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Evaluation of polyethersulfone highflux hemodialysis membrane in vitro and in vivo

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Abstract A new hemodialysis membrane manufactured by a blend of polyethersulfone (PES) and polyvinylpyrrolidone (PVP) was evaluated in vitro and in vivo. Goat was selected as the experimental animal. The clearance and the reduction ratio after the hemodialysis of small molecules (urea, creatinine, phosphate) for the PES membrane were higher in vitro than that in vivo. The reduction ratio of β_2-microglobulin was about 50% after the treatment for 4 h. The biocompatibility profiles of the membranes indicated slight neutropenia and platelet adhesion at the initial stage of the hemodialysis. Electrolyte, blood gas, and blood biochemistry were also analyzed before and after the treatment. The results indicated that PES hollow fiber membrane had a potential widely use for hemodialysis.

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Introduction

Hollow fiber membranes are widely used in blood purification, such as hemodialysis, hemofiltration, plasma separation, and membrane oxygenation. Among them, hemodialysis is a widely used treatment modality for kidney failure. The treatment is intermittent, generally three times weekly for periods between 3 and 5 h depending upon the patient clinical requirements. The first hollow fiber hemodialyzers were used clinically in the 1960s [1, 2], and the material of the hollow fiber membrane is unmodified cellulose. Cellulose membranes are still widely used for hemodialysis because their hydrogel structure and small thickness provide very effective removal of small solutes like urea and creatinine. However, these membranes provide relatively little clearance for "middle" molecules like β_2-microglobulin that is known to be associated with many dialysis-related disorders. In addition, the cellulosic structure contains a high density of hydroxyl groups, which are known to cause complement activation upon contact with blood [3].

The shortcomings in cellulose membranes have led to the development of hemodialyzers based on a variety of synthetic polymers and modified cellulosic materials, including polysulfone (PSF), polyamide (PA), polyacrylonitrile (PAN), cellulose triacetate (CTA), and Hemophan (HP). Among the materials, PSF is one of the most important polymeric materials and is widely used; PSF-based membranes show outstanding oxidative, thermal, and hydrolytic stability as well as good mechanical and film-forming properties. The PSF membranes also showed high permeability for low molecular weight proteins when used for hemodialysis. The commercial product of PSF hollow fiber hemodialyzer was produced by Germany (Fresenius Polysulfones, Fresenius Medical Care, Bad Homburg,

Germany), and was widely acknowledged as providing an optimal biocompatibility in terms of solute removal and complement activation [4, 5]. PSF can also be used as bone joint screws due to its biostable [6].

Polyethersulfone (PES) is a parent material of PSF, with a better chemical resistance, thermal stability, mechanical properties as well as a better hydrophilicity compared to PSF. The PES plasma separation membrane was evaluated by animal experiments, and showed good blood compatibility [7]. In the present study, we prepared a new hollow fiber dialysis membrane by blending PES and polyvinylpyrrolidone (PVP), and then evaluated the performance of the membrane in vitro and in vivo. Goat was selected as the experimental animal. Experiments were performed to evaluate the solute clearance and the blood compatibility.

Materials and methods

PES hemodialyzer

The PES hollow fiber membrane hemodialyzer was prepared by us. Both PES (BASF Aktiengesellschaft) and PVP (PVP-90K, BASF Aktiengesellschaft) were dissolved in *N*,*N*-Dimethyl acetamide (DMAc, Chengdu Chemical Reagent, Inc., China) to obtain the spinning solution. The hollow fiber was spun by the dry–wet spinning method [8], based on a complex process involving phase inversion or precipitation. The specifications of the hemodialyzer are shown in Table 1.

For scanning electron microscopy (SEM) observation, the hollow membrane samples were broken in liquid nitrogen, attached to the sample supports and coated with a gold layer. The SEM images were recorded using an S-2500C microscope (voltage = 20 kV, Hitachi, Japan).

