culture flask with sterile PBS and 0.05 % trypsin/EDTA solutions, and the culture medium was changed every day.

The PES and surface assembled PES membranes were cut into 1 × 1 cm^2 to suit the size for 24-well cell-culture polystyrene plates, and pre-wetted by immersion in the culture medium for 3 h in a 37 °C incubator. And then the membranes were placed

into the cell-culture plates, rinsed with PBS and sterilized by γ -ray.

Cell Morphology on the Membranes

For the SEM observation, the HUVECs were seeded onto the membranes at a density of approx. 2.5×10^4 cells/cm^2. After 5 d, the seeded membranes were immediately rinsed with PBS and fixed with 2.5% (wt. %) glutaraldehyde in PBS at 4 °C for 12 h. For morphology observation, the fixed samples were subjected to a drying process by passing them through a series of graded alcohol-PBS solutions (10, 30, 50, 70, 80, 90, 95 and 100%, 15 minutes for each time) and then dehydrated through isoamyl acetate-alcohol solutions (10, 30, 50, 70, 90, and 100%, 15 minutes for each time). The critical point drying of the specimens was done with liquid CO_2. The specimens were sputter-coated with a gold layer and examined by a FE-SEM (JSM-7500F, JEOL, Japan).

MTT Assay

After cell culture for 2, 4, and 6 days, the viability of the vein endothelial cells was determined by MTT assay. The endotheliocytes were seeded onto the membrane at a density of approximately 2.5×10^4 cells/cm^2. Cells cultured in the wells without membrane served as controls in this study. After predetermined time intervals, 45 μL MTT solution (1 mg/mL in the test medium) was added to each well and incubated for 4 h at 37 °C. Mitochodrial dehydrogenases of viable cells selectively cleave the tetrazolium ring, yielding blue/purple formazan crystals. Then, 400 μL of ethanol was added to dissolve the formazan crystals. Thus, the quantity of the formazan dissolved

in the ethanol reflects the level of cell metabolism. The solution was shaken homogeneously for about 15 min. The sample solution was aspirated into microtiter plates and the optical density was read in a Microplate reader (model 550, Bio-Rad) at 492 nm. All the experiments were repeated three times, and the results were expressed as means ±SD. The statistical significance was assessed by Student's t-test with the level of significance set at $P < 0.05$.

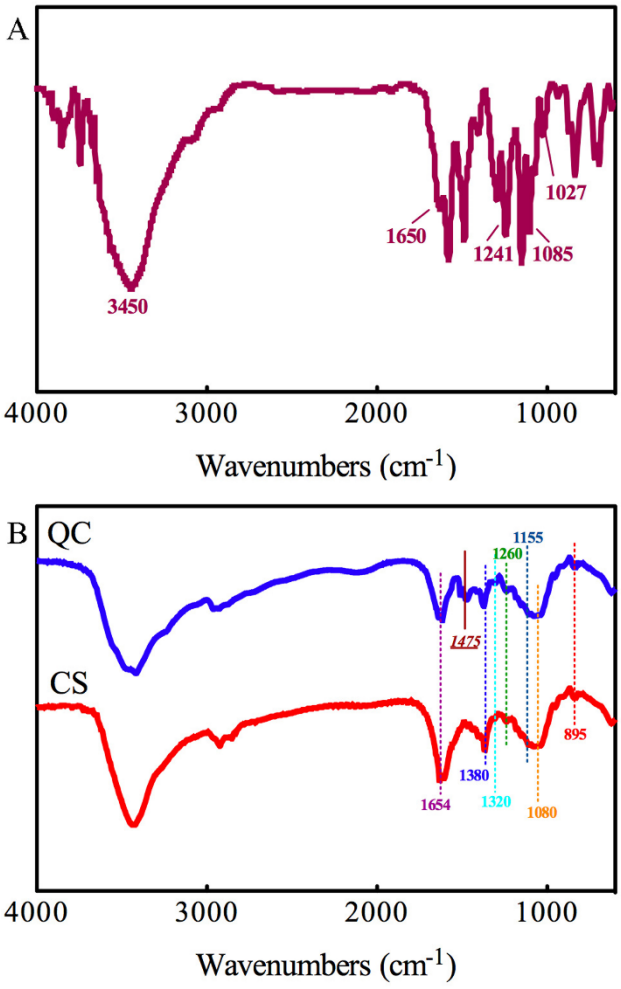

Figure S1. FT-IR spectra for WHP (A), CS and QC (B).

