Rapid Perfusion System for Inhibition Investigation of Membrane Proteins in Planar Lipid Bilayer

Y. Tsuji[1,3], R. Kawano[1], T. Osaki[1], K. Kamiya[1], N. Miki[1,3] and S. Takeuchi[1,2]

[1]Kanagawa Academy of Science and Technology, Japan

[2]Institute of Industrial Science, The University of Tokyo, Japan, [3]Keio University, Japan

ABSTRACT

This paper describes measurement system of membrane proteins with a rapid perfusion capability using microfluidic channels. In the previous report, we successfully recorded channel conductance of alpha-hemolysine (αHL) in bilayer lipid membranes (BLMs) using droplets contacting method with PDWC (parylene double well chip). This method is one of the easiest ways for preparing the BLMs, just pipetting droplets of the solution. The drawback of the droplet-based systems is the incapability of exchanging solution. To efficiently test the effect of inhibitors and promoters of membrane proteins in drug discovery, the solution exchange is mandatory. In this study, we proposed the concept of solution exchange in the system using microfluidic channel. We demonstrated rapid perfusion that is capable of exchanging the solution within 20 s in this droplets contacting system. We experimentally evaluated the system in terms of the flow rate and the BLM stability and demonstrated the binding assay of a membrane protein using its blockers. We believe that this method is necessary for efficient drug screening in our droplet-based system.

KEYWORDS

Droplets contacting method, Parylene Double Well Chip, Bilayer Lipid Membranes, Solution Exchange

INTRODUCTION

Recently, a drug screening exploiting an artificial cell membrane has been extensively studied. This method is expected to reduce cost and measurement time compared to the live-cell-based screening. For more efficiency, various analyses using exchanging the solution on single chip are required[1]. Artificial BLM techniques are of great importance in membrane protein research or high-throughput drug screening. Hence, easy and stable BLMs formation methods are absolutely imperative. Extensive researchers have developed the methodology of the BLMs preparation. The first trial to form artificial cell membrane was done by M. Montal and P. Mueller in 1972[2]. Since then, various methods have been invented[3,4]. However, these BLM formation techniques could not satisfy throughput and reproducibility required for drug screening and biological sensor applications.

We proposed a rapid and simple BLMs forming process using droplets contacting method[5] with PDWC[6]. BLMs are formed as follows; the PDWC is filled *n*-decane containing a phospholipid and two droplets of buffer solution are dropped into lipid solution in each well. A lipid monolayer forms at the interface between an aqueous droplet and the organic solution. When two monolayers contact each other, the BLM spontaneously forms at the interface of two contacting monolayers through the parylene micropores[7,8] after several minutes. The parylene micropores prevent the two droplets from fusing each other. This method is advantageous over other methods in stability and reproducibility of BLMs. We investigated the long-term stability of BLMs formed with PDWC. BLMs containing membrane protein alamethicin were maintained for 2 weeks. αHL was reconstituted in PDWC at the yield of 76%[6]. For these advantages of high stability and reproducibility, we could array the PDWC with electrodes and reconstitute αHL in parallel for multiple analysis in the previous work.

While PDWC can produce BLMs stably at high throughput, it lacks the capacity to exchange buffer solution, that is, we investigate only some solution dispensed in the well before BLMs formation. PDWC with capability of adding or removing membrane proteins and inhibitors or promoters will greatly enhance the efficiency of experiments. In this paper we propose PDWC with microfluidic channels to exchange solutions. We experimentally verify the effectiveness of this system by observing the electrical signal of αHL when its blockers are injected after forming BLM.

DEVICE FABRICATION

The solution is injected to a droplet from an inlet while the inner solution of the droplet is withdrawn at the same flow rate from the outlet. The solution exchange is performed with two syringe pump equipped out of the faraday cage. Droplet solution exchange can be gradually achieved through the microfluidic channel with keeping BLM stable (Figure 1a). Device fabrication process is as following; Chamber part: a fabricated parylene film which has five pores was sandwiched between PMMA films and separated the two round well. Base

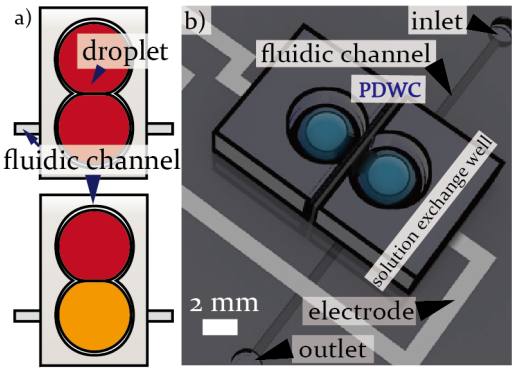

Figure 1a) BLMs can be formed using droplets contacting method. The solution of bellow droplet is exchanged with two microfluidic channels. b) Diagram of solution exchange device. A parylene with micropores was sandwiched between two chambers. The base has two fluidic channels (square 500 mm on a side) for injection and withdrawing the solution.

part: we integrated two microfluidic channels (square 500 μm on a side) at the bottom of the PMMA chamber. These channels allow for the solution exchange. One of the channel works as the inlet and the other is the outlet. Ag/Cr was wired by vapor deposition and patterned on a PMMA plate as electrodes from the chambers to a patch-clamp amplifier for the electrical recording of channel proteins. Connecting: the chambers with parylene films and the wired plate with microfluidic channels were connected by thermocompression bonding for 20 minutes at 120 degrees Celsius while applying a force of approximately 300 N. Finally, the bottoms of the chamber which contacts droplets were coated with Ag/AgCl paste for electrical measurement. Figure 1b shows the completed device. The advantages of this device include simple processes, high stability of BLMs, ease of electrical recording, and high signal-to-noise ratio in addition to solution exchange capacity. We have completed a platform for solution exchange of the droplet contacting lipid bilayer system.

