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Permeability and Blood Compatibility of Nanoporous Parylene Film-Coated Polyethersulfone Membrane Under Long-Term Blood Diffusion

Gunawan Setia Prihandana,[1] Hikaru Ito,[1] Ippei Sanada,[1] Yuya Nishinaka,[1] Yoshihiko Kanno,[2] Norihisa Miki[1]

[1]Department of Mechanical Engineering, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama, Kanagawa 223-8522, Japan
[2]Department of Nephrology, Tokyo Medical University, 6-7-1 Nishi-Shinjuku, Shinjuku-ku, Tokyo 160-0023, Japan
Correspondence to: G. S. Prihandana (E-mail: g_prihandana@a2.keio.jp)

ABSTRACT: Nanoporous polyethersulfone (PES) membranes are widely used in dialysis systems due to their permeability and diffusion characteristics. However, PES membranes lack blood compatibility, which influences their permeability performance when employed in blood contact devices. Parylene film was deposited on a PES membrane surface and the membrane permeability and blood compatibility were investigated by long-term blood diffusion testing. After 28 days of testing, 90% of a bare PES membrane was covered with platelets, while the parylene film coated PES membrane had improved biocompatibility with a platelet coverage of only 20–30%. The permeability of the bare PES membrane significantly declined during the first 7 days of the blood diffusion and became stable after 8 days. In contrast, the permeability of the parylene film coated PES membrane exhibited more consistent performance during the entire test. Thus, parylene film coating on PES membrane has potential for application in hemodialysis systems.
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INTRODUCTION

In recent decades, polymeric membranes have been widely used in the field of water filtration, blood purification, and as membranes in medical devices and hemodialysis systems. In the field of medical devices for hemodialysis systems, we have successfully designed and fabricated a multilayered microfilter for use as the dialyzer of a wearable artificial kidney.[1] The wearable artificial kidney needs to operate dialysis treatment for a long time. For that reason, the PES membrane was selected as a separation membrane for wearable artificial kidney due to its good mechanical strength, thermal stability, and chemical resistance.[2]

However, the poor blood compatibility of the PES membrane when in contact with blood causes adsorption of protein and further platelet adhesion, aggregation, and coagulation, which limits the application of PES membranes to blood-contacting devices.[3–5] In order to improve the blood compatibility and biocompatibility of PES membrane, numerous researches have been conducted, such as surface coating,[2] blending,[3,5,6] and surface grafting.[4]

We have also previously developed a new technique to coat parylene film onto PES membrane surfaces using glycerin vapor and control of the amount of parylene dimer to improve the diffusivity of the coated membrane.[7] The parylene deposition process utilizes the passage of glycerin vapor through the membrane to create a nanoporous parylene film. The nanoporous parylene film formed on the membrane is thus considered to be aligned with the pores of the PES membrane.

Parylene film has been used as a protective layer on PES membranes, due to its excellent blood and biocompatibility.[8–11] However, the use of parylene film as a layer on PES membrane surfaces had not been studied, especially the reliability of a PES membrane coated with parylene film in a system involving blood contact. Therefore, platelet adhesion to parylene film coated PES membrane during long-term diffusion tests was investigated in this study. Long-term diffusion tests (0–28 days) were conducted to clarify the extent of time that the modified PES membrane could be applied in a blood contact device. In addition, the permeability performance of the modified membrane during the long-term diffusion tests was also examined.

EXPERIMENTAL

Membrane Preparation

Flat sheet PES membranes were prepared from PES (molecular weight: 4800, Sumitomo Chemical Co., Japan), polyvinylpyrrolidone (PVP; molecular weight: 35,000, Wako Pure Chemical

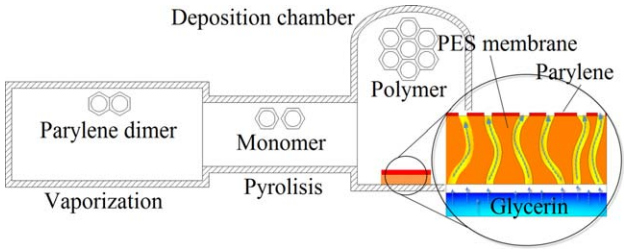

Figure 1. Parylene deposition process assisted by glycerin vapor. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Industries, Japan), and 1-methyl-2-pyrrolidone (NMP; Wako Pure Chemical Industries, Japan), as solute, solvent, and additive components, respectively. PES, PVP, and NMP were mixed in a ratio of 20 : 20 : 60 (wt %), respectively, and kept at room temperature for approximately 48 h to form a transparent casting solution. The PES casting solution was then poured onto a glass substrate. The PES membrane was then prepared by spin coating at 3500 rpm followed by direct immersion into distilled water. As soon as the glass substrate was immersed in distilled water, a thin layer of white membrane was observed forming at the interface between the casting solution and the distilled water. The as-formed PES membranes were then stored for further use in distilled water at room temperature for more than 24 h to remove the PVP solvent.

Membrane Modification

Surface modification of the PES membrane was conducted by deposition of parylene-C onto the membrane using a Labcoter® 2 parylene deposition unit (SCS Coating Center Parylene Japan, LLC). During deposition, the parylene dimer charge was vaporized at 175°C and 133.3 Pa, then decomposed to its monomer (paraxylylene) at 690°C and 66.7 Pa, and finally condensed and polymerized onto the PES membrane at room temperature in a deposition chamber at 6.7 Pa, as illustrated in Figure 1. Parylene deposition is achieved by utilizing the passage of glycerin vapor through the membrane to create a nanoporous parylene film. The low vapor pressure of glycerin (0.2 Pa[12]) ensures vaporization during the deposition process. Figure 1 shows a schematic of the process, where glycerin is vaporized and passes through the membrane pores, which prevents parylene deposition above the pores and subsequent blocking.

Platelet Adhesion and Aggregation

Platelet adhesion and aggregation play an important role in the formation of a blood clot on blood-contacting membranes applied in medical devices. Therefore, platelet adhesion must be assessed to determine the blood compatibility of a membrane intended for use in a hemodialysis system.

After blood diffusion experiments were conducted for 7 and 28 days, the diffusion chambers were opened, and the membranes were removed for scanning electron microscopy (SEM). The membranes were washed with phosphate-buffered saline (PBS; pH 7.4) and platelets adhered onto the membrane surface were fixed by treatment with freshly prepared 1% glutaraldehyde for 1 h at room temperature. After fixation, the samples were washed and dehydrated in a series of graded ethanol solutions (20, 40, 60, 90, and 100%) for 10–15 min each. Dehydrated membranes were then placed in a vacuum chamber and dried overnight. The completely dried membranes were coated with osmium and investigated using SEM (Quanta 200 SEM, Field Emission). In order to determine the percentage of membranes coverage by platelets, only two samples ($n = 2$) were used in the experiments, due to the long period of diffusion test (28 days). One sample was used on diffusion test from day 1 to day 7, whilst the second sample was used continuously from day 1 to day 28.

Long-Term Diffusion Test Experiments

Much research has been conducted to characterize molecular transport through membranes by evaluation during filtration processes.[13,14] One of the methods commonly used to measure membrane permeability is diffusion based separation. Diffusion-based separation adopts the principle of the dialysis process, where a dialysis membrane, which is permeable to water and nanomolecules, is used as an ion exchange transport membrane. In the dialysis process, the membrane exploits the difference in concentration between the blood and the dialysate to remove accumulated nitrogenous waste products in urine, excess water and ions from the patient by the dialysate solution. The dialysate solution is also used to supply ion deficiencies in the blood.

In this study, defibrinated bovine blood (Kohjin Bio Co.) was used as a blood solution and NaCl solution, which follows the medical standard, was used as the dialysate solution. Urea was added to the blood to achieve concentrations of blood urea nitrogen (BUN) to 100 mg/dL. The concentrations of Na, K, and Cl in the blood and dialysate were measured using an electrolyte analyzer (SPOTCHEM EZ SP-4430, Arkray, Japan), while the urea concentration was measured using an automated analyzer for clinical chemistry (SPOTCHEM EZ SP-4430, Arkray, Japan). The detailed concentrations of the defibrinated bovine blood and dialysate solution are presented in Table I.

