Interfacial Formation of Porous Membranes with Poly(ethylene glycol) in a Microfluidic Environment

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Received 23 May 2007; accepted 24 December 2007
DOI 10.1002/app.27890
Published online 25 July 2008 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: In a microfluidic environment, the liquid–liquid interface, formed by laminar flows of immiscible solutions, can be used to generate thin membranes via interfacial polymerization. Because these thin nylon membranes have a very small pore size or lack porosity entirely, their utilization in some biological applications is greatly limited. We introduce an *in situ* fabrication method using the interfacial reaction of a two-phase system to generate a porous nylon membrane. The membranes were characterized with scanning electron microscopy and fluorescent beads. Scanning electron microscopy micrographs verified the asymmetrical structure of the porous membrane, and the membrane pore sizes ranged from 0.1 to 1 μm. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 110: 1581–1589, 2008

Key words: biological applications of polymers; nanotechnology; nylon

INTRODUCTION

The interface between two phases has been of interest in microfluidics.[1–5] A liquid–liquid interface can be formed by laminar flows of immiscible solutions.[5] Another relatively simple method for creating this interface involves a surface treatment to create different surface wetting properties (i.e., hydrophobic and hydrophilic) using self-assembled monolayers (SAM).[6] Octadecyl trichlorosilane (OTS) is commonly used to make a hydrophobic SAM on glass surfaces. The conventional method of creating a hydrophobic region inside a microchannel is to first modify the selected part of the substrate with SAM and then assemble substrates to form microchannel networks.[7] This process involves complicated and time-consuming steps, including aligning and bonding. A more convenient method for modifying the surface is to use multistream liquid laminar flow and SAM chemistry.[2] This process can be done quickly (within several minutes) and *in situ*. An alternate method is to use photocleavable SAMs. Treated hydrophobic regions of various shapes can be created inside microchannels with this method.[2,3] Once a channel is divided into hydrophobic and hydrophilic regions, a liquid–liquid interface forms along the boundary of each region. This liquid wall can be used to conduct an interfacial polymerization, which then fabricates a thin membrane.[8–17]

In conventional, bulk interfacial polymerization, a microporous support membrane is typically used to provide an interfacial barrier that allows only transport of monomers (e.g., amine and chloride dissolved in immiscible solvents) by diffusion. Once the support is impregnated with the aqueous amine solution and placed on top of the organic chloride solution, the interfacial polymerization occurs via a polycondensation reaction between two monomers. A thin film is promptly formed at the interface and grows on the organic side of the interface because there is negligible solubility of chlorides in water and higher solubility of amines in organic solvents.[9,10,15,16,18] In this work, we provided the interface by taking advantage of microfluidic phenomena (i.e., surface tension and laminar flow).[2,3,8]

The membranes interfacially formed *in situ* will enable realization of microfluidic filtration devices and be suitable for biological applications such as cell migration and invasion devices. The current alternative, formed interfacially, is a thin nylon membrane that is nonporous; that is, it has a very small pore size (a few nanometers or few tens of nanometers in diameter). A porous membrane would be useful for providing a convection barrier to establish

Additional Supporting Information may be found in the online version of this article.

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Contract grant sponsor: U.S. Department of Homeland Security, National Center for Food Protection and Defense at the University of Minnesota; contract grant number: N-00014-04-1-0659.

TABLE I
Materials Used To Make the Nylon Membranes

Membrane	Aqueous solution	Organic solution
A	0.01 mL of a 60% 1,6-diaminohexane solution in 1 mL of DI water	0.006 mL of adipoyl chloride in 1 mL of toluene
B	0.01 mL of a 60% 1,6-diaminohexane solution and 10 mg of PEG (MW = 20,000) in 1 mL of DI water	0.006 mL of adipoyl chloride in 1 mL of toluene
C	0.01 mL of a 60% 1,6-diaminohexane solution and 20 mg of PEG (MW = 20,000) in 1 mL of DI water	0.006 mL of adipoyl chloride in 1 mL of toluene
D	0.05 mL of a 60% 1,6-diaminohexane solution in 1 mL of DI water	0.03 mL of adipoyl chloride in 1 mL of toluene
E	0.05 mL of a 60% 1,6-diaminohexane solution and 100 mg of PEG (MW = 10,000) in 1 mL of DI water	0.03 mL of adipoyl chloride in 1 mL of toluene
F	0.05 mL of a 60% 1,6-diaminohexane solution and 100 mg of PEG (MW = 8000) in 1 mL of DI water	0.03 mL of adipoyl chloride in 1 mL of toluene

MW, molecular weight.

a static gradient or cell substratum in cell migration and invasion devices.[19] Thus, to expand the applications and usefulness of interfacially formed membranes, it is necessary to develop methods that generate porous nylon membranes while also maintaining control of the pore size.