Hemodialysis using a simulation solution

The test solutions were prepared according to the international standard ISO 8637 [9]. The molar concentrations of urea, creatinine, and phosphate in the simulation solution were 15 mmol/L, 500 μmol/L, and 1 mmol/L, respectively. The test procedure was accordant to the procedure in ISO 8637. The ultra-filtration coefficient was calculated as the unit mL/mmHg h. The clearance (K) of small molecules (urea, creatinine, phosphate) were established by sampling from the inlet and outlet segments of the extracorporeal circuit 1 h after the initiation of the treatment, and was calculated using the following formula.

$$K = \left(\frac{C_{BI} - C_{BO}}{C_{BI}}\right) Q_{BI} + \frac{C_{BO}}{C_{BI}} Q_F$$

where C_B is the solute concentration in the blood (here is the simulation solution); I, O refer to the inlet and the outlet to the device; Q_{BI} is the blood flow rate at the dialyser inlet; Q_F is the filtration rate.

Urea was determined by a reagent Kit for Urea Determination (Diethyl-Monoxime, Beijing chemical regent factory, China); creatinine was quantified by the absorption at 235 nm using an UV–Vis spectraphotometer U-200A (Hitachi Co., Ltd., Tokyo, Japan) through a standard curve. Phosphate was determined using the molybdate blue method: phosphate reacts with ammonium molybdate and is then reduced by stannous chloride to form a blue complex, and then measured at 670 nm with the UV–VIS spectraphotometer U-200A.

Table 1 Specifications of the PES hemodialyzer

Hollow fiber	
Internal diameter (μm)	205
Wall thickness (μm)	50
Effective length (mm)	240
Membrane area (m^2)	1.5
Potting material	Polyurethane
Sterilized method	γ-ray

Hemodialysis using pig blood in vitro

Pig fresh blood was collected using a glass tank, containing citrate/phosphate/dextrose/adenine-1 mixture solution (CPDA-1) as an anticoagulant (anticoagulant to blood ratio, 1:7). The dialysis procedure was the same as the Sect. "Hemodialysis using a simulation solution", and the solute clearance was calculated using the same formula as described in the Sect. "Hemodialysis using a simulation solution". The concentrations of urea, creatinine, and phosphate were determined using an Auto Biochemistry Analyzer 7170A (Hitachi Co., Ltd., Tokyo, Japan).

Hemodialysis in vivo

Three adult hybrid goats weighing about 20 kg were used in the experiment. All animals underwent local anesthesia with 1.0% procaine hydrochloride by injection into the neck muscle. The hair on the neck was cleared away carefully. The animal was laid on its back and fixed on the experimental table.

Extracorporeal circuits were primed with 500 mL normal saline solution to remove the bubbles in the circuits and in the dialyzer, then primed with 500 mL saline solution containing 10,000 IU heparin. About 150 mg urea and 50 mg creatinine were injected to the animal blood before the treatment. At the initiation of the treatment goats received a loading dose (3,000 IU) of heparin, and

followed by continuous infusion (3,000 IU/h). The infusion was terminated at 30 min prior to the end of the dialysis.

Extracorporeal circuits with left–right neck intravenous cannulation were created on the animal using B. Braun blood tubing lines for hemodialysis. The clearance (K) of small molecules (urea, creatinine, phosphate) were established by sampling between the inlet and outlet segments of the extracorporeal circuit 1 h after the initiation of the treatment, and was calculated using the formula described in Sect. "Hemodialysis using a simulation solution". The fluid removal rate during these measurements was maintained at (3 mL/min).

The levels of urea, creatinine, phosphate, total proteins (TP), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were determined using an Auto Biochemistry Analyzer 7170A (Hitachi Co., Ltd., Tokyo, Japan).

Removal of β_2-microglobulin was established from the changes in plasma β_2-microglobulin levels during the treatment at different time intervals (30, 60, 120, 180, and 240 min). Plasma β_2-microglobulin levels were determined using a commercially produced ELISA assay (Cambridge Life Sciences, Cambridge, UK).