The diffusivity of the membranes was evaluated over a long term of 28 days. A loop system and diffusion chamber, as illustrated in Figure 2, was designed and fabricated to represent a portable dialysis system for long-term diffusion tests on the membranes. In this system, a peristaltic pump (Peri-Star Pro, World Precision Instruments) was used to circulate the blood and dialysate into the diffusion chamber.

The diffusion chamber was made of poly(methyl methacrylate) (PMMA) plates, where grooves in the chamber chips acted as channel connections for the blood and dialysate, which were separated by the membrane. The channel in the chamber layer

Table I. Urea, Na, K, and Cl Concentrations in the Defibrinated Bovine Blood and Dialysate

Solution	Urea (mg/dL)	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)
Defibrinated bovine blood	100	128	4.4	89
Dialysate	<5	141	2.3	88

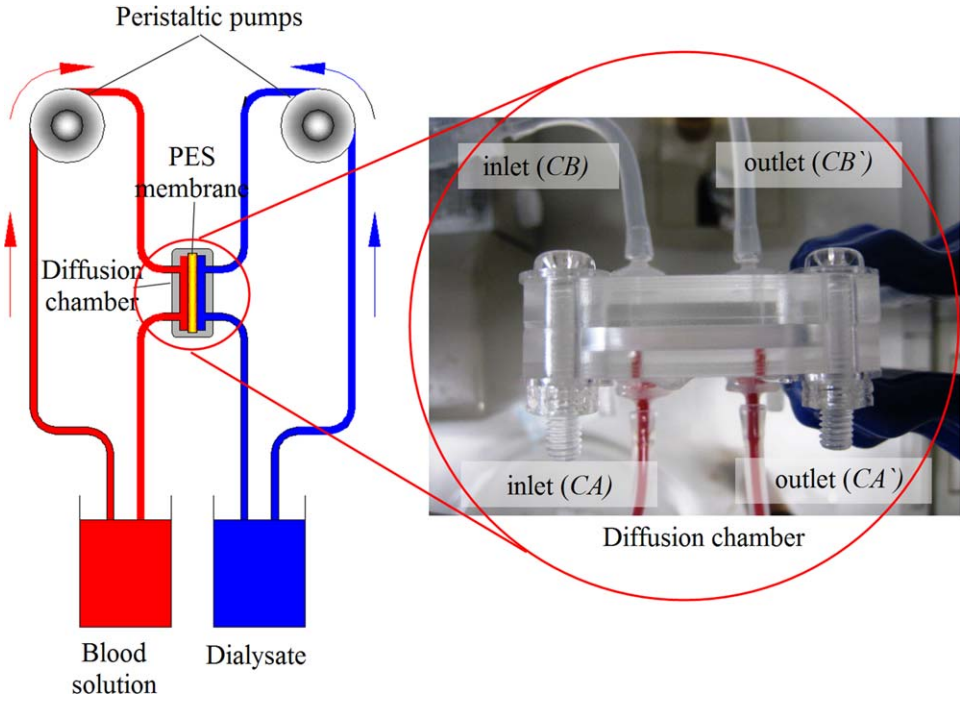

Figure 2. Loop system and diffusion chamber. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

was 14 mm long, 2.8 mm wide and 200 μm deep. Figure 3 shows the detailed dimensions of the diffusion chamber.

Based on our previous results,[7] the bare PES membrane and PES membranes coated with parylene dimer (10 and 20 mg) were selected for comparison during a long-term diffusion test. The methodology for calculation of the membrane diffusion coefficient has been described previously,[1,15] and the diffusion coefficient was determined based on the following equation:

$$D = \frac{Q \times H}{A} \times \ln\left[\frac{C_B - C_A}{C_{B'} - C_{A'}}\right] \quad (1)$$

where C_A is the initial concentration of urea in solution A and C_B is that in solution B, which is initially the dialysate ($C_B = 0$). H is the thickness of the membrane, A is the diffusion area of the chamber, and Q is the flow rate of the blood and dialysate, which were set to be the same and constant (10 μL/min). By supplying blood solution and dialysate into the dual inlets of the device, molecules smaller than the mean pore size of the membrane can diffuse through the membrane into outlet B ($C_{B'}$). The extent of solute diffusion through the membrane can be determined by measuring the concentration of the solution collected in outlet B and that of the blood solution in outlet A ($C_{A'}$). The diffusivity performance of the membranes was measured daily during the 28 days of diffusion testing. The results of these measurements are expressed as the mean of three replicates and the corresponding standard deviation.