Poly(ethylene glycol) (PEG) has been previously used to create porous structures in chitosan and polycarbonate membranes, which are formed by phase separation with heat and supercritical CO_2.[20,21] Because interfacial polymerization is similar to phase-separation methods, it is expected that PEG will contribute to the porous structure formation of an interfacially polymerized nylon (polyamide) membrane when it is incorporated with the polymer. However, the domain of interest is different for the interfacial polymerization. More specifically, the surface property of the membrane is the key factor in interfacial polymerization, especially for the thin membranes used in microfluidic applications. In contrast, the bulk property of the membrane will be more important for other phase-separation methods.

In this article, a methodology to create thin porous nylon membrane using PEG in a microfluidic environment is introduced. PEG dissolves in an aqueous solution and does not bind within the membrane during interfacial polymerization. Rather, PEG occupies space inside the membrane. Once the membrane has been formed, PEG can be dissolved with methanol (MeOH) or other solvents, leaving a porous membrane. Alternatively, it is possible to create a porous membrane by an increase in the reaction time; however, this membrane tends to be thicker, and this may make it unsuitable for microfluidic applications.

EXPERIMENTAL

Porous membranes were formed under several different experimental conditions to determine the effects of the chemical composition, solvents, and dissolving time. They were then examined with scanning electron microscopy (SEM) and image processing software to evaluate the porosity. As the last step, a microfluidic filtering device was made and characterized with fluorescent beads to prove the porosity of the porous membrane.

Porous membrane formation

To create porous nylon membranes, PEG samples of different molecular weights (PEG with molecular

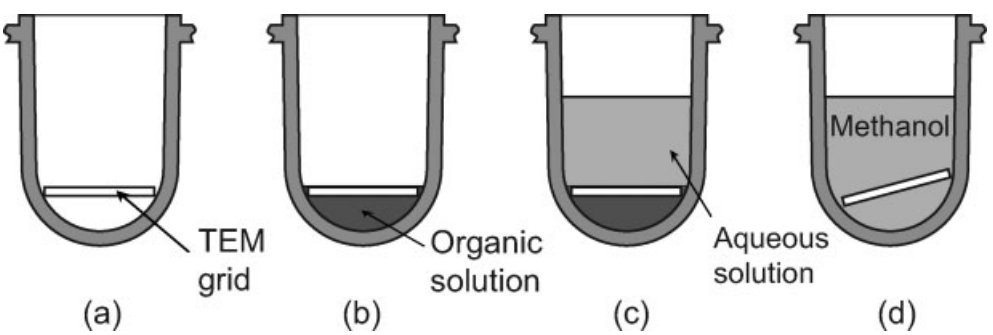


Figure 1 Membrane formation with the TEM grid: (a) the TEM grid was placed in a microwell; (b) the organic solution filled the bottom; (c) the aqueous solution filled the top of the TEM grid; and (d) after 15 min of reaction, the TEM grid was washed with MeOH and dipped in MeOH to remove PEG.

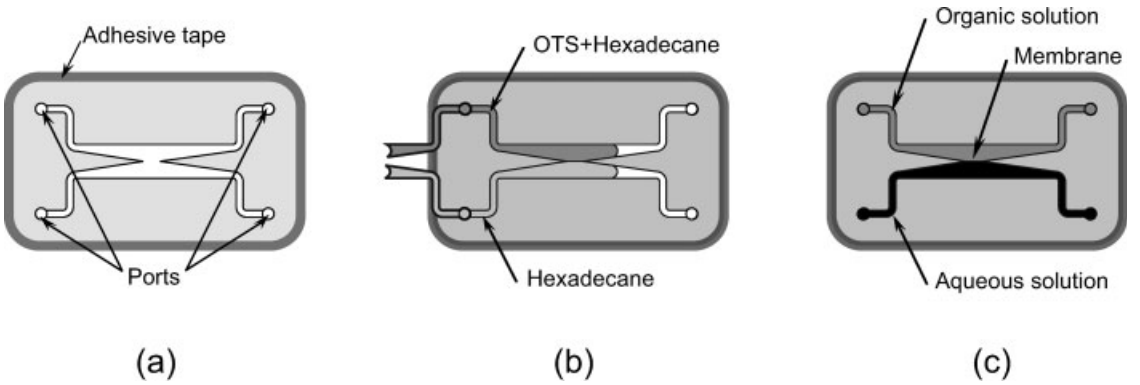


Figure 2 Procedures of interfacial membrane fabrication: (a) channels were fabricated with photopolymerization; (b) a part of the channel surface was rendered hydrophobic by a flowing OTS solution; and (c) a membrane was formed by interfacial polymerization at the interface between the organic solution and aqueous solution.

