Preparation and evaluation of heparin-immobilized poly (lactic acid) (PLA) membrane for hemodialysis

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A R T I C L E   I N F O

Article history:
Received 30 August 2013
Received in revised form 8 October 2013
Accepted 9 October 2013
Available online 19 October 2013

Keywords:
Poly (lactic acid) membrane
Hemodialysis
Heparinization
Bio-based material

A B S T R A C T

Bio-based poly (lactic acid) membrane with asymmetric porous structure was developed for hemodialysis via phase inversion for the first time. Heparin was immobilized to PLA membrane surface through the strong adhesion ability of dopamine, as confirmed by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy and X-ray photoelectron spectroscopy (XPS) respectively. The morphologies variations of PLA membranes induced by dopamine coating and heparin immobilization were analyzed by scanning electron microscope (SEM) and atomic force microscopy (AFM). Hydrophilicity and permeability of PLA membranes before and after modification were characterized. Particularly, platelet adsorption, plasma recalcification time and hemolysis ratio were executed to evaluate the blood compatibility of PLA membranes decorated by heparin. The in vitro results demonstrated that surface heparinization improved the hemocompatibility of PLA membrane, suppressed the adhesion of platelet, extended plasma recalcification time, and also decreased hemolysis ratio. The dialysis simulation experiments including urea and lysozyme clearance as well as bovine serum albumin (BSA) rejection were implemented to determine the dialysis performances. The results showed that 79% of urea and 18% of lysozyme were cleaned and over 90% of BSA was retained. This study disclosed a window of opportunity to produce novel hemodialysis membranes using bio-based materials.

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1. Introduction

Biomaterials have played an enormous role in the success of biological and medical applications, such as medical devices and drug delivery systems, tissue engineering, diagnostics and array technologies etc. [1].

Hemodialysis membrane which removes blood toxins by diffusion and convection across the porous membrane and restoring fluid and solute balance within the patient to some extent, also called as artificial kidney, is the most important material to treat the renal failure, especially for the end-stage renal disease (ESRD). Cellulose and its derivatives are the first generation of polymers which were used in dialyzers [2,3]; however, the weak hydraulic permeability and low molecular weight cutoff near 2000 daltons limits the prospect of cellulose-based materials. Synthetic polymers such as polysulfone, polyether sulfone, polyacrylonitrile, polyamide and poly(methylmethacrylate) were widely used and researched, which can be regarded as the second generation of dialysis membrane. Compared to cellulose membrane, synthetic fibers are 10 times more permeable and can be tailored to a range of molecular weight cutoffs. Nevertheless, the intrinsic hydrophobicity of synthetic polymer has adverse influences on the biocompatibility, which would directly result in the blood clotting. Therefore, surface grafting, coating or blending are usually needed to render the membrane hydrophilic and improve the biocompatibility [4–6]. Therefore, novel membrane materials with more comprehensive properties including better dialysis performances, biocompatibility, and controlled degradation processing after use will be developed in an urgent need.

Poly (lactic acid) or poly(lactide) (PLA) is the most extensively researched and utilized biodegradable and renewable thermoplastic bio-based polyester, with great potential to replace conventional petrochemical-based polymers [7–9]. However, few studies on PLA porous membrane preparation and application have been conducted. PLA and its copolymers, e.g. poly (lactic-co-glycolide), were fabricated into porous membranes for tissue engineering due to its eminent biocompatibility via thermally induced phase separation [10–13]. Tanaka et al. attempted to prepare PLA depth filter microfiltration membranes serving as screen filters and bacterial retaining via a combined process including nonsolvent induced and thermally induced phase separation [14,15]. In addition, PLA and its copolymers have also been converted into hollow fiber ultrafiltration membranes with asymmetrical structures, which exhibited high water permeability and good separation performances [16].
The biocompatibility of bio-based PLA also stirred up considerably discussion for the extensive application of sutures [17], devices for bone fracture fixation [18], and meshes for tissues engineering [19,20]. Although, previous studies have suggested wild response between PLA device and human tissue, the outcomes may alter with the change of material feature and special use-pattern. Thus, to regulate response condition, a number of modification technologies have been presented, in which, surface heparinization is one of effective ways to improve anticoagulant and antithrombotic property [21–23]. Researches stated that, with the synergistic effect of platelet factor III (AT3), heparin could not only act on thrombin to suppress the conversion reaction from fibrinogen to fibrin, but also prevent platelet adhesion and aggregation on the materials surface [24]. Both ionic adsorption and covalent bonding have been explored to fix heparin. For example, plasma processing was adopted to make the surface electropositive, followed by combine with electronegative heparin [25]. Besides, reactive groups such as amidogen or hydroxyl were also introduced to bind heparin covalently [26–28]. To avoid the complex implementation process of methods summed up above, polydopamine-coating based on its self-polymerization and strong adhesion feature attracted much attention as the most practical way to functionalize membrane surfaces [29–32].