EXPERIMENT

A hydrodynamic simulation in the droplet and microfluidic channels was performed by COMSOL (COMSOL, Sweden). We prepared the model imitated the PDWC equipped with microfluidic channels in the software and introduced the solution from the inlet at various flow rate (dilution species transport study mode). In particular, we simulated the flow velocity distribution, pressure distribution and shear velocity distribution.

At bellow experiment described as follow, we used fluorescent solution (calcein, 5 μM) for visualizing the solution exchange. This experiment was to observe the transition of the fluorescence intensity in the droplet at several injection flow rate. It makes known the relationship between the solution exchange time and the concentration of the desired solution. This experiment was carried out with single well chip (one well of the double well chip) equipped two microfluidic channels.

We tested a solution exchange experiment with one chip using αHL and its blocker with PDWC under the following condition. BLMs were initially formed using two buffer droplets which contain αHL (0.3 μmol protein/L in 1 M KCl buffer solution) and we monitored the channel conductance of αHL at 50 mV applied. Then, s_7βCD solution (50 μM) was added as the blocker after reconstituting αHL channel at the trans-side for 20 seconds. After observing αHL channel conductance and the current blocking events by s_7βCD molecules, the droplet was washed by exchanging the solution through the fluidic channel for 20 seconds.

RESULT and DISCUSSION

We decided the experimental condition such as the flow rate or the solution exchange time through simulating the flow velocity distribution in a droplet using COMSOL (Figure 2). We found that the flow effect hardly reaches at the bilayer formation area and the disturbance in a droplet was almost negligible. We also investigated the transition of the fluorescent intensity in a droplet. We estimated the relationship between the solution exchange time and the concentration of desired solution at each injection speed. In this double well system, BLMs were sometimes ruptured before the initial solution was fully exchanged. The reason may be a first impact by pump starting or some disturbance which doesn't appear in COMSOL simulation. So, we optimized the injection speed would be 15 μL/s if you wanted to exchange the solution at highest speed.

We describe about the demonstration using membrane protein αHL and its blocker s_7βCD[9]. αHL is an exotoxin secreted by the bacterium *Staphylococcus aureus* and is polypeptide of 293 amino acids[10]. The monomers are considered to spontaneously assemble in the BLM and forms oligomeric cylindrical pores which is approximately 1-2 nm in internal diameter[11]. When voltage gradient is applied, the ion could flow through the pore and we can observe the protein channel conductance from αHL. βCD[12,13] is a cyclic molecule with a hydrophobic cavity, comprising seven d-glucose units. When βCD or other cyclodextrins like s_7βCD are lodged in the lumen of the αHL pore, they alter the unitary conductance (from 658 pS to 240 pS in 1 M NaCl (pH 7.5)), which is observed as a reversible stepwise change in the signal, change the ionic selectivity and act as binding sites for channel blockers by contributing their host properties.[14,15,16] βCD is a neutral molecule on the other hand s_7βCD is the negatively charged molecule. The dwell time of s7βCD is far greater than βCD[16]. By using s7βCD, we can observe the blocking events more easily. So, we used s7βCD instead of neutral βCD.

In this experiment, the channel conductance was observed by path clamp amplifier at first. Then, one of the droplets was transposed to s_7βCD solution as the blocker. Then the blocking current was observed, verifying that s_7βCD worked as the blocker, and the blocker solution was washed up with pure buffer solution and no more blocking was observed. It indicated that s_7βCD molecules were fully removed from the droplet and replaced with pure buffer solution.

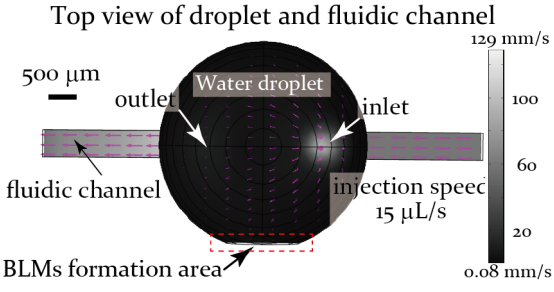

Figure 2 Flow velocity distribution in the water droplet and fluidic channel simulated with simulated with simulation software COMSOOL. The arrows indicate the speed and the direction of the flow.

CONCLUSION

Owning to investigating the flow velocity distribution and real impact or disturbance which occurs when pumps are running, we could optimize the solution injection speed. We succeeded in exchanging the solution in a droplet with maintaining BLMs stable. Membrane protein and its blocker (s_7βCD) were successfully introduced into the chamber and then s_7βCD was removed from droplet after observing the blocking events in the one chip.
The rapid perfusion demonstrated equipped with the PDWC can be readily applicable to high-throughput drug screening and other biological researches.

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CONTACT

Yutaro Tsuji,
Kanagawa Academy of Science and Technology,
KSP EAST 303, 3-2-1 SAKADO, TAKATSU-KU, KAWASAKI, KANAGAWA, 213-0012, JAPAN
tel: +81-44-819-2037; yutaro1210@a7.keio.jp