weights of 10,000 and 20,000, Avocado Research Chemicals, Lancashire, United Kingdom, and PEG with a molecular weight of 8000, Alfa Aesar, Ward Hill, MA) were dissolved in an aqueous solution and interfacially reacted with an organic solution. The concentrations of the aqueous and organic solutions used for the experiment are shown in Table I. Adipoyl chloride and a 1,6-diaminohexane 60% solution were obtained from Acros Organics (Geel, Belgium).

The experiment was carried out under several different conditions, such as the prepolymer concentration (see Table I), molecular weights of PEG (see Table I), dissolving time (1 vs 12 h), and solvents (MeOH vs NaOH). This allowed for the determination of the effects of various parameters on the pore size. As an alternate for MeOH, a solution of NaOH (pH 11) was used because it is capable of decomposing PEG.[22]

To examine the porosity with SEM, the membranes were formed as described in Figure 1. A transmission electron microscopy (TEM) grid (75-mesh Pelco Grids, Ted Pella, Redding, CA) was placed in a polypropylene microwell (96-well standard microplate, ABgene, Epsom, United Kingdom), and 5 μL of the organic solution was gently introduced into the microwell to wet the TEM grid and fill the bottom of the microwell. Next, 50 μL of the aqueous solution was poured on top of the TEM grid. After a 15-min reaction, as determined by the preliminary experiments to achieve membrane thicknesses of 2–5 μm, the aqueous solution was removed with Kimwipes, and the TEM grid was removed from the microwell with tweezers. Then, the TEM grid was dipped into a microwell filled with MeOH for a short time (10 s) to wash out prepolymers. The rinsed TEM grid was dipped into a microwell filled with either deionized water or other solvents according to the experimental purpose. After a designated period of dissolution time, the TEM grid was taken out of the microwell, dried for 30 min at room temperature, and attached to an SEM specimen mount (Ted Pella). Next, gold was sputtered on the membrane with a CVC 601 dc sputterer and imaged with a LEO DSM 1530 field emission scanning electron microscope. NIH ImageJ (image processing and analysis in Java; see http://rsb.info.nih.gov/ij/) was used to compute the porosity of the membranes from the SEM images. Images were subjected to a threshold value to remove background and subsequently analyzed with the particle analysis tool of NIH ImageJ (see supplemental Fig. S.1). Porosity was determined by calculation of the ratio of the area of all pores over the total image area. Small pores less than 0.1 μm were regarded as noise and filtered out during image processing.

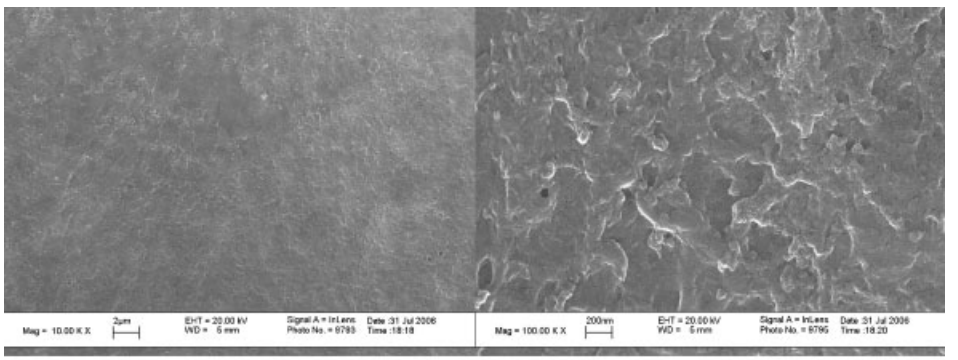
TABLE II
Porosity of the Nylon Membranes (%)

	Solvent		
Membrane	MeOH	NaOH	Dissolving time (h)
A	0	n/a	1
B	1.39	n/a	1
C	0.66	n/a	1
D	0	0	12
E	1.47	1.88	12
F	0.74	1.8	12

