Surface modification of polyethersulfone membrane with a synthesized negatively charged copolymer

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Supporting Information

Synthesis of negatively charged copolymer

Synthesis of poly (styrene-alt-maleic anhydride) (PSMA)

In a typical procedure, MA (0.1 mol) and St (0.1 mol) were dissolved in adequate solvent DMF in a 100 mL flask, then AIBN was added with the amount of 0.5 mol. % relative to the total monomers. Then the flask was sealed after bubbling for 0.5 h with nitrogen, and the polymerization reaction was carried out for 24 h at 60 °C. The product was precipitated by cold diethyl ether, then dissolved in acetone; the solution was filtrated to remove the polystyrene (the by-product of the polymerization). After evaporating the acetone from the filtrate by a rotary evaporator, the product was put into a vacuum oven to be dried at 25 °C.

Synthesis of negatively charged PES membrane

Synthesis of aminated PES (PES-NH₂)

The synthetic route is shown in Scheme. S1. Nitration reaction was used to synthesis PES-NO₂. The main preparation route was as follows. H₂SO₄ (40 mL) and HNO₃ (30 mL) were mixed evenly in a 250 mL flask; after the mixed solution was cooled, 10 g PES was slowly added. Then, the nitration reaction was lasted at 65 °C with continuously stirring for 8 h. After the reaction, the product was washed for a couple of times using double-distilled water, and then the final yellow product of PES-NO₂ was obtained. The obtained PES-NO₂ was dried under vacuum at 25 °C for 24 h and then crushed into powders for the next step.
The obtained powdered PES-NO₂ (8 g) was dissolved in DMF (160 mL) thoroughly, and then SnCl₂ (40 g) and several drops of HCl solution were added. The mixed solution changed from yellow to purple gradually. After that, the solution was stirred for 4 h at 60 °C, the product was deposited in double-distilled water, and then washed with double-distilled water and HCl solution for a couple of times, respectively. The resulting purple PES-NH₂ was dried under vacuum for 24 h.

Scheme. S1 Synthetic scheme. (1) Synthesis of PES-NO₂, (2) synthesis of PES-NH₂ and (3) synthesis of PES-N⁺₂.

Preparation of surface diazotized PES membrane

As shown in Scheme. S1, the PES-NH₂ PES and were dissolved thoroughly in DMF with the PES-NH₂ concentrations of 0, 4 and 8 wt. %, respectively. The total concentration of the polymers was 16 wt. %. After vacuum degassing, by using spin-coating couple with a liquid–liquid phase separation technique, the casting solutions were prepared into membranes at room temperature. Then, the prepared membranes were washed thoroughly using double-distilled water for a couple of times in order to remove the residual solvent. The thickness of the final obtained
membranes controlled at about 60–70 µm, and the prepared membranes were termed L-16-0 (PES 16%, PES-NH₂ 0%), L-12-4 (PES 12%, PES-NH₂ 4%), and L-8-8 (PES 8%, PES-NH₂ 8%), respectively.

The prepared PES/PES-NH₂ membranes were immersed into 100 mL NaNO₂ aqueous solution (0.132 g/mL) at 0-5 °C; then 300 mL HCl solution (18%) was added slowly. The diazo-reaction was lasted for 2 h at the temperature of 0 and 5 °C. The diazotize PES membranes (PES/PES-N₂⁺) were then washed for several times with cold double-distilled water to remove impurities.

**Coating density of copolymers onto membranes**

In this study, coating yield (mg/cm², the increased weight after coating) was used to express the coating density of the copolymers onto the membranes. To measure the coating yield, 6 pieces (5 × 5 cm² for each) of the membranes were used. The weights of the L-12-4 and L-8-8 were 0.4783 g and 0.5367 g, respectively; after coating NaSPSMA onto the membranes, the total weight increased to 0.4919 g and 0.5568 g for the M-12-4 and M-8-8, respectively. The coating densities of copolymers onto the membranes were 0.09 mg/m² and 0.13 mg/m² for the membrane M-12-4 and M-8-8, respectively.
**Fig. S1** FT-IR spectra for the PSMA and NaSPSMA.

**Fig. S2** $^1$HNMR spectra for the PSMA and NaSPSMA.
**Fig. S3** FT-IR spectra for the PES, PES-NO$_2$, PES-NH$_2$, and the modified PES (M-8-8) membranes.

**Fig. S4** XPS spectra for the PES and modified PES membrane (M-8-8) surfaces.
**Morphology of the modified membranes (SEM micrographs)**

*The formation mechanism of the membrane morphology*

We could summarize the formation mechanism as follows: The membrane casting solution was a homogeneous phase and consisted of PES, PES-NH$_2$ and DMAC. Because of the amphiphilic property, the PES-NH$_2$ tended to move toward the interface of water and dope solution when phase separation occurred. Therefore, abundant PES-NH$_2$ might be concentrated and formed a layer of PES-NH$_2$ on the membrane surface, and the top skin of the modified membranes became denser.
**Fig. S5** Cross-sectional and surface SEM micrographs for the PES and modified PES membranes. (A), (a) and (A’) are M-16-0; (B), (b) are M-12-4; (C), (c) and (a’) are M-8-8.