RESULTS AND DISCUSSION

Platelet Adhesion and Activation

In hemodialysis systems for the treatment of blood diseases, the PES membrane has been well acknowledged due to its good chemical and mechanical properties. Therefore, it was important to evaluate the blood compatibility and biocompatibility of the PES membrane for further application in dialysis systems.

Protein absorption from the blood into the membrane is the fundamental process in the formation of blood clotting and

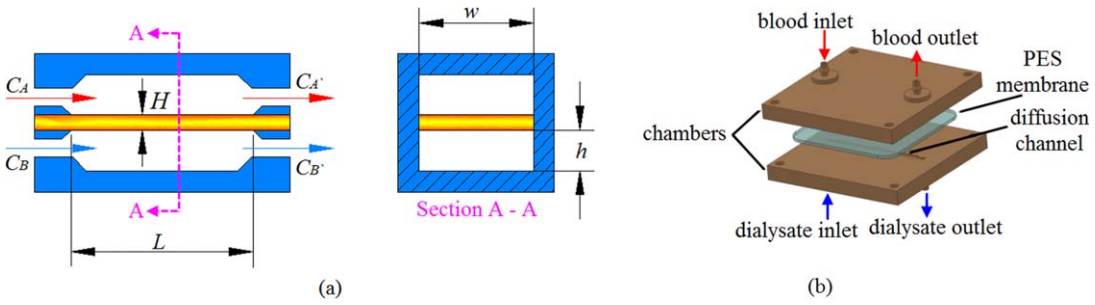

Figure 3. Design of the diffusion chamber. (a) Side view, where H is the membrane thickness, L is the channel length, h is the channel height, and w is the channel width. (b) Isometric view. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