Electrolyte levels were determined before and after hemodialysis. K^+, Na^+, and Cl^- were determined using electrolyte analyzer (NOVA CRT-4, US), and Ca^{2+} was determined using an Auto Biochemistry Analyzer 7170A (Hitachi Co., Ltd., Tokyo, Japan).

Blood cells including red blood cell (RBC) and white blood cell (WBC), and blood components including hemoglobin (HGB) and platelet were determined using a blood cell analyzer (BC-3000peus, Shenzhen Mairui Biomedical Device Co. Ltd., China). Blood gas was determined using a blood gas analyzer (CORNing 238, US).

For complement and WBC activation investigation, various membranes were used from different companies, Cuprophane (Nephross, Netherlands), Cellulose acetate (Nissho, Japan), Hemophane (Ningbo-Yatai, China), Polysulfone (PSF, Fresenius, Germany). Polycarbonate (PC) was obtained from BASF Co. Ltd., and the PC membrane was prepared in our Lab. Complement C3 activation was determined in vitro by enzymelinked immunosorbent assays (ELISA) [10]. For comparing the results, activation for Cuprophane membrane was used as control.

Results

Morphology of the PES hollow fiber membrane

Figure 1 shows the SEM images of the cross-section of the PES hollow fiber membrane used to prepare the hemodialyzer. As shown in the figure, the pore structure of the fiber was a figure-like structure that started from the inner edge of the fiber and went through the whole cross-section. And a skin layer with a thickness of about 3 μm was found at the internal surface of the hollow fiber.

The skin layer and the figure-like structure were useful for using as blood purification membrane. Additionally, from the SEM pictures for the internal surface and the outer surface (data not shown), we could found that there was no visible pores at the inner edge of the fiber at a magnification of 10,000×, while there was a network of pores of 0.3 μm at the outer edge.

Solute transport

Table 2 summarizes the clearance data and the reduction ratio after the dialysis for small molecules in vitro and in vivo. It was clearly that the clearances and the reduction ratios for all the solutes were larger using the simulated solution than that for blood. Changes in β_2-microglobulin during the dialysis for the goats are plotted in Fig. 2. The reduction ratio was about 50% after the treatment for 4 h.

The ultrafiltration coefficient was obtained by the hemodialysis process using the simulated solution with a value of 81 mL/h mmHg, from which we can conclude that the PES membrane was a high-flux hemodialysis membrane. The removal of small molecules during dialysis is governed by hydrodynamic conditions within the dialyser rather than membrane structure since the major resistance

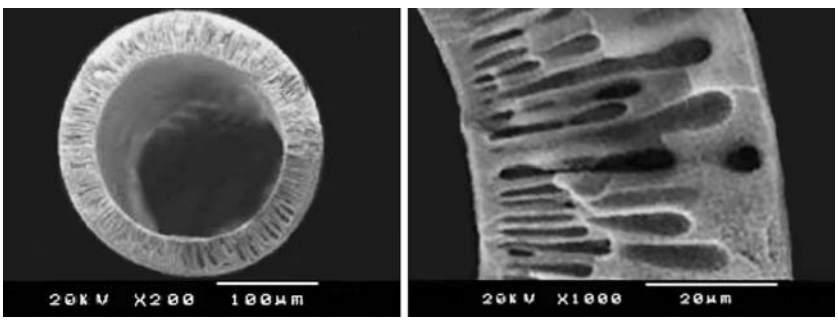

Fig. 1 SEM images of the PES hollow fiber membrane

Table 2 Small molecular clearance at a blood (or simulated solution) flow rate of 180 mL/min and dialysate flow rate of 500 mL/min

	Clearance (mL/min)			Reduction ratio (%)	
	Urea	Creatinine	Phosphate	Urea	Creatinine
Simulated solution	174.0 ± 6.0	169.0 ± 5.0	170.0 ± 6.0	94.3 ± 3.8	92.4 ± 4.1
Blood in vitro	157.5 ± 7.4	143.6 ± 6.8	144.5 ± 7.2	71.2 ± 3.9	69.9 ± 4.0
Blood in vivo	153.6 ± 9.4	141.6 ± 8.2	142.5 ± 7.3	69.2 ± 4.5	68.9 ± 5.2

