Low Cost Fabrication of Micro- and Nanopores in Free-Standing Polymer Membranes for Study of Lipid Adsorption

Junseo Choi, Anish Roychowdhury and Sunggook Park*

*Department of Mechanical Engineering and Center for Bio-Modular Multiscale Systems
Louisiana State University, Baton Rouge, LA70803, USA, sunggook@me.lsu.edu

ABSTRACT

This study presents low cost fabrication of free-standing membranes in polymer with perforated pores down to sub-μm diameter, which provides platforms for fundamental studies of many biosystems. For the fabrication, a combination of imprint lithography and a sacrificial layer technique was employed in order to obtain a clean, fully released, and mechanically stable membrane with perforated pores. Lift-off resist (LOR) was used as a sacrificial layer first while SU-8 resist spin-coated on the LOR layer was used as the active membrane layer in which micro- and nanopores patterns are formed via a combined thermal- and UV-imprint process. With this method, we could achieve a large area, free-standing SU-8 membrane with micropores up to 4 inch diameter.

As a demonstration of the use of the membrane in the study of a biosystem, the membrane was exposed to a solution with lipid vesicles. Lipid vesicles preferentially adsorb at the pore sites in the membrane, the extent of which depends on a surface treatment with poly(L-lysine)-graft-poly(ethylene glycol) (PLL-g-PEG) performed prior to the lipid adsorption. We will also show integration of the polymer membrane into microfluidic devices made of polydimethysiloxan, which allows for in-situ study of lipid adsorption.

Keywords: thermal- and UV-imprint process, perforated micro- and nanopores, polymer membranes, lipid adsorption, conductivity measurements

1 INTRODUCTION

The ability to mimic micro- and nanostructures existing in biosystems is important because it provides tools and platforms to answer many relevant fundamental questions without necessity of performing time-consuming and costly in-vivo experiments. One interesting structure is micro- and nanoscale pores. As an example, a cell membrane consists of a lipid bilayer and different proteins, both sides of which are exposed to different chemical and biological environments. Many cell functions are mediated via transportation of materials and signals through numerous nanopores existing in the membrane. Naturally occurring nanopores in a cell membrane, however, suffer from a number of inherent limitations for use in the study of biosystems. This includes poor thermal, mechanical and chemical stabilities, and poor controllability over the pore sizes and locations. Thus, an artificial micro- and nanopores in a synthetic membrane will allow for overcoming such limitations as well as controlling environments of both sides of the membrane.

Artificial membranes have been used in modeling cell membranes to study specific biological phenomena such as the formation and structure of lipid micro-domains of rafts [1], peptide/lipid interactions [2], cell-adhesions [3]. Lipid bilayers in artificially fabricated micro- and nanopores are also useful in understanding chemical and mechanical properties of the lipid bilayers, such as surface tension effects on the mechanical stability of the lipid bilayers. Furthermore, nanopores can be instantaneously formed upon introduction of α-haemolysin, into the lipid biolayer. Such naturally-formed nanopores have been used to study DNA transport through the nanopores [4].

Membrane structures with perforated micro- and nanoscale pores have been produced by a number of methods. Synthetic polymer membranes with random sizes and pore locations are most widely used in separation. Also commercially available are membranes fabricated by ion track etching in polycarbonate [5] and anodization of aluminum [6], which produce randomly distributed and a hexagonal array of nanopores, respectively, with pore diameter as small as 10 nm. Perforated micro- and nanopores at designated locations with controlled pore sizes can be produced using high end nanofabrication techniques. Lo et al. demonstrated fabrication of sub-5 nm pores using focused ion beam and electron beam techniques [7]. Electron beam in the transmission electron microscope followed by a size shrinkage by laser heating was also used to produce nanoscale pores in SiO_2 [8]. However, those methods do not allow for control over both the size and location of pores and the high yield of production. Therefore, it is needed to develop a flexible method to fabricate such membrane structures with micro- and nanoscale pores at designated locations with high throughput.