Based on the state of art reviewed above, we developed a novel hemodialysis membrane originated from bio-based PLA for the first time, which can be regarded as the next generation of dialysis membrane. The PLA porous membrane was fabricated via the conventional phase inversion, and then immobilized with heparin onto the PLA membrane via polydopamine-coating. Surface chemistry, morphology, water contact angle, and water flux of PLA membranes were studied as a contrast after polydopamine-coating and surface heparinization were studied. The blood compatibility of PLA membrane was investigated in terms of platelet adhesion, extended plasma recalcification time, and hemolysis ratio in details. Finally simulation experiment including urea, lysozyme and bovine serum albumin osmosis was conducted to evaluate the dialysis performances of our PLA membranes.

2. Experimental

2.1. Materials

Poly (lactic acid) (crystalline < 5%, Mn=130–140 K Dolton, Natural works, US) was used to prepare dialysis membrane. Poly (ethylene glycol) (PEG, CP, Mv20000, Sino pharm, China) was used as pore-forming agent and flexibilizer and 1–methyl-2-pyrrolidinone (NMP, AR, Aladdin, China) as solvent. Dopamine hydrochloride, tris hydroxymethyl amino methane (tris), lysozyme, and bovine serum albumin obtained from Aladdin with the purity of AR were all directly used. Urea with the purity of AR was purchased from Sino pharm of China. Heparin sodium (BR) was supplied by Solar brio, China. The anticoagulant sheep whole blood was purchased from Beijing Pingrui Biotechnology Company, China.

2.2. Preparation of PLA porous membrane

PLA porous membrane was prepared via phase inversion. 18 g PLA and 5 g PEG were dissolved in 77 g NMP at 80 °C. After the air bubbles removed by vacuum deaeration at 70 °C, the formed homogenous solution was immediately casted onto a clean glass plate with a thickness of 150 μm blade, and coagulated in water bath at room temperature for 15 min. After that, PLA porous membranes were obtained for further modification and characterization after phase inversion and then soaked in deionized water to remove any residual solvent. Polysulphone (PSf) membrane as the control sample was also prepared via phase inversion with the quality ratio of PSf/PEG/DMAc = 18/5/77.

2.3. Heparinization of PLA porous membranes

A certain amount of dopamine was first dissolved in tris buffer solution (10 mM, pH8.5) to obtain dopamine solution (2.0 g/L, 1.0 g/L, 0.5 g/L), subsequently, PLA membranes (25 cm × 15 cm) were immersed in 300 mL dopamine solution and roundly shaken at 20 °C for 8 h. Subsequently, the membranes were taken out and washed with deionized water for 30 min. In the alkaline aqueous solution, the pyrocatechol group of dopamine will be oxidized into benzoquinone under the effect of dissolved oxygen. There is a disproportionation reaction between pyrocatechol and benzoquinone. The semiquinone radical is produced consequently and followed by the couple reaction. The result polymerization is called polydopamine. Polydopamine was finally coated on PLA membrane surface. The resultant polydopamine-coated PLA membranes were marked by DA2-M, DA1-M, DA05-M corresponding to the various dopamine concentration, and used for characterization and further surface heparinization, while the native PLA membrane without polydopamine coating was labeled as PLA-M.