![Fig. S5](image)

**Fig. S6** The number of the adhering platelets onto the membranes from platelet-rich plasma.

![Fig. S6](image)

**Experimental section**

*Platelet activation and complement activation*

We used Commercial enzyme-linked immunosorbent assays (ELISA) to investigate the platelet activation (Human Platelet Factor 4 (PF-4), Cusabio Biotech Co. Ltd., China) and complement activation (Human Complement Fragment 3a (C3a) and 5a (C5a), Cusabio Biotech Co. Ltd., China). Firstly, human fresh blood (man, 25 years old, containing sodium citrate as an anticoagulant) was incubated with the membrane for 2 h, and then centrifuged at 2000g (4 °C) for 20 min to obtain plasma. The plasma was mixed thoroughly with specific inhibitor and centrifuged in accordance with the
respective instruction manuals for each assay. Simultaneously, the control experiment was carried out according to the same process without adding the sample. The experiments were repeated 3 times, and the results were expressed as mean ± SD, and the statistical significance was assessed by Student’s t-test, and the level of significance was chosen as p < 0.05.

**Results and discussion**

*Platelet activation and complement activation*

When platelet activation occurs, the platelets will release platelet factor 4 (PF-4) which has great influence on injury response and inflammation\(^2\); and then form a central core of innate immune to defense against the foreign surface. After complement activation, the anaphylatoxins C3a and C5a are liberated, and then combined with specific cell surface receptors and further give rise to leukocyte activation\(^3\). In this study, in order to investigate platelet activation and complement activation for the modified PES membranes, the concentrations of PF-4, C3a and C5a were measured, respectively; and the data are shown in Figs. S7 and S8.

From Fig. S7, it was observed that for the pristine PES membrane, the PF-4 concentration was the highest, and the concentration of PF-4 decreased with increasing the NaSPSMA amounts grafted onto the modified membranes. Moreover, there is no obvious difference among the control sample and the modified membranes was observed (<4%, \(p > 0.05\)); and slightly decrease for the modified membranes was found compared to the pristine membrane. The results indicated that compared with
the pristine PES membrane, the negatively charged PES membranes could suppress the platelet activation.

**Fig. S7** The concentrations of PF-4 for the samples with whole blood. (Values are expressed as mean ± SD, n = 3. #p > 0.05 compared with plasma and pristine PES membrane, respectively.)

In Fig. S8A, we could observe that the C3a concentration for the control sample was the lowest, and the C3a concentration for the pristine PES membrane was a little higher than that of the negatively charged PES membranes. In addition, the concentration of C3a decreased with the increase of the NaSPAMA amounts grafted onto PES membrane. Moreover, nearly no obvious difference was observed between the control sample and M-8-8 (<0.5%, *p > 0.05). For C5a (Fig. S8B), the concentrations for the negatively charged PES membranes decreased obviously compared with that of the pristine PES membrane, and no obvious difference between the control sample and M-8-8 was observed (<0.5%, *p > 0.05). The lower activation
of platelet and complement for the modified PES membrane might be resulted from the grafted functional negatively charged groups.

Fig. S8 (A) Concentrations of C3a for the samples with whole blood. (Values are expressed as mean ± SD, n = 3.) (B) Concentration of C5a for the samples with whole blood. (Values are expressed as mean ± SD, n = 3. *p > 0.05 compared with plasma and pristine PES membrane, respectively.)
In our previously study\(^1\), complement C3 activation in vitro was tested by ELISA for different kinds of membranes, including Cuprophane membrane (Nephross, Netherlands), Polysulfone membrane (PSF, Fresenius, Germany), Hemophane membrane (Ningbo-Yatai, China), Cellulose acetate membrane (Nissho, Japan), Polycarbonate (PC, obtained from BASF Co. Ltd.) membrane (prepared in our lab), and PVP/PES membrane (prepared in our lab). In the test, we used the activation for Cuprophane membrane as the control (100%). As shown in Fig. S9, the PVP/PES membrane had the lowest complement activation, since the hydrophilicity of PES membrane was increased by PVP. In this study, the hydrophilicity of the PES membrane increased considerably after grafting NaSPSMA, and the water contact angle decreased from 76.5° to 27.3°; the negatively charged surface might decrease the complement activation within a limited value and lower the activation of blood coagulation system\(^5-6\).

![Fig. S9 Complement activation for various membranes](image)
Reference:


