CONTINUOUS EXCHANGE OF BUFFERS OVER A LIPID BILAYER MEMBRANE FORMED IN A GLASS MICROFLUIDIC DEVICE

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ABSTRACT

In this study, we described a glass microfluidic device that enables exchange of the buffer solution without breaking the formed lipid bilayer membrane (LBM) for a long period of time (over 24 hrs). We confirmed that the glass device contributed long-term stability of the formed LBM that allows monitoring transmembrane phenomena in the LBM: the phenomena of the spike-like emission were observed by using calcium indicators (Fluo3) under a fluorescent microscope. This result suggest that α-hemolysin known as nano-pore membrane protein was successfully reconstituted into the LBM. We expect that these results are important for the long-term analysis of the membrane proteins which is useful for drug screening and drug kinetics research.

KEYWORDS: Lipid bilayer membrane, Glass microfluidic chip, Membrane protein

INTRODUCTION

The analysis of the membrane protein is important for drug screening and drug kinetics research. The membrane proteins keep their activities only within lipid membranes such as cell membranes or organelles [1], which is a technically tough hurdle to used them for the membrane protein study. Therefore, we have been investigating a method using microfluidics technology to achieve ease of analysis of the membrane proteins. We recently developed a PDMS-microfluidic device consisting of micrometer-sized arrayed chambers, on which lipid membranes are formed by incorporating with membrane proteins such as α-hemolysin [2]. Moreover, we improved to use glass substrate as a material of a microfluidic device that prevents absorption of fluid into the substrate, providing a stable membrane formation and inserting a membrane protein (α-hemolysin) by microfluidic techniques in the device [3]. To analyze the activity of the membrane protein (i.e. ion-channels and transporters) inserted into the formed membrane, the device need to have a function of altering the chemical/physical condition of both side of the membrane. However, conventional device allows only one-way access to the formed membrane. Therefore, in this study, we designed the microfluidic glass device having a feature of two-way access to the formed LBM (Fig. 1). We also performed LBM formation over the apertures and analyzed transmembrane phenomena by using calcium indicators.

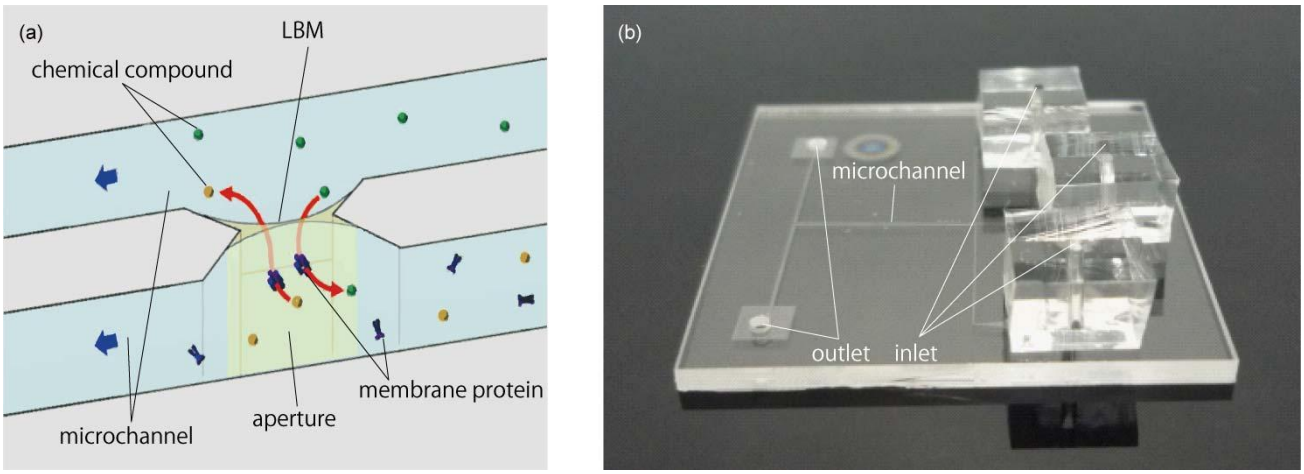

Figure 1: Concept of our two-way access glass microfluidic device. (a) Schematic diagram of the device. The LBM formed on the aperture between two main channels enables visualization of reagent transportation through membrane proteins incorporated in the LBM. (b) Macroscopic view of the fabricated glass microfluidic device.