We have previously developed a fast and high-throughput process to produce free-standing polymer membranes down to sub-μm pore diameter by using modified imprint lithography and a sacrificial layer technique [9, 10]. The self-supporting mechanical stability was achieved by using a UV-curable polymer SU-8. In this work, we extend the process to the fabrication of large area membranes up to 4 inch diameter and a systematic study on the adsorption behavior of lipid vesicles at the membrane

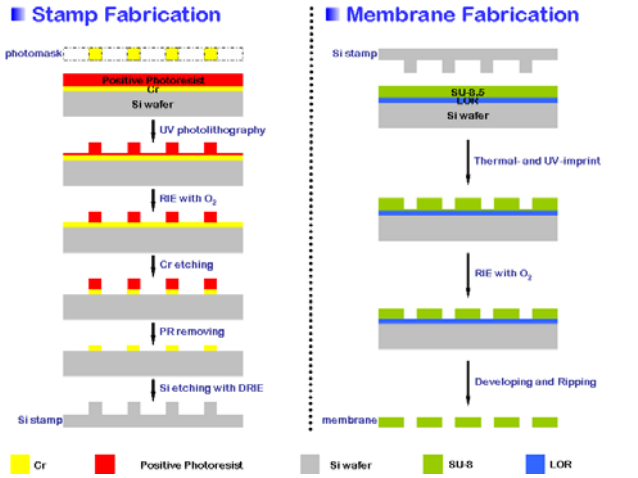


Figure 1. Process schematics for fabricating imprint stamps (left) and SU-8 membranes with perforated micro- and nanopores (right).

surface upon exposure to a lipid solution. In addition, we will demonstrate a concept of how to integrate the membrane with a fluidic device that can be used for in-situ study of lipid adsorption.

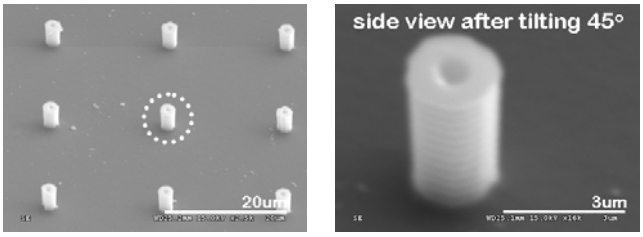
2 EXPERIMENTAL

Figure 1 shows the process schemes for fabricating imprint stamps and SU-8 membranes with perforated micro- and nanopores. The process involves a number of sub-processes: photolithography and deep reactive ion etching (DRIE) for stamp fabrication, and modified imprinting process using a double resist layer and lift-off for the membrane fabrication.

2.1 Stamp Fabrication

Si stamps were fabricated using a combination of photolithography and semiconductor micromachining techniques. First, photolithography was done with a custom designed photomask in a 'Quintel' UV exposure station at the Center for Advanced Microstructures and Devices, Louisiana State University. The resist patterns were transferred down to Si substrate using a DRIE process which was performed at the Micro Electronics Research Center, Georgia Institute of Technology. The DRIE process involved alternating etch and sidewall passivation cycles so as to maintain a high degree of anisotropy, resulting in almost vertical sidewall profiles. Figure 2 shows scanning electron microscopy (SEM) images for a Si stamp containing micro-posts of 1.5 μm diameter. Nearly vertical sidewalls with scallop-like features resulting from the DRIE process are seen.

Prior to imprinting, the stamp surface was treated with a fluorinated silane in the vapor phase in a home-made chemical vapor deposition chamber in order to reduce the adhesion to the resist.



(a) (b)

Figure 2. SEM Images for Si posts with 2μm produced by photolithography and DRIE.

