GLASS MICROFLUIDIC CHIPS FOR LONG-TERM
LIPID BILAYER FORMATION
Y. Watanabe1,3* and S. Takeuchi1,2
1 Life BEANS Center, BEANS Project
2 IIS, The University of Tokyo
3 Corporate R&D Center, Olympus Co, JAPAN

ABSTRACT
In this paper, we describe a fabrication method of a glass microfluidic chip for long-term and repeatable lipid bilayer formation. We etched a glass substrate by magnetic neutral loop discharge plasma (NLD) (Fig. 1a). Using this method, we fabricated a glass microfluidic chip with microchambers forming lipid bilayer membranes (Fig.1b,c). Since the glass chip prevents absorption of fluids, the formed membrane was maintained more than 4 days. We also found that the formation process is repeatable by washing the channel with organic solvents; these properties show great advantages over the PDMS chips that absorb aqueous solutions and swells by organic solvents. As a demonstration, we successfully formed lipid bilayer membrane that reconstituted nanopore membrane protein (α-hemolysin), and observed a transmembrane phenomenon at the chip.

KEYWORDS: Bilayer lipid membrane, Glass microfluidic chip, Membrane protein

INTRODUCTION
Together with recent progress of biological science and technology, the functionality of membrane proteins became significantly important for drug discovery. The membrane proteins, however, keep their activities only within lipid membranes such as cell membranes or organelles [1], which is a technically tough hurdle on the membrane protein study. Historically the study is performed with the patch-clamp techniques using thin glass capillaries on living cells [2], yet it often suffers not only from the requirement of experienced, well-trained operators but also low throughput of data [3]. To overcome those problems, several research groups have recently innovated a well controlled, artificial membrane platform that applies the principle of the Langmuir-Blodgett technique combined with microfluidic systems; lipid-oil solution and aqueous buffer are infused into microchannels or microchambers [4-6]. In our group, we recently developed a microfluidic device consisting μm-sized arrayed chambers on which lipid membranes is formed with membrane proteins (Fig 1a) [7]. The device provided a highly integrated platform for membrane protein study designed for varieties of optical techniques. However, since the PDMS chip absorbs aqueous buffer solutions to the chambers, the formed bilayer breaks / disappears within hours unless we use special lipid components and surface treatments onto PDMS. Moreover, the PDMS chip was not reusable: we can only make a fluid flow just one time in each channel otherwise the channel is clogged by the residual lipids; they can be washed only by organic solvents but may damage the PDMS surface as well. To solve the problems, we focused on a glass channel that prevents the absorption of fluids and would provide stable membrane formation. Also glass will be applicable to wash with most of organic solvents. In order to fabricate such glass chips that has deep and perpendicular wall of channels, we applied NLD plasma dry etching process. Firstly we tried finding a suitable condition for borosilicate-glass microfluidic chips (Fig. 1b).

Figure 1: (a) Schematic diagram of the arrayed microchamber chip. The bilayer lipid membranes confine aqueous solutions at the chambers from the main channel, enabling visualization of reagent transportation through membrane proteins incorporated in the membranes. (b) Photo of the developed glass microfluidic chip.

EXPERIMENTAL
The schematic diagram and the image of the glass microfluidic chip are shown in Fig. 1. The sample microchambers (19×17×10 μm3) were arrayed aside the main straight channel. The microchambers and main straight channels were etched by magnetic neutral loop discharge plasma (NLD). The schematic diagrams of the NLD plasma etcher is shown in Fig. 2a [8]. Due to the magnetic neutral loop, glass etching plasma of carbon fluoride gas can be high density
at gas low pressure. After the etching, we thermally bonded the glass substrate with another flat glass substrate (Fig. 2b). Lipid bilayer membrane was formed by a sequential injection of (1) a nanopore protein (α-hemolysin at 1 mg/ml) and fluorescent dye (calcein at 0.5 mM)-containing aqueous buffer solution (PBS; 2.7 mM KCl, 137 mM NaCl, pH 7), (2) a lipid-hexadecane solution (15 mg/ml of DPhPC), and (3) a pure buffer (Fig. 2c). The respective injection rates were 0.7, 0.3, and 0.15 μL/min. Since the α-hemolysin forms an open-state nanopore, we expect that transmembrane phenomena will be observed in response to the number of the incorporated α-hemolysin.

RESULTS AND DISCUSSION

First, we examined the etching conditions of the bias power and C₂F₈ gas mass flow rate, described in Fig. 3. After determining the condition, we demonstrated the advantage of the glass as a chip material. As shown in Fig. 4, the formed lipid membrane was stable for four days at the glass chip whereas the PDMS chip was unable to form the membrane for such a long term due to the absorption problem. Moreover, we found that the glass chip was reusable several times by washing the channels with an organic solvent (Fig. 5a). Fig. 5b represents the reconstitution of α-hemolysin into the formed membrane. With the mixture of α-hemolysin, the decrease of the fluorescence intensity at the chambers became faster than photobleaching, demonstrating the successful bilayer lipid membrane formation and the reconstitution of the nanopores at the membrane.
Figure 5: (a) The device washed by an organic solvent recovers for reuse. (b) Change of the fluorescence intensity at the microchambers over time. After the reconstitution of α-hemolysin, the intensity decreased faster, indicating the diffusion of calcein molecules through the α-hemolysin nanopores.

CONCLUSIONS

In this study, we succeeded in fabricating a glass microfluidic chip for formation of a bilayer lipid membrane microarray. Magnetic neutral loop discharge plasma (NLD) technology was applied to finely etch the channel design onto a glass substrate at a micrometer length scale. We demonstrated that the formed membrane array was stable over a long period of time at the developed glass chip, in comparison to a PDMS chip, by preventing the absorption of aqueous and organic fluids. Moreover, we were able to reconstitute nanopore protein (α-hemolysin) into the formed lipid membranes and to observe a transmembrane phenomenon at the chip. We believe that glass microchips fabricated with the NLD method will be suitable not only for membrane ion-channel study but also for various biological/chemical assays.

ACKNOWLEDGEMENTS

A part of this work was supported by New Energy and Industrial Technology Development Organization (NEDO), Japan.

REFERENCES


CONTACT

Y. Watanabe, Life Beans Center, BEANS Project; 4-6-1 Komaba, Meguro-ku, Tokyo 153-8505, JAPAN;
Tel +81-3-5452-6545; Fax +81-3-5452-6544;
Email ywatanabe@beanspj.org