EXPERIMENTAL

Fabrication of the glass microfluidic device

The schematic diagram and the image of the glass microfluidic device are shown in Fig. 1 and Fig. 2a. We etched a glass substrate by magnetic NLD (neutral loop discharge, NLD-5700Si, ULVAC Inc.). Due to the magnetic neutral loop, higher density of glass-etching plasma of carbon fluoride gas is realized even at a lower pressure [4]. After the etching, we thermally bonded the etched glass substrate with another bare glass substrate (Fig. 2b). The concept figure of the two-way access glass microfluidic device is shown in Fig. 2a.

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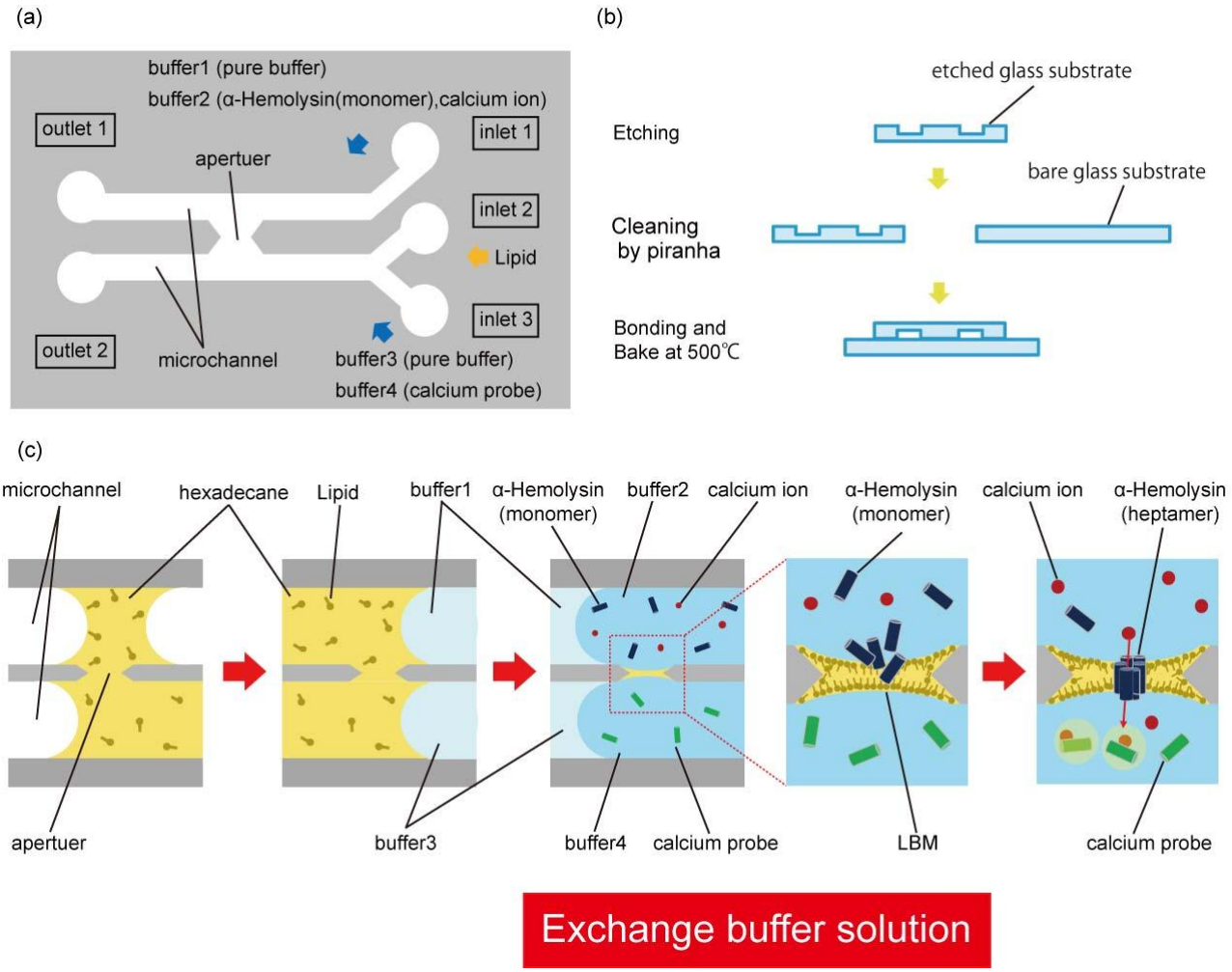

