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Decoration of heparin and bovine serum albumin on polysulfone membrane assisted via polydopamine strategy for hemodialysis

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ABSTRACT

Renal failure brings about abnormality of waste and toxins and deposition in the body. In clinic, the waste and toxins *in vitro* are eliminated by hemodialysis device with polysulfone (PSF) porous membranes. In the work, decoration of heparin (Hep) and bovine serum albumin (BSA) on PSF membranes would be beneficial to improve the hemocompatibility and reduce the anaphylatoxin formation during hemodialysis. The PSF porous membranes are surface-modified by simply dipping them into dopamine aqueous solution for 8 h. Then, Hep and BSA are immobilized covalently onto the resultant membrane. Attenuated total reflectance Fourier transform infrared spectra (ATR-FTIR) confirms that Hep and BSA are successfully introduced onto the surface of PSF membranes. Scanning electronic microscopy (SEM) and atomic force microscopy (AFM) display the changes of surface morphologies after modification. The result of water contact angle measurement shows that the hydrophilicity of PSF membranes is remarkably improved after coating polydopamine (pDA) and binding Hep and BSA. The experiments of hemocompatibility indicate that Hep and BSA grafted onto membranes suppress the adhesion of platelet and enhance the anticoagulation ability of PSF membranes. Furthermore, the protein adsorption tests reveal that Hep and BSA immobilized onto membranes depress the protein adsorption and develop antifouling-protein ability of pristine membrane. This study proves a convenient and simple approach to graft two functional organic polymers which, respectively, play a vital role and then improve the hemocompatibility and biocompatibility of PSF membranes for their biomedical and blood-contacting applications.

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1. Introduction

With development of porous polymeric membranes in advanced separation technology, PSF membranes, due to their good chemical resistance, thermal stability, mechanical, and film-forming properties, have been widely used in multitudinous fields such as blood purification and water treatment. For blood purification, dialysis membrane (PSF) plays a key role in clearing urine poison such as creatinine and urea nitrogen from the blood as same as kidney. However, clotting, protein fouling, and anaphylatoxin bring limitation of clinical application for PSF membrane. At present, numerous researches have been performed to modify PSF membranes, such as physical treatment, grafting and coating, and much effort have been made to enhance the fouling resistance and blood compatibility of PSF membranes.[1–4] Therefore, surface modification of membranes has been widely employed to develop the hemocompatibility and biocompatibility of membranes for its application.

Hep, a kind of highly sulfated glycosaminoglycan, is widely utilized as a common and efficient anticoagulant. The Hep plays a dominating role in the blood coagulation resistance properties for its combination with antithrombin III (ATIII) which results in rapid inactivation of coagulation factors. Many studies have been made on the surface heparinization of hemodialysis membranes which showed improved blood compatibility. Fu et al. [5] grafted Hep onto the surface of polylactic acid (PLA) porous membrane via self-polymerization of dopamine in the aqueous solution. Zhu et al. [6] immobilized Hep onto the surface of PE membranes based on the self-polymerization and strong adhesion characteristic of dopamine, and the results of *in vitro* hemocompatibility tests proved that surface modification improved the permeability and biocompatibility of inert polyethylene (PE) porous membranes for their biomedical and blood-contacting application.

Serum albumin manufactured by liver is a protein of the highest concentration in plasma, which plays an important role in maintaining the osmotic pressure of blood. Otherwise, serum albumin, a highly specific functional biopolymer, can be used as a polymeric material. Yang et al. [7] modified polyacrylonitrile (PAN) membrane by covalently immobilizing human serum albumin (HSA) onto the surface of membrane to evaluate the hemocompatibility and anaphylatoxin formation. Zhao et al. [8] grafted acrylonitrile-co-acrylic acid to PSF membrane, followed by immobilizing BSA onto the surface, then the absorption protein of the modified membrane significantly decreased.

In conclusion, previous researches either utilized Hep for reducing blood clotting or employed BSA or HSA for avoiding absorption protein. There is no research simultaneously focusing on blood clotting, protein fouling, and anaphylatoxin during the dialysis. Inspired by the strong adhesion and cohesion behaviors of mussel proteins, it was found that dopamine was able to self-polymerize in aqueous solution and forms strong attachment to the substrates.[9,10] What was far more important, the active layer of pDA provided a versatile platform for further surface modification. Therefore, a facile and convenient approach for surface modification of substrates was put forward. In the present study, PSF porous membranes are coated by immersing the membranes into dopamine aqueous solution for a certain time, and then both Hep and BSA are simultaneously grafted onto the surfaces of PSF/ pDA composite membranes via coupling to the active layer of pDA. The effects of surface modification on morphology, hydrophilicity, hemocompatibility, and biocompatibility of PSF membranes are investigated in detail.

2. Materials and methods

2.1. Materials

Pristine PSF (MWCO = 30,000) was purchased from SEPRO (Georgia, USA). 3,4-dihydroxyphenethylamine (dopamine) and Tris (hydroxymethyl) aminomethane were bought from Sigma-Aldrich (St. Louis, USA). Phosphate buffer (PBS), Bovine serum albumin (BSA, purity of $\geq 96\%$), and Hep sodium (185 USP) were from Aladdin (Shanghai, China). All other chemicals were commercially analytical grade and were used as received unless noted. All aqueous solutions were prepared with deionized water (DI water).

2.2. Surface modification of porous PSF membranes

Dopamine was dissolved in Tris-HCl buffer solution (10 mM, pH 8.5) (2.0 g/L). The clean PSF membranes were immersed into the dopamine solution and shaken at 37 °C for 8 h. Then, the pDA-modified membranes were taken out and washed with DI water to remove the unattached dopamine molecules (denoted as PSF/pDA). The pDA-modified membranes were used for characterization and further surface modification. Hep (2 g/L) and (or) BSA (1 g/L) solution were beforehand prepared by dissolving Hep and (or) BSA in PBS buffer solution (pH 7.4). The PSF/pDA membranes were immersed in the mixed solution and single solute solution for 24 h at 37 °C in a constant temperature shaker (ZHWY-103B, Zhicheng Analytical Instrument Manufacturing CO. Ltd, China). Then, the membranes were taken out and rinsed with DI water sufficiently so as to remove physically adsorbed Hep and BSA (named as PSF/pDA-Hep, PSF/pDA-BSA and PSF/pDA-Hep/BSA). After dried under nitrogen influx, the resultant membranes were used for characterization.

