HIGHLY EFFICIENT FORMATION OF DROPLET INTERFACE BILAYERS BY USING A MICROPERFORATED SEPARATOR

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ABSTRACT

This study reports an efficient droplet interface bilayer (DIB) formation using an open chamber with an external separator that can divide an aqueous solution to two regions. This approach facilitates reagent-saving and simplification of the device fabrication. Amphiphobic modification of the separator surface enables us to handle the device easily without liquid spill, and assists bilayer lipid membrane (BLM) formation. Furthermore, we found that pretreatment of lipid deposition on the separator increased the success rate of BLM formation. We are assured of this method's availability for various research and development fields based on DIB applications.

INTRODUCTION

Bilayer lipid membranes (BLMs) have been utilized for making artificial cell membranes owing to a simplicity of the method for preparation [1]. That kind of synthetic membrane is recently used for analyses and applications of a large variety of membrane proteins that are reconstructed in BLMs [2]. Hence, more efficient and convenient method for droplet interface bilayers (DIBs) formation is required in these days.

Typical DIBs are generally formed at boundary face between two droplets of aqueous solution surrounded by a hydrophobic solvent containing lipid molecules [3, 4]. Therefore, the formed BLMs in aqueous solution are usually isolated from the outside. Such a system is unsuitable for applications of membrane proteins (e.g. olfactory, and gustatory receptors) functionally associated with extraneous chemical stimuli. As a solution for the drawbacks, there is a report that half-sunken droplets in oil were used for DIB formation [5]. And also, we recently reported that hydrogel was useful in substitution for oil-covered solution in a membrane protein reconstructed BLM system [6, 7]. The hydrogel part was sufficiently tall for upcropping out from oil layer, and thus the hydrogel had air-gel interface.

However, in these approach, we still have difficulties in configurations of electrodes for membrane potential measurements, and repeatable BLMs formation. To overcome the problems, and for easy DIB formation with high reproducibility, we here newly design a fluidic device, and attempt to use of small solvent by controlling the wettability of the device surface.

EXPERIMENTAL

Figure 1 schematically shows a design and a process flow of BLM formation in this study. A perforated T-shaped separator (hole: 600 µm in diameter, and 75 µm thickness) and the well (3 mm depth, and 24.7 mm^2 area) were designed for the manual assembly. All parts were made of polymethylmethacrylate (PMMA), and fabricated with an end milling machine as same as previous work [6]. The separator was partly modified to amphiphobic surface using a fluorinating agent (SFE-B002H, AGC Seimi Chemical) before deposition of oil with lipid.

We totally used 60 µL buffer solution (5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid pH 7.6, 96 mM NaCl, 2 mM KCl, 5 mM $MgCl_2$, 0.8 mM $CaCl_2$) and 1.5 µL oil (*n*-decane) with 20 mg/mL lipid mixture (1,2-dioleoyl-*sn*-glycero-3-phosphocholine and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine at the weight ratio of 3:1) in this study. We additionally used a hydrophobic stick for DIB formation for reducing the thickness of oil layer by repainting when bulky oil with lipid remained at the hole. The success and failure of BLM formation were confirmed by microscopic observation through an outside wall of the well device as shown in Figure 1(vi). All experiments were carried out at room temperature in this study.

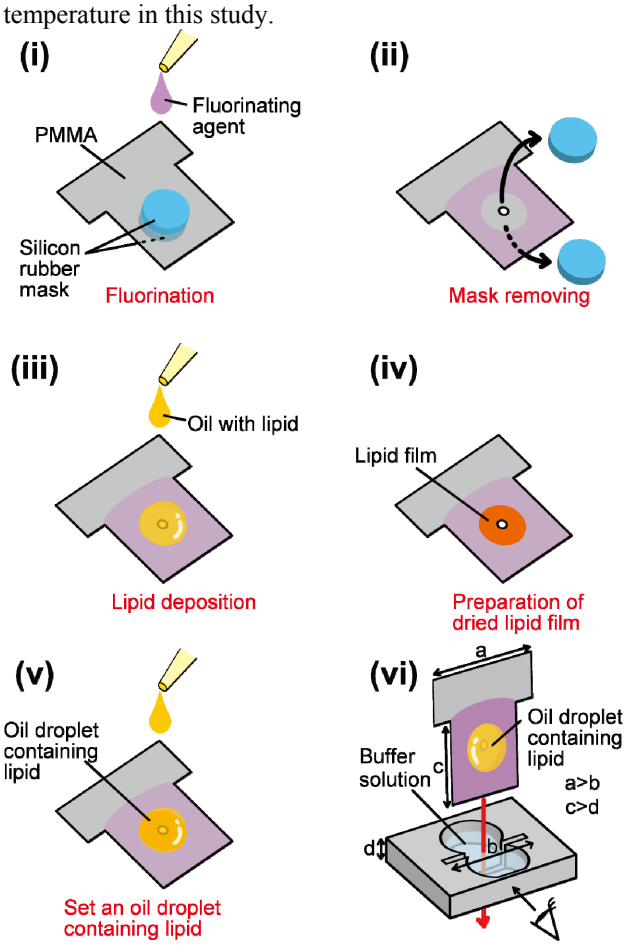

Figure 1: Schematic process flow for BLM formation by insertion of a perforated separator. (i and ii) procedure of the separator modification for obtaining water-repellent and greaseproof surface, (iii-v) preparation of lipid source, and (vi) illustration of T-shaped separator insertion into a well device.