Figure 2: Our two-way access glass microfluidic device enabling continuous exchange buffers. (a) The concept figure of a device. Lipid flow in the device from a inlet2. Buffer1 and buffer2 flow in the device from a inlet1. Buffer3 and Buffer4 flow in the device from a inlet3. (b) Thermal bonding is performed after the surface cleaning of glass. (c) Procedures of the LBM formation incorporated with membrane proteins at the apertures and analysis of transmembrane phenomena by using calcium indicators (Fluo3).

Fabrication of the lipid bilayer membrane

The LBMs were formed on apertures as follows. First, a lipid-hexadecane solution (15 mg/ml of DPhPC, Avanti Polar Lipids, Inc.) was injected into the glass device from inlet 2. Second, a pure buffer solution (buffer1 and buffer3) were injected into the glass device from inlet1 and inlet 3. Third, a protein-containing (a nanopore protein α-hemolysin at 10 μg/ml, Sigma-Aldrich) aqueous buffer (HBSS-14025, 1.26 mM $CaCl_2$, 5.33 mM KCl, pH6.7-7.8, Life Technologies Corporation.) solution (buffer2), and the Fluo3-containing (calcium indicator Fluo3, 200μg/ml, Dojindo Molecular Technologies, Inc.) aqueous buffer (HBSS-14190, 5.33 mM KCl, pH7.0-7.2, Life Technologies Corporation.) solution (buffer4) were injected into the glass device from inlet1 and inlet3 (Fig. 2c). The respective injection rates were 1 μl/min (lipid), 0.1μl/min (buffer1,2,3,4). Then membranes were formed on apertures in the microfluidic device. Since the α-hemolysin forms an open-state nanopore, we expect that transmembrane phenomena can be observed by using calcium indicators (Fluo3) by analyzing fluorescent intensity in the main channels under the fluorescent microscope (Fig. 2c).

RESULTS AND DISCUSSION

First, we observed the life time of the LBM formed between apertures using a microscope. As shown in Fig. 3a, we confirmed that the formed LBM was stable for over 24 hours at the glass device. Next, we carried out the protein reconstitution experiment. Briefly, we evaluated nano-pore formation of α-hemolysin on the LBM by fluorescent emission caused by combination of Fluo-3 and calcium ions. We observed fluoresence in the micorchannel near the apertures, and measured the fluorescent intensity (Fig. 3b). We detected three-spike like fluorescent emission, indicating in transportation of calcium ions into the buffer 4 side through the nanopores of α-hemolysin from the buffer 2 side. We suggest that this result indicates successful incorporation of α-hemolysin into the LBM observed. However, fluorescence intensity fell rapidly after fluorescence about 20 seconds. α-hemolysin may be de-constituted out from the LBM.