2.3. Surface characterization of the resultant membranes

The morphologies of the samples were observed by a scanning electron microscope SEM (JSM-7701F, JEOL, Tokyo, Japan). An atomic force microscopy (AFM, BRUKER-Dimension ICON, USA) was applied to determine the surface roughness. Attenuated total reflectance Fourier transform infrared spectra (ATR-FTIR, Bruker-Vetor 22, Germany) were used to detect any changes of functional groups on PSF membrane surfaces before and after modification. The hydrophilic properties were investigated using the static water contact angles. Static water contact angles were measured at room temperature, by the sessile drop method with Dataphysics OCA20 contact angle system (Filderstadt, Germany). The average of six parallel specimens was taken.

2.4. Protein absorption assays

The fouling behaviors of all membrane samples were studied by BSA adsorption and filtration experiment. In the BSA adsorption experiment, the pristine and modified membranes were immersed in PBS (pH 7.2, with hydrochloric acid) solutions containing BSA (1 mg/mL), and incubated at 37 °C for 60 min, subsequently the incubated membranes were taken out. The protein concentrations of incubated solutions were measured by Pierce BCA protein kit. The adsorbed protein amounts were calculated via standard curve of protein.

Protein adsorption was calculated as follows:

$$\text{Protein adsorption} = (C1 - C2)/S$$

where C1 is the protein concentration of pristine solutions, C2 is the protein concentration of incubated solutions, and S is the area of the membranes.

Moreover, fluorescence staining assay for BSA adsorption was also used for the evaluation of BSA adsorption. The membranes were immersed in PBS (pH 7.2, with hydrochloric acid) solutions containing fluorescent-labeled BSA (1 mg/mL). After 60 min incubation at 37 °C, the membranes were taken out, and enwrapped in aluminum-foil paper. Then confocal laser scanning microscopy (LSM5, Carl Zeiss, Oberkochen, Germany) was used to observe the samples.

During the filtration of BSA solution, BSA solution (1 mg/mL) was fed into the filtration apparatus (Mosu, Shanghai) and the filtration was performed for 10 min under 0.15 MPa at room temperature. The membrane permeate flux was obtained by measuring the volume of permeate liquid at the interval of 10 min.[11]

2.5. Hemolysis test

Healthy human blood from volunteers containing sodium citrate (3.8 wt. %) at a ratio of 9:1 was taken and diluted with normal saline (4:5 ratio by volume). The pristine and modified membranes were dipped in separate standard tubes containing 10 ml normal saline and incubated at 37 °C for 30 min. Then, 0.2 ml of diluted blood was added to these standard tubes and the mixtures were incubated at 37 °C for 60 min. Similarly, normal saline solution was used as a negative control, and DI water as a positive control. After this period, all membranes were removed and all the tubes were centrifuged at 800 g for 5 min. The supernatant from each tube was transferred to a well in a 96-well plate (Corning, USA) where the absorbance was measured with a microplate reader (Bio-Rad 680) at 545 nm. In addition, the hemolysis percentage was calculated based on the average of three replicates and calculated as follows:

$$\text{Hemolysis percentage} = (\text{OD (test)} - \text{OD (negative control)}) / (\text{OD (positive control)} - \text{OD (negative control)}) \times 100\%, \text{OD (optical density)}$$

2.6. Platelet adhesion

Platelet-rich plasma (PRP) was prepared by centrifuging whole human blood at 1000 rpm for 15 min. The PRP covered the surfaces of the experimental samples (1 cm × 1 cm) and then the samples were incubated at 37 °C for 1 h. After 1 h, the samples were gently rinsed with phosphate buffered saline (PBS) to remove non-adherent platelets. They were then fixed in 2.5% glutaraldehyde solution for 2 h at 4 °C, followed by dehydration in a gradient ethanol/distilled water mixture from 30 to 100% in 10% increment for 10 min each, and finally freeze-dried for 2 days. After the surfaces of samples were coated with gold, they were observed by SEM.

2.7. In vitro coagulation time measurement

Platelet-poor plasma (PPP) was prepared by centrifuging the whole human blood at 4000 rpm for 15 min. Then, 0.5 mL PPP was dropped onto the surfaces of the samples

(1 cm × 1 cm). After incubation for 30 min at 37 °C, the APTT (activated partial thromboplastin time) and TT (thrombin time) measurements were performed by adding 500 µl incubated PPP solution in a test tube with automatic blood coagulation analyzer (ACL TOP, USA). The average of three measurements was taken.

2.8. Cytocompatibility

2.8.1. Cell culture and Cell viability assay

The pristine and modified PSF membranes (diameter = 15.6 mm) were placed in a 24-well cell culture plate (Corning, USA), human umbilical vein endothelial cells (HUVECs, union cell bank, Beijing, China) were then seeded in the 24-well plate at a density of 5×10^4 cells in each well filled with 800 µl of culture medium supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco, USA) and 1% (v/v) antibiotics mixture. Subsequently, the plate was incubated in a humidified atmosphere of 5% CO₂ at 37 °C (incubator, MCO-18AIC, Japan).

Cell proliferation behavior of the samples was evaluated by the cell counting kit-8 assay (CCK-8, Dojindo, Japan). After incubating the cells in a humidified atmosphere with 5% CO₂ at 37 °C for 1, 3, and 5 days, the samples were gently rinsed with PBS for three times, then 300 µl mixture supplemented with DMEM and CCK-8 (v/v, 10:1) was added to each well and incubated for 2 h at 37 °C. After 100 µL incubated medium was added to each well of a 96-well plate, the measurement of the absorbance value was performed by a spectrophotometer (Model 680, Bio-Rad, Hercules, Canada) at a 450 nm wavelength. Three parallel specimens were used to provide an average and standard deviation.

2.8.2. SEM for cell morphological examination

For SEM observation, the cells (HUVECs) were cultured for 5 days on the surfaces of modified membranes. The membranes with cells cultured rinsed with PBS buffer to remove the unbound cells and then soaked in the fixative of 2.5% glutaraldehyde at 4 °C for 2 h. The samples were then dehydrated by replacing a series of buffer solutions with increasing concentrations of ethanol (35, 50, 70, 80, 90, 95 and 100%) for 10 min, respectively, and dried at room temperature. After the surfaces of all samples were coated with gold, SEM images were obtained by the SEM (JSM-7701F, JEOL, Tokyo, Japan).