The above polydopamine-coated PLA membranes were immersed into 200 mL heparin solution (2.0 g/L, PBS buffer solution as solvent, pH 7.4) at 4 °C for 24 h, a long time enough for the covalent bonding between heparin and polydopamine. Then the membranes were taken out and rinsed with deionized water for 30 min to remove instable heparin. Similarly, the resultant heparin-immobilized PLA membranes were named by HE2-M, HE1-M, and HE05-M. All membranes obtained were dried in the oven at 40 °C for any further characterization.

2.4. Characterization of PLA membranes

2.4.1. Surface chemistry and morphologies

Attenuated total reflectance Fourier transform infrared spectra (ATR-FTIR, Thermo-Nicolet 6700, US) were used to detect any change of functional groups on PLA membrane surface before and after modification. And the chemical compositions on surface were analyzed by the X-ray photoelectron spectroscopy (XPS, Shimadzu Axis Ulttratrdl, Japan), with Mg-Kα as radiation resource.

The morphologies of the cross section fractured in liquid nitrogen and top surface of the membranes were characterized by a scanning electron microscope (SEM, Hitachi S-4800, Japan). All samples were sputtered with gold for 2 min for observation. An atomic force microscopy (AFM, Dimension 3100V, Veeco, US) was carried out to determine the surface roughness.

![Fig. 1. Schematic diagram of single membrane dialysis system.](image-url)
2.4.2. Hydrophilicity and permeability measurement

Contact angle change with the drop age (2 µL) of PLA membranes was recorded by a water contact angle system (OCA20, Data physics, Germany) to measure the effects of surface modification on the hydrophilicity of membranes.

The pure water flux was measured by a commercial filtration apparatus (Saifei Company, China) with an effective membrane area of 24 cm². Membranes were pre-pressured at 0.2 MPa for 30 min before the flux was recorded every 5 min at 0.1 MPa until a stable water flux value obtained. The average value from three tests was obtained for each sample.

Fig. 2. Schematic diagram showing dopamine-coating and heparin-grafting onto PLA porous membranes.

2.4.3. Blood compatibility

2.4.3.1. Platelet adhesion. 10 ml anticoagulant sheep whole blood was added into centrifuge tube, followed by centrifuging at 1000 rpm for 10 min. Taking out supernatant with tubularis, the platelet rich plasma (PRP) was obtained. All membrane samples (1 cm × 1 cm) after washed with PBS buffer solution (pH 7.4) were added into the 24-well plate. 100 µL PRP was dropped on each sample using pipette, and then maintained at 37 °C for 1 h. When incubation

Fig. 3. FTIR spectra of the PLA-M, DA05-M, and HEP05-M.

Fig. 4. XPS spectra of PLA-M, DA05-M and HEP05-M.
was finished, the samples were rinsed in PBS twice to remove unstable platelet. 2.5 wt% glutaraldehyde was added into the solution for one night to fasten the adsorbed platelets. The samples were then dehydrated step by step with 50%, 75%, 85%, 95%, 100% (v/v) ethanol/water solution for 10 min in sequence. Platelet adhesion on PLA membrane was observed by SEM after freeze drying with liquid nitrogen.

### 2.4.3.2. Plasma recalcification time (PRT).

10 ml anticoagulant sheep whole blood was added into centrifuge tube and then centrifuged at 3000 rpm for 15 min. Taking out supernatant, the platelet-poor plasma (PPP) was. Drop 200 µL PPP on each sample (0.2 cm × 0.2 cm) in a 48-well cell culture plate and incubate the culture plate in water bath at 37 °C for 10 min. Then, 100 µL preheated 0.025 mol/L CaCl₂ aqueous solution (37 °C, water bath) was added to each sample. The mixture was stirred and when any fibrin threads formed, the time consumed was recorded as the plasma recalcification time. Experiment of each kind of sample was carried out 3 times and the average value was figured out.

