Dielectrophoretic introduction of the membrane proteins into the BLM platforms for the electrophysiological analysis systems

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Abstract—This paper proposed a technique to introduce the membrane protein into the lab-on-chip analysis system having a planar lipid bilayer. The proposed technique utilized a dielectrophoretic (DEP) force generated by the asymmetric configuration of the solid electrodes on the aqueous buffer separator. By applying the alternating current to the separator and the counter electrode, we manipulated liposomes that could host the membrane proteins on the surface. The key point for the dielectrophoretic manipulation on this system was the effective configuration of the droplet separator having the taper-edge on the contour of the micropore. This configuration made a strong interpenetrating DEP force at the lipid bilayer, and prompted the fusion of liposome into the lipid bilayer. The separator was fabricated by micromachining techniques. Using the separator, we formed the lipid bilayer without evading the solid electrode on the surface. Finally, we elucidated the introduction of the liposome by monitoring with the optical microscopy.


I. INTRODUCTION

This research proposed a reliable method to introduce the membrane proteins into the electrophysiological measurement device. Membrane proteins play important roles in the transportation of the chemical substances and the signal reception and the transduction inside the cell system [1]–[3]. In particular, the ion channels and the conjugated receptor proteins are extensively studied to elucidate their function of chemoreception using the patch-clamp method [4]. Although the patch-clamp method allows us to directly measure the ionic current from these ion channels, the experimental throughputs are highly depending on the human skills. In addition, false positive signals are frequently measured due to the interruption of unexpected ion channels, making it difficult to quantitatively evaluate the functions of the specified proteins. For these reasons, some of the recent studies incorporated the lab-on-chip platforms that mimic the environment around the cell membrane. Namely, these devices fabricated the artificially configured cell membrane with the lipid bilayer on the microfluidic devices to host the extracted and purified membrane proteins. Our group developed the droplet contact method (DCM) [5] to easily form the lipid bilayer on the microfluidic device, and contributed to the growth of this research field. The DCM, depicted in Fig. 1, has been further improved over a decade, and many applications and its peripheral technologies were proposed [6]–[8]. However, the platform using DCM still has a latent difficulty on the introduction of the membrane proteins into the bilayer membrane. Therefore, we offer a dielectrophoretic method that induces the membrane proteins to fuse into the lipid bilayer. Using this technique, we improve the introduction rate and the duration of the membrane proteins.

II. OVERVIEW OF THE MEASUREMENT SYSTEM

We briefly explain the overview of the experimental system. In the similar way of the previous studies, we utilized microwell devices and systems as depicted in Fig. 2(a). The device was configured by the machinery milled acrylic microwells whose contours are intersected with each other. When we soured the organic oils containing the lipid molecules and a couple of aqueous buffer solution, the lipid bilayer was formed at the interface of the droplets. Since the lipid bilayer was the same conformation of the cellular membrane, the membrane protein could be hosted on the surface of the lipid bilayer. The membrane proteins such as the nanopores, or ion channels could be characterized by their gating behavior against their chemical receptions. We therefore installed the Ag/AgCl buried electrodes on each side of the microwells and measured the ionic current passing through the membrane proteins. The electrodes are then connected to the IV converting amplifier having the same configuration of the patch clamp systems. Thus, we measured the quite small ionic current on the order of 1 pA. Since the conductance of the membrane proteins took the invariant conductance depending on the gating states, the measured
ionic current became the discrete steps or the rectangular waves. To make the success rate of the bilayer formation, the recent studies have utilized the droplet separator with some micropores. These were established under and beside the optical microscopes for the monitoring.

In this study, we modified the structure of the aqueous buffer separator and added the dielectrophoretic control units. The previous experiments expected that membrane protein could be reconstituted spontaneously into the lipid bilayer by just souring them into the droplet. Contrary to our prospects, the membrane proteins are scarcely fused in the empirical raw. This drawback caused the low throughput of the membrane protein analysis. To overcome this problem, some studies tried to modify the device configuration [9] or tool for the introduction of membrane proteins. These trials were, in certain viewpoint, succeeded. However, these required the sophisticated experimental setups such an acute needle electrode or the automated manipulators. For the practical use of DEP force, we took a simplest and compatible way to the existing DCM-based platforms.