2.9. Statistical analysis

All data were expressed as mean standard deviations of three similar experiments carried out in triplicate. The *T*-test method was used to determine the significant differences among the groups, and *p*-values less than 0.05 were considered statistically significant.

3. Results and discussion

3.1. Preparation of the resultant membranes

PSF membrane, pristine membrane, simply immersed into dopamine aqueous solution were first coated by pDA through its strong adhesion, and then the Hep and BSA were immobilized on the surface of PSF membrane coated by pDA via the covalent bonding between phenolic hydroxyl groups of pDA and imino or amino groups from Hep and BSA. The possible decoration-mechanism was depicted in Figure 1.

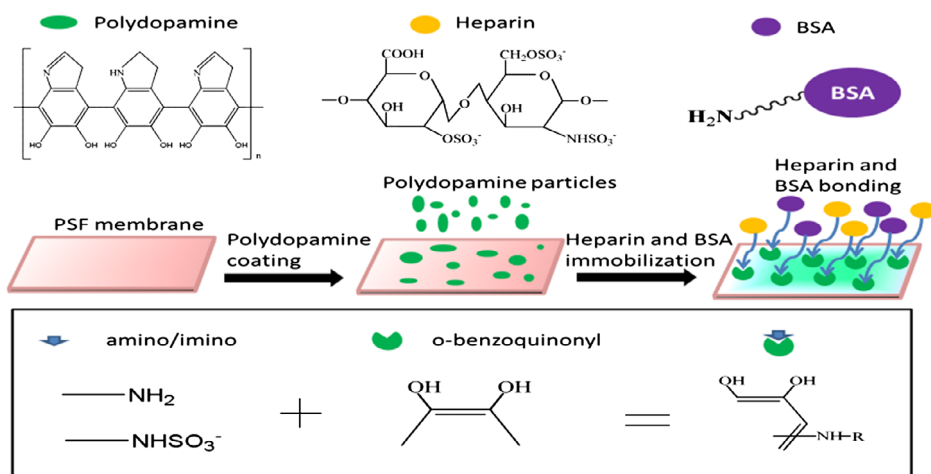


Figure 1. Schematic diagram showing dopamine-coating and heparin/BSA-grafting onto the surface of PSF membrane.

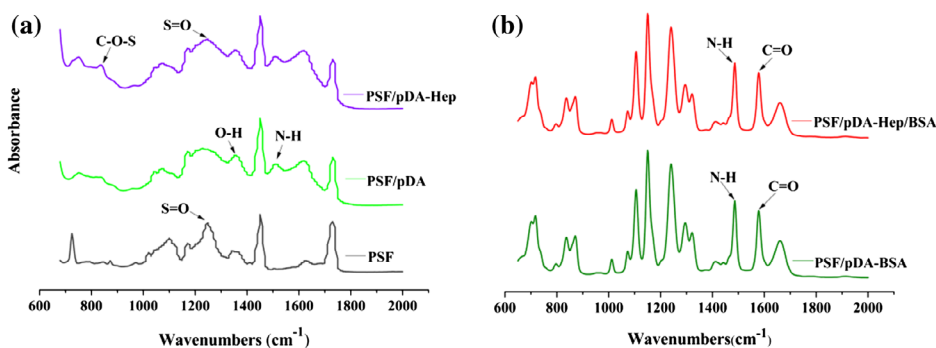


Figure 2. The spectrum of the different membranes by ATR-FTIR.

3.2. Characterization of surface

3.2.1. Membrane surface compositions

The changes in surface chemistry of the pristine and modified membranes were detected by ATR-FTIR. As shown in Figure 2, the spectrum of pristine PSF membrane displayed characteristic absorption peak at about 1250 cm^{-1} , which was attributed to S=O bond. After coating dopamine, new absorption peaks at about 1350 cm^{-1} (O-H bending vibration) and 1510 cm^{-1} (N-H bending vibration) indicated the existence of dopamine on the PSF membrane surface. Compared with the PSF membrane coated by dopamine, the characteristic peaks at about 600 cm^{-1} and 1250 cm^{-1} were caused by C–O–S and S=O vibrations, which suggested Hep was successfully grafted onto the surface of PSF membrane. As seen from the PSF/pDA-BSA and PSF/pDA-Hep/BSA curves in Figure 2, the characteristics of the two curves had no obvious difference and new absorption peaks appeared at about 1550 and 1650 cm^{-1} , which was attributed to N–H and C=O of peptide bond. These results might

imply that when both Hep and BSA were simultaneously immobilized on the surface of PSF membrane, the spectrum of BSA molecules showed stronger characteristic bands than Hep.

3.2.2. Membrane surface morphology

The coating process had some effect on the membrane surface morphology. SEM and AFM were used to observe the changes in surface morphology, which are presented in Figure 3. It could be found that there were no coating layer for the pristine PSF membrane and it seemed to be smoother than other membranes in the Figure 3(a). However, compared with pristine PSF membrane, the pDA particles could be clearly observed, owing to the coating of pDA layer. Via pDA grafting Hep, the SEM images exhibited some wrinkle-like structures by contrast with the pristine PSF membrane. Ma, Lang, et al. reported that the wrinkle-like structure became more and more dense as the grafting-Hep time increased via dopamine.[12] Meanwhile, there were some larger particles when BSA was grafted on the pDA-coating membrane. After Hep and BSA simultaneously grafting, large particles alternated with smaller particles and some wrinkle-like structures seemed to disappear. It also could be seen that the membranes modified by Hep or (and) BSA became rougher than pristine PSF membrane, and heparin and BSA simultaneously immobilized, Hep was covered by BSA molecule, which was in accordance with the results of ATR-FTIR.

The three-dimensional AFM surface topography pictures for different membranes are presented in Figure 3(b). It could be observed that the surface roughness of the membrane coated by pDA increased by 49.4% versus the pristine PSF membrane control surface, which might be caused partial aggregates of polymerized dopamine. After Hep or (and) BSA immobilization on the surface of PSF/pDA, the surface roughness of would descend to 35.8, 64.2, and 44.3 nm, respectively. These phenomena suggested that the surface modification including pDA coating and Hep or (and) BSA grafting had significant influence on the surface topology and roughness of PSF membranes and BSA molecule played a crucial role in membrane roughness rather than Hep, which was in agreement with the results of SEM. Therefore, the modification by Hep and BSA smoothened the PSF membrane surface and improved hemocompatibility and antifouling ability of PSF membrane.