### 2.4.3.3. Hemolysis ratio (HR).

Samples (1 cm × 1 cm) were rinsed with deionized water and 0.9 wt% NaCl aqueous solutions for 10 min in sequence, and then soaked in 0.9 wt% NaCl in water bath at 37 °C for another 30 min. 200 µL sheep whole blood was added to the NaCl solution, maintaining 37 °C for 1 h. After centrifuging at 1500 rpm for 10 min, the absorbance of top clear layer was measured by Ultraviolet spectrophotometer (Lambda 950, Perkin Elmer, US) at 545 nm. Pure water was used as positive reference, while 0.9 wt% NaCl aqueous solution as negative reference. The HR was calculated using the following equation:

\[
HR = \frac{(A_S - A_N)}{A_P - A_N}
\]

where \(A_S\) is the absorption value of samples, \(A_N\) is the absorption value of negative reference, and \(A_P\) is the absorption value of positive reference.

### 2.5. Dialysis simulation experiment

The dialysis performance of the membranes (PLA-M, HEP05-M) was measured in terms of urea and lysozyme clearance, and BSA retention. The home-made testing system was shown in Fig. 1. The total effective area of every membrane is 64 cm². 1.5 g/L urea aqueous solution, 0.04 g/L lysozyme and 1.0 g/L BSA aqueous solution were used as simulative blood, traversing through the dialysis mold (flow rate 200 ml/min). And 1.5 g/L dextrose solution played the role of simulative dialysate, traversing through the mold from the opposite direction (flow rate 500 ml/min). The experiment was continually run for 4 h and 10 mL test solution was taken out every h from the simulative blood. The concentration change of urea solution was evaluated using TOC (Multi N/C 2100-Jena, Germany) by measuring the organic nitrogen content, and that of BSA and lysozyme were detected with Ultraviolet spectrophotometer at 278 nm and 280 nm respectively. The urea clearance is calculated using the following equation:

\[
\text{Urea clearance percentage} = \frac{(C_t - C_0)}{C_0} \times 100\%
\]

where \(C_0\) and \(C_t\) are the urea concentrations in the testing solution reservoir at time \(t=0\) and \(t=1, 2, 3, 4\) h respectively.

The lysozyme clearance is calculated using the following equation:

\[
\text{Lysozyme clearance percentage} = \frac{(A_t - A_0)}{A_0} \times 100\%
\]

where \(A_0\) and \(A_t\) are the urea concentrations in the testing solution reservoir at time \(t=0\) and \(t=1, 2, 3, 4\) h respectively.

### 3. Results and discussion

#### 3.1. Surface chemistry

PLA membrane was first coated by polydopamine due to its strong adhesion, and then heparin was immobilized on PLA membrane through the covalent bonding between phenolic hydroxyl group of polydopamine and amino-group on heparin. The possible reaction mechanism was depicted in Fig. 2.

The surface chemistry of PLA membrane (PLA-M), as well as dopamine coating (DA05-M) and heparinization one (HEP05-M), were characterized by ATR-FTIR. The result is shown in Fig. 3. In terms of polydopamine-coated PLA membrane, a new broad absorbance 3550–3100 cm⁻¹ assigned to N–H/O–H stretching vibrations appeared compared to PLA membrane (PLA-M). The peaks appeared at 1625 and 1519 cm⁻¹ were attributed to the overlap of C= C resonance vibrations in the aromatic ring and the N–H bending vibrations, respectively. The weak absorbance at 806 cm⁻¹ was caused by the C–H out-plane bending vibrations. All new appeared peaks indicated the successful coating of polydopamine on PLA membrane, which is in accordance with the results reported previously [22]. As reference to heparin-immobilized PLA membrane, all peaks mentioned above appeared and just migrated slightly.

The characteristic peaks of SO₃⁻ ascribed to heparin could not be identified from FTIR spectra of HEP2-M in Fig. 3, due to the overlap with PLA membrane at 1205, 1158 and 1037 cm⁻¹. Therefore, XPS was further employed to characterize the heparin component on PLA membrane surface. XPS spectra of PLA-M, DA05-M and HEP05-M are shown in Fig. 4, and their corresponding elemental compositions are shown in Table 1. PLA membrane had no nitrogen and sulfur elements just as shown in Fig. 4, whereas, the nitrogen element (mass ratio 2.51%) was observed for polydopamine-coated membrane DA-05, and both nitrogen (mass ratio 3.22%) and sulfur elements (mass ratio 0.54%) were observed for heparin-immobilized membrane HEP05-M. All results confirmed the successful covalent immobilization of heparin on PLA membrane through polydopamine anchor effect.

