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2006 Biomed. Mater. 1 170
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An albumin-fixed membrane for the removal of protein-bound toxins

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Received 5 July 2006
Accepted for publication 9 August 2006
Published 25 August 2006
Online at stacks.iop.org/BMM/1/170

Abstract
Established methods for kidney dialysis do not work for liver failure because kidney dialysis removes only water-soluble toxins, while the liver normally removes albumin-bound toxins. In the present study, a polysulfone dialysis membrane with a $-\text{OH}$ reactive group was prepared by hydrolyzing the chloromethylated polysulfone membrane, and the bovine serum albumin molecules were fixed into the membrane with 1,1′-carbonyldiimidazole activation. The content of albumin of the albumin-fixed membrane was 121.3 mg (g membrane)$^{-1}$. The albumin-fixed dialysis membranes were used to remove protein-bound toxins, bilirubin, from the bilirubin–albumin solution. The transfer rate of bilirubin of the albumin-fixed membrane was obviously higher compared to the normal dialysis membrane. The clearance of bilirubin with the albumin-fixed membrane was 49.8%. The albumin-fixed membrane can easily be regenerated by the bovine serum albumin and NaOH solution. Regeneration of the membrane suggested good mechanical and chemical stability, as well as good clearance of bilirubin. In addition, the effects of membrane thickness and bilirubin initial concentration on the removal of bilirubin were discussed. (Some figures in this article are in colour only in the electronic version)

1. Introduction
Uremic syndrome and hepatic failure are characterized by the retention of a large number of toxins with different molecular masses and chemical properties [1]. Some toxins are water-soluble and non-protein bound, whereas others are partially lipophilic and protein bound. There is increased evidence that protein-bound toxins are responsible for the biochemical and functional alterations present in uremic syndrome and hepatic failure. The removal of water-soluble substances is well established by dialysis procedures. However, an unsolved problem is the low dialyzability of lipophilic toxins including middle chain fatty acids, aromatic amino acids, free phenols and bilirubin [2].

Many attempts have been made to remove the protein-bound toxins directly from plasma of patients suffering from uremic syndrome and hepatic failure such as hemoperfusion and affinity membrane separation. Hemoperfusion, i.e., circulation of blood through an extracorporeal unit containing an adsorbent system for protein-bound toxins [3], is the main method for the removal of the protein-bound toxins from the plasma. Successful hemoperfusion requires the adsorbents to be specific, of high adsorption capacity, blood compatible and non-poisonous. Recently, the affinity membrane process has become an attractive and competitive method for the removal of toxins from the plasma [4, 5]. Common limitations such as pressure drop, channeling and intra-bed diffusion are minimized by convection through the small pores of the membranes [6]. We prepared the polylysine-attached affinity membranes, and they were used to remove the protein-bound toxin, bilirubin, from the bilirubin–phosphate solution and the bilirubin–albumin solution. The experimental results showed that these membranes exhibited a high adsorption capacity for bilirubin [7–11].

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are not specific enough, because they also remove essential substances such as hormones, which are linked to their own transport proteins.

It has long been known that albumin molecules are able to exchange their ligands over short distances. According to this principle, Stange et al. [12] extend the applicability of dialysis to the removal of albumin-bound toxins by using an albumin-impregnated dialyzer and an albumin containing dialysate. However, albumin molecules cannot be immobilized into the polysulfone membrane due to the absence of any convenient functional groups for linking surface modifiers in the polysulfone membrane. Therefore, this system would inevitably cause the loss of albumin molecules on the membrane surface and in the membrane pores. If the albumin molecules can be covalently bound to the membrane backbone, the leaching and loss of albumin can be prevented effectively.

In this paper, we choose bilirubin as a representative of protein-bound toxins, and the mixture solution of albumin and bilirubin is regarded as 'simulation plasma'. The hydroxylated asymmetric polysulfone membrane with the −OH reactive group was obtained by hydrolyzing the chloromethylated polysulfone membrane. Bovine serum albumin (BSA) molecules were immobilized as carriers of the fixed-site carrier polysulfone membrane. Bovine serum albumin (BSA) molecules were immobilized as carriers of the fixed-site carrier membrane, using 1,1′-carbonyldiimidazole as a crosslinking reagent. The BSA-fixed membrane was used to remove the bilirubin from the mixture solution of BSA and bilirubin.