Data are expressed as the means ± SD, $n = 3$

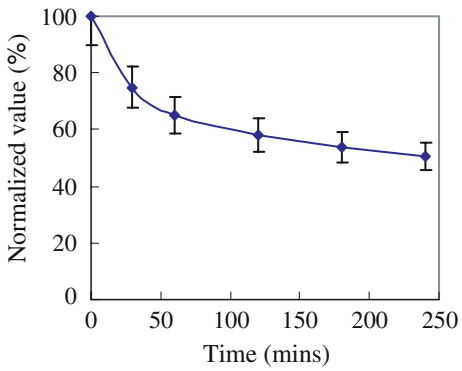

Fig. 2 Changes in β_2-microglobulin during the dialysis Data are expressed as the means ± SD, $n = 3$

to transport from the blood into the dialysis fluid lies not in the membrane but boundary layers adjacent to the membrane. Thus, the data of clearance and the reduction ratio (Table 2) for the simulated solution were higher than that for the blood due to the proteins in the blood, which may induce concentration polarization.

The PES membrane was able to reduce the plasma burden of β_2-microglobulin during the treatment are shown in Fig. 2. The data were analyzed by consideration of actual values and the percentage reductions achieved. The reduction ratio was about 50% after the treatment for 4 h, this value is comparable compared to that for PSF membrane and polyflux [4]. More recently, clinical study was performed to compare the PSF and the PES membrane, the β_2-microglobulin reduction ratios were 51.70 ± 15.75 and 53.13 ± 20.41, respectively ($p = 0.52$).

Table 3 shows the electrolyte values in the goat blood before and after the dialysis process. Among the these ions, only the K^+ exhibited statistically significant decreases after the dialysis, whereas Na^+, Cl^- and Ca^{2+} did not change.

Table 3 Electrolyte values pre- and post-dialysis

	Pre-dialysis	Post-dialysis
K^+ (mmol/L)	3.71 ± 0.37	2.98 ± 0.17
Na^+ (mmol/L)	144.0 ± 3.8	142.8 ± 1.8
Cl^- (mmol/L)	105.1 ± 4.1	101.0 ± 1.2
Ca^{2+} (mmol/L)	2.12 ± 0.16	2.02 ± 0.11

Data are expressed as the means ± SD, $n = 3$

Biocompatibility

Figure 3 summarizes the changes in the goat blood observed during the dialysis in respect of white cells (WBC) and platelets. Both WBC and platelet counts have been normalized to pretreatment levels and expressed as a percentage of these values. A small decline in both was noted at the first 30 minutes, and returned to the initial levels after about 2 h.

The complement and WBC activation for various membranes were investigated after contacting to blood for 1 h, as shown in Table 4. The data show the correlation between the complement and WBC activation. We also found that the concentration of C3a increased rapidly at the beginning of the contact between the blood and the PES membrane and remained constant after 90 min, which was consistent with the decrease of WBCs [7].

Hemolysis ratio was also determined for the pig blood in vitro and for the goat blood in vivo. Data showed that there was only a slightly hemodialysis phenomena (about 1.7%) in vitro, while the hemolysis ratio was zero in vivo (The absorption value for (+) is 0.832, but for the sample is 0).

The RBC and hemoglobin (RGB) levels were also determined during the dialysis. The RBC was $(2.04 \pm 0.12) \times 10^{12}$/L and $(1.96 \pm 0.10) \times 10^{12}$/L respectively before and after the hemodialysis. And the HGB was 115.0 ± 8.0 and 110.5 ± 8.0 g/L before and after the hemodialysis, respectively.

Biochemistry for the blood was analyzed before and after the hemodialysis, and the data were summarized in Table 5. Only the ALP level increased. And the others, including ALT, AST, TP and plasma albumin (ALB) were slightly decreased. The blood gas was also analyzed as shown in Table 6. And the data showed no statistically change during the dialysis. The urine solution was also analyzed before and after the hemodialysis, the pH value, urine protein and urine glucose had no change before and after the hemodialysis. The concentrations of urobilinogen were 3.5 and 3.7 mmol/L before and after the hemodialysis, respectively.

