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RESEARCH ARTICLE

Polysulfone hemodialysis membrane incorporated with Fe_2O_3 for enhanced removal of middle molecular weight uremic toxin

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Abstract

Removing middle molecular weight uremic toxin remains as one of the most challenging tasks in hemodialysis. Hence, in this study a high performance polysulfone (PSf) hemodialysis membrane was developed by incorporating iron oxide (Fe_2O_3) nanoparticles. The PSf/Fe_2O_3 hemodialysis membrane and pristine PSf membrane were prepared via dry-wet spinning process. The membranes were characterized by scanning electron microscopy, water contact angle, average pore size, and porosity measurements. The biocompatibility profiles of the membranes were also evaluated in terms of protein adsorption and blood coagulation time. Next, the performance of the membranes was determined by measuring pure water permeability (PWP), bovine serum albumin rejection, and removal of various solutes such as urea and lysozyme. The incorporation of Fe_2O_3 resulted in significant increment of the PWP from 40.74 L/m^2/h/bar to 58.6 L/m^2/h/bar, mainly due to the improved water transport properties of the membrane. Moreover, the percent removal of urea and lysozyme was reported to be 75.1% and 35.6%, respectively. PSf/Fe_2O_3 hemodialysis membrane is proven to have a bright prospect for enhanced blood purification process.

Keywords: Iron oxide nanoparticles, middle molecular weight uremic toxin, hemodialysis membrane, polysulfone membrane

INTRODUCTION

The number of chronic kidney disease (CKD) patients has increased drastically from year to year. One of the prevalent treatments for CKD is hemodialysis. In hemodialysis, the most important component that governs the quality of the treatment is called dialyzer, where ten thousand of hollow fiber membrane are situated. The membranes are usually made up of synthetic polymers such as polysulfone (PSf) and polyethersulfone (PES) [1,2]. However, the membrane surface hydrophobicity and low removal of middle molecular uremic toxin are among the issues currently faced in hemodialysis application [3]. Studies showed that larger uremic toxins are difficult to be removed by diffusion. The accumulation of these middle molecular weight uremic toxins may lead to the formation of amyloid, an abnormal protein that deposits on internal organs and causes organ failure [4]. Apart from that, the hydrophobic nature of the membrane would promote protein loss via adsorption on the membrane surface [5].

To address the mentioned problems, additives that have excellent biocompatibility were added to form a membrane with high permeability and surface hydrophilicity. The high permeability can actually improve convective separation of middle molecule uremic toxins, whereas the surface hydrophilicity can minimize the interaction between membrane surface and hydrophobic proteins [6]. Incorporation of inorganic nanomaterials in membrane matrix has become an emerging trend to enhance the membrane permeability, selectivity, and physicochemical properties. The recent progress in the development of hemodialysis membrane involved the uses of carbon nanotubes (CNTs) and graphene [7,8], where the membranes showed excellent water transport properties and uremic toxin removal compared to that of pristine polymeric membrane.

Nevertheless, the use of other potential nanomaterials like metal oxide in hemodialysis membrane and their potency to improve the dialysis performance have never been reported before. Iron oxide (Fe_2O_3) nanoparticles are among the unique metal oxide nanoparticles due to their nanoscale dimension, chemical inertness, good hydrophilicity, and remarkable total surface area [9,10]. The Fe_2O_3

nano-structure behaves as a good liquid transport medium, thus producing membranes with good interconnectivity and porosity which could facilitate water movement across the membrane at minimal operating pressure. The presence of the hydrophilic Fe_2O_3 at the membrane surface could minimize the amount of protein adsorbed on the membrane. Additionally, in biomedical applications, Fe_2O_3 are known to be biocompatible and non-toxic [11]. Thus, they have a great potential for incorporation in hemodialysis membrane.

