ELECTRIC STRESS PRODUCES BILAYER LIPID MEMBRANES BY EXCLUSION OF EXCESSIVE OIL LAYER

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ABSTRACT

This paper proposes a formation method for oil-layer-free bilayer lipid membranes (BLM) by using a compressive force of DC electric field. Using a droplet contact device previously developed, constant-voltage steps were applied to the pre-bilayer membrane and carefully controlled the electric stress in response to the membrane features, based on optical and current monitoring. We examined the pathways of the membrane formation and disruption, and discovered a qualitative protocol for oil-layer-free BLM formation. We succeeded in BLM formation using 1:1-mixture of hexadecane and *n*-decane as an oil example, which was hardly available for the former studies using the device. The method would contribute to produce *vivo*-like membranes appropriate for membrane protein functionalities.

INTRODUCTION

Droplet contact methods (DCM) are a robust approach to form BLM simply by contacting two aqueous droplets in lipid-dispersed oil [1, 2]. BLM formation is commonly confirmed by monitoring the electrical properties of the membrane, for example, membrane conductivity and capacitance. Alternatively, a pore-forming membrane protein, α-hemolysin, is applied for the confirmation, by infusing the protein to the DCM device and observing the current signals. Once BLM is formed, the toxic membrane protein α-hemolysin (α-HL) monomers are spontaneously integrated into the BLM and form heptameric nanopores. The nanopore consists of a β-barrel structure with a narrow constriction of 1.4-nm diameter, and represents 1 nS conductance in 1 M KCl solution; thus a stepwise increase of the current, 100 pA at 100 mV, clearly exhibits the BLM formation as well as the integration of α-HL pore.

In DCM devices, *n*-decane (the number of the carbon chain is 10; C_{10}) have been commonly applied for the oil because BLM formation occurs autonomously and rapidly. A possible problem of using the *n*-decane for DCM is that the oil layer of *n*-decane might be remained between the lipid monolayers, and this remaining oil would interfere the functions of membrane proteins. On the other hand, it is known that the length of hydrocarbons of oil indicates trade-off between the remaining oil-layer thickness and the difficulty in BLM formation by DCM: Long hydrocarbons would form oil-layer-free BLM, although the system required larger activation energy to exclude hydrocarbons from the lipid monolayer (Figure 1) [3].

A previous research demonstrated the formation of BLM with squalene (C_{30}) as the solvent of lipids by using a pressure-control system [4, 5]. Although the principle of BLM formation was worthwhile, the system apparatus was rather complex and bulky, and would not applicable for practical applications. In this study, we focus that the BLM formation process was assisted by a compressive force; instead of the hydraulic pressure control, we apply electric stress [6] and aim to form oil-layer-free BLM with a mixture of a long hydrocarbon (hexadecane; C_{16}) and *n*-decane (C_{10}).

CONCEPT

In DCM, BLM formation by using oil with long hydrocarbon chain is difficult. This is because the oil layer

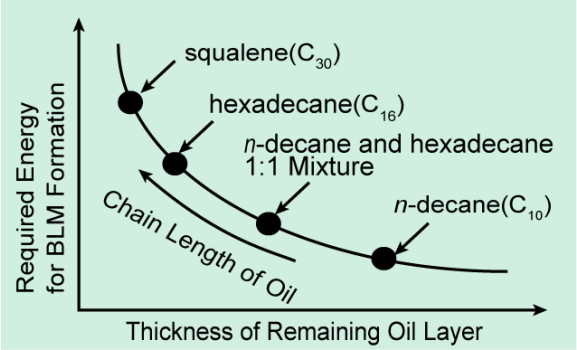

Figure 1: Relationship between activation energy for BLM formation and thickness of remaining oil layer in various length of hydrocarbons

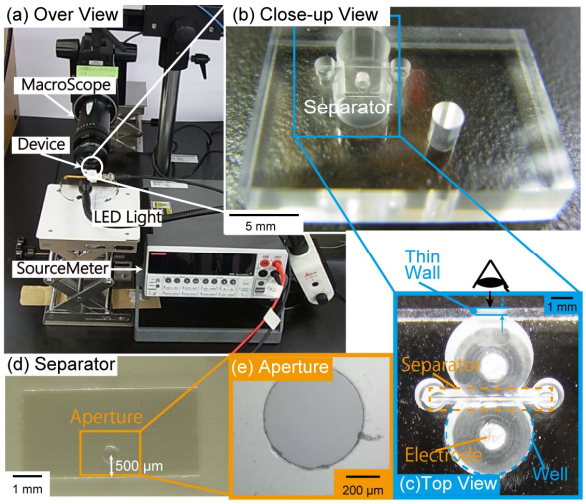

Figure 2: (a) Experimental setup. The electric stress was applied with a source meter, simultaneously monitored the current. (b-c) Ag/AgCl electrodes were wired at the bottom of the wells. Transparent wall of 0.25 mm thick was fabricated at the side of the well for the membrane observation. (d-e) The aperture was placed at 500 μm from the bottom edge.