3.2.3. Water contact angle

The wetting or non-wetting property was important for biomaterials, particularly for materials contacting blood for hydrophobic materials might cause thrombosis and inflammation when contacting blood. Water contact angle was a regular method to characterize the hydrophilicity and hydrophobicity of materials. The results of water contact angle of all membranes are shown in Figure 4. It could be observed that the contact angle of all coated membranes decreased in some degree, which indicated that they became more hydrophilic after the modification, and the water contact angle of membrane immobilizing Hep and BSA (PSF/pDA-Hep/BSA) was placed in the middle of PSF/pDA-Hep and PSF/pDA-BSA. These results suggested that both Hep and BSA molecules had hydrophilic characteristic and Hep was more hydrophilic than BSA.

3.3. Hemolysis

Hemolysis, an especially significant test detecting the negative influence for erythrocyte, was used to assess the hemocompatibility of materials. A high plasma hemoglobin level

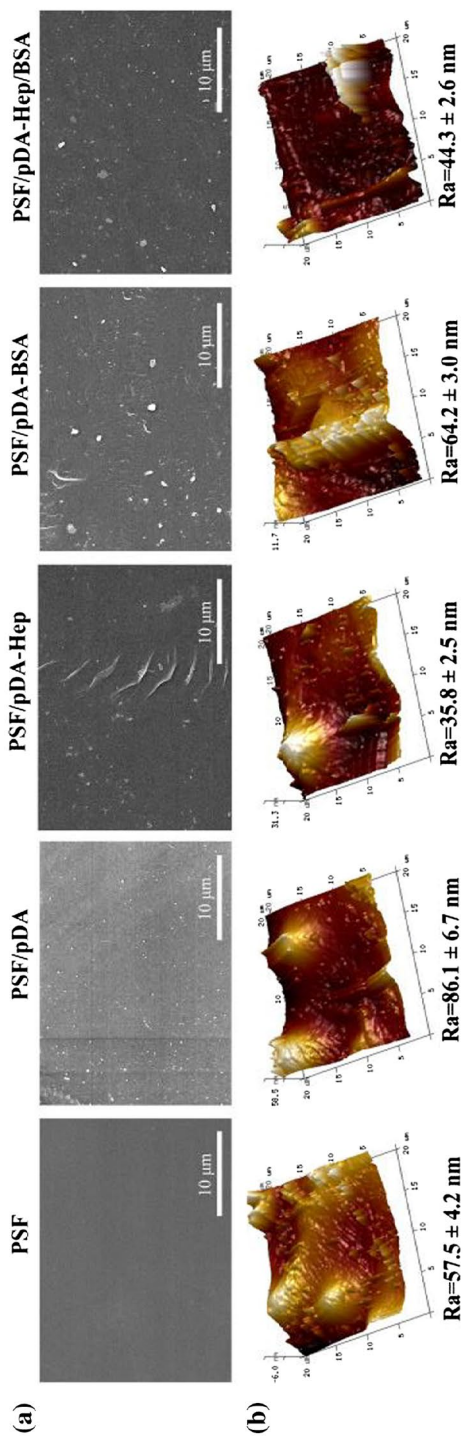


Figure 3. Surface SEM images and Surface AFM topography of the different membranes.

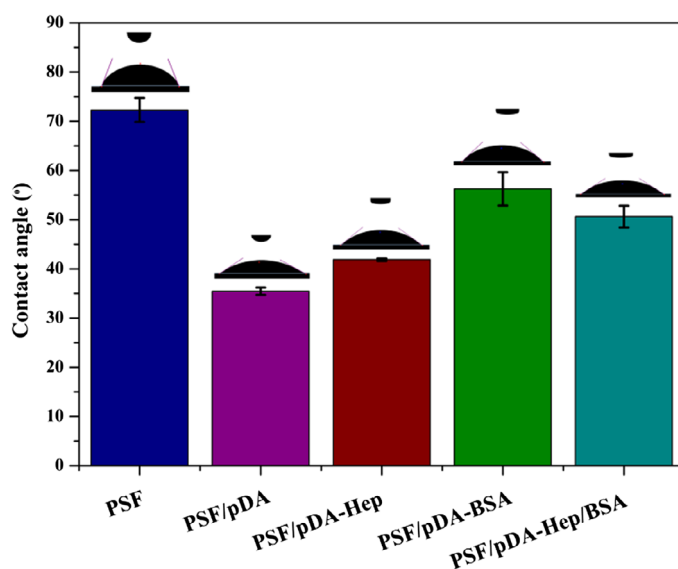


Figure 4. Water contact angle of the different membranes.

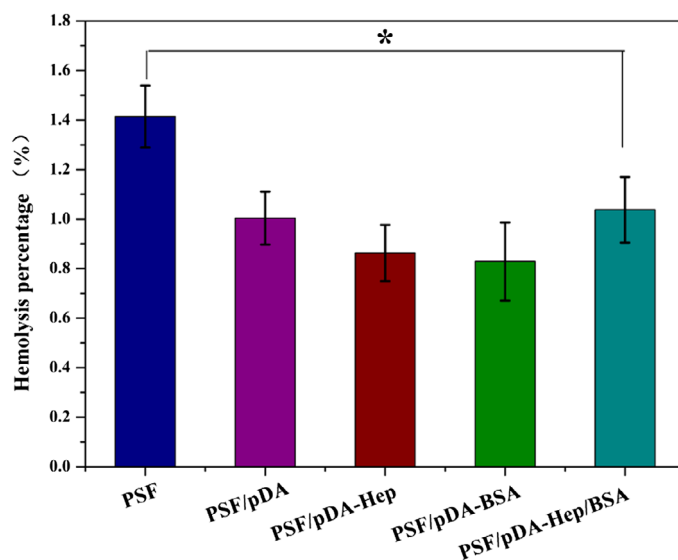


Figure 5. Hemolysis ratio for the different membranes.

normally indicated fragility of erythrocyte membrane in contact with materials. Figure 5 showed hemolysis ratio (HR) of all samples. It was obvious that the HR of all modified membranes was approximately 1% and had no visible difference with each other. These indicated that Hep and BSA could weaken the damage to erythrocyte and further the membranes immobilizing Hep and BSA possessed good hemocompatibility in a way.