In our previous study, we have investigated the impacts of Fe_2O_3 addition on membrane liquid separation characteristics and antifouling resistance [12], where the nanocomposite membrane was more superior compared to the pristine PSf membrane. A particle stabilizer known as citric acid (CA) was utilized to improve Fe_2O_3 dispersion stability during membrane fabrication. In a following work [13], the optimum weight ratio of Fe_2O_3 and CA was determined to be 1:20. Here, the main objective of this work is to study the effects of Fe_2O_3 addition as membrane nanofillers on hemodialysis separation performance, focusing on middle molecular weight uremic toxin removal. Pristine PSf membrane, which is commonly regarded as hemodialysis membrane was used as a reference. The morphological and surface properties of the two membranes were compared. The biocompatibility profile of the fabricated membranes was also evaluated in terms of protein adsorption and blood coagulation time. In addition to urea separation, the clearance of lysozyme which represents the middle molecular weight toxin was also determined in this study.

EXPERIMENTAL

Materials

The reagent-grade chemicals were used. PSf polymer was purchased from Solvay Advanced Polymers; PVP-K90, CA, human serum albumin (HSA), bovine serum albumin (BSA), sodium dodecyl sulfate (SDS), urea, and lysozyme were purchased from Sigma-Aldrich; and Fe_2O_3 was purchased from Nova Scientific; N-methyl-2-pyrrolidone (NMP, 99.5 %) and glycerol were supplied from Merck. Calcium chloride, Actin FS, and Thromborel S were procured from Siemens; BCA™ protein assay reagent kit was purchased from Thermo Scientific, while QuantiChrom™ urea assay kit was obtained from BioAssay Systems; phosphate buffer saline (PBS) was a product of Amresco.

Fabrication of PSf/Fe_2O_3 hollow fiber membrane

The PSf pellets were pre-dried in oven before use. PVP was dissolved in NMP at 70 °C by mechanical stirring. Next, CA was added and blended for 30 min. Then, Fe_2O_3 was mixed and the solution was stirred for 2 h to obtain a homogeneous mixture. Finally, PSf was added into the solution with continuous stirring for 24 h. The dope solution compositions for the pristine PSf and PSf/ Fe_2O_3 membranes are shown in Table 1. The dope solutions were degassed to remove air bubbles. Dry-wet spinning technique was used for the fabrication of the hollow fiber membranes. The air gap was fixed at 50 cm. The membrane dope solution was extruded from a spinneret with an inner/outer diameter of 0.4/0.8 mm at a speed of 1 mL/min. The bore fluid flow rate of 1 mL/min was streamed into the spinneret needle by a constant-flow syringe pump. The collection drum speed of 10 m/min was used to collect the hollow fiber membranes. The fabricated hollow fiber membranes were immersed in water for two days and treated in 10 wt% glycerol for one day.

Table 1 Dope solution compositions (wt%) of pristine PSf and PSf/Fe_2O_3 membranes.

Membrane	PSf	PVP	Fe_2O_3	CA	NMP
Pristine PSf	18.0	4.8	-	-	77.2
PSf/Fe_2O_3	18.0	4.8	0.1	2.0	75.0

Characterization of the membranes

The membrane morphologies were observed using scanning electron microscopy (SEM, Hitachi TM3000). The contact angle analysis was conducted to determine the membrane hydrophilicity using contact angle goniometer from Dataphysics, United States of America. Membrane porosity and average pore size were measured using methods described by Abidin *et al*. [3].

Biocompatibility assessment

HSA was chosen as model protein for adsorption test. The 1 cm^2 membrane sample was pre-immersed in PBS for 12 h, followed by incubation in 2 mL of 1 mg/mL protein solution for 2 h. The sample was then rinsed with PBS solution and water to eliminate excess protein. SDS was then used to extract the protein from membrane surface. Protein amount adsorbed on membrane sample was measured using BCA™ protein assay reagent kit.

The membranes biocompatibility was further evaluated by determining blood clotting time. Around 10 mL of fresh human blood was withdrawn and stored in the 2.7 mL vacutainer tubes that contain sodium citrate. The blood was centrifuged at 3500 rpm for 15 min to obtain PPP. The test was performed using a semi-automated blood coagulation analyzer (Sysmex, CA-104) to measure the activated partial thromboplastin time (APTT) and prothrombin time (PT). These parameters show the time taken before the blood coagulates once it is exposed to surrounding or foreign materials. Membrane sample (3 pieces x 0.5 cm x 0.5 cm) was immersed in PBS solution for 1 h before the incubation in platelet poor plasma (PPP). Next, the membrane was incubated in 0.5 mL of PPP for 30 min. To measure the APTT, 50 µL of the incubated PPP was mixed with 50 µL of Actin FS and incubated for 180 seconds. 50 µL of calcium chloride, $CaCl_2$ was then added and the clotting time was taken. For PT test, 50 µL of PPP was added into the cuvette and incubated for 60 seconds. Next, 100 µL of Thromborel S was mixed and the clotting time was taken.

Separation performance experiment

Membrane module (25.0 cm x 1.3 cm) was made consisting of 60 fibers, potted using epoxy glue with both ends cut open. The module was equipped with 2 sets of inlet and outlet connected to feed and dialysate containers.

The membrane pure water permeability (PWP) and BSA rejection were first performed as both represent the ultrafiltration capacity of the membrane and its ability to retain albumin in blood. The PWP of the fabricated membranes was measured at a TMP of 0.7 bar using Eq. (1)

$$PWP\ (L/m^2\ h\ bar) = (V/A \times \Delta t \times \Delta P) \quad (1)$$

where V is the volume of permeate (L), A is the effective surface area (m^2), Δt is the permeation time (h), and ΔP is the TMP. The percent rejection was calculated using following Eq. (2)

$$Rejection\ (\%) = (1-C_p/C_f) \times 100 \quad (2)$$

where C_p and C_f are the BSA concentrations of permeate and feed, respectively. The concentration of BSA was measured using UV-vis spectrophotometer (Hach DR 5000™).

The prepared module was equilibrated with pure water for overnight and pressurized with water at 1 bar for at least 30 min before the experiments. The feed tank was filled with a mixture of solutes containing urea (1500 ppm) and lysozyme (40 ppm) in water, while dialysate tank was filled with water. The feed flow rate was fixed at 15 mL/min, while dialysate was fixed at the flow rate of 37.5 mL/min. The experiment was conducted for 4 h and the sample was collected every 1 h. The concentration of samples was measured using UV-vis spectrophotometer where the urea concentration was identified using QuantiChrom™ urea assay kit. The amount of toxins removed from feed solution was defined as percent clearance, which was calculated using Eq. (1)

$$Clearance\ (\%) = (1- C_f/C_i) \times 100 \quad (3)$$

where C_i and C_f are the initial and final concentrations of the solute in feed solution (mg/L), respectively.

RESULTS AND DISCUSSION

Morphology of the fabricated membranes

FESEM images of the pristine PSf and PSf/Fe_2O_3 membranes are shown in Fig. 1. The membranes produced common asymmetric structure which consists of a dense skin layer and a porous sub layer. The porous sublayer extends from the compact like structure along the finger-like macrovoids and ends with sponge-like macropores. The permeation rate depends on the skin layer thickness. The porous sublayer mainly acts as a mechanical support of the membrane that prevent it from collapsing.

The PSf/Fe_2O_3 membrane exhibited more finger-like structures than the PSf membrane due to faster phase inversion process. The thinner skin layer was also formed due to the presence of Fe_2O_3 which induced the instantaneous phase inversion as soon as the PSf/Fe_2O_3 dope solution gets in contact with water.

The analysis was continued with the observation on the membrane outer morphological surface. It can be seen that the pore distribution on the membrane surface has increased in size due to free movement of Fe_2O_3, CA, and PVP molecules near the membrane-moisture interface. Besides, the CA and PVP tend to diffuse out of the solvent and move into the water bath during phase inversion process, leaving empty spaces in the nascent hollow fibers [14].

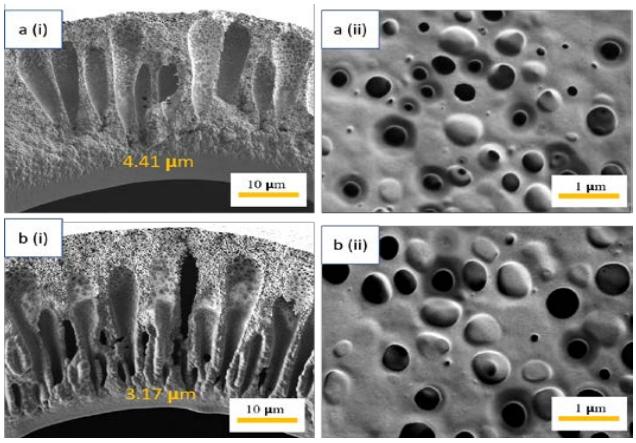

Fig. 1 The FESEM images of (a) pristine PSf and (b) PSf/Fe_2O_3 membranes showing (i) cross-section and (ii) outer surface.

Water contact angle, porosity, and average pore size of membranes

Contact angle is known as a first indication of the interaction between the water, protein, and the membrane surface. By improving membrane hydrophilicity, the interaction between membrane and albumin will be weakened. Eventually, the membrane has better chance of rejecting albumin and reducing the tendency of protein fouling. Meanwhile, membrane porosity should be as high as possible to enable high transmembrane flux. The results of water contact angle, porosity, and average pore size are shown in Table 2. The incorporation of Fe_2O_3 resulted in substantial increase of the PSf/Fe_2O_3 membrane surface hydrophilicity, where hydrophilic Fe_2O_3 migrated spontaneously to membrane or water interface to reduce their surface energy. On top of that, the membrane hydrophilicity was improved due to the presence oxygen species in the Fe_2O_3, allowing better interaction with water molecules.

Table 2 Contact angle, porosity, and average pore size of pristine PSf and PSf/Fe_2O_3 membranes (n=3).

Membrane	Contact angle (°)	Porosity (%)	Pore Size (nm)
Pristine PSf	66.81 ± 0.21	43.29 ± 0.11	63.51 ± 0.07
PSf/ Fe_2O_3	55.20 ± 0.16	67.36 ± 0.18	38.84 ± 0.12

As shown in Table 2, the PSf/Fe_2O_3 membrane is more porous compared to the pristine PSf membrane. The increase in the number and size of finger-like structures has enhanced the membrane porosity. Nevertheless, the average pore size of the membrane became smaller after the addition of F e_2O_3, most probably due to the rapid precipitation of polymer at the membrane skin layer. The smaller average pore size may permit only uremic toxins to pass through the membrane, while preventing large proteins like albumin from crossing the membrane.

Biocompatibility of the membrane

In hemodialysis treatment, the membrane must be biocompatible. The PSf/Fe_2O_3 and pristine PSf membranes were assessed in terms of protein adsorption and blood coagulation time. Protein adsorption contributes to health complication after hemodialysis by causing protein depletion in blood. Besides, the adsorbed protein molecules on membrane surface during hemodialysis treatment can activate the blood coagulation cascade thus causing biofouling. The results of HSA adsorption are shown in Table 3.

The PSf/Fe_2O_3 membrane adsorbed less HSA compared to the pristine PSf membrane. Generally, hydrophilic membranes have a lower tendency to adsorb proteins compared to hydrophobic membranes. This has been proven by several studies, confirming that the amount of adsorbed protein per unit area of membrane decreased with increasing surface hydrophilicity of membrane [15].

Table 3 Protein adsorption results of the PSf/Fe_2O_3 and pristine PSf membranes (n=3).

Membrane	HSA adsorption (µg/cm^2)
Pristine PSf	8.23 ± 1.13
PSf/Fe_2O_3	6.68 ± 0.71

Blood coagulation is among the fatal problems that can occur during hemodialysis treatment. This problem is usually caused by the interaction of blood with blood-incompatible materials. Membrane with poor blood-compatibility may induce the blood to clot rapidly and be deposited on the membrane surface. The clotting of blood promotes excessive loss of blood, which poses health risk on the CKD patients especially those who suffer from anemia. Therefore, it is important to maintain the normal range of blood coagulation time when the blood is in contact with membrane surface. APTT and PT are the established measurements used to predict the intrinsic pathway and the bioactivity of blood coagulation factors [1].