2.2 Fabrication of SU-8 Membranes

A double resist layer was used for imprinting: Lift-off Resist (LOR, MicroChem) as a sacrificial layer and SU-8 as the active membrane layer. First, a 4 inch quartz wafer was sequentially spin-coated with 1 μm and 5 μm thick LOR and SU-8 layers, respectively. Imprint lithography was performed with a commercial nanoimprinter (Obducat 6") which allows for both thermal and UV imprinting. In order to define pore structures in SU-8 which is a UV-curable resist, a modified imprinting process combining thermal and UV imprint was employed. Imprinting was carried out at 65°C, which is close to the glass transition temperature (T_g) of 55°C for uncured SU-8. An imprint pressure of 5 MPa was used. These conditions are comparable to those used in a reversal imprint process (40-85°C and 1-5 MPa) reported by Hu et al. [11]. After the temperature was reduced to 50°C, which is slightly lower than T_g, the sample was exposed to UV light for 10 sec. For a UV imprint process, UV light is usually shone through a transparent stamp to harden a UV-curable resist. However, in our process, we could avoid the requirement of fabricating a transparent stamp by using a transparent quartz wafer as substrate. Then, the quartz substrate was separated from the Si stamp at 40°C and baked at 95°C for 5 min to complete the cross-linking of SU-8.

Finally, a free-standing SU-8 membrane with perforated micropores was lifted-off by dissolving the LOR sacrificial layer with a MF319 solution. It took ~ 3 hours to complete the lift-off process.

2.3 Selective Immobilization of Lipid Vesicles

The feasibility of using the perforated micro- and nanopores in the SU-8 membrane to mimic a cell membrane was examined by studying the adsorption behavior of lipid vesicles to the membrane surface. Prior to the lipid adsorption, some of the SU-8 membrane samples were treated with a poly-L-lysine-grafted-(polyethylene glycol) (PLL-g-PEG) solution in order to prevent non-specific adsorption of lipid vesicles at the membrane

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surface. For synthesis and treatment of PLL-g-PEG, we followed a synthetic path described in [10].

A di-palmitoylphosphatidylcholine lipid solution (Avanti Polar Lipids) was mixed with chloroform (Purity=99.9%, Fisher Scientific) at different volume ratios. After staining with a fluorescent dye (5'-Cy5-oligonucleotide-amine-3'), the solution was dispensed on the membranes. Excessive lipid vesicles were then washed off with DI water and the membrane was observed under a fluorescence microscope (Leica).

2.4 Integration of the Membrane into a PDMS Microfluidic Devices

The SU-8 membrane in a free-standing form is very versatile in many applications. One example is a fluidic interconnecting component in modular microfluidic devices. As a demonstration of this concept, we have fabricated a modular microfluidic device containing the SU-8 membrane with micro- and nanopores, which allows for in-situ study of lipid layer formation at the pore sites. In order to fabricate simple microfluidic channels, SU-8 negative photoresist was patterned on silicon via photolithography, which was replicated by casting PDMS (10:1 mass ratio of silicone elastomer to curing agent). After curing overnight at room temperature, the PDMS replica was peeled off from the master. The SU-8 membrane was then sandwiched between the two PDMS microchannels. The PDMS devices were bonded at 100°C for 2 hr on a hot plate and connected with inlet and outlet ports.

3 RESULTS AND DISCUSSIONS

The free-standing SU-8 membranes with micropores were fabricated by a combination of a modified imprinting process and a sacrificial layer technique. The most important requirement for the fabrication involved selection of polymer materials that allow for imprinting of high aspect ratio structures with good replication fidelity and at the same time had enough mechanical stability to be free-standing. Although thermoplastic polymers such as PMMA and PC are widely used for imprinting, it does not provide enough mechanical stability. Therefore, SU-8 was chosen as the membrane layer because it is the resist widely used for high aspect ratio microstructures in the LIGA process and thus high mechanical stability was expected. The Young's modulus of PMMA and SU-8 is reported to be in the range of 1.8-3.1 GPa and 3.5-7.5 GPa, respectively [12].