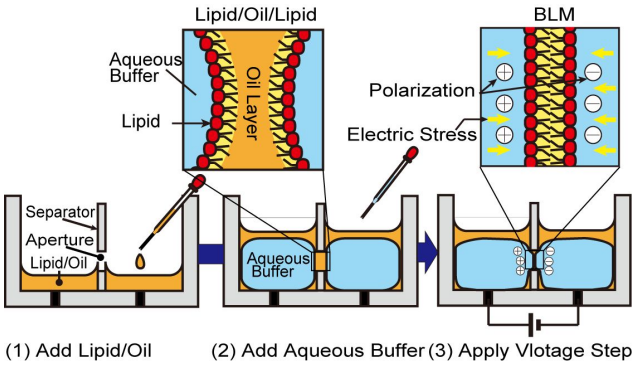

Figure 3: Conceptual diagrams of BLM formation using the electric stress.

between lipid monolayer membranes cannot be excluded, therefore inhibits the contact of two lipid monolayers. In this study, we applied external voltage to generate electric stress – compression force to the BLM, on purpose to exclude the remaining oil layer. The proposed method has advantages in the simplicity since only using a pair of electrodes integrated in the former devices. This approach will be useful for the formation of BLM with desired types of oil.

DEVICE FABRICATION

As shown in Figure 2, the base device was fabricated with PMMA (poly(methyl methacrylate) plates using a micromilling machine (Mini Miller MM-100, Modia Systems, Japan). Two cylindrical wells with 4 mm in diameter and 3 mm in depth were constructed in the plate. One sidewall of a well was polished for optical observation of BLM formation through the sidewall by using a macroscope (Fastcam Mini Ax50, Photron, Japan). Two electrodes with Ag/AgCl paste were embedded through the bottom of the wells for application of DC electric field. The base device was bonded to a BNC terminator and connected to a sourcemeter (type 2400, Keithley, USA). A PMMA thin film of 75-µm thickness, with an aperture of 600 µm in diameter, was used as a separator, placed between the two wells.

EXPERIMENTAL

We explored the BLM formation protocol using electric stress generated by DC electric field. We observed the optical features of lipid membranes and the ionic current through the membrane under various voltage-step situations. Figure 2 shows the setup. The DCM device consists of two wells and a separator with an aperture; at the bottom of the wells, a pair of electrodes was wired for the voltage application. We used 1:1 mixture of *n*-decane and hexadecane as the oil (Sigma-Aldrich, USA), dispersing 20 mg/mL DPhPC (1,2-diphytanoyl-*sn*-glycero -3-phosphocholine, Avanti Polar Lipids, Inc., USA) as the lipid, 1M KCl prepared with 10 mM phosphate buffer as the aqueous buffer solution. The general procedure of BLM formation is shown in Figure 3. First, lipid-dispersed 1:1-mixture of hexadecane and *n*-decane and an aqueous buffer were sequentially infused to the device. Then, a

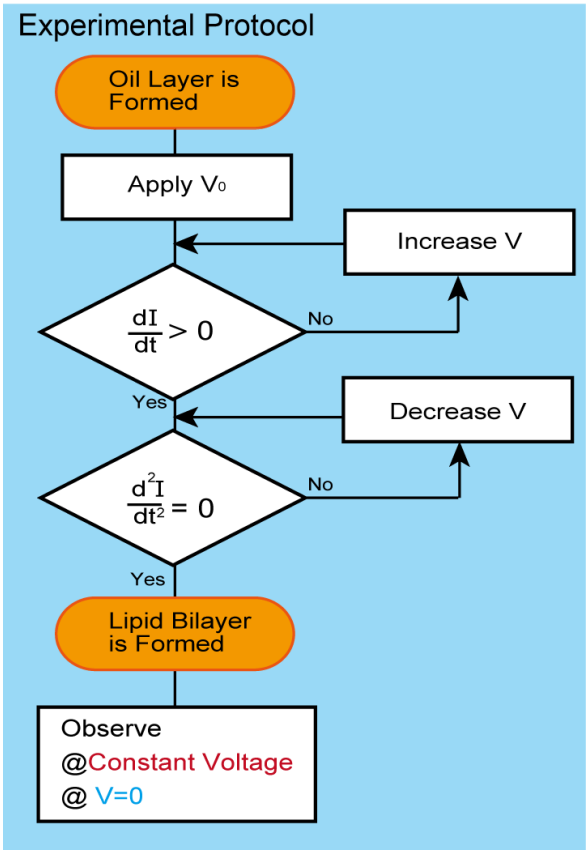

Figure 4: BLM formation protocol using the electric stress. The voltage step was controlled in response to the optical and current monitoring. Orange panel: the states of the membrane; square: the actions of the voltage control; diamond: the decisions for changing the voltage.

thick lipid/oil/lipid layer was formed between the droplets at the aperture. Finally, constant-voltage steps were applied to the membrane *via* the electrodes. The applied voltage generated an electric stress, which compressed and excluded the oil layer, resulting in thinning the membrane. This process was followed by optical observation and current monitoring, and the voltage was manually controlled to form BLM. The qualitative protocol is shown in Figure 4.