The results of APTT and PT of the pristine PSf and PSf/Fe_2O_3 membranes compared to the control sample are shown in Fig. 2. The membranes fall within the normal range value, in which the PSf/Fe_2O_3 membrane prolonged the APTT and PT value as compared to the pristine PSf membrane. The findings proved that the materials used to produce the membrane was compatible since the membrane did not speed up the blood coagulation time excessively.

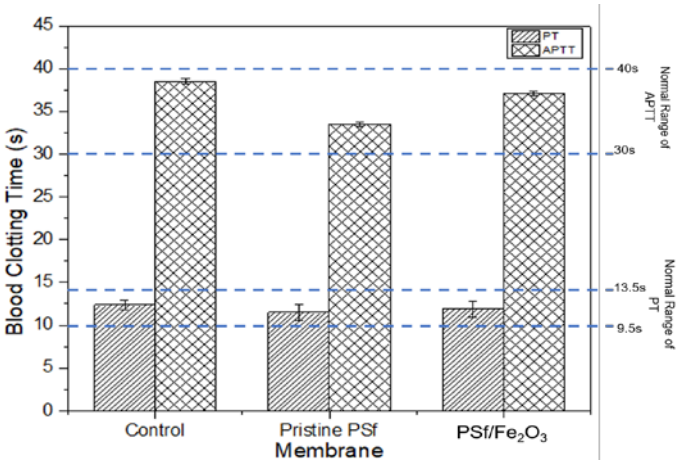

Fig. 2 Blood clotting time of the control, pristine PSf and PSf/Fe_2O_3 membranes (*n*=3).

PWP and BSA rejection

The results of PWP and BSA rejection of the pristine PSf membrane and the PSf/Fe_2O_3 membrane are shown in Table 3. The PSf/Fe_2O_3 membrane expressed better PWP which could be attributed to its increased porosity and surface hydrophilicity, which vitally enhanced the membrane's water transport properties. The role of the high surface area Fe_2O_3 embedded in membrane matrix was to accommodate and facilitate more water molecules. On top of that, the PSf/Fe_2O_3 membrane morphologies which comprised of more finger-like structures and well-distributed pores promoted more water passages through the membranes compared to the pristine PSf membrane.

The BSA (~67 kDa) separation experiment was conducted to determine the amount of albumin that can be retained in blood during hemodialysis treatment. This study showed that the BSA rejection of the PSf/Fe_2O_3 membrane was higher compared to the pristine PSf membrane. This was due to improved route of water molecules to pass through, leaving the large and hydrophobic BSA molecules behind. As the water moved faster, the BSA had a lesser tendency of getting close to the hydrophilic membrane surface. The recorded BSA rejection certainly exceeded most results reported in literature which were below than 97 % [7,16].

Table 3 PWP and BSA rejection of the pristine PSf and PSf/Fe_2O_3 membranes.

Membrane	PWP (L/m^2h bar)	Rejection (%)
Pristine PSf	40.7	91.2
PSf/ Fe_2O_3	58.6	98.1

Urea and lysozyme separation

The results of urea and lysozyme clearance are displayed in Fig. 3 and Fig. 4, respectively. Urea is known as the classical marker for uremic toxins, where it is used to define the quality of hemodialysis membrane. A similar trend was observed on the two membranes, where the urea diffusion took place at the highest rate in the first one hour before it got slower.