We have previously reported an optimal condition for thermal imprinting into SU-8 layers [9, 10]. Best imprint results were obtained when imprinting was performed at 135°C. However, high thermal stress and adhesion generated during molding and cooling often resulted in a peeling of imprinted SU-8 from the substrate at the demolding step. This problem becomes more significant for large area imprinting and we hardly obtained good imprint results when a 4 inch stamp fully covered with micropillars

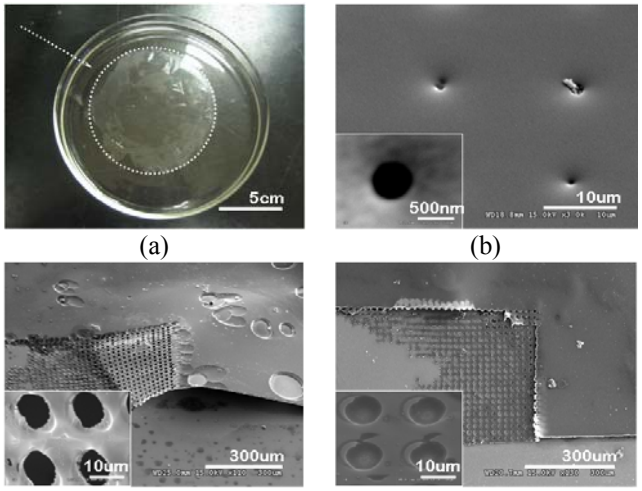


Figure 3. (a) Photograph for a SU-8 membrane after release from substrate and (b), (c) and (d) are SEM images from the top surface of membrane with nanopores, top and bottom surfaces of the released membrane with micropores, respectively.

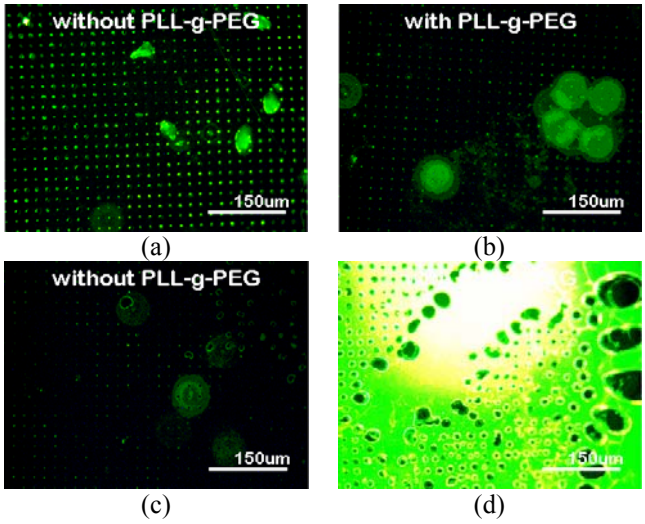
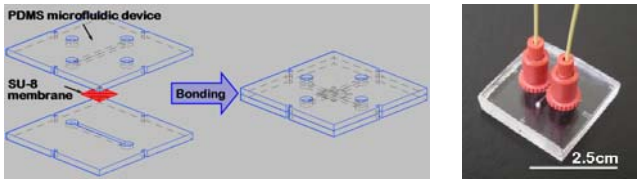


Figure 4. Fluorescence images after exposure to a lipid solution for SU-8 membranes with or without PLL-g-PEG treatment at a different volume ratio of lipid to chloroform. The volume ratio is 1:100 (a), (b) and 1:10 (c), (d).

was used. In this experiment, the thermal imprint process was combined with UV-imprint in order to reduce the imprinting temperature. We were able to achieve high aspect ratio micropores over 4 inch diameter at a low imprint temperature close to T_g of SU-8. Figure 3(a) shows a photograph for a 4 inch diameter, free-standing SU-8 membrane after release from substrate. Figure 3(b), (c) and (d) show SEM images from the top surface of membrane with sub-micrometer pores, top and bottom surfaces of the released membrane with micropores, respectively.



(a) (b)

Figure 5. Schematic image (a) and photograph (b) of a fluidic device integrated with the membrane.