RESULTS AND DISCUSSION

We obtained appearances categorized into four main types (droplets are not contacting, BLM is formed, droplets breaking the membrane, and droplets squeezing out the oil containing lipid). Although mostly observed type was successful example of BLM formation, we observed no BLM appearances in some cases, especially when the separator was not treated with the fluorinating agent and lipid precoating.

There were often leakage cases in the absence of the fluorination process of the separator. The leakage was thought to be due to lipid molecules that had amphiphilic properties worked as surfactant agents, and accordingly increased PMMA surface's wettability. Hence, we thought that the amphiphobic modification of the separator was necessary for stable BLM formation.

Finally, we confirmed that success rate of BLM formation depending on each treated process of separator, and with or without repainting (Figure 2). Figure 2B(d) indicates that BLMs are definitely formed with high efficiency when the separator is fully treated as shown in Figure 1(i-v). Since we did not apply a voltage to the BLM in this experiment, the success rate of membrane formation would be changed when BLMs were stressed by the applied electric pressure. And the lifetime of formed BLMs is need to be investigated as the next step for obtaining a stable BLM. BLMs formation with high reproducibility at the initial process is always required for experiments using BLMs. Thus, we think that highly efficient membrane formation method is need for BLM-associated researches.

In our suggested approach of BLM formation, the separator is detachable from a well device. Therefore, it is easy to exchange with new separator if there are some failures due to separators' consumption. This convenience will contribute to the enhancement of experimental cycles.

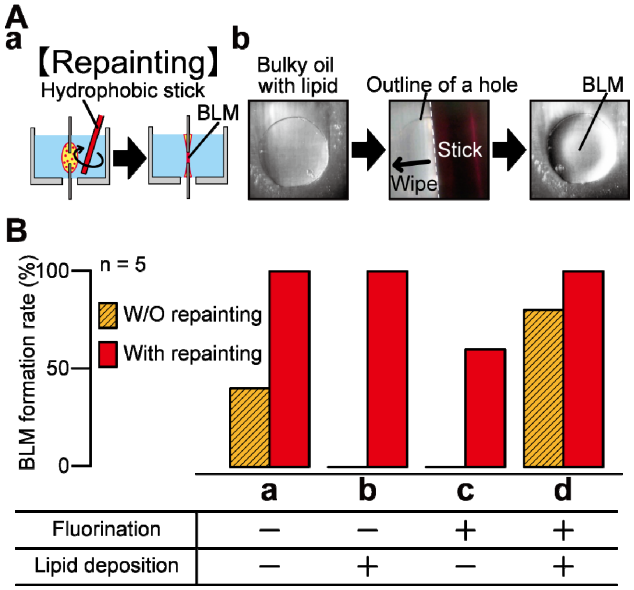

Figure 2: (A) Thinning of oil layer by repainting for BLM formation using a hydrophobic stick: (a) schematic view of the procedure, and (b) each step micrograph of BLM formation by repainting. (B) BLM formation rate depending surface modification (with or without fluorination), and lipid source preparation.

CONCLUSION

We showed an easy BLM formation method using a lipid-coated separator and a well device. It was found that precoating of dried lipid film, and fluorination on the detachable separator's surface improved the efficiency of BLM formation. Consequently, we think that this simple method facilitates BLM formation and applications using various membrane proteins.

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REFERENCES

[1] M. Montal, and P. Mueller, "Formation of Bimolecular Membranes from Lipid Monolayers and a Study of Their Electrical Properties", *Proc. Nat. Acad. Sci. USA*, vol. 69, no. 12, pp. 3561-13566, 1972.

[2] T. Gutsmann, T. Heimburg, U. Keyser, K. R Mahendran, and M. Winterhalter, "Protein reconstitution into freestanding planar lipid membranes for electrophysiological characterization", *Nat. Protoc.*, vol. 10, no. 1, pp. 188-198, 2015.

[3] K. Funakoshi, H. Suzuki, and S. Takeuchi, "Lipid bilayer formation by contacting monolayers in a microfluidic device for mem brane protein analysis", *Anal. Chem.*, vol. 78, no. 24, pp. 8169-8174, 2006.

[4] H. Bayley, B. Cronin, A. Heron, M. A. Holden, W. L. Hwang, R. Syeda, J. Thompson, and M. Wallace, "Droplet interface bilayers", *Mol. BioSyst.*, vol. 4, pp. 1191-1208, 2008.

[5] J. B. Boreykoa, G. Polizos, P. G. Datskos, S. A. Sarles, and C. P. Collier, "Air-stable droplet interface bilayers on oil-infused surfaces", *Proc. Nat. Acad. Sci. USA*, vol. 111, no. 21, pp. 7588-7593, 2014.

[6] A. Nobukawa, T. Osaki, Y. Morimoto, and S. Takeuchi, "Agarose Gel – Air Interface Enables Direct Chemical Vapor Sensing through Artificial Lipid Bilayer Systems" in *Digest Tech. Papers Micro Total Analysis Systems 2015 Conference*, Gyeongju, Korea, October 25-29, 2015, pp. 1577-1579.

[7] N. Misawa, S. Fujii, K. Kamiya, T. Osaki, Y. Miyama, T. Takaku, Y. Takahashi, K. Saito, and S. Takeuchi, "Odorant Sensor using an Insect Olfactory Receptor Reconstructed in Artificial Cell Membrane" in *Digest Tech. Papers Micro Total Analysis Systems 2016 Conference*, Dublin, Ireland, October 9-13, 2016, pp. 37-38.

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