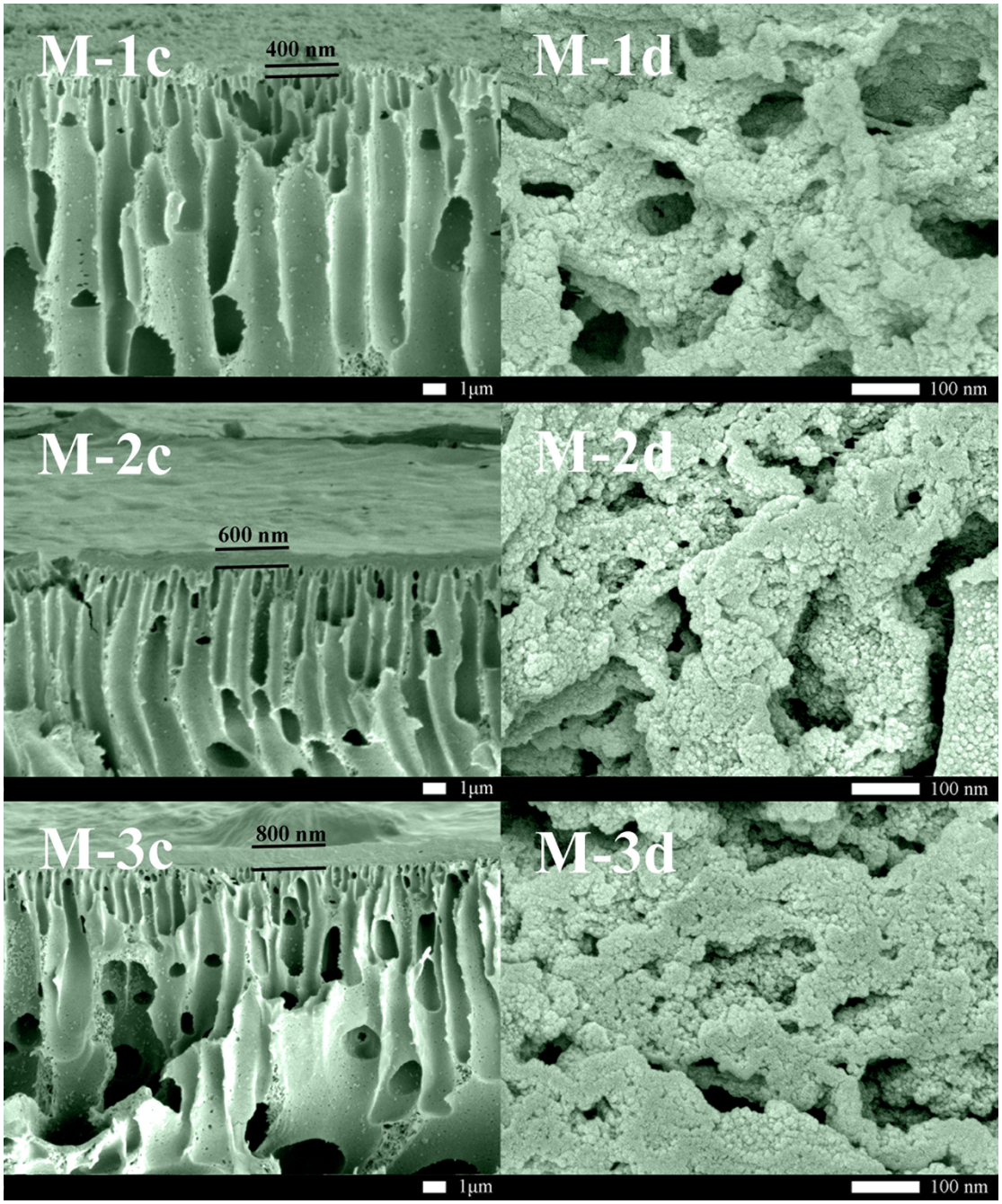

Figure S2. Cross-sectional SEM images of the WHP/QC LBL (M-1, M-2, M-3) membranes. (c, × 3000; d, × 80000)

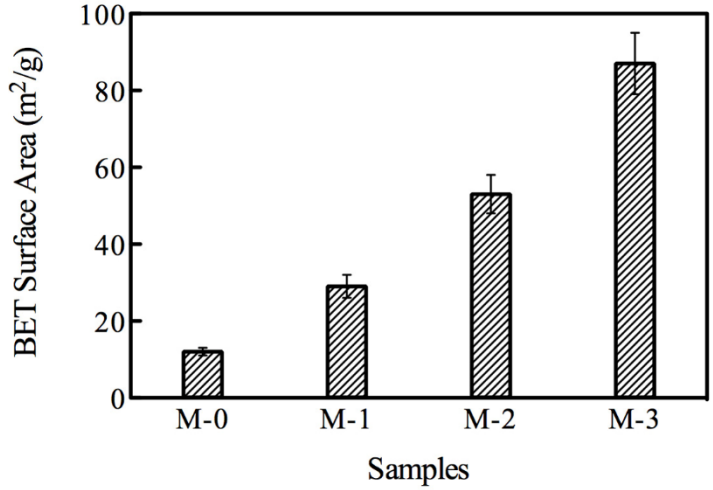

Figure S3. Specific surface areas of the pristine PES and WHP/QC LBL membranes.

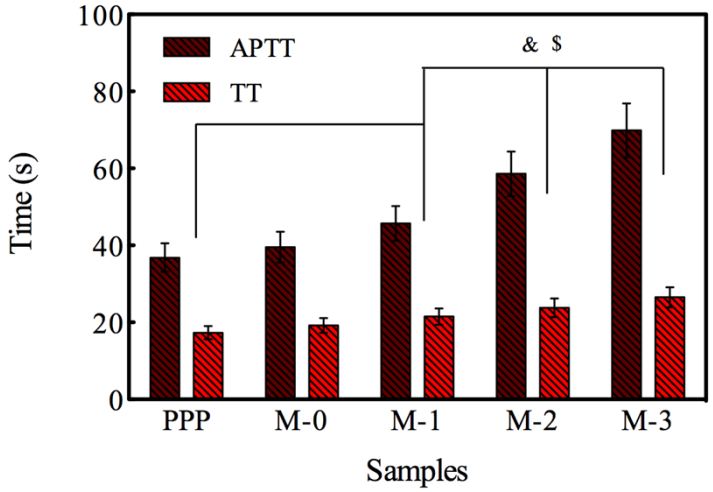

Figure S4. Activated partial thromboplastin times (APTTs) and Thrombin times (TTs) values of poor platelet plasma (PPP), pristine PES (M-0) and WHP/QC LBL (M-4, M-5 and M-6) membranes (Values are expressed as means ± SD, n=3. $P < 0.05$ compared with plasma and pristine PES membrane, respectively).

References:

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2. A. Sieval, M. Thanou, J. Verhoef, J. Brussee and H. Junginger, *Carbohydrate*

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