#### 3.2. Surface morphology of membranes

SEM images were used to observe the surface morphology as shown in Fig. 5. It can be seen that the native PLA membrane ((A) PLA-M) possesses smooth and compact surfaces and finger-like cross sections, which is a typical asymmetric structure induced by an delayed liquid–liquid demixing mechanism. In comparison, all polydopamine-coated membranes ((C) DA2-M; (E) DA1-M; (G) DA05-M) exhibited porous surfaces despite the dopamine concentration. Furthermore, all heparin-immobilized PLA membranes ((D) HEP2-M; (F) HEP1-M; (H) HEP05-M) showed much larger pores on the surface after dipping in 2.0 g/L heparin.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Element mass concentration determined by XPS.</th>
</tr>
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<tbody>
<tr>
<td>Samples</td>
<td>C (%)</td>
</tr>
<tr>
<td>PLA-M</td>
<td>70.54</td>
</tr>
<tr>
<td>DA05-M</td>
<td>60.78</td>
</tr>
<tr>
<td>HEP05-M</td>
<td>59.63</td>
</tr>
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</table>
Fig. 5. SEM images of the surface morphologies and cross-section views for different membranes. (A) PLA-M; (B) cross-section views of PLA-M; (C) DA2-M; (D) HEP2-M; (E) DA1-M; (F) HEP1-M; (G) DA05-M; (H) HEP05-M.
buffer solution for 24 h. Chong et al. have reported similar phenomenon that polyethersulfone (PES) ultra-filtration membrane modified by polydopamine displayed more porous surface [33], in that article the reason was deemed to the deposition of polydopamine spherical nanoparticles. However, different possible reasons were first unveiled for the appearances of more porous surfaces. In our experiments, different pH value phosphate buffer solutions (0.2 M, pH 5.5, 7.0, 8.5) without addition of dopamine were prepared. Subsequently, PLA membranes were put into the buffer solution, maintaining at the same time and temperature as polydopamine-coating process. The membranes were dried and observed by SEM. As shown in Fig. 6, significant pores appeared on the membrane surfaces despite the pH value of buffer solution. It can be inferred that the surface pores were formed due to the
buffer solution. The possible mechanism was that the salt ingredients in buffer solution caused polymer degradation and corrosion for both PES and PLA membranes. Some oligomer or molecules were evolved to induce the formation of pores due to the mild degradation of PLA substrate. More importantly, the membrane performance was maintained and due to the brief action time by buffer solution. It is worth noting that acidic buffer solution seems to create more porous surfaces, indicating the various structure regulation way and possible controlled membrane degradation post-treatment.

The surface roughness ($Rq$) of native PLA membrane and heparin-immobilized membranes are shown in Fig. 7. The native PLA membrane surface is mainly flat with a roughness $Rq$ of 8.71 nm. However, the roughness increased slightly after polydopamine coating and subsequent heparinization. Large bulges emerged as 1 g/L dopamine solution deposited to form plentiful polydopamine aggregation. However, there is no absolutely positive correlation between the roughness $Rq$ and the dopamine solution. The roughness of HEP2-M decreased to 11.7 nm compared to HEP1-M (14.2 nm), implying that excess polydopamine may smooth the membrane surface to some extent.

Fig. 10. SEM images of platelets adhesion to all the membrane samples.
3.3. Hydrophobicity and water flux

For materials contacting human blood, the balance between hydrophilic and hydrophobic was important. The dynamic water contact angle variation of PLA membranes before and after modification was measured and shown in Fig. 8. The contact angle of all membranes decreased slightly with drop age, despite the addition of hydrophilic polymer PEG. The native PLA presents an initial contact angle of $84^\circ$, and that of polydopamine-coated PLA membranes decreased to DA05-M ($73^\circ$), DA1-M ($75^\circ$), and DA2-M ($74^\circ$) with the increase of dopamine concentration due to the hydrophilic group benzene hydroxyl on polydopamine. The contact angle of heparin-immobilized PLA membranes decreased significantly to 41, 43, and 51 for HEP2-M, HEP1-M, and HEP05-M respectively due to the introduction of more hydrophilic groups, such as hydroxyl, carboxyl, and sulfuric acid groups. The hydrophilic surface of PLA membrane by heparinization was in favor of the improvement of blood compatibility.

