DOUBLE BILAYER LIPID MEMBRANE (dBLM) CHIPS FOR STUDIES OF BIOMEMBRANE INTERACTION AND FUSION

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ABSTRACT

Here we report a microfluidic double bilayer lipid membrane (dBLM) chip designed to study interactions between two contacting on-chip membranes. The rapid fabrication of the five-layer device consists of direct milling of thermoplastic, hot needle patterning of PVDC thin film, and double adhesive bonding of cover glass. It supports a previously reported *in situ* method for simultaneous formations of two parallel facing bilayers. With a FET based membrane selection circuit, we can continuously monitor the capacitance and conductance of the two bilayers by electrical measurements while monitoring their growth by confocal fluorescence imaging. By precisely controlling their bulging states and (bio)chemical boundary conditions via rapid three way perfusions, this device is potentially useful to extend our understanding of membrane interaction and membrane fusion.

KEYWORDS

Double bilayer lipid membrane, lipid rafts, confocal imaging, membrane fusion

INTRODUCTION

Lipid membrane fusion plays a range of important roles in cellular processes. Despite extensive studies of membrane interactions, the mechanisms controlling the association and fusion of membranes remains unclear [1]. While giant unilamellar vesicles (GUVs) are a natural choice of model membrane for fusion studies, they are enclosed elements, with no direct fluidic access to the inner compartment of the vesicle such that the chemical or biochemical composition can only be readily changed at the outer compartment. Similarly, isolation of the inner vesicle compartment also prevents effective electrical characterization of the membrane, which would otherwise offer an orthogonal measurement dimension to elucidate details of average membrane structure. This is particularly important for studies of membrane fusion, where electrical characterization may provide new insight into the formation and propagation of defects at the interfacing membranes that ultimately lead to fusion. Planar BLMs offer an excellent alternative model system to address these limitations. Previously, we reported a microfluidic BLM system that supports reliable *in situ* "kiss and retreat" membrane formation, rapid perfusion, and simultaneous electro-optical characterization [2]. Here we extend the design from three-layer chip to five-layer structure to enable simultaneous dBLM formation. Aided by fluorescence labeled lipid phase separation, we successfully observed the growth of planar bilayer, jumping out of its supporting PVDC aperture and bulging into hemisphere. In addition, a membrane selection circuit is developed to monitor the electrical properties of membranes continuously.

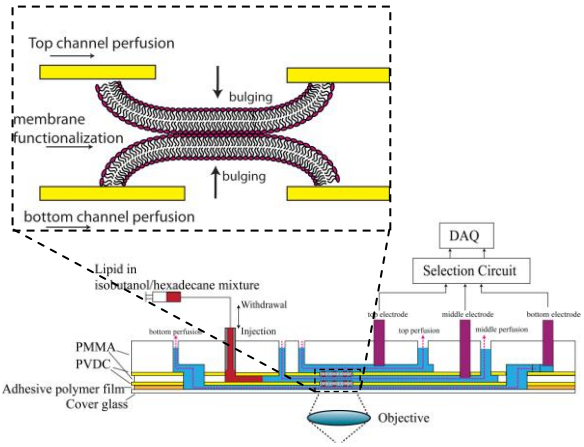

Figure 1: Schematic of a double BLM microchip.

FABRICATION

Chip Fabrication. The schematic of a dBLM microchip is shown in Fig. 1. Microfluidic channel networks are directly milled in a 4.5 mm thick PMMA substrate and through a 200 µm thick PMMA film. A BLM hosting aperture was burnt by hot needle in a PVDC film. They are then thermally bonded with PVDC film in between. After a careful alignment to ensure the overlapping of two BLM apertures, a second BLM-hosting PVDC film is thermally bonded to the assembly. A 100 µm thick double side adhesive polymer film is pattern and used to bond a 85 ~ 130 µm cover glass to the bottom of second PVDC film. A "kiss and retreat" manipulation of lipid solution in the middle channel forms two bilayers facing each other at one time. All of the top, middle, and bottom channels support rapid perfusion that could be used to control the bulging of the two membranes or the (bio)chemical conditions in each of the three chambers [3].

Electronics. In order to measure the capacitance and conductance of two bilayers independently in real time, a FET-based membrane selection circuit was developed. As shown in Fig. 2, three Ag/AgCl electrodes connect the top,

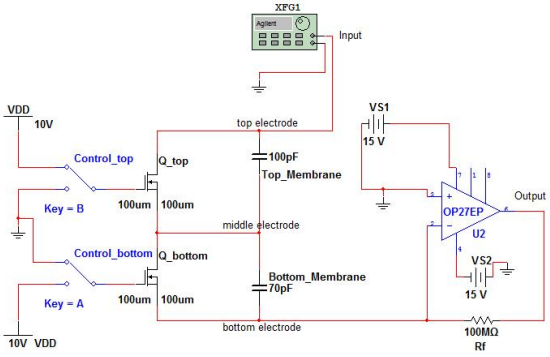

Figure 2: Diagram of the membrane selection circuit.

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middle and bottom channels to two independent MOSFETs, which are controlled separately by two voltage switches. By selectively shorting one membrane, we can make continuous electrical measurements of the lower membrane, upper membrane, or series combination of both membranes in real time. Because the MOSFET effectively eliminates the charge shock of voltage switches to bilayers, the membranes remain stable in the experiments.

RESULTS AND DISCUSSION

Lipid Solution. To obtain better fluorescence images of lipid bilayers, an equal molar mixture (1:1:1) with total lipids 10 mg/mL of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC) and cholesterol with 1 molar % Texas Red labeled 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (TR-DHPE) is chosen in all experiments. TR-DHPE has been shown to effectively partition into the liquid disordered phase while being excluded from both the liquid ordered phase and gel phase. All fluorescence images presented here were taken at 22 ^{O}C, at which the lipid bilayers exhibit co-existence of liquid disorder phase and gel phase, as shown in Fig. 3.

Double Bilayer Lipid Membrane Formation. Fig. 3 shows fluorescence images of two membranes simultaneously formed after a lipid solution is swept past the apertures by the "kiss and retreat" method. After membranes were formed, confocal images may be taken for each membrane alternately to monitor the continuous evolution of gel phase over time. Fig. 4 shows a typical observation of gel phase growth under transmembrane pressure. The expanding gel phase reveals that DPPC is pulled out of supporting annulus as a result of applied transmembrane pressure.

Bilayer Bulging. The controllable perfusion in top and bottom microfluidic channel can bulge bilayers inward for membrane interaction and fusion, while z-scanning confocal microscope allows the sequential process of membrane aggregation, contact, dehydration, defect formation, and finally defect growth and membrane destabilization to be optically observed with high spatial and temporal resolution. Application of differential perfusion rates through the channels on either side of a membrane leads to membrane formation, forcing the BLM out of the plane of its supporting aperture. Scanning confocal microscopy along z axis in Fig. 5 reveals the bulging state of the membrane. The membrane was formed at a PVDC aperture with diameter of ~ 70 μm, while its bulged hemisphere has a height over ~ 110 μm. Continuous bulging of membranes up to 200 μm has been achieved, enabling two mating membranes to be brought into contact across the 100 μm thick middle perfusion