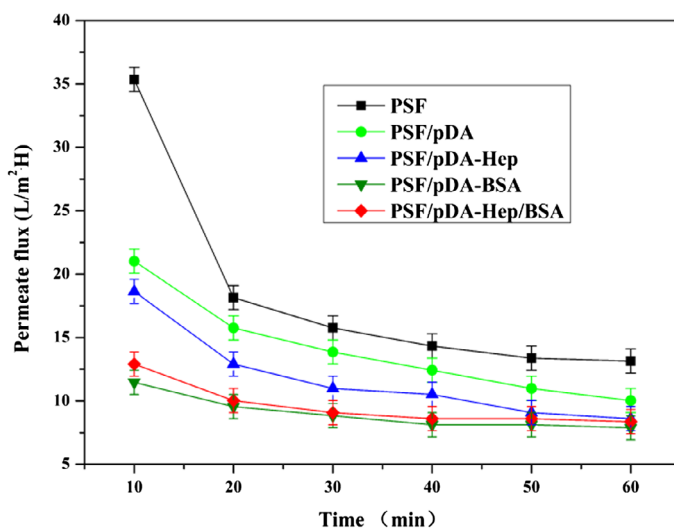


Figure 6. Permeate flux decline behavior during filtration of BSA solution. BSA concentration: 1 g/L. TMP: 0.15 MPa.
Note: Room temperature.

3.4. Protein adsorption

When blood for purification came into contact with the membrane surface, plasma proteins from blood, such as albumin, rapidly adhered to the interface between blood and membrane, which might bring back further negative influence for hemodialysis. Protein adsorption, a dominant factor for fouling, was also considered to be one of key factors to evaluate the blood compatibility of materials. It was universally known that there were many factors affecting the interaction between membrane surface and protein, such as surface charge, surface free energy, surface structure, solution environment, and protein characters. Many researchers have put forward the solution idea to increase the hydrophilicity of membrane and reduce protein adsorption, because they thought that the hydrophilic membrane surface preferentially adsorbed water rather than plasma proteins.[13,14]

In the current work, the interface between blood and studied membrane was related to the adsorption of BSA *in vitro*, since BSA was one of typical plasma proteins which might represent direct connection with the blood compatibility of membrane. As shown in Figure 6, the typical permeate flux declined for all membranes during BSA solution filtration and the membrane coated by BSA or Hep/BSA had a significantly slower flux decline rate than the others, which implying lower protein adsorption. In Figure 7, it was observed that the intensity of fluorescence in the PSF/pDA-BSA and PSF/pDA-Hep/BSA membranes was weaker in contrast with other samples, which suggested that they had less protein fouling. Figure 8 displayed the amounts of BSA adsorbed on the membrane surfaces from the respective PBS solutions. The BSA adsorption amounts for the pristine PSF membrane were higher, while that was relatively low for the membranes decorated by BSA or Hep/BSA. Hence, these results demonstrated that grafting BSA molecules could depress the protein adsorption for the PSF membranes.[8] It might be because grafting

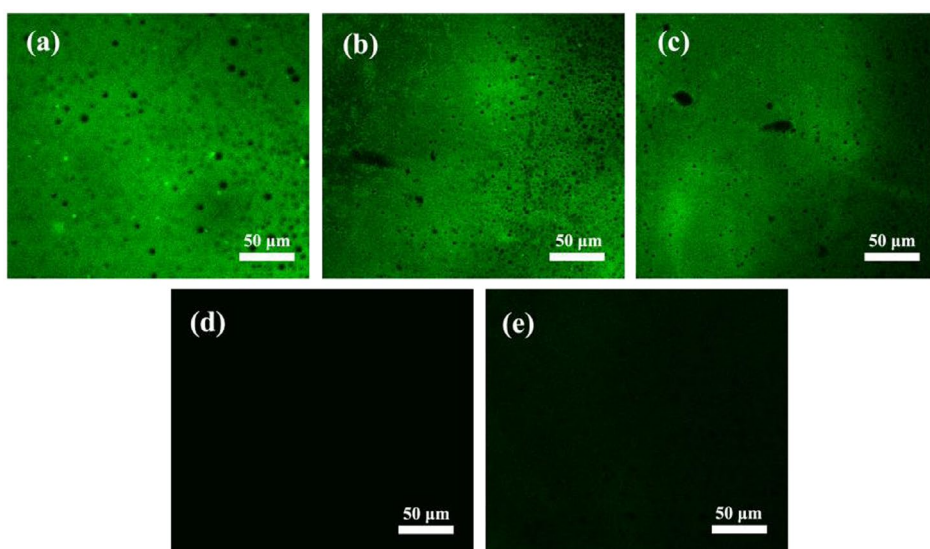


Figure 7. Fluorescence staining for BSA adsorption on the surfaces of pristine PSF (a), PSF/pDA (b) PSF/pDA-Hep (c), PSF/pDA-Hep (d) and PSF/pDA-Hep/BSA (e).

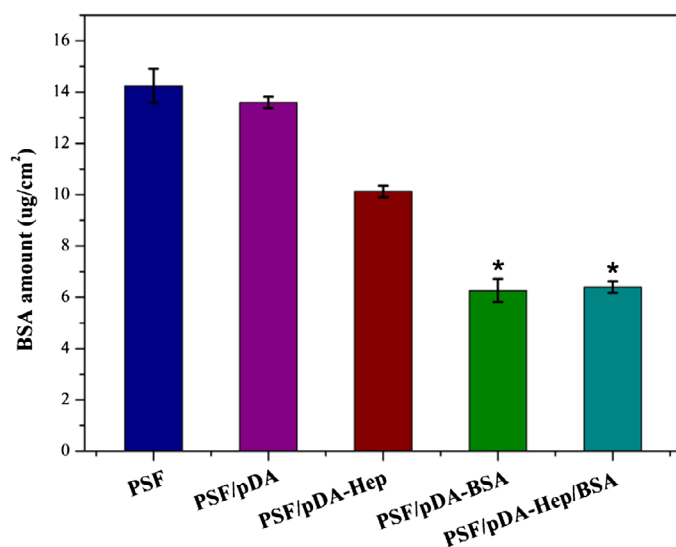


Figure 8. Amounts of BSA adsorption on the surfaces of different membranes.