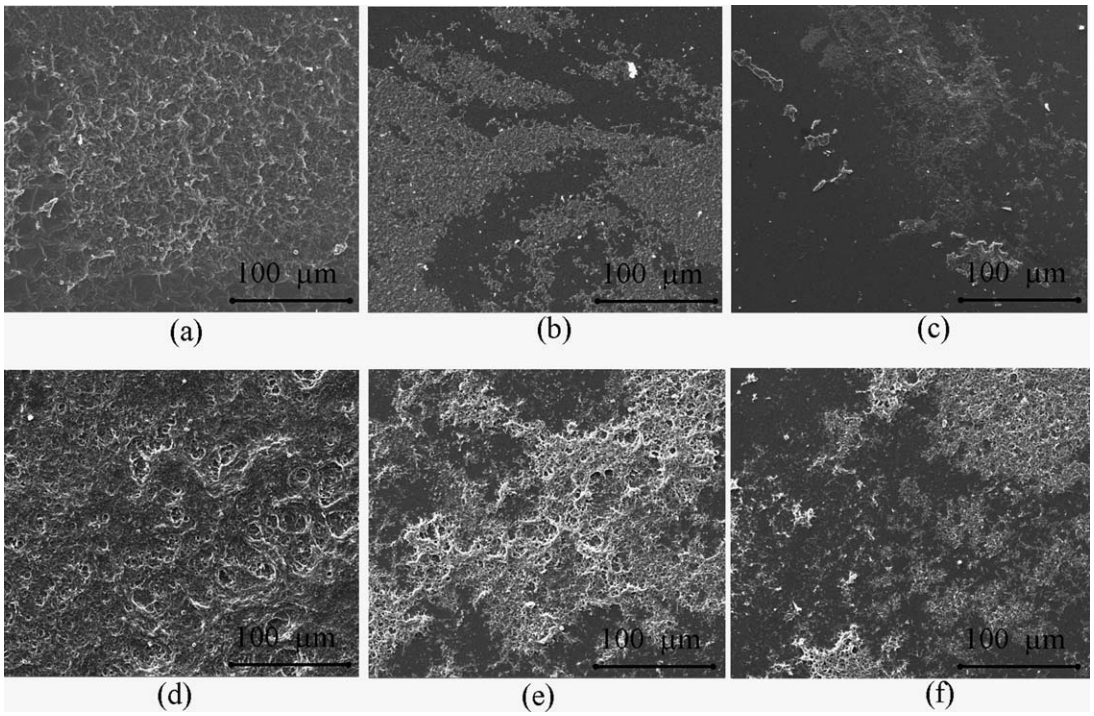

Figure 4. SEM images of platelets adhered onto the surfaces. (a) Bare PES membrane after 7 days diffusion, (b) PES membrane coated with 10 mg of parylene dimer after 7 days diffusion, (c) PES membrane coated with 20 mg of parylene dimer after 7 days diffusion, (d) bare PES membrane, after 28 days diffusion, (e) PES membrane coated with 10 mg of parylene dimer after 28 days diffusion, and (f) PES membrane coated with 20 mg of parylene dimer after 28 days diffusion.

tissue formation on the membrane surface used in dialysis devices.[16] Therefore, platelet adhesion and tissue growth due to long-term diffusion (0–28 days) was investigated. On day 7 of the diffusion test, the diffusion chambers used for the dialysis system were opened and three membranes were prepared for SEM analysis.

Based on the SEM analysis, after 7 days of diffusion testing, the surface of the bare PES membrane started had some platelets adhered to it, as shown in Figure 4. Approximately 20% of the membrane surface was covered by platelets. The appearance of platelets should correspond to the long-term diffusion performance of the bare PES membrane; the membrane diffusivity was significantly decreased by 60% after 7 days of blood diffusion.

In contrast, the PES membranes coated with parylene film showed promising results, where the membrane surfaces were relatively free from adhered platelets and only 5–10% of the membrane surface was covered by platelets, as shown in Figure 4(b) and (c). The percentage of the membrane diffusion area covered by platelets within the first 7 days and after 28 days of diffusion testing is shown in Figure 5. The results confirm that the parylene film was successfully deposited on the PES membrane surface by employing the passage of glycerin vapor through the membrane pores during parylene deposition (Figure 1). The coating of parylene onto the PES membrane surface resulted in improved blood compatibility of the PES membrane and the membrane permeability was maintained, as shown in Figures 4 and 5.

Diffusion testing was continued until 28 days to evaluate the long-term reliability of the membranes. After the diffusion tests were completed on day 28, the three membranes were prepared for the platelets adhesion analysis. Figure 4(d) shows that platelets have completely covered the bare PES membranes surface, with only some limited areas free of platelets. Approximately 90% of the bare PES membrane surface was covered with adhered platelets. Numerous platelets were aggregated on the bare PES membrane, the numbers of which increased with the diffusion test period. However, the addition of platelets accumulated on the membrane surface was not directly related to

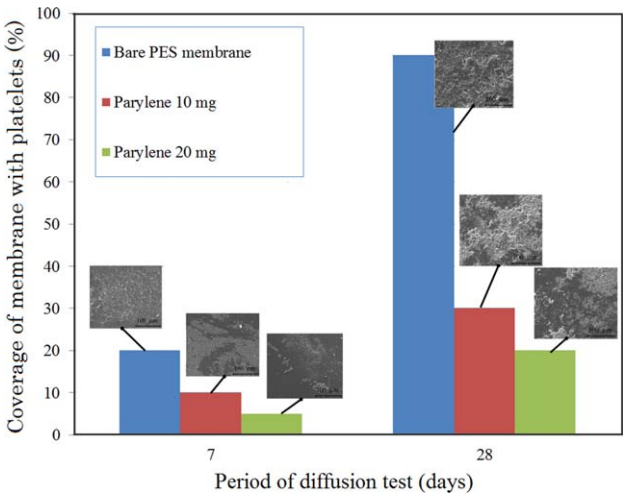

Figure 5. Coverage of membrane with platelets. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