The pure water flux of different dry membranes was measured. The flux of native PLA membrane is around 120 L/h m$^2$, and decreased gradually with increasing dopamine content in buffer solution (DA05-M 98 L/h m$^2$, DA1-M 90 L/h m$^2$, DA2-M 82 L/h m$^2$) after polydopamine coating. HEP05-M presents better hydrodynamic permeability performance compared to the rest. Effects of polydopamine nano-layer to the pure water flux of polymeric membranes have been studied before; however, the results were inconsistent. It might decrease sharply only after coating for several hours [22], or also decrease mildly [34]. For PLA dialysis membrane, 70% flux is kept after coating for 8 h, which is due to the slightly improved hydrophilic and newly formed nano-scale pores. As shown in Fig. 9, the surface immobilized heparinization is helpful to improve water flux, which is mainly benefited from the improvement of hydrophilicity.

3.4. Blood compatibility

PLA has been used as tissue engineering materials for a long time. So its histocompatibility has been researched in depth, including cell adhesion, anti-inflammatory, cytotoxicity etc. Those results proved that PLA shows outstanding biocompatibility property. However, the biocompatibility research on PLA dialysis membrane was not reported before. As relating to hemodialysis, platelet thrombus forming caused by platelet adhesion and activation was the most crucial interaction between material surface and blood ingredients. In this study, platelet adhesion and plasma recalcification time were employed to evaluate the anticoagulation properties of native PLA membrane and the heparin-immobilized PLA membranes. PSf ultrafiltration membrane was prepared and adopted as the control sample due to its extensive application in hemodialysis.

Platelet adhesion results of all samples are revealed in Fig. 10. PSf membrane adsorbed more platelets in the same time quantum and more serious aggregation occurred compared to native PLA membrane, whereas, the shape of platelets adsorbed on PLA membrane seemed more irregular. Notably, the amount of platelets did not decrease for DA2-M, DA1-M and DA05-M, which is quite different from the previous reports. They assumed that the membranes with polydopamine layer will adsorb fewer platelets [22], but stronger platelet adhesion was occurred to all polydopamine-coated PLA membrane within our study. In consideration of the strong adhesion

Table 2
Hemolysis ratio of different membrane samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorbance (545 nm)</th>
<th>Hemolytic rate (%)</th>
</tr>
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<tbody>
<tr>
<td>Positive reference</td>
<td>0.986 ± 0.012</td>
<td>7.012</td>
</tr>
<tr>
<td>Negative reference</td>
<td>0.032 ± 0.0020</td>
<td>7.012</td>
</tr>
<tr>
<td>PLA-M</td>
<td>0.063 ± 0.0015</td>
<td>7.012</td>
</tr>
<tr>
<td>PSF-M</td>
<td>0.061 ± 0.0020</td>
<td>7.012</td>
</tr>
<tr>
<td>DA2-M</td>
<td>0.054 ± 0.0025</td>
<td>7.012</td>
</tr>
<tr>
<td>DA1-M</td>
<td>0.057 ± 0.0015</td>
<td>7.012</td>
</tr>
<tr>
<td>DA05-M</td>
<td>0.052 ± 0.0005</td>
<td>7.012</td>
</tr>
<tr>
<td>HEP2-M</td>
<td>0.046 ± 0.0010</td>
<td>7.012</td>
</tr>
<tr>
<td>HEP1-M</td>
<td>0.048 ± 0.0015</td>
<td>7.012</td>
</tr>
<tr>
<td>HEP05-M</td>
<td>0.045 ± 0.0010</td>
<td>7.012</td>
</tr>
</tbody>
</table>

Fig. 11. Plasma recalcification time of different membranes samples.