The PSf/Fe_2O_3 membrane showed better urea removal rate compared to the pristine PSf membrane. The higher water retention capacity of PSf/Fe_2O_3 membrane due to higher porosity eased the urea diffusion process by drawing dialysate to fill in the membrane wall faster. In general, the urea clearance achieved the minimum requirement set by the Ministry of Health Malaysia (>65 %). Besides, the value was higher compared to previously published hemodialysis membranes like heparin-immobilized poly (lactic) acid membrane (74 %) by Goa *et al.* [16] and MWCNTs-incorporated PES membrane (56 %) by Irfan *et al.* [7].

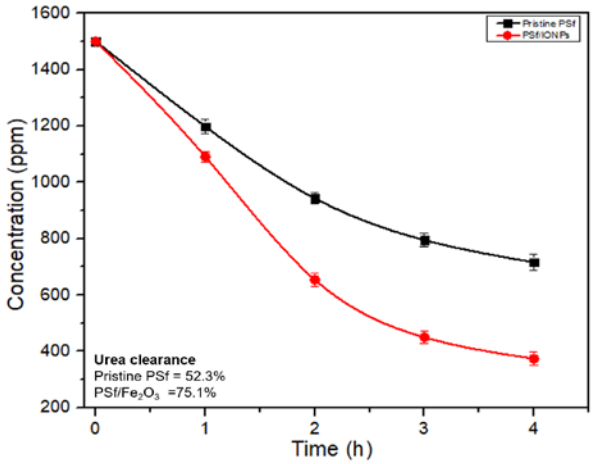

Fig. 3 The results of urea percent clearance for pristine PSf and PSf/Fe_2O_3 membranes over 4 h of operation (*n*=3).

Meanwhile, a steady reduction of lysozyme concentration in the feed solution was achieved by both membranes. The PSf/Fe_2O_3 displayed a superior performance over the pristine PSf membrane with lysozyme clearance of 35.6 %. Having a good transport property has influenced the PSf/Fe_2O_3 to obtain the better separation, as middle molecules removal can be improved using high flux and porous membranes. The lysozyme, dissolved in water, was carried along with water via convection and passed through the membrane, following a hydrostatic pressure gradient.

From this study, the lysozyme percent clearance marked a better value compared to the previous studies by Gao *et al.* [16] and Irfan *et al.* [7] which were 18 % and 28 %, respectively.

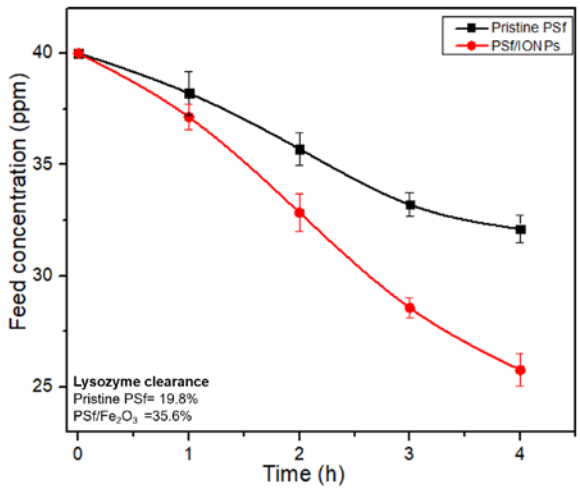

Fig. 4 The results of lysozyme percent clearance for pristine PSf and PSf/Fe_2O_3 membranes over 4 h of operation (*n*=3).

CONCLUSION

The effects of Fe_2O_3 incorporation on the hemodialysis membrane morphology, surface property, and separation performance were studied. The PSf/Fe_2O_3 membrane was confirmed to be more hydrophilic and had a highly porous structure with better pore distribution compared to the pristine PSf membrane. The PSf/Fe_2O_3 membrane was more biocompatible, proved by the lesser HSA adsorption and normal blood coagulation time. The PSf/Fe_2O_3 membrane achieved PWP of 58.6 L/m^2 h bar and BSA rejection of 98.1 %, while achieving excellent separation performance of urea (75.1 %) and lysozyme (35.6 %). Overall, the results showed that the PSf/Fe_2O_3 membrane is an outstanding candidate for hemodialysis treatment since it could retain almost all albumin while showing high percent clearance of urea and lysozyme.

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