The free-standing membranes with micro- and nanoscale pores having access from both sides can be used as a platform to mimic a cell membrane. The first step to mimic a cell membrane is to selectively immobilize lipid bilayer at the pore sites. Figure 4 shows that fluorescence images after exposure to a lipid solution for different SU-8 membranes with or without PLL-g-PEG treatment. For the SU-8 membrane with well-defined micropores (Figure 4(a) and (b)), lipid vesicles preferentially adsorb at the pore sites in the membrane. However, when the membrane surface was treated with PLL-g-PEG prior to the lipid adsorption, the fluorescence signal becomes weaker. Given that PLL-g-PEG prevents non-specific adsorption of the lipid vesicles at the membrane surface, it would be surface tension force by which the lipid vesicles can remain at the pore sites stably. This in turn gives an insight to a bilayer formation at the pore sites. The adsorption behavior is dramatically changed where a strong fluorescence signal emanates from the corrugated SU-8 surface created due to failed imprints, as shown in Figure 4(c) and (d). This result indicates the importance of achieving good imprinted patterns in using the free-standing SU-8 membranes to selectively immobilize the lipid vesicles at the pore sites, more broadly to mimic the cell membrane.

Figure 5(a) shows a schematic diagram of how to integrate the free-standing SU-8 membrane into a modular microfluidic device. The membrane with perforated micropores was sandwiched by two PDMS microfluidic devices. The microchannels in the two PDMS devices were so aligned to be perpendicular to each other. One advantage of such a crossed orientation is that only micropores in the membrane which are located within the overlapped area between the two microchannels will be active in the transportation of substances through the pores. Therefore, the number of active pores can be controlled simply by using microchannels of different widths. This will also alleviate the requirement of high accuracy in aligning two PDMS devices for bonding. The fabricated PDMS device integrated with a SU-8 membrane is shown in Figure 5(b).

4 CONCLUSIONS

A low-cost and flexible method was developed using all parallel processes to produce large area, free-standing polymer membranes up to 4 inch diameter which contain perforated micropores. For the fabrication, a combination of imprint lithography and a sacrificial layer technique were used. Selective adsorption of lipids at the pore sites in the SU-8 membrane was achieved using lipid vesicles stained with a fluorescent dye and PLL-g-PEG chemistry, demonstrating that the membrane can be used as platforms to study transport behavior through lipid layers. In addition, we have developed a concept of how to integrate the membrane structure into microfluidic devices.

This technique developed in this study can be extended to produce a membrane with smaller pore size. Of course, this requires high aspect ratio imprinting of nanostructures in order to achieve enough mechanical strength for free-standing, which is still challenging. However, an improved imprinting process combined with high quality stamp fabrication will enable fabrication of free-standing membranes with nanosized pores at low cost and with high throughput.

ACKNOWLEDGEMENT

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REFERENCES

[1] C. Yuan, J. Furlong, P. Burgos, and L. Johnston, Biophys. J, 82, 2526, 2002
[2] D. Takamoto, M. Lipp, A. Nahmen, K. Lee, A. Waring, and J. Zasadzinski, Biophys. J., 81, 153, 2001
[3] S. Sivasankar, W. Brieher, N. Lavrik, B. Gumbiner, D. Leckband, Proc. Natl. Acad. Sci. U.S.A., 96, 11820, 1999
[4] C. Dekker, Nature Nanotechnology, 2, 209, 2007
[5] Y. Komaki and S. Tsujimura, Science, 199(4327), 421, 1978
[6] J. O'Sullivan and G. Wood, Proceedings of the Royal society of London Series A, Mathematical and Physical Sciences, 317(1531), 511, 1970
[7] C. Lo, T. Aref, and A. Bezryadin, Nanotechnology, 17(13), 3264, 2006
[8] S. Wu, S. Park, and X. Ling, Nano Lett., 6(11), 2571, 2006
[9] A. Roychowdhury and S. Park, Proceedings for NSTI Nanotech Conference 07, 453, 2007
[10] A. Roychowdhury, J. Choi, A. Yi, F. Xu, and S. Park, Proceedings for ASME International Mechanical Engineering Congress & Exposition (IMECE) 07, IMECE2007-43813, 2007
[11] W. Hu, B. Yang, C. Peng, and S. Pang, J. Vac. Sci. Technol. B, 24(5), 2225, 2006
[12] H. Khoo, K. Liu, and F. Tseng, J. Micromech. Microeng., 13, 822, 2003