Fig. 12. The surface roughness of PSF and PLA native membranes.
and combination property of polydopamine, it is also reasonable that polydopamine nano-layer can promote the platelet adhesion although the improvement of the hydrophilicity for a certain extent. As expected, there were only very few platelets adsorbed on HEP2-M, HEP1-M and HEP05-M as shown in Fig. 10. The surface heparinization significantly suppressed the aggregation of platelets and improved the biocompatibility of PLA membranes due to the good anticoagulation ability of heparin and better hydrophilicity [35].

Plasma recalcification time (PRT), also named partial thromboplastin time (PTT), was used to monitor the time taken for clotting of blood or to determine the deficiency of factor responsible for clotting. If coagulation factor VII is activated, the thrombin will be produced from thrombinogen via the stepped active process. And then the thrombin will spur the transform from fibrinogen in the plasma into fibrous protein, which is the foundation of thrombus formation. With the assistance of Ca$^{2+}$ and activated factor VIII, fibrous protein will be crosslinking, to be steady and insoluble.

From Fig. 11, we can see that native PLA membrane exhibited shorter plasma recalcification time (223 s) compared to PSf membrane (272 s), possibly attributed to the higher roughness as shown in Fig. 12 and release of bits of acid substances produced by the degradation of native PLA. However, heparin-immobilized PLA membranes exhibited excellent prolongation of plasma recalcification time (270–280 s) compared to native PLA membranes, PSf membranes and polydopamine-coated PLA membranes. The sulfonic groups of heparin have been thought to play an important role to restrict the transform of fibrinogen [23].

Hemolysis ratio (HR) is another aspect of blood compatibility. Generally, it is used to detect the erythrocyte damage caused by materials. Table 2 shows the HR value of different membrane samples. We can find that PSf and PLA native membrane have considerable HR, 3.24% and 3.04% respectively, less than 5%, within safety levels for biomaterials. The HR of polydopamine-coated PLA membranes decreases slightly, and that of heparin-immobilized PLA membranes decreases to 1.46%, 1.68%, and 1.36% for HEP2-M, HEP1-M and HEP05-M respectively. All results indicate that heparin can also weaken the damage to erythrocyte in addition to preventing platelet adhesion and blood clotting.

3.5. Dialysis performance

The urea and lysozyme clearance, BSA retention of HEP05-M and PLA-M were determined to evaluate the dialysis performances of PLA membranes. From Fig. 13, we can figure out that 79% of urea has been cleaned for HEP05-M after simulating dialysis for 4 h, which is slightly higher than 74.6% of PLA-M. With respect to middle molecule clearance, 13.7% of lysozyme was cleaned out for PLA-M after simulating dialysis for 3 h, and 18.5% for HEP05-M. After running for 4 h, lysozyme concentration was raised to some extents for both membrane samples as shown in Fig. 13(b), indicating a saturation clearance for lysozyme. Whereas, the BSA rejection value of PLA-M was slightly higher than that of HEP05-M (93.7% and 90.8%) possibly due to its denser membrane surface.

4. Conclusion

In this work, PLA porous membrane aimed to hemodialysis was prepared firstly via the phase inversion process. The surface heparinization was realized through the self-polymerization and strong adhesion of dopamine. It can be concluded from the results that: (1) the surface heparinization chemistry was confirmed by both FTIR and XPS, all PLA membranes exhibited asymmetric finger-like pore structure; (2) the heparin-immobilized PLA membranes showed smooth surface, better hydrophilicity and permeability; (3) the native PLA membrane exhibits better platelet adhesion, shorter plasma recalcification time and considerable hemolysis ratio compared to PSf membrane; (4) the surface heparinization on PLA...
membrane improved the platelet adhesion, plasma recalcification time and decreased hemolysis ratio significantly; (5) the dialysis simulation test revealed that heparin-immobilized PLA membrane cleaned almost 80% urea, 18% lysozyme and retained over 90% BSA. All results demonstrate there is a great potential of heparin-immobilized PLA membranes in the application of hemodialysis, and more work e.g. membrane micro-structure regulation, sterilization, controlled degradation post-treatment etc. need to be done to achieve the clinical application of PLA dialysis membranes in near future.

Acknowledgment

The authors would like to thank National Natural Science Foundation of China (51273211), National 863 Foundation of China (2012AA03A605), the International Cooperation Project from Ministry of Science and Technology of China (2012DFR50470).

References