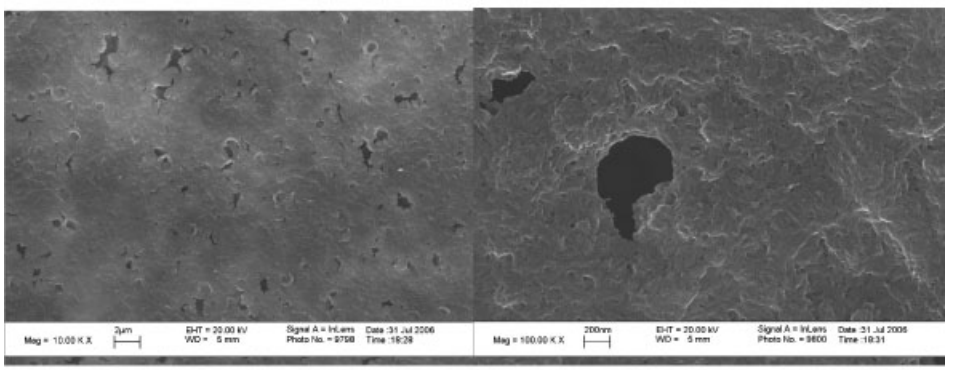
n/a, not applicable.

Microfluidic filtering devices

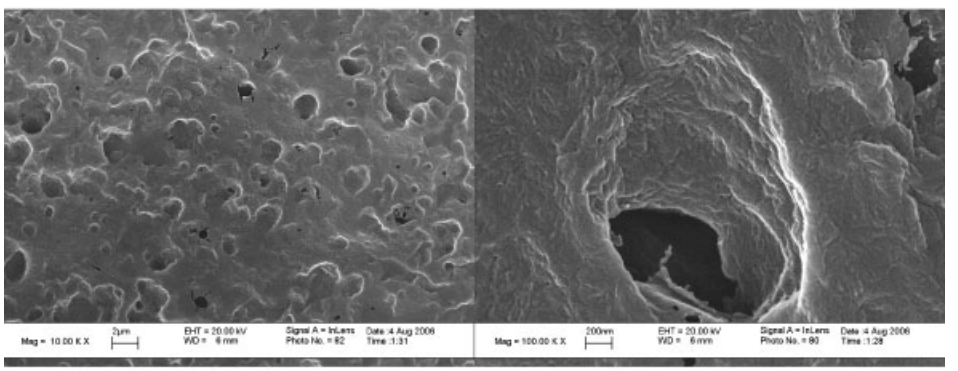
Two microfluidic filtering devices were prepared with *in situ* liquid-phase photopolymerization (LP^3).[23,24] A glass microscope cover with double-sided adhesive tape at the boundary (125 μm thick for the corresponding channel thickness) was cored to have four ports and attached to a microscope slide glass [see Fig. 2(a)]. The cavity was filled with UV-curable epoxy (NOA73, Norland Products, Cran-



(a) Membrane A



(b) Membrane B



(c) Membrane C

Figure 3 Comparison between membranes A, B, and C dipped in MeOH for 1 h. The surface of membrane A was relatively smooth, whereas the surfaces of membranes B and C were porous. The magnifications of the left and right micrographs are 10,000 and 100,000×, respectively.

bury, NJ). A film photomask with the channel design was placed on top of the device and exposed to UV light (EXFO Acticure 4000, Mississauga, Ontario, Canada) for 12 s with a wavelength of 365 nm and intensity of 24 mW/cm^2. After removal of the unpolymerized epoxy with a vacuum pump, the channel was flushed with 4 mL of MeOH. Figure 2 illustrates the channel network of the device with two inlets and two outlets. After washing and drying, the two inlets were connected to two separate syringe pumps (74900 series, Cole-Parmer, Vernon Hills, IL), which contained an OTS–hexadecane mixture and a hexadecane-only solution [see Fig. 2(b)]. Both chemicals were obtained from Acros Organics. The OTS formed a hydrophobic SAM, dividing the channel surface into hydrophobic [gray part of Fig. 2(c)] and hydrophilic [black part of Fig. 2(c)] regions.

An aqueous solution from Table I was introduced into the inlet of the hydrophilic side of the channel. Next, an organic solution from Table I was intro-

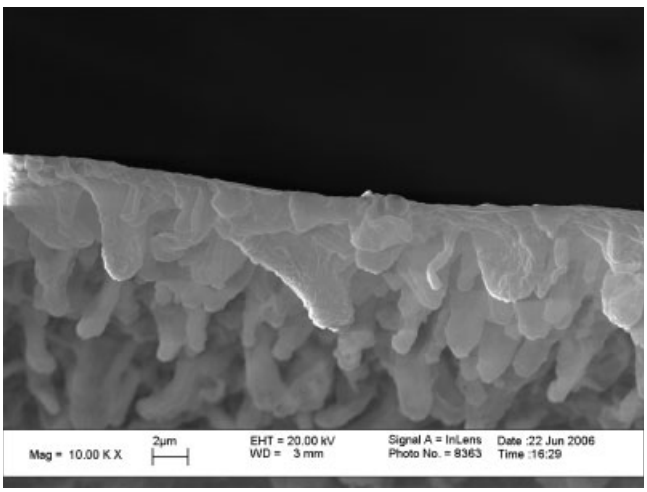


Figure 4 Angled view of a typical nylon membrane formed by interfacial polymerization. The asymmetric membrane showed the ridge-and-valley structure on the organic side (bottom) and a smooth and thin skin on the aqueous side (top). The magnification of the micrograph was 10,000×.