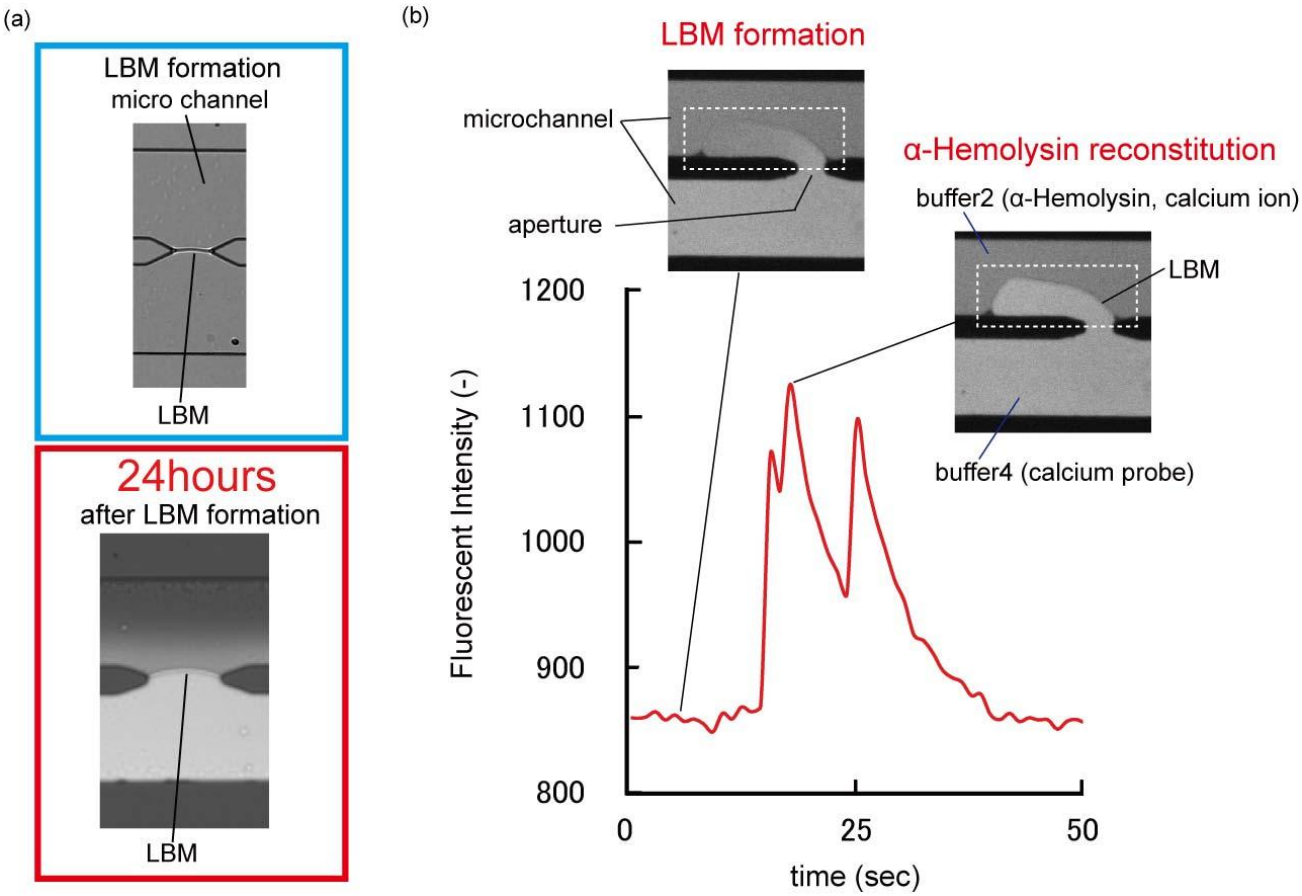

Figure 3: Formation of a LBM at the aperture in the device and characterization of transmembrane phenomena in the LBM. (a) Microscopic views of the formed LBM after 0 and 24 hours. (b) Time-dependent fluorescent intensity of the micro channel of buffer1 side (in dotted white line region) after forming the membrane incorporating membrane proteins. Higher fluorescence intensity was observed. The events might correspond to the incorporation of an α-hemolysin into the membrane.

CONCLUSIONS

In this study, we confirmed that the glass device contributed to long-term stability of the formed lipid bilayer membranes that enables monitoring transmembrane phenomena in the LBM in microcfluidic device. Since our two-way access device allows altering the chemical/physical condition of both side of the LBM, we believe that our device will be useful for multifunctional analysis of membrane proteins.

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