III. METHODOLOGIES OF DIELECTROPHORETIC FUSION OF MEMBRANE PROTEIN INTO LIPID BILAYER

A. Dielectrophoretic control of the membrane proteins

The overview of the membrane protein introduction toward the lipid bilayer is illustrated in Fig. 2(b). The proposed technique generates the dielectrophoretic attractive force on the membrane proteins toward the lipid bilayer. As a container of the membrane proteins, we embedded the membrane proteins on liposomes, which are the spherical vesicle formed by lipid bilayer. This technique was broadly utilized for the safekeeping of membrane proteins in buffer solutions. Here, we briefly consider the principle of the dielectrophoresis, and subsequently design the dielectrophoretic system compatible with the DCM platforms. The dielectrophoretic force acting on the spherical particle \( F_{DEP} \) was expressed as the following equation [10]:

\[
F_{DEP} = 2\pi r^3 \varepsilon_p \text{Re} \left[ f_{CM}(\omega) \right] \cdot \nabla E^2,
\]

where \( r \) is the radius of the particle, \( \varepsilon \) is the permittivity of the medium. \( f_{CM}(\omega) \) is so called a Clausius Mossotti factor expressed as

\[
f_{CM}(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*},
\]

where \( \varepsilon^* = \varepsilon - j(\sigma/\omega) \) is the complex permittivity, including the factor of conductivity \( \sigma \) as a dissipative term. The \( p, m \) in the subscription indicates the particle and medium, respectively. Eqn. 1, definitely illustrates that we need to designed and fabricated the separator of the aqueous buffer solution to generate the asymmetric gradient of the electrical field.

B. Design and fabrication of the separator

In order to exert an attractive force on the liposome toward the lipid bilayer, we designed the electrical field around the micropore on the droplet separator. In this study, we employed a solid electrode on the surface of taper-shaped micropore to generate the gradient of the square of electrical field. As illustrated in Fig. 3, the solid electrode on the taper-shaped micropore could generate the electrical field gradient. In the case of negative DEP, the dielectric force around the micropore are always looking toward the micro pore. In addition, due to the solid electrode on the opposite side of the separator, the dielectric force are penetrating across the lipid bilayer. The large interpenetrating DEP force are only attained by fabricating the taper structure on the sidewall of micropore. We calculated the effect of the taper angle for the electrical field gradient at the center axis of the micropore as depicted in Fig. 4. By decreasing the corner angle of the micropore, the electrical field gradient was interpenetrated across the lipid bilayer. In this study, we set the taper angle less than 60°, typically 58.4° from the cross-sectional image. We would like to notice the following two point; first, the solid electrode should be fabricated on both side of the separator, but the both electrodes should be completely insulated with each other. This insulaion was required to apply a voltage on the lipid bilayer and to measure the ionic current passing through the membrane proteins. If we fabricated the electrode by means of the simple bulk fabrication technique,
Fig. 3. The result of FEM analysis on the electrical field gradient. The geometrical parameters are applied by using the nominal value of the design. The electrical filed was the maximal value when 1 Vpp was applied between the solid electrode on the surface and the counter electrode. At the bilayer region, the 5 nm gap are set to mimic the behavior of the bilayer. The relative permittivity of aqueous phase and oil phase was 80 and 1.8, respectively.

Fig. 4. The dependence of the electrical field gradient at the center axis of the micropore against the taper angle of the micropore.

those two electrodes would have been connected due to the step coverage of the fabrication process. Second, the interruption of the oil around the micropore would distort the electrical field gradient. Since the distribution of the oil in the microwell was uncontrollable, this interruption could be an obstacle to achieve the high reproducibility. Therefore, we formed the microslit inside the separator to eliminate the electrode at the intermediate area of the micropore and to enclose the lipid-in-oil phase.