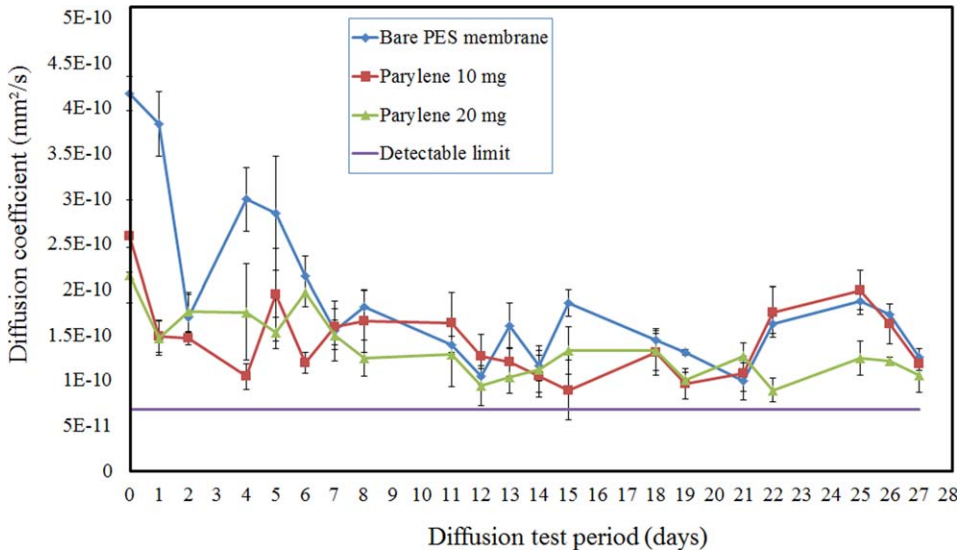

Figure 6. Diffusion coefficients for urea transport through the different membranes. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the diffusion performance of the membrane, because the diffusivity of the membrane was relatively stable.

In contrast, PES membranes coated with parylene film (10 and 20 mg of parylene dimer) had only a very sparse adherence of platelets, as shown in Figure 4(e) and (f). The parylene film on the membrane surface improved the biocompatibility and blood compatibility of the membrane, due to a reduction of adsorbed protein, thereby preventing the activation of platelets from adhering and accumulating on the membrane surface. Approximately 20–30% of the parylene coated membranes were covered by platelets. Although the parylene coated PES membrane surfaces were relatively free from adhered platelets, the diffusivity performance of these membranes were in the same range as that of the bare PES membrane.

Figure 5 shows that platelet adherence to the membrane surface was increased with the period of diffusion testing. In the first 7 days, only 20% of the bare PES membrane surface was covered with platelets. However, after 28 days of diffusion testing, almost all of the membrane diffusion area was covered with platelets. In contrast, the PES membranes coated with parylene films had improved blood compatibility, where only 20–30% of the membrane diffusion area was covered with platelets after 28 days of diffusion testing.

Diffusion Coefficients of the Membranes After Long-Term Diffusion Tests

Figure 6 shows the diffusivity of urea for the bare PES membrane, and the PES membrane coated with 10 and 20 mg of parylene dimer. The detectable measurement limit shown in Figure 6 represents the minimum detection limit of urea diffusion through the membrane porous. The concentration measurements were performed three times and the error bars indicate the standard deviation of the mean from three measurements. The bare PES membrane gave the highest diffusivity on the first day, followed by the PES membranes coated with 10 and 20 mg of parylene dimer, due to the absence of parylene coating on the PES membrane surface.

The diffusivity of the bare PES membrane was significantly decreased during the first 7 days, due to membrane fouling.

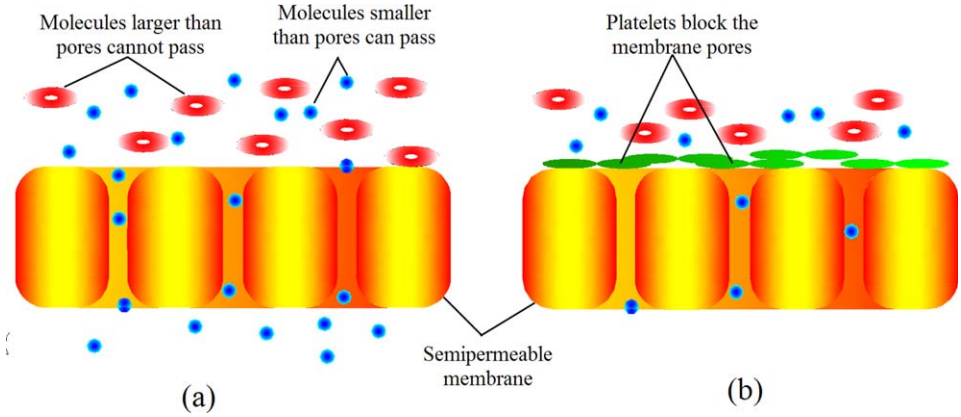

Figure 7. Filtration process. (a) Diffusion based separation using porous membrane, and (b) fouling mechanism for a semipermeable membrane. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Membrane fouling may occur immediately upon membrane contact with blood or other biological materials.[17] Furthermore, in a semipermeable membrane, the pores act as a separator and allow molecules smaller than the pore to pass through the membrane, while larger molecules cannot pass through, as illustrated in Figure 7(a). If biolayers or platelets in the blood foul or cover the permeable membrane, then fewer molecules will diffuse through the membrane, which results in a reduction of membrane diffusivity, referred to as membrane fouling [Figure 7(b)].

The blood diffusion experiments were continued from day 8 to day 21, during which time the diffusivity of urea through the bare PES membrane had a tendency to fluctuate. The diffusivity of the bare PES membrane remained stable until day 28 with some amount of urea diffused through the membrane, and the diffusion coefficient was still above the detectable limit line. This explained the growth of biolayers in the form of tissue on the membrane surface[18,19] during the long-term diffusion test, as shown in Figure 4. The growth of tissue occurred in the first 7 days of the diffusion test and the density of tissue increased with the duration of contact between the membrane and the blood.[17] However, the higher density of tissue had no significant effect on the membrane permeability. The same amount of urea particles could diffuse through the adhered tissue and porous membrane after 8 days of diffusion testing due to the porosity of the tissue. Therefore, from day 8 until day 21, the permeability of the bare PES membrane became constant, even though the intensity of tissue growth increased. This result implies that even if stable bio-layers form, some molecules will still be able to permeate through.

For the PES membranes coated with parylene film (10 and 20 mg), the diffusivity for urea began as lower than that for the bare PES membrane, and then declined within the first 7 days. The reduction in membrane diffusivity was not as significant as that for the bare PES membrane. This is apparently due to the presence of the parylene film on the PES membrane surface, where the coated parylene layer improves the biocompatibility and blood compatibility of the membranes. The diffusivities of these membranes had similar trends from day 8 to day 21, and the membrane diffusivity reached the highest value for the 10 mg parylene dimer coated PES membrane. However, during this time period of blood diffusion, both coated membranes also experienced their lowest diffusivity values.

The diffusion test was continued until day 28. During day 22 to day 28, the coated membrane (10 mg of parylene dimer) exhibited diffusivity similar to that for the bare PES membrane. The 10 mg parylene coated PES membrane even had the highest diffusivity of all the membranes at times during this period. However, at the end of long-term diffusion test (day 28), the three membranes exhibited diffusivities within the same range, where the bare PES membrane gave the highest diffusion coefficient, followed by the membranes coated with 10 and 20 mg of parylene dimer.

The membrane diffusivities are within an acceptable range, because they were above the detectable limit. Based on the long-term diffusion tests, it was concluded that although the diffusivity of the coated membrane was well below that for the bare PES membrane on the first day, after 28 days diffusion in a dialysate system of blood and dialysate solution, the membrane performance remained relatively stable at the same diffusivity value. After 28 days, the diffusivity performance of the bare PES membrane had declined up to 61%, while the decline in diffusivity performance of the 10 and 20 mg parylene dimer coated PES membranes was approximately 45%.

The final goal of this study was to create a membrane that could be used in a dialysis system. Principally, implanted membranes in blood contact devices such as dialysis systems rely on maintaining diffusion of a stable amount of molecules through the membrane pores, regardless of the diffusion period or the blood contact time. The modified PES membrane had a significantly reduced number of adhered platelets and the amount of tissue growth on the membrane surface. In addition, the diffusivity performance of the coated membranes was more stable than that of the bare PES membrane, which indicates that the parylene coated PES membrane has significant potential for application as a membrane in the dialyzer of a wearable artificial kidney. The capacity of PES membranes coated with parylene has been demonstrated with long-term blood diffusion tests (0–28 days), during which period the membranes gave consistent permeability performance and the number of adhered platelets and amount of tissue growth was significantly reduced.