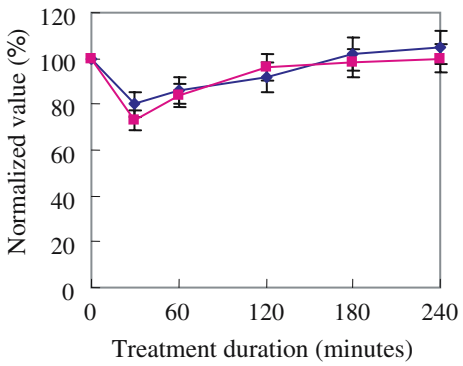

Fig. 3 Changes in WBC and platelet during the dialysis in vivo ◆ Platelet; ■ WBC Data are expressed as the means ± SD, $n = 3$

Table 4 Complement and WBC activation for various membranes

Membrane	Complement activation (%)	WBC activation (%)
Cuprophane	100	100
Cellulose acetate	67	43
Hemophane	60	62
Polycarbonate	40	53
PSF	27	15
PES	24	12

Table 5 Data for biochemistry analysis pre- and post-dialysis

	Pre-dialysis	Post-dialysis
ALT (IU/L)	29.0 ± 3.0	26.5 ± 1.5
AST (IU/L)	189.5 ± 9.5	173.0 ± 15.5
ALP (IU/L)	284.0 ± 33.0	266.0 ± 13.0
TP (g/L)	70.2 ± 1.4	63.8 ± 1.8
ALB (g/L)	29.2 ± 0.4	27.6 ± 0.4

Data are expressed as the means ± SD, $n = 3$

Table 6 Blood gas values pre- and post-dialysis

	Pre-dialysis	Post-dialysis
pH	7.43 ± 0.0	7.47 ± 0.02
PCO_2 (Kpa)	4.5 ± 0.7	4.3 ± 0.1
PO_2 (Kpa)	9.7 ± 0.4	9.2 ± 0.3
HCO_3(mmol/L)	24.5 ± 2.4	25.6 ± 1.5

Data are expressed as the means ± SD, $n = 3$

Discussion

PES membrane

Synthetic membranes are manufactured by blending of normally hydrophobic polymers with hydrophilic materials to make them suitable for use in renal replacement therapy. As we know, hydrophobic polymer membrane surface adsorb much more proteins from plasma when they come into contact with blood [11–13], and this is so-called "membrane fouling," which can induce flux decrease. The blending of hydrophilic polymer materials is one practical approach to modify hydrophobic membrane surface.

To modify PSF and PES membrane, PVP is usually blended into them to increase the hydrophilicity of the resultant membranes. The commercial product of PSF hollow fiber membrane produced by Germany was modified by blending of PVP. Qin et al. [14] prepared PES/PVP blended hollow fiber membranes with enhanced flux for humic acid removal. Hoenich et al. [4] evaluated the clinical performance of a hemodialysis membrane manufactured from a blend of polyamide, polyarylethersulfone and PVP. Huang et al. [15] evaluated two high-flux dialyzers, dialyzer A (cellulose triacetate) and dialyzer B (PES), in the study water solution with urea and creatinine were made as "blood," and pure water was used as dialysate. Gerdemann et al. [16] studied the advanced glycation end products (AGEs) removal by high-flux dialysis; they used a DIAPES membrane prepared from PES, which was similar to the PES membrane in our study. The difference was the ultra-filtration coefficient, in this study the ultra-filtration coefficient is 81 mL/mmHg h, while that for the DIAPES membrane was 35 mL/mmHg h. The higher ultra-filtration coefficient indicated that higher water removal rate could be obtained, but the plasma protein level was not significantly decreased as mentioned above. These could contribute the higher molecular weight of PVP used in this study, which significantly increased the hydrophilicity of the membrane.