duced into the inlet of the hydrophobic side of the channel. The diaminohexane and adipoyl chloride reacted at the interface of the solutions to form a membrane. After a 15-min reaction time, the organic solution was removed, and the hydrophobic side was washed with toluene. The aqueous solution was also removed, and the hydrophilic side was washed with MeOH. Both sides were washed with MeOH and dried with nitrogen gas as the final washing step.[2,3,8]

Membranes A and B were formed *in situ* within the devices with the methods described previously, and the devices were filled with MeOH for 1 h. The filtering characteristics were examined with polystyrene fluorescent beads of different sizes (0.1-, 1-, and 6-μm diameters; Fluorescent FluoSpheres, Invitrogen, Carlsbad, CA) under an inverted microscope (IX70, Olympus, Center Valley, PA). Florescent images were taken with Metamorph imaging software (Molecular Devices, Downingtown, PA).

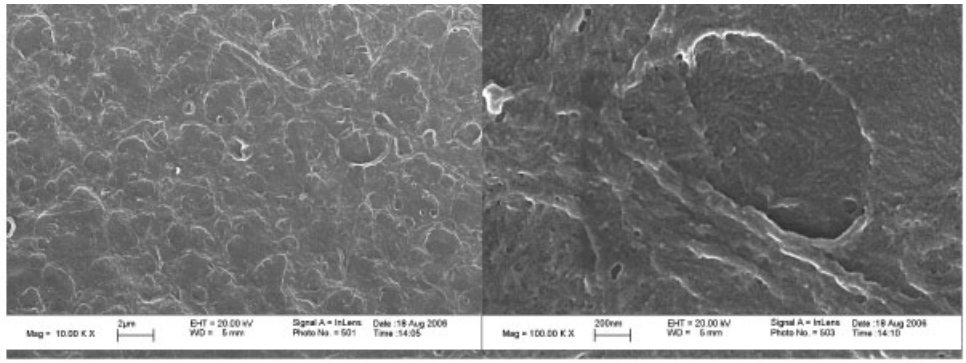
RESULTS AND DISCUSSION

Membranes A, B, and C, dipped in MeOH for 1 h, showed different porosities as expected (see Table II). The surface of membrane A was relatively smooth and had small pores less than 0.1 μm, whereas the surfaces of membranes B and C contained pores ranging in size from 0.2 to 0.8 μm, as shown in Figure 3. There was little notable difference in pore size despite different membrane formation environments (the concentration of PEG used for membrane C was 2 times that of membrane B).

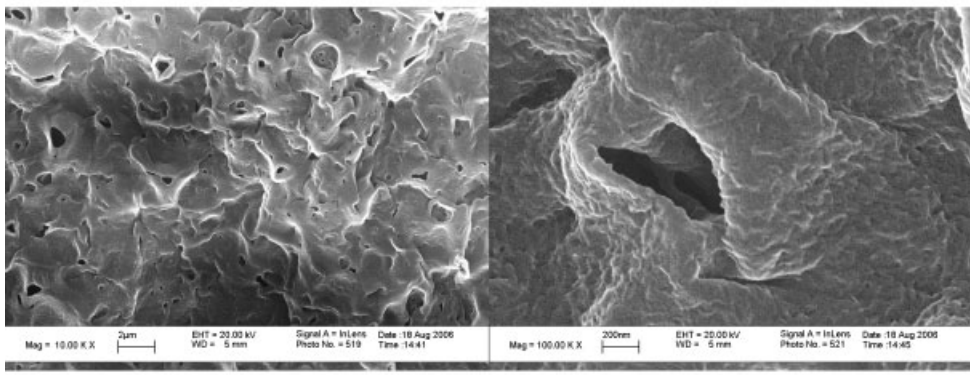
The aqueous and organic sides of the membranes exhibited different morphologies, as shown in Figure 4, and this was consistent with previous findings of asymmetric membranes with bulk formation.[18] The organic side had a ridge-and-valley structure (2–5 μm), whereas the aqueous side showed a smooth skin surface (~ 0.5 μm). The smooth surface indicated the transport of the polymer from the aqueous solution into the organic solution.[18]

