BILAYER LIPID MEMBRANE FORMATION BY ELECTROWETTING-ACTUATED ENCAPSULATED DROPLETS

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ABSTRACT

We demonstrate the formation of a bilayer lipid membrane (BLM) by contacting two electrowetting-actuated encapsulated droplets across an aperture. The encapsulated droplets contained KCl cores surrounded by lipid/decane shells. A suspended BLM was formed at the contacting interface across an aperture between two encapsulated droplets. The BLM formation was reproducible, while the BLM remained over hours under optical and electrical inspections. An ion channel peptide, α-hemolysin, was incorporated into the BLM, showing the capability of electrophysiological studies of membrane proteins.

KEYWORDS: Bilayer lipid membrane, Electrowetting, Encapsulalted droplet

INTRODUCTION

Artificial bilayer lipid membranes (BLMs) are essential to the study of membrane proteins. Several methods of BLM formation have been reported including microfluidic-assisted means [1-3]. However, generating isolated BLMs with independent environments is chanllanging. Here encapsulated droplets are demonstrated to achieve isolated environments on both sides of the BLM.

THEORY

Lipids are amphiphilic molecules, which spontaneously form a monolayer at a water-oil interface. In our system, a BLM is formed by contacting two lipid monolayers by two encapsulated droplets. After the BLM is formed, ion channels are incorporated and verified by transmembrane current measurements.

EXPERIMENTAL

As shown in Fig. 1(a), coplanar electrowetting electrodes were defined on a glass substrate by patterning Cu/Ti (1500 Å/ 200 Å). The electrodes were covered with a 5.6 μm-thick AZ P4620 as a dielectric layer and then spun a 55-nm-thick Teflon layer to be hydrophobic. The prepared glass plate was assembled in a BLM formation system as a top plate shown in Fig. 1(b). The middle plate was a 250-μm-thick silicon substrate with a 100-120 μm-wide square aperture prepared by KOH wet etching. The silicone substrate was electrically passivated by parylene (1 μm). The bottom layer was a glass substrate with a hydrophobic Teflon coating. Two 1.5 μl and 0.1 M KCl droplets encapsulated with 0.1 μl lipid/decane solution were placed above and beneath the middle plate. Because of the amphiphilic nature, the lipid molecules formed a monolayer at the interface between KCl and decane. After actuating one of the droplets towards the aperture to touch the other one, two lipid

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monolayers would contact each other and then form bilayer spontaneously. The process of BLM formation is shown in Fig. 2. At the beginning, the bottom droplet was placed below the aperture (Fig. 2(a)). Figure 2(b)-(d) are snapshots of the encapsulated droplet moving through the aperture until two encapsulated droplets contacting together (Fig. 2(d)). After the thinning process of excess lipid, BLM was formed with clear Plateau-Gibbs border shown in Fig. 2(e).

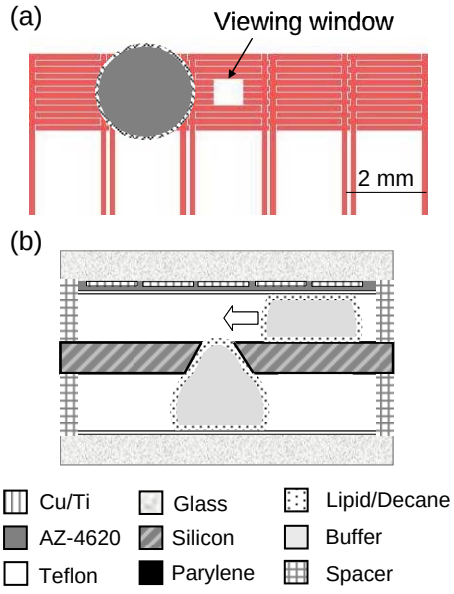

Figure 1. BLM formation device configuration. (a) Top view of interdigitated electrowetting electrode design for encapsulated droplet actuations. (b) Cross-section of the device containing three parallel plates.

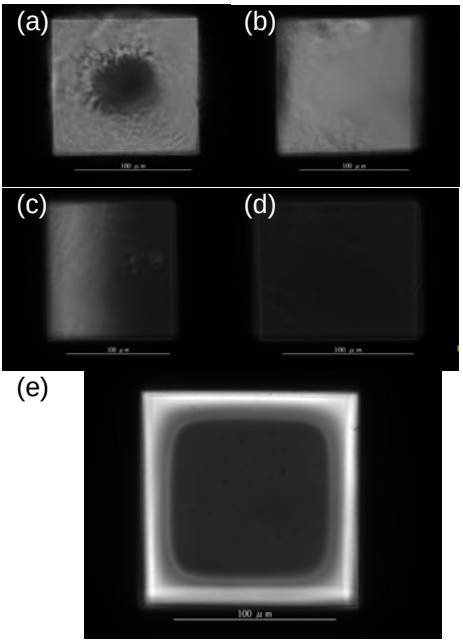

Figure 2. BLM formation. (a) Bottom droplet at the aperture. (b)-(d) Top droplet passing the aperture. (e) BLM formed.

RESULTS AND DISCUSSION

The capacitance of the BLM was recorded through Ag/AgCl electrodes with a square wave (2 mV_{PP}, 200 Hz) applied. The specific capacitance and thickness of the BLM were calculated from the measured current (Fig. 3) to be 0.5 $\mu F/cm^2$ and 6.8 nm, respectively, which are in accordance with the literature [4]. α-hemolysin was incorporated into the formed BLM. The ion current across the membrane was recorded under the voltage clamp at 80 mV and shown in Fig. 4. Each step was considered incorporation of a single channel into the BLM. The conductance was detected as 0.72-1 nS, agreeing with reported values [5].

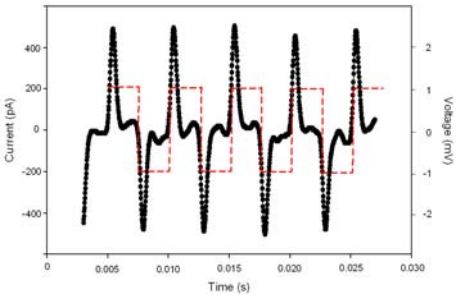

Figure 3. Electrical transient current recording of BLM by applying a 2 mVpp and 200 Hz square wave.

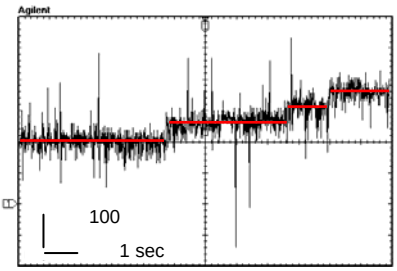

Figure 4. Recording of single channel current through α-hemolysin incorporated into the formed BLM.

CONCLUSIONS

We successfully demonstrated a novel system to form artificial BLM and also incorporate ion channel protein, α-hemolysin, into the membrane. This system, capable of reproducible BLM formation, can be used to study various membrane protein with isolated liquid droplet environments.

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