BSA molecules on membrane surface prevented more proteins from contacting the PSF substrate membrane by the chain movement. Besides, the improvement in the membrane hydrophilicity by BSA grafting weakened membrane protein fouling. The lower protein adsorption for membranes decorated by BSA or Hep/BSA implied that they might have good blood compatibility, which would be further examined by platelet adhesion and clotting time.

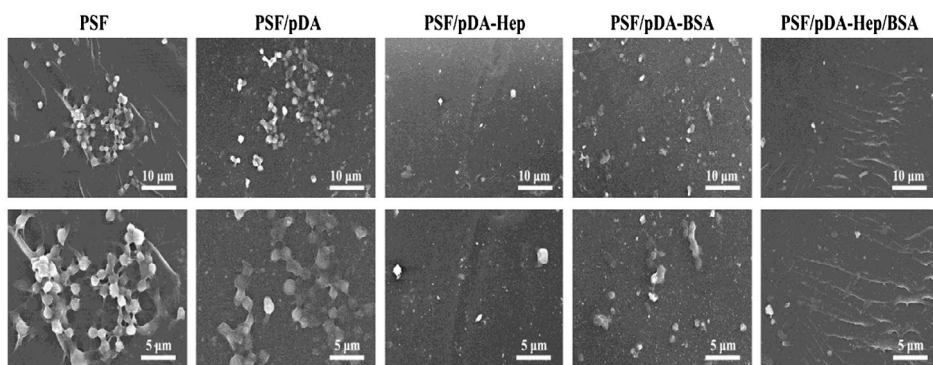


Figure 9. Representative SEM images of human blood platelet adhesion on the surface of the different membranes with the magnification of 2000 $\times$ and 4000 $\times$.

3.5. Platelet adhesion

Platelet adhesion on the surface of membrane was another main factor in evaluating hemocompatibility. When blood contacted with the surface of membrane, the preliminary response of blood was the adsorption of plasma proteins, then platelets adhered to the surface and were activated, leading to thrombus. However, for some specially modified membranes, the platelet deformation of outspread and pseudopodium was obviously suppressed. As exhibited the representative SEM images (with magnification of 2000 $\times$ and 4000 $\times$) of human blood platelet adhesion on the membrane surface in Figure 9, many adhered platelets with deformation and pseudopodium were observed and they were aggregated and spread in irregular sharps on the surface of pristine PSF and PSF/pDA membranes, which suggested that the thrombus formation had occurred possibly. However, the adhered platelets with deformation and pseudopodium were rarely observed on the surface of other membranes. The adhesion of platelets for PSF/pDA-Hep/BSA was not different from PSF/pDA-Hep and PSF/pDA-BSA. It was worth noting that on the surface of membrane Hep or BSA immobilized, no spherical morphology of platelets was seen. Moreover, it was obvious that the number of adhering platelets on the PSF/pDA-Hep or PSF/pDA-BSA was less than pristine PSF membrane after 1-h incubation in Figure 10. These results suggested that the PSF membrane-surface-attached Hep and BSA not only formed a natural barrier which resisted non-specific protein adsorption, but also significantly suppressed the adhesion, activation, and deformation of platelets, hence showing improved hemocompatibility.[5,6,15]

3.6. Blood clotting time

Blood clotting time was usually applied for examining the hemocompatibility of hemodialysis membranes. Generally, APTT was used to display the intrinsic pathway of blood coagulant system. TT was utilized to measure the clotting formation time that thrombin converted fibrinogen into fibrin during the blood coagulation. Both APTT and TT for the pristine and modified PSF membranes are presented in Figure 11. It was shown in Figure 11 that the clotting times of PSF/pDA-BSA did not remarkably increase than the control or pristine membrane. It implied that BSA molecules exhibited a low anticoagulant activity. However, PSF/pDA-Hep prolonged the clotting times. Also, the clotting times of PSF/

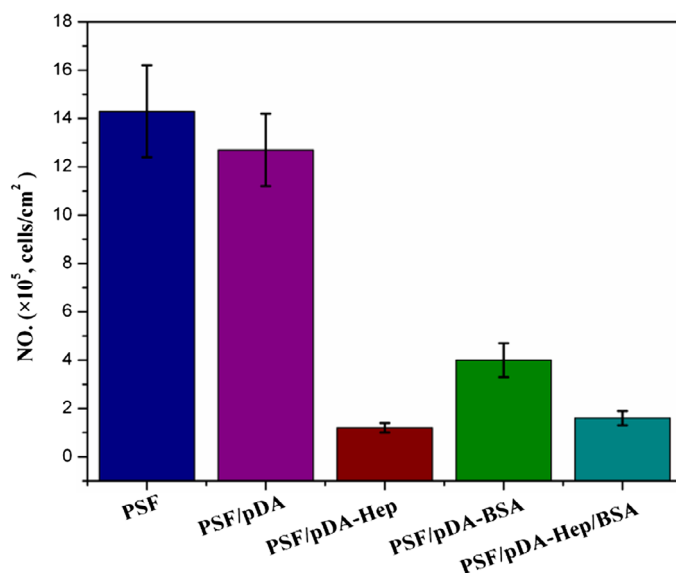


Figure 10. The number of adhered platelets of the different membranes.