However, the elution of the blended hydrophilic polymer should be noticed. Thus, the molecular weight of the hydrophilic polymer and the miscibility of the two polymers are important when we prepare the membranes. In this study, we chose one kind of PVP with high molecular weight (PVP-90K, BASF, Germany) to modify PES. The resultant PES membrane has an increased hydrophilicity, the contact angle decreased to about 30°, and correlated to the PVP amount. The dynamic contact angle decreased with time. Also, when high molecular weight PVP was used, the PVP is stable in the membrane compared to low molecular weight ones PVP-30K, this can be easily testified by incubating the membranes in normal saline solutions.

The PES hollow fiber membrane was prepared using a dry-wet spinning method based on the common phase inversion and precipitation technique, and has an inner skin as shown in Fig. 1. The inner skin layer was obtained when a strong coagulant, water was used as a bore liquid to speed up the instantaneous phase separation from the inner surface of the nascent fiber and at the meantime the phase separation at the outer surface was delayed by the air gap [14].

Solute transport

As shown in Table 2, the reduction ratio for the β_2-microglobulin was smaller than that for the urea and creatinine due to the higher molecular weight ($p < 0.05$). The alteration of β_2-microglobulin in plasma levels may not simply be a result of trans-membrane transport; the adsorption to the membrane may also play a role in the observed plasma changes [4]. For the removal of β_2-microglobulin, cellulose derived membrane is impermeable to β_2-microglobulin due to its dense symmetrical structure which does not permit the easy diffusion or convection of proteins through the membrane, while polyacrylonitrile (PAN), polysulfone and polymethylmethacrylate (PMMA) membrane could be used [17]. The PMMA membrane could also adsorb β_2-microglobulin. To remove β_2-microglobulin more efficiency form plasma, hemodialysis membranes must therefore not simply be considered as filters of low-molecular-weight metabolites but should be equally assessed for their capacity to eliminate potentially deleterious low-molecular-weight plasma proteins. For the PES membrane, β_2-microglobulin adsorption is not an important mechanism of removal. The large solute removal by the membrane is mainly caused by the asymmetric structure and the higher ultra-filtration coefficient, which was presumably caused by the larger pore size and the hydrophilicity of the membrane.

For the electrolyte, only the K^+ exhibited statistically significant decreases during the dialysis ($p < 0.05$), whereas Na^+, Cl^-, and Ca^{2+} did not change ($p > 0.05$). The electrolyte balance could be adjusted by the dialysis fluid, and the accurate K^+ values are critically important for the management of patients with little or no residual kidney function [18, 19].

Biocompatibility

Decreases in WBCs and platelets were observed immediately after the start of the hemodialysis, as shown in Fig. 3. These phenomena have been reported frequently in hemodialysis, hemofiltration, and plasma separation. The decrease of WBC was caused by complement activation, as the data in Table 4; and the activation of the complement system results in the release of anaphylatoxins into the circulation which have potent physiological effects, thus complement activation has been the most widely used parameter to evaluate hemocompatibility.

RBCs and hemoglobins (HGB) decreased slightly after the treatment, and both of the reduction ratios were about 5%. Slightly decreases in ALT, AST, TP and plasma albumin (ALB) were also observed. The reduction ratios for all of them ranged 3–10%, which were presumably caused by the dilution of the blood by normal saline solution infused after the hemodialysis process. ALP is produced primarily in the liver and in bone, the result for ALP indicted that the PES membrane had no effect on the liver.

Conclusion

A new hemodialysis membrane manufactured by a blend of PES and PVP was evaluated in vitro and in vivo. Goat was selected as the experimental animal. The PES hollow fiber hemodialysis membrane could effectively remove water and waste products not only small molecular weight solute such as urea and creatinine, but also "middle" molecular solute as β_2-microglobulin. Slight neutropenia and platelet adhesion were observed at the initial stage of the hemodialysis and no significantly differences were found in electrolyte, blood gas and blood biochemistry before and after the treatment. Some performance were compared some commercial membranes. Clinical characterization is now undertaken in our university hospital. The PES hollow fiber membrane hemodialyzer may be a good commercial product in the future.

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