Microfluidic Platforms with Micro- and Nanopore Membranes for In-Situ Formation of Lipid Bilayer

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ABSTRACT

This study presents a stable and flexible method for fabricating an integrated microfluidic system with lipid bilayers formed in a free-standing SU-8 membrane. For the fabrication of the SU-8 membranes, 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. The membrane was then sandwiched between two simple polydimethysiloxane chips with microchannels to produce integrated microfluidic devices. Using the method developed by Suzuki et al. (2004), lipid bilayers were successfully formed at micropore sites in the SU-8 membrane within the microfluidic system.

Keywords: perforated micro- and nanopores, polymer membrane, microfluidic platforms, lipid bialyer formation, capacitance

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 the 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. Therefore, in the past decade those nanopores have come into the spotlight as the basis for technologies to analyze and sequence single nucleic acid molecules. Particularly, the alpha-hemolysin (α-HL) ion channel embedded in a lipid bilayer across micro- and nanoscale apertures is useful for the detection of nucleic acids because it is a water-soluble protein with a ~ 1.5 nm diameter inner pore that allows translocation of single-stranded DNA [1]. During translocation, the ion current flowing through this ion channel is selectively blocked, depending on the presence of the biopolymer inside the channel. Current blockades are sufficiently sensitive to measure the properties of the biopolymers such as their nucleotide base composition, length, and secondary structure, and to physical parameters (e.g. the driving field intensity, temperature, and ionic strength). However, in order to use the α-HL as biological nanopore sensors for single molecule analysis, bilayer lipid membranes (BLMs) should firstly be synthesized to be used as its supporting materials.

The method to form BLMs can be classified by supported BLMs [2-6] and non-supported (suspended) BLMs [7-11]. Supported BLMs are formed over patterned micro- and nanostructures in polymers, hydrogels and solid substrates. Suspended BLMs are formed across micro- and nano apertures in thin hydrophobic materials. Due to the coupling of the bilayer to the rigid substrate surface, the supported BLMs configuration experiences many challenges, which include interfacial suppression of thermally activated membrane fluctuations, limitation of incorporating membrane proteins, and hindrance of translational mobility. Another drawback in using the supported BLMs is the difficulty in providing appropriate environments for both sides of the membrane. Access to both sides of the membrane is important because both intercellular and extracellular environments, such as concentrations of individual ions, often determine biophysical properties. Therefore, suspended BLMs architecture, which is formed in mechanically stable, free-standing membranes, that facilitates access from both sides will provide a more flexible platform to elucidate cell functions, such as chemical and mechanical stability of the cell membrane, transportation through ion channels, and signaling/regulation functions of membrane proteins. Furthermore, the suspended BLMs architecture has huge potential to be integrated into micro- and nanofluidic systems for portable and disposable biosensors and bioanalytic devices. Integration of lipid bilayer membranes into microfluidic platforms allows for minimal use of reagents, decrease of the analysis time, and portability of the fabricated devices. However, this has seldom been carried out. Therefore, it is needed to develop a stable and flexible method for integrated microfluidic system with lipid bilayer membranes that have access to both sides of the membrane. In this paper, we will present novel microfluidic devices with micro- and nanopore membranes fabricated by a modified imprinting process and a sacrificial layer technique and show in situ formation of lipid bilayers within this microfluidic platform.

2 EXPERIMENT

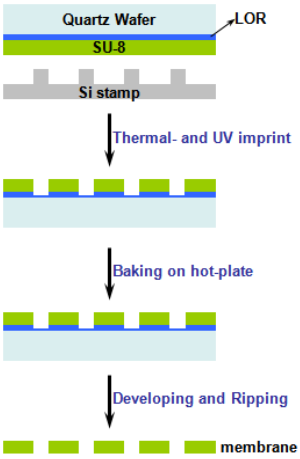

Figure 1. The process scheme for fabricating a polymer membrane with perforated micro- and nanopores.

2.1 Fabrication of SU-8 Membranes

Figure 1 shows the process scheme for fabricating a polymer membrane with perforated micro- and nanopores. A double resist layer was used for imprinting: lift-off resist (LOR-7B, MicroChem Corp.) as a sacrificial layer and SU-8 (SU-8.5, MicroChem Corp.) 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 using a commercial nanoimprinter (Eitre® 6, Obducat) 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 [12]. 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. 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 achieved by dissolving the LOR sacrificial layer with a MF319 solution. It took ~ 3 hours to complete the lift-off process.