The fabrication of the separator was illustrated in Fig. 5. To sustain the slit structure with the sufficient mechanical strength, we used the borosilicate glass plate as a separator material. The slit regions were fabricated using the wet etching, whereas the taper-shaped micropores were fabricated by the blast etching. In order not to evade the aqueous phase into the unexpected region, the wettability of the surface was controlled by coating the perfluorinated polymers. Using the silane-coupling reagents and deposition under the plasma, the polymer membrane was strongly adhered on the glass and durable to the post cleaning. Since the glass-transition temperature of the perfluorinated polymer is around the 230 °C, the bonding of the glass substrates were performed at lower temperature using the surface activation method. In order to confirm the bilayer formation at the micropore of the separator, we also prepared several samples on which solid electrodes was not deposited.

The proposed configuration of the separator formed the lipid bilayer in a different manner of DCM. First, we introduced the lipid-dispersed oil (DPhPC 20 mg/ml in decane) into the slit of the separator. Since the demanded quantity of the oil was as small as 100 nL, we utilized the polytetrafluoroethylene (PTFE) stick with the hanging oil drop. By imposing the stick tip into the upper side aperture of the slit, the oil was automatically infiltrated into the slit and stopped at the acute edge of the micropore. At this moment, the micropore worked as a capillary-stop valve for the oil. We subsequently soured the aqueous buffer solution in one microwell. Due to the intervention of the aqueous buffer solution at the micropore, the capillary stopping effects were disabled and the lipid-dispersed oil were satisfied at the micropore. Finally, by sowing the aqueous buffer solution in the opposite microwell. Immediately after the oil were sand-
Fig. 6. The bilayer formation scheme using the fabricated separator. (a) schematic view of the bilayer formation; (b) The microscopic image of the lipid bilayer; (c) The timelapse image of the bilayer. Since the capillary force were acting on the oil, the oil were evacuated from the bilayer region. This effect could be useful for the fusion of liposome to dispose the abundant lipid or oil after introduction.

Fig. 7. Experimental device for the dielectrophoretic introduction of the membrane protein into the lipid bilayer; (a) the Foothold of the experimental device used for the membrane protein analysis; (b) the expanded image of the DEP region. The separator and the counter electrodes were connected to the function generator (Wavestation2012, Teledyne LeCroy Co. Ltd) and the amplifier (BA4825, NF circuit block Co. Ltd). The applied voltage was \( V_{PE} \) and the frequency was 1 MHz; (c) the side view and the top view of the liposome introduction. The trajectory was a little bended due to the shape of the counter electrode.

wiched by the aqueous buffer solution, the oil were evacuated by the capillary force inside the slit of the separator. Thus, the lipid bilayer was obtained at the micropore of the separator, avoiding the organic contamination on the solid surface. The existence of the lipid bilayer was confirmed by the image of the microscopy. Since the bilayer membrane satisfied the condition of \( \phi_0 \) order Newton-Ring, the color became gloom, as shown in Fig. 6.

IV. DEMONSTRATION OF THE MEMBRANE PROTEIN INTRODUCTION USING DEP

To demonstrate the liposome introduction into the lipid bilayer, we monitored the behavior of the liposome using the optical microscopy from both the sidewall and the top of the separator. To clearly see the separator, we used the glass plate on the sidewall of the microwell. The experiment was performed in the same configuration of the membrane protein analysis system by adding a couple of electrodes for the DEP manipulation. Since these functions were, for the time being, interrupting with each other and the applied voltage range of the DEP was four order of the magnitude larger than that used for the membrane protein analysis circuit, we mechanically insulated the circuit. Figure 7 illustrated the experimental view of the liposome introduction. From the sideview, we could clearly see that the liposome was attracted to the lipid bilayer formed on the micropore of the separator. The topside image also showed the trajectory along which the liposome was introduced. In this demonstration, the trajectory of the liposome at the neighbor of the solid electrodes are repulsive against the micropore. If we could suppress this effect, the proposed technique could be further effective for the high throughput introduction.

From the experimental result, we achieved the liposome introduction to the planar lipid bilayer using DEP force. Therefore, by embedding the membrane protein, this technique could improve the throughput of membrane protein analysis platform. The remaining problem is the integration of the operation circuit of DEP and the analysis circuit of membrane proteins that deal with the different magnitude of the electrical current. Using the upcoming circuits, we would like to demonstrate both the liposome introduction and the membrane protein analysis in series.

REFERENCES