The membranes that were formed with higher concentrations of prepolymers and dipped in MeOH for 12 h also showed similar surface morphologies, as shown in Figure 5. The surface porosity of membrane D was almost identical to that of membrane A (i.e., zero porosity; see Fig. 3 and Table II), even though membrane D was exposed to MeOH for 12 times longer than membrane A. Because both membranes A and D were made with the aqueous solution lacking PEG, these results indicate that the presence of PEG has a much greater effect on the pore size in comparison with the dissolving time. Although membrane E was dipped in MeOH for 12 times longer than membrane B, the surface scan showed similar porosities (see Table II). This indicates that the molecular weight of PEG did not affect the size of the pores significantly either, as shown in Figure 5(b,c). Membranes C, E, and F were formed from different prepolymer concentrations with different molecular weights and concentrations of PEG; however, all three had similar surface porosities, in contrast to previously reported results that showed a pore size dependence on the concentration of the prepolymer and PEG and the molecular weight of PEG.[20,21] The differences may be a result of the difference in membrane formation (i.e., interfacial vs bulk) and the fact that the membrane skin that formed during the initial reaction acted as a rate-limiting barrier for the transport of the polymer, including PEG into the organic solution.[18] Also, there may have been some hindrance for PEG to reach the interface during the initial reaction because of the hydrophilic nature of PEG. Figure 6 shows the results for membranes dipped in NaOH for 12 h. Interestingly, the porosities were not significantly different in comparison with the results from those membranes dipped in MeOH (see Table II). NaOH is known to decompose PEG, and this is consistent with the result.[22] Although only a small number of samples for each type of membrane were fabricated and analyzed here, the porous structure formation on the membranes with PEG was still deterministic in comparison with the membranes without PEG (i.e., positive vs zero porosity, respectively; see Table II). Optimization of porous membrane formation may be necessary when microfluidic applications using the porous membranes are designed.

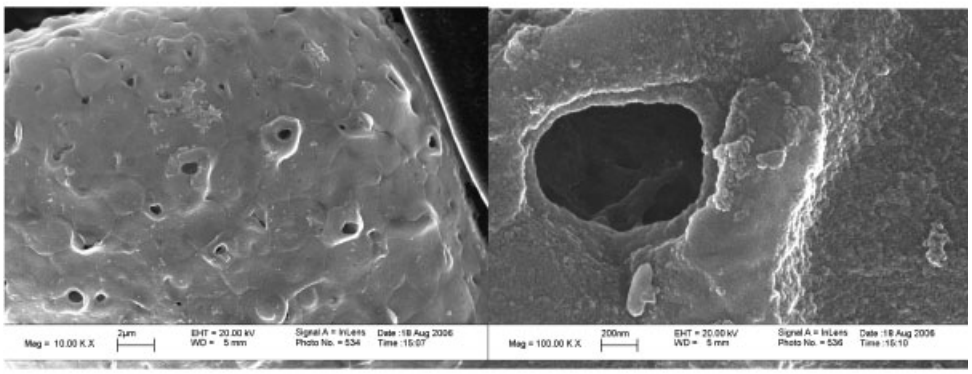
The results of the filtering experiment using membranes A and B are shown in Figure 7. All three sizes of beads (0.1, 1, and 6 μm) were unable to pass through membrane A [Fig. 6(d)]. For membrane B,