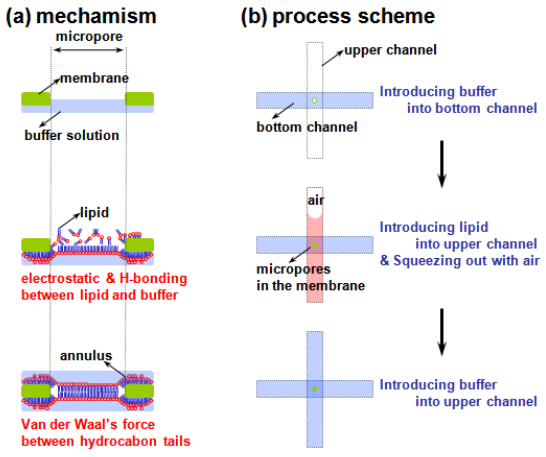

Figure 2. The mechanism and process scheme for the formation of lipid bilayers.

2.2 Chip Design, Procedure, and Fabrication

In order to fabricate an integrated microfluidic platform, the SU-8 membrane with 5 μm diameter pores was sandwiched between two crossed PDMS microchannels. 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.

One of the PDMS replicas having microchannel was slightly stamped onto a substrate spin coated with curing agent as an adhesive material between PDMS replicas. The other was punched out with a hole-puncher to form the reservoirs. The PDMS replicas were then bonded at 70 °C for 1 hr in a vacuum oven after putting the membrane between them [13].

2.3 Lipid Bilayer Formation

Lipid bilayers were formed at miropore sites within mcirofluidic platform following the sequence used by Suzuki et al [14]. The process scheme and the mechanism for the formation of lipid bilayers are shown in Figure 2. Firstly, 0.1 M potassium chloride (KCl) buffer obtained by dilution of 1 M KCl solution (BioUltra, Sigma-Aldrich) is filled into the bottom channel at a flow rate of 2 μl/min. The lipid solution is then introduced to the upper channel and squeezed out with air at a flow rate of ~0.1 μl/min. For the lipid solution, 20mg of phosphatidylcholine dissolved in chloroform (1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphocholine (DPPC), Avanti Polar Lipids) is added to 1 ml of n-decane (99%, Alfa Aesar). Then, 0.02mg of Rhodamine B-labeled phosphatidylethanolamine dissolved in chloroform (1,2-Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-(Lissamine Rhodamine B Sulfonyl), Avanti Polar Lipids) is added to 1 ml of lipid solution for the fluorescent observation. Lastly, the buffer is gradually introduced into the upper channel at a flow rate of ~0.1 μl/min.

2.4 Optical Monitoring

The fluorescence imaging of lipid bilayers was optically monitored on an inverted optical microscopy stage (IX-81, Olympus) with a green excitation filter (U-MWG2, Olympus). The images of the lipid bilayers at miropore

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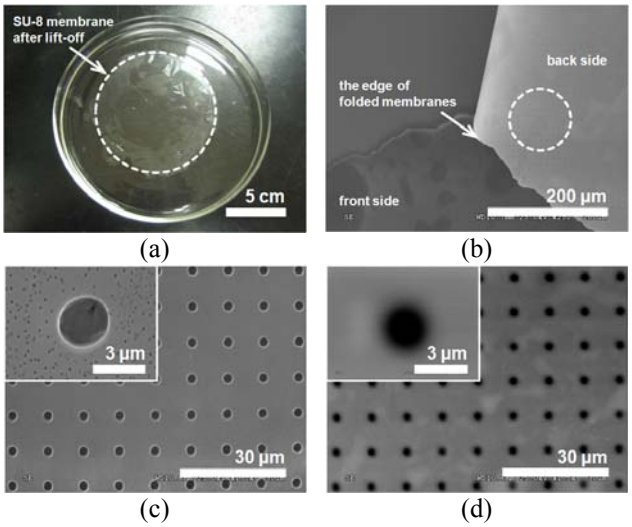

Figure 3. (a) Photograph for a SU-8 membrane after release from substrate and (b), (c) and (d) are SEM images from the surface of membrane after folding and its magnification of front- and back-side surfaces, respectively.

sites were captured by using a EMCCD Digital Camera (Rolera-MGi Plus, QImaging®) and imaging software (Image-Pro Express 6.0, Media Cybernetics).

3 RESULTS AND DISCUSSIONS

We have previously developed a fast and high-throughput process to produce free-standing polymer membranes to be sandwiched between two crossed microfluidic channels. It was fabricated by a combination of a modified imprinting process and a sacrificial layer technique. SU-8 was also chosen as the membrane layer because of its high mechanical stability. In actuality, it was widely used for high aspect ratio microstructures in the LIGA (Lithographie, Galvanoformung, and Abformung) process and has higher young's modulus than common thermoplastic polymers such as PMMA, PC, and so on. Also, the properties of its high optical transparency and sensitivity to UV radiation are suitable to the thermal imprint process combined with UV-imprint in order to reduce high thermal stress and adhesion generated during

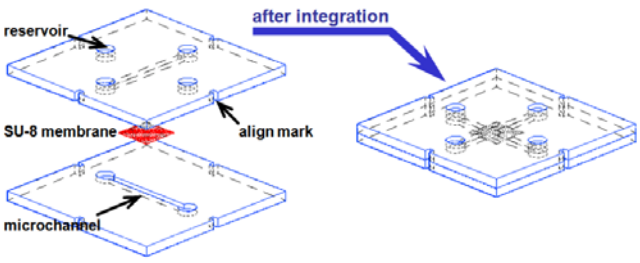

Figure 4. A schematic diagram of a microfluidic device integrated with the free-standing polymer membrane.

molding and cooling.