RESULTS

Figure 5 shows the specific membrane features resulting from the optical and electrical monitoring. We observed dynamic changes of the membrane feature in response to the voltage-step applications followed by the protocol shown in Figure 4. First, the DC voltage was manually increased in a stepwise manner until the membrane resistance decreased at 100 GΩ (Figure 5a-b). From the microscopic view, the membrane at the aperture was homogeneous and significant change was not observed. Then, the voltage was switched to decrease to control the decreasing rate of the resistance more gradually (Figure 5b-c). During this step, the membrane was still homogeneous. By keeping the voltage decrease, however, we optically

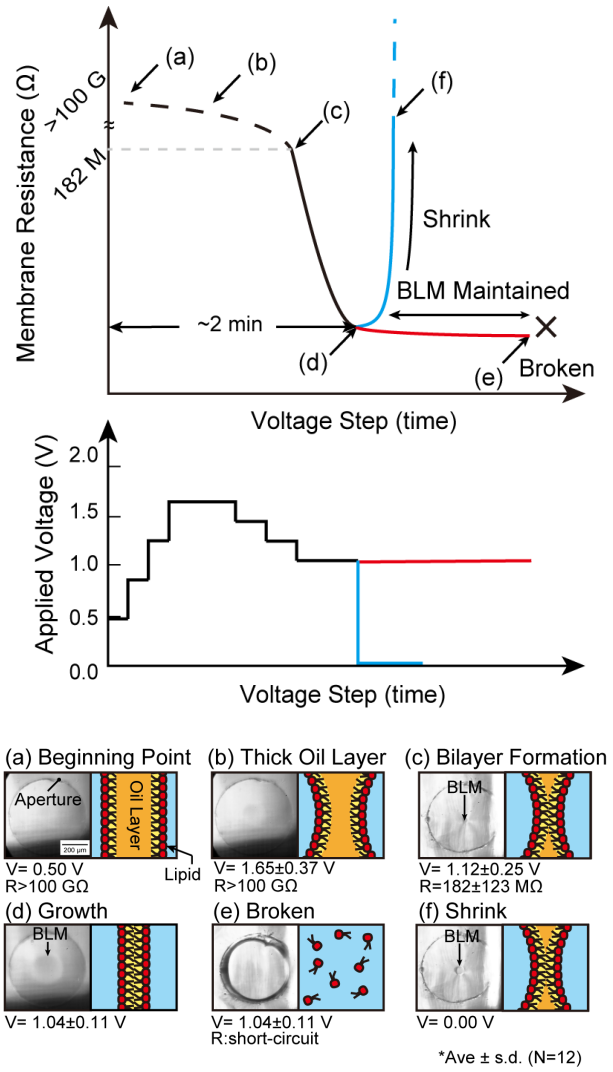

Figure 5: (Upper schematic) The relationship between the membrane resistance and the applied voltage-step sequence. (a-f) Specific states of the membranes (macroscope image and its schematic) at the voltage step applied. The membrane resistance was calculated from the voltage and the monitored current. The voltage was switched at arbitrary timing, in response to the observation of the membrane feature and the current.

observed that BLM started forming at the aperture, which was confirmed by the emersion of a circular boundary between BLM and the surrounding annulus. The formed BLM was kept growing by occupying a half diameter of the aperture (Figure 5c-d). Electrically, the resistance drastically dropped together with the beginning of the BLM formation and probably depended on the BLM area. Keeping the voltage at 1 V allowed to remain BLM for 20 s in maximum and broke (Figure 5e), while turning off the voltage made BLM shrink (Figure 5f). Further studies are required to explore the optimal condition that keeps BLM stable for longer time, and ideally, stable after eliminating the application of the DC voltage. In this work, we determined the qualitative BLM formation protocol using the electric stress for facilitating oil-layer-free BLM (Figure 4, Figure 5).

CONCLUSIONS

In this study, we proposed a method to form oil-layer-free BLM by applying DC voltage steps. We determined the sequential protocol of the voltage applications, and succeeded in forming the BLM in a mixture of hexadecane and *n*-decane. Although the results were still preliminary level, we believe that the protocol obtained by the future works will help oil-layer-free BLM formation in various types of oil, which will, in turn, be applied for membrane protein studies without the influence of the oil. We have started developing the automated logging setups for electrical monitoring, which would correct and optimize the protocol.

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