(a) Membrane D



(b) Membrane E

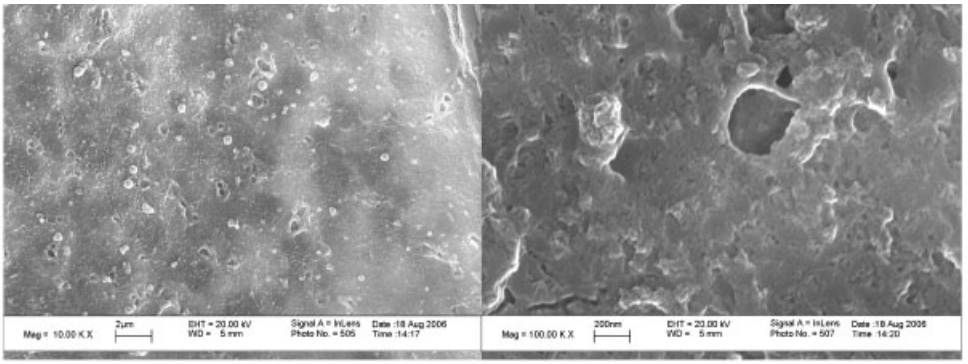


(c) Membrane F

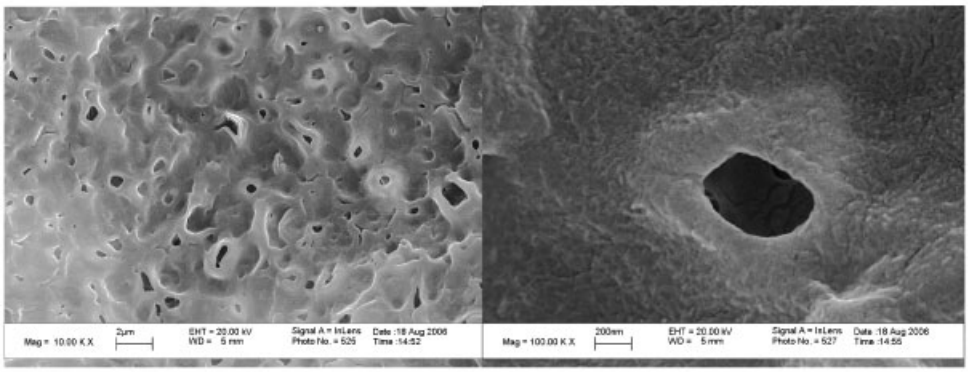
Figure 5 Comparison of membranes D, E, and F dipped in MeOH for 12 h. The membranes that were formed with higher concentrations of prepolymers showed surface morphologies similar to those of the membranes formed with lower concentrations of the prepolymer (Fig. 3). The magnifications of the left and right micrographs are 10,000 and 100,000×, respectively.

0.1-μm beads were able to pass through the membrane, whereas 1- and 6-μm beads were filtered [Fig. 7(a–c)]. Thus, the pore size of membrane B was thought to be greater than 0.1 μm and smaller than 1 μm, and this was consistent with the SEM data, although the full range of pore sizes was not tested.

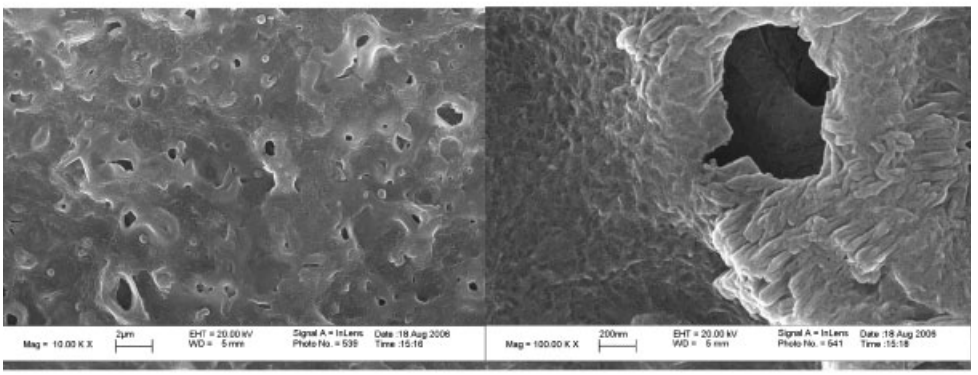
To build a robust filtration device, it is important to choose materials compatible with organic solutions for the channel network. The channel wall will be swollen during the fabrication process if incompatible materials (e.g., polydimethylsiloxane) are used. This increases the chances that the membrane will delaminate from the channel wall because of the swelling. To achieve a robust standing membrane, the interface between the hydrophobic and hydrophilic surface should be patterned to have a sharp



(a) Membrane D



(b) Membrane E



(c) Membrane F

Figure 6 Comparison of membranes D, E, and F dipped in NaOH for 12 h. The surface morphology was not significantly different from the result with MeOH (Fig. 5). It is thought that NaOH decomposed PEG. The magnifications of the left and right micrographs are 10,000 and 100,000×, respectively.

and clear interface by careful adjustment of the flow rate of the syringe pump.

It is known that the permeability of the membrane changes depending on the degree of the hydrostatic and osmotic pressure induced on the membrane.[25,26] This is called the compaction effect, and it is not explored in this article because the membrane developed in this work is applicable under low pressure, as demonstrated by the microfluidic filtering device. Besides, the membrane developed in this work is relatively thin compared to the membranes generally being used (i.e., 2–5 vs 20–30 μm, respectively). Thus, the compaction effect could be subsequently further minimized.