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, improving the problem during a demolding process. 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 surface of membrane after folding and its magnification of front- and back-side surfaces, respectively. SEM images were generated by sputter coating a thin layer of gold onto the SU-8 membrane. Therefore, there were numerous spots around the micropore in figure 3(c) due to the heat occuring during gold sputtering for the SEM measurement. Thus they do not appear in the backside of the membrane as shown in figure 3(d). Moreover, the back-side image was not clear compared to the front-side view due to non-treated with gold layer.

Figure 4 shows the assembled microfluidic device in which the free-standing SU-8 membrane with perforated micropores was sandwiched between two PDMS microfluidic chips. 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.

Microscope and fluorescence images of the membrane sandwiched two microfluidic channels during the process of lipid bilayers formation are shown in figure 5(b)-(d). Firstly, the buffer solution was filled into the bottom channel at a flow rate of 2 μl/min. At this stage, the buffer remained at the front of the micropores and did not flow out into upper channel due to the surface tension. As shown in figure 5(b), the color of image between bottom and upper channel was slightly different due to the refractive index of water. Secondly, the lipid solution was introduced to the upper channel and squeezed out at a flow rate of ~0.1 μl/min. For fluorescent observation, an inverted optical stage with a green excitation filter was used, as shown in figure 5(a). At this step, a thin lipid layer might stay over the buffer surface at the micropores in a crossed area by two microchannels, resulting with numerous fluorescent spots. However, the spots in upper channel excepted by the crossed area seem to be adsorption of lipid vesicles at micropores, as shown in figure 5(c). Lastly, the buffer was gradually introduced into the upper channel at a flow rate of ~0.1 μl/min. After passing the buffer the lipid bilayer was spontaneously formed at the micropores. As shown in figure 5(d), it was observed with weaker fluorescence signals compared to figure 5(c).

However, Plateau-Gibbs border, that commonly appears when lipid bilayers are formed, could not be observed because the micropores in the membrane were too small.

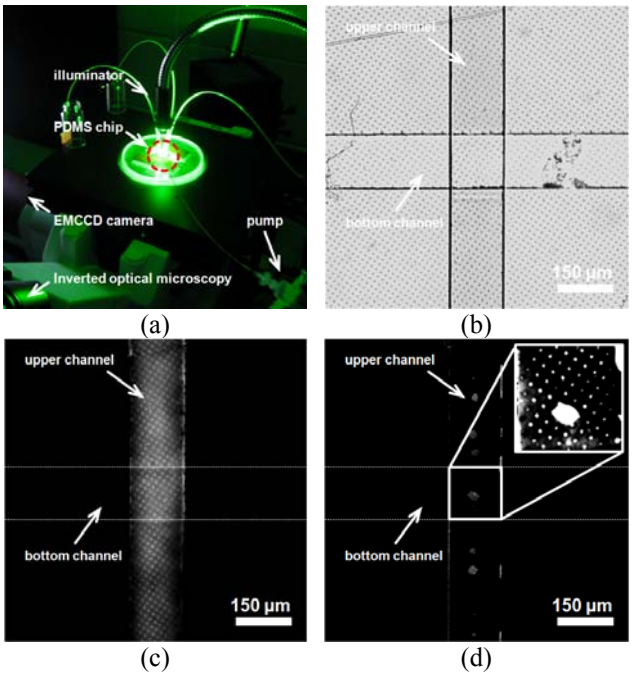

Figure 5. (a) Photograph for optical system with PDMS chip (a), microscope image after introducing buffer solution into bottom channel (b), fluorescence images after introducing lipid solution into upper channel (c), and introducing buffer solution into upper channel (d).

Although it was not detected, we believe that it was not lipid vesicles but lipid bilayers because lipid was placed between two buffer layers. In order to confirm the existence of lipid bilayers at micropores, however, the experiment to measure the capacitance and transmembrane current before and after its formation is currently being carried out.

4 CONCLUSIONS

A stable and flexible method for integrated microfluidic system with lipid bilayers membranes was developed. At first, 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. It was then integrated with simple polydimethysiloxan microfluidic devices, which allows for in-situ study of lipid bilayers formation.

With this chip, lipid bilayers were formed at micropore sites in the membrane within microfluidic system. Of course, in order to confirm the existence of lipid bilayers at micropores, the capacitance and transmembrane current should be measured before and after its formation. However, this method developed in this study can be widely used for the study of lipid bilayers formation because it can be easily made, having the advantage of a low-cost and high throughput.

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