CONCLUSION

The diffusivity performance of PES membranes coated with parylene was evaluated during long-term diffusion tests (28 days). The modified membranes were prepared by controlling the amount of parylene dimer and deposition was accomplished with the assistance of glycerin vapor. After 28 days of diffusion testing, the tested membranes (bare PES membrane, and 10 and 20 mg parylene dimer coated PES membranes) had similar diffusion coefficients. However, the membranes coated with parylene had a very sparse amount of adhered platelets on the surface compared with that on the bare PES membrane surface. Approximately 90% of the bare PES membrane surface was covered with platelets, whereas the coated membranes had only ca. 20–30% platelet coverage of the membrane surface. This is attributed to the biocompatible parylene layer coated on the PES membrane surface. Thus, the coated PES membranes are considered to have potential for application in hemodialysis systems.

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REFERENCES

1. Gu, Y.; Miki, N. *J. Micromech. Microeng.* **2009**, *19*, 065031.
2. Cheng, C.; Li, S.; Zhao, W.; Wei, Q.; Nie, S.; Sun, S.; Zhao, C. *J. Membr. Sci.* **2012**, *417–418*, 228.
3. Huang, J.; Xue, J.; Xiang, K.; Zhang, X.; Cheng, C.; Suna, S.; Zhao, C. *Colloids Surf., B.* **2011**, *88*, 315.

4. Fang, B.; Ling, Q.; Zhao, W.; Ma, Y.; Bai, B.; Wei, Q.; Li, H.; Zhao, C. *J. Membr. Sci.* **2009**, *329*, 46.
5. Nie, S.; Xue, J.; Lua, Y.; Liu, Y.; Wang, D.; Suna, S.; Ran, F.; Zhao, C. *Colloids Surf., B.* **2012**, *100*, 116.
6. Li, L.; Cheng, C.; Xiang, T.; Tang, T.; Zhao, W.; Sun, S.; Zhao, C. *J. Membr. Sci.* **2012**, *405–406*, 261.
7. Prihandana, G. S.; Ito, H.; Nishinaka, Y.; Kanno, Y.; Miki, N. *J. Microelectromech. Syst.* **2012**, *21*, 1288.
8. Cieślik, M.; Kot, M.; Reczyński, W.; Engvall, K.; Rakowski, W.; Kotarba, A. *Mater. Sci. Eng. C* **2012**, *32*, 31.
9. Zhang, C.; Thompson, M. E.; Markland, F. S.; Swenson, S. *Acta Biomater.* **2011**, *7*, 3746.
10. Baskin, S. G.; Navarro, L. T.; Sybers, H. D. *Polym. Sci. Tech.* **1981**, *14*, 143.
11. Kanda, Y.; Aoshima, R.; Takeda, A. *Electron. Lett.* **1981**, *17*, 558.
12. Ernest, W. *Industrial Solvents Handbook*; Noyes Data Corporation: Westwood, NJ, **1998**.
13. Leypoldt, J. K. *ASAIO J.* **1996**, *42*, 133.
14. Langsdorf, J. L.; Zydney, A. L. *J. Biomed. Mater. Res.* **1994**, *28*, 573.
15. Gu, Y.; Miki, N. *J. Micromech. Microeng.* **2007**, *17*, 2308.
16. Liu, Z.; Deng, X.; Wang, M.; Chen, J.; Zhang, A.; Gu, Z.; Zhao, C. *J. Biomater. Sci. Polym. Ed.* **2009**, *20*, 377.
17. James, M.; Anderson, J. M.; Rodriguez, A.; Chang, D. T. *Semin. Immunol.* **2008**, *20*, 86.
18. Wisniewski, N.; Klitzman, B.; Miller, B.; Reichert, W. M. *J. Biomed. Mater. Res.* **2001**, *57*, 513.
19. Sharkawy, A. A.; Klitzman, B.; Truskey, G. A.; Reichert, W. M. *J. Biomed. Mater. Res.* **1997**, *37*, 401.