Fouling caused by materials that accumulate on and/or inside the membrane could also affect the membrane permeability.[27–29] Once the application of the membrane is specifically determined, fouling

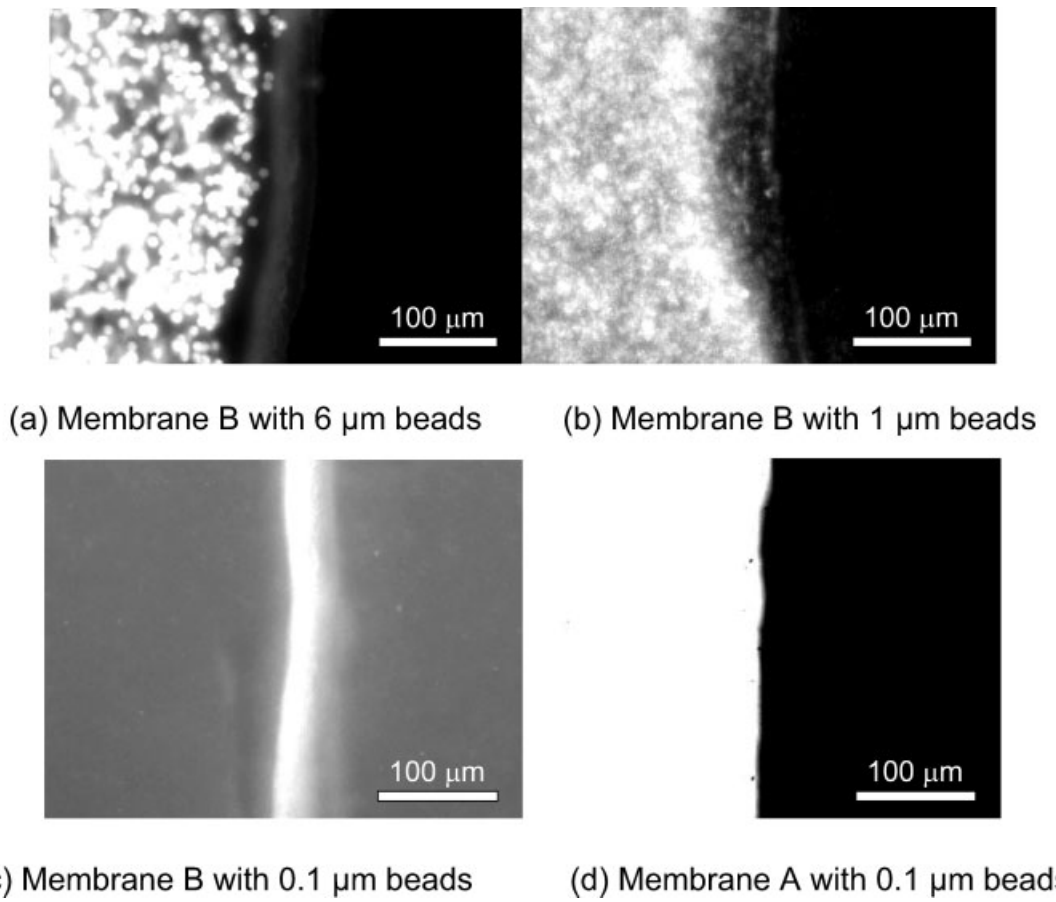


Figure 7 Filtering test for membranes with fluorescent beads of different sizes. (d) None of the three sizes of beads could pass through membrane A. (a–c) For membrane B, 0.1-μm beads were able to pass, whereas 1- and 6-μm beads were filtered. Thus, the pore size of membrane B was thought to be greater than 0.1 μm and smaller than 1 μm; this was consistent with the SEM data.

characteristics of the membrane will need to be studied on the basis of the substances involved in the application.

CONCLUSIONS

An *in situ* fabrication method using the interfacial reaction of a two-phase system to realize a porous nylon membrane was developed with LP^3 and interfacial polymerization. The resulting membranes were characterized with SEM imaging and fluorescent bead filtering. SEM micrographs verified the asymmetrical structure of the porous membrane. The size of the pores on the surface of the membranes ranged from 0.1 to 1 μm.

The porous membrane, interfacially formed *in situ*, will enable relatively easy fabrication of microfluidic filtration devices. The porous membrane may also be applicable to biological applications, such as cell migration and invasion devices[19] and detection devices.[30,31] However, further study should be done to investigate the biocompatibility of the porous membrane. As an example of detection devices, the porous membrane can be used as a scaffold for a less physically robust sensing material. If the porous membrane is formed inside the platform and the micropores are filled with a toxin-degradable material, it will be possible to detect a specific toxin by observation of the degradation of the membrane and subsequent increase in the porosity.

The benefit of using this technology is that one can create a very thin porous membrane already fixed inside a microchannel so it does not require additional bonding processes. Generally, this is hard to achieve with conventional fabrication methods because of its very high aspect ratio and difficulties in manipulating such a small and thin membrane.

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