2. Materials and methods

2.1. Materials

Polysulfone was a gift from the Tianjin Institute of Textile Science and Technology (People’s Republic of China). 1,1′-Carbonyldiimidazole (CDI) was obtained from the Sigma Chemical Company (USA). Bovine serum albumin (BSA) was purchased from the Beijing Chemical Reagent Company (People’s Republic of China). Bilirubin was provided by the Shanghai Weihui Chemical Factory (People’s Republic of China). The other reagents used were bought from other Chinese factories.

2.2. Preparation of the BSA-fixed membrane

Chloromethyl polystyrene membranes with finger-like pores were prepared according to our procedure [13]. These membranes were shaken in 15% (w/v) NaHCO₃ for 6 h at 85 °C. Then, the membranes were washed several times with distilled water at room temperature. After hydrolysis of chloromethyl, the hydroxylated polystyrene (HPS) membranes were obtained. A reaction scheme for the preparation of the HPS membrane is shown in figure 1.

The HPS membranes were shaken in a solution of 20 mg CDI per 10 ml acetone at room temperature for 1 h. After activation, the membranes were washed three times for 20 min with acetone at room temperature and then dried in a vacuum. The CDI-activated membranes were stored in a dry atmosphere until further use to avoid hydrolysis of the imidazole groups.

100 mg BSA was dissolved in a 10 ml NaHCO₃ solution, pH 8.4. One CDI-activated HPS membrane was fed into the reaction solution and shaken overnight at room temperature. Afterwards, the membrane was washed in 1 M NaCl and water extensively. The amount of BSA bound was determined by the variation of the absorbance of the BSA solution at 280 nm before and after crosslinking. A reaction scheme for the preparation of the BSA-fixed membrane is shown in figure 2.

2.3. Characterization of the membranes

The flux of pure water was measured via a membrane filter holder, which can hold one piece of membrane with an effective diameter of 47 mm. Pure water was pumped through the membrane holder at 0.1 MPa and 20 °C for 0.5 h. The porosity of the membranes was measured by determining the amount of water adsorbed by the membranes. The average pore size was measured by the permeation rate method, which was based on the Poiseuille principle. The flux (J), porosity (ε) and average pore size (r) were then calculated, respectively, by the following equations:

\[
J = \frac{Q_m}{A_{eff} t},
\]

\[
ε = \frac{W_1 - W_2}{D_{water}} \times 100\% ,
\]

\[
r = \sqrt{\frac{8\eta J L_d}{\varepsilon A P}},
\]

where \(Q_m\) is the amount of water through the membrane in a given time \(t\), \(A_{eff}\) is the efficient membrane area, \(W_1\) and \(W_2\) are the weights of the membrane in wet and dry states, respectively, \(V\) is the effective volume of the measured membrane in the wet state, \(D_{water}\) is the density of pure water at room temperature,
Table 1. The characteristics of the HPS and BSA-fixed membranes.

<table>
<thead>
<tr>
<th></th>
<th>Flux (J) (ml cm(^{-2}) h(^{-1}))</th>
<th>Porosity (ε)</th>
<th>Average pore size (r) (nm)</th>
<th>BSA content (mg (g membrane))(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPS membrane</td>
<td>54</td>
<td>0.62</td>
<td>30</td>
<td>–</td>
</tr>
<tr>
<td>BSA-fixed membrane</td>
<td>47</td>
<td>0.59</td>
<td>27</td>
<td>121.3</td>
</tr>
</tbody>
</table>

\(L_d\) is the thickness of the wet membrane, \(η\) is the viscosity of water and \(ΔP\) is the pressure drop across the membrane.

2.4. Transport experiments

Bilirubin is easily destroyed by exposure to direct sunlight or any other source of ultraviolet light, including fluorescent light; all transport experiments are carried out in a dark room. The BSA-fixed membrane was put on the joint mouth of the diffusion cell, with the permselective layer towards the bottom and the thicker microporous support layer towards the top. The bottom cell contained the mixture solution of bilirubin and albumin; the other was filled with the albumin solution. Thus, the bilirubin molecules were transported by the albumin molecules in the membrane under the driving force of a concentration gradient. At a given time interval, the solution concentration of the upper cell was determined spectrophotometrically by measuring the absorption at 460 nm.

3. Results and discussion

3.1. Characteristics of BSA-fixed membranes

The characteristics of the HPS and BSA-fixed membranes are summarized in table 1; no remarkable differences in the water flux, porosity and average pore size were found between the HPS and BSA-fixed membranes. The BSA content is 121.3 mg BSA (g membrane)\(^{-1}\) and BSA molecules are uniformly distributed in the membrane (as shown in figure 3).

3.2. Bilirubin removal

A comparison of clearance between the blank membrane (no BSA-fixed membrane) and the BSA-fixed membrane is shown in figure 4. The clearance is defined as

\[
CL = \left(1 - \frac{C_A}{C_{A0}}\right) \times 100\%.
\]

where \(C_{A0}\) and \(C_A\) are the initial concentration of bilirubin and the bilirubin concentration in the mixture solution of BSA and bilirubin, respectively. As shown in figure 4, the clearance of bilirubin with the blank membrane is only 3.32%, and it proves again that the normal dialysis membrane cannot remove the protein-bound toxin effectively. When the BSA-fixed membrane is used, the clearance of bilirubin is greatly enhanced and is 49.8%. The HPS membrane which we prepared is an asymmetric membrane, which consists of a thin dense skin about 0.5–0.6 \(µm\) thick (usually called the permselective layer) which is formed over a much thicker microporous layer supporting the skin [13]. The carriers, BSA, are fixed in the pores of the microporous layer and on the surface of the membrane. Because albumin molecules are able to exchange their ligands (protein-bound toxins) over short distances, bilirubin molecules in the BSA solution are transported over the dense skin of the membrane by the BSA molecules in the pores of the support layer.

3.3. Effect of membrane thickness

Figure 5 shows the effect of membrane thickness on bilirubin removal. The clearance of bilirubin with a 115 \(µm\) thick membrane is only 3.32%.

Figure 4. A comparison of CL between the blank membrane and the BSA-fixed membrane (\(C_{A0} = 20 \mu mol l^{-1}\); membrane thickness: 106 \(µm\)).

transported over the dense skin of the membrane by the BSA molecules in the pores of the support layer.
An albumin-fixed membrane for the removal of protein-bound toxins

3.3. Effect of membrane thickness

The effect of membrane thickness on bilirubin removal is shown in Figure 5. The highest transmembrane rate is obtained with the 115 µm thick membrane, followed by the 70 µm thick membrane, and the lowest is with the 138 µm thick membrane. We offer two reasons for the results. On one hand, the flux decreases with increasing membrane thickness because the diffusion rate in the membrane is inversely proportional to the membrane thickness. On the other hand, the number of carriers increases with the membrane thickness due to membrane structures almost regardless of the thickness, and the facilitation factor increases with increasing number of carriers.

3.4. Effect of bilirubin initial concentration

In the clinic, the molar ratio of bilirubin to albumin is beyond 1 in the patient blood of hyperbilirubinemia. We adopted the molar ratios of bilirubin to albumin of 3:1, 2:1 and 1:1. The effect of bilirubin initial concentration on bilirubin removal is presented in Figure 6, which shows that the bilirubin transmembrane rate increases with increasing bilirubin concentration. Albumin is the natural carrier for bilirubin in serum and each albumin molecule can strongly bind only 1–2 bilirubin molecules and loosely binds an additional 9–13 bilirubin molecules [14, 15]. When the molar ratio of bilirubin to albumin is greater than 1, more bilirubin molecules would be bound weakly on albumin molecules, and the free bilirubin concentration would increase with increasing bilirubin initial concentration. This will conduce to increasing transmembrane rate of bilirubin. In addition, the transmembrane rate is proportional to the concentration gradient. However, we also noted that the equilibration time was extended with increasing bilirubin initial concentration.

3.5. Regeneration and reuse of the membranes

The membranes have very preferable mechanical and chemical properties. Both BSA and NaOH were used to regenerate the membrane, and the regenerated membrane seems to have a comparative performance of clearance of bilirubin (48.1%) similar to the initial BSA-fixed membrane. The clearance of bilirubin was still at a relatively high level, and the physical character of the membrane remains nearly unchanged.

4. Conclusions

The BSA-fixed membrane was prepared by immobilizing albumin molecules into the HPS membranes with finger-like pores using CDI as a crosslinking reagent. BSA molecules were uniformly distributed in the membrane and their content was 121.3 mg (g membrane)$^{-1}$. This membrane can well remove the protein-bound toxins, bilirubin, from the bilirubin–albumin solution, and the clearance of bilirubin was 49.8%. The transmembrane rate of bilirubin increased with increasing bilirubin initial concentration. The BSA-fixed membrane can easily be regenerated, and the loss of carriers can be prevented effectively.

Acknowledgments

This work was supported by the Natural Science Foundation of Fujian Province (no C0510005) and the National Nature Science Foundation of China (no 3050127).

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