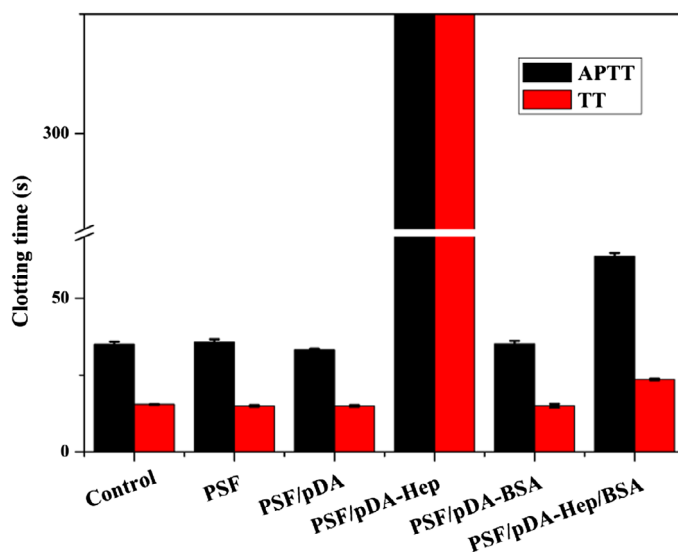


Figure 11. APTT and TT values of the different membranes. The results are expressed as means $\pm$ SD ($n = 3$). Notes: The marks (*) meant that the clotting time in PSF/pDA-Hep/BSA was significantly increased compared to the pristine PSF. * $p < 0.05$.

pDA-Hep/BSA slightly increased, in comparison with the control or pristine PSF membranes. It is well known that Hep, a clinical anticoagulant, inhibits platelet adhesion and aggregation and suppresses coagulation enzyme and thrombus, which play a vital role in coagulation of blood.[16,17] For PSF/pDA-Hep/BSA, it might be heparin covered by BSA macromolecules resulted in that the most of active groups of heparin, having dominant role on blood clotting, lost function. Therefore, there was the extreme prolongation of

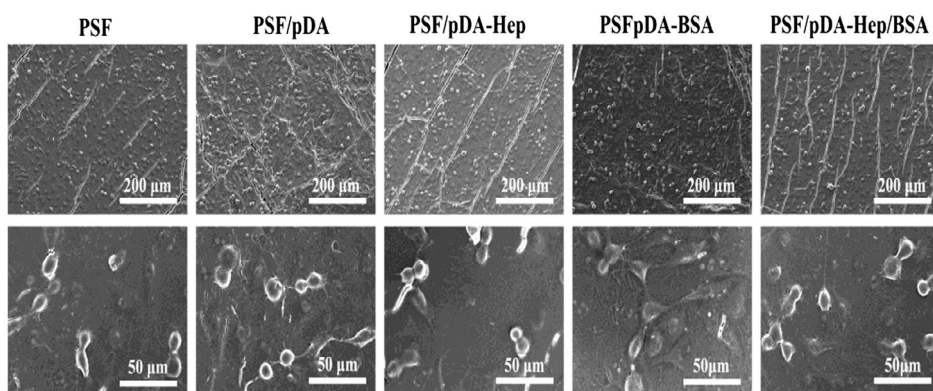


Figure 12. The morphologies of the HUVECs observed by SEM.

APTT and TT for PSF/pDA-Hep compared with PSF/pDA-Hep/BSA.[5,15] In this study, the approach immobilizing Hep and BSA on membrane surface might be an effective and facile way to prolong the clotting time and suppress plasma proteins, leading to significant improvement of the hemocompatibility of membrane for hemodialysis.

3.7. Cytocompatibility

PSF, one of desired biomaterials has been widely employed in the field of blood purification in clinical for its excellent biocompatibility, physical properties, and chemical properties. In this study, the PSF membranes immobilizing Hep and BSA via the strong adhesion of pDA were expected to exhibit good cytocompatibility. HUVECs were selected for estimating the cytocompatibility of the modified membranes. In general, when contacting with biomaterials, cells would undergo their morphological changes for fitting the interface between cells and biomaterials. For the good cytocompatibility of decorated membranes, the HUVECs easily adhered and spread on the membrane surface.

3.7.1. Cell morphology

Figure 12 presented the morphologies of HUVECs cultured on the pristine and modified PSF membranes at 37 °C for 4 days. The figure showed that the number of HUVECs grown on the pristine PSF membrane was the least and there was little difference for other samples, which indicated that the adhesion of the pDA, Hep, and BSA on the PSF membrane surface could promote cell attachment and growth. That might be because some bioactive groups from Hep and BSA containing good biological compatibility facilitated the adherence of HUVECs to PSF membranes. In addition, dopamine, the mussel-inspired molecules also could help HUVECs adhere on the PSF membrane. The results implied that the modified membranes might promote the adhesion and spreading of HUVECs. Zhao et al. [12] reported similar finding via pDA-grafted heparin-like polymers and heparin.

3.7.2. CCK-8 assay

CCK-8 assay was a common method to measure cell cytotoxicity, proliferation, and activation used *in vitro*. The light absorbance indicated that the HUVECs seeded onto all the

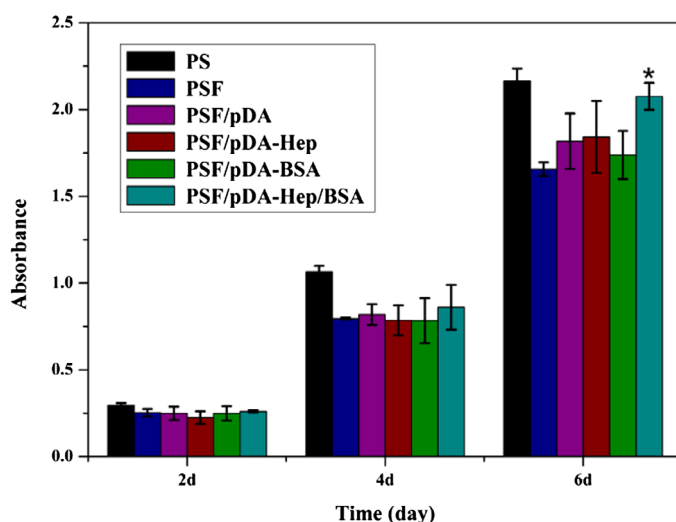


Figure 13. The CCK-8 assay of the cell cultured membranes. The values were expressed as means $\pm$ SD ($n = 6$).

Notes: The marks (*) meant that the difference between PSF/pDA-Hep/BSA and PSF was statistically significant for 6 days. $*p < 0.05$.

samples were able to convert CCK-8 into a colored product. The results of the CCK-8 assay on the second, fourth, and sixth days for all the samples are shown in Figure 13. It was observed that on the second and fourth days, the viability of the HUVECs for pristine and decorated membranes had no significant difference. However, the PSF/pDA-Hep/BSA membrane exhibits the best cytocompatibility among all the membranes on the sixth day. It was widely believed that surface of substrate with hydroxyl and amine groups was often beneficial to the adhesion, spreading and growth for cells, having a positive influence on the cytocompatibility of the substrate. BSA, Hep, and pDA contained abundant hydroxyl and amine groups possessing the special adhesion function.[12,15] Therefore, it could be concluded that PSF/pDA-Hep/BSA may have the potential to be used for various biological applications.

3.7.3. Fluorescence staining

The viability of cells for the pristine membrane and decorative membranes was evaluated with fluorescent microscopy [18]. Figure 14 showed the fluorescence staining of the HUVECs cultured for 1 day and 5 days on the all samples. It could be easily observed that the amounts of HUVECs on the pristine PSF membranes were relatively low for 1 day and for 5 days; the number of cells on the membranes grafting BSA was different from other coated membranes. This could be explained as follow: the cell attachment and growth have been improved with the coating of pDA and grafting of Hep/BSA for the special adhesion behavior of hydroxyl and amine groups [15] and BSA molecules seemed to play greater role than Hep for cell viability. Thus, it could be concluded that the PSF/pDA-Hep/BSA membrane exhibited good cytocompatibility and had the potential to be used for blood purification, hemodialysis, or other biological applications.

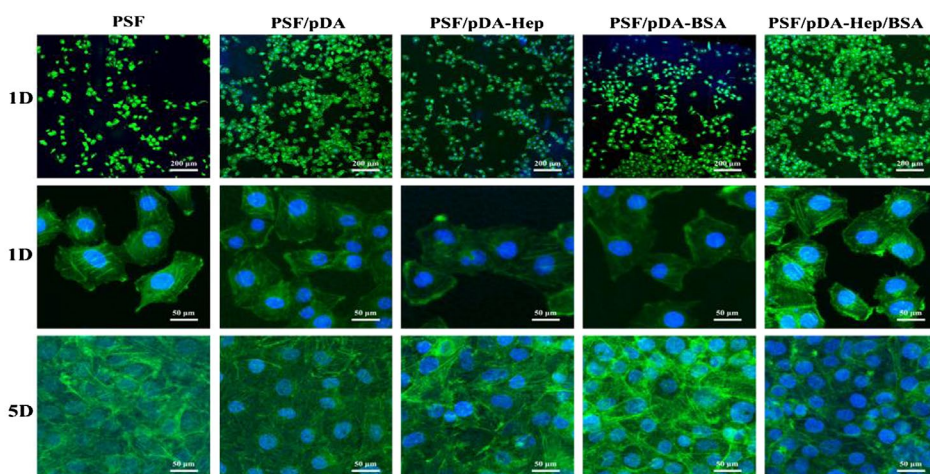


Figure 14. Confocal micrographs of the human umbilical vein endothelial cells (HUVECs) cultured for one day and five days on the different membranes with diameters of 50 and 200 μm . (Please see the online article for the colour version of this figure: <http://dx.doi.org/10.1080/09205063.2016.1169479>.)

Note: Phalloidin was colored green and nuclei were colored blue.

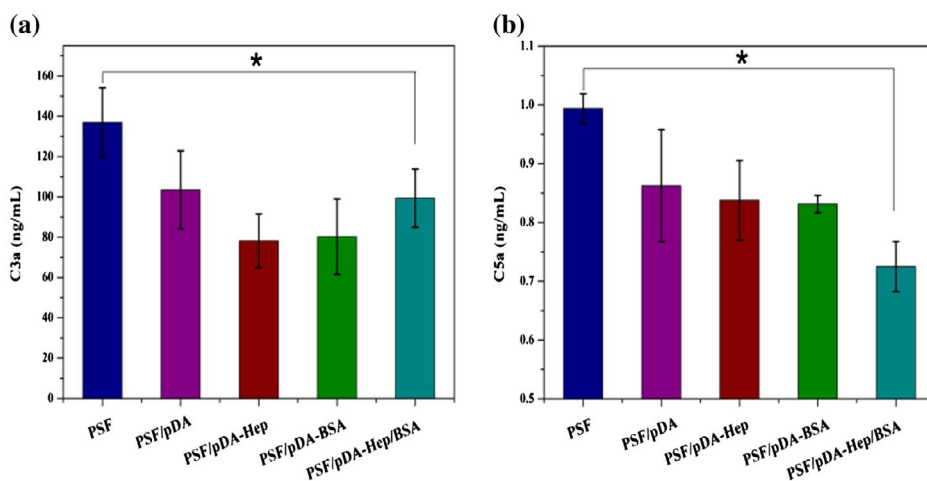


Figure 15. Concentrations of serum C3a and C5a in the samples of whole blood. The results were expressed as means $\pm$ SD ($n = 3$).

Note: The marks (*) meant that the difference between PSF/pDA-Hep/BSA and PSF was statistically significant. $*p < 0.05$.

3.8. Serum complement study

Serum complement activation which played a key role in immunity was used to assess the hemocompatibility of hemodialysis membranes *in vitro*. As shown in the Figure 15, the serum C3a and C5a concentrations for the modified membranes were different from the pristine membrane. The result indicated that both Hep and BSA immobilization could reduce the leukopenia for serum C3a and C5a. Yang et al. [7] modified polyacrylonitrile (PAN) membrane by covalently immobilizing human serum albumin (HSA) onto the

membrane surface and the decorated membrane induced less increase of serum C3a and C4a, which demonstrated that the immobilization of HSA and onto the surface of PAN membrane would be beneficial to reduce the anaphylatoxin formation and to improve the hemocompatibility during hemodialysis. Therefore, the grafting of Hep and albumin might not only mask the complement-activating sites leading to inhibition of the complement activation, but also reduce thrombosis during hemodialysis.

4. Conclusion

These results presented here displayed that dopamine was able to self-polymerize, cross-link on the surface of substrates in aqueous solution and then form a reactive layer tightly attaching to the surface of substrates. Through the reactive pDA layer, Hep and BSA were covalently immobilized on the membrane surface. After both Hep and BSA were simultaneously immobilized, the resultant membrane obtained good hemocompatibility regarding reduction in the platelet adhesion and protein adsorption, extension of the blood coagulation times, and decrease of anaphylatoxin formation. On the other hand, the hybrid membrane exhibited excellent cell compatibility in terms of cell adhesion, growth, and proliferation. Therefore, in order to obtain good hemocompatibility and cytocompatibility, this approach described above may be suitable for the surface decoration of blood-contacting materials.

Disclosure statement

No potential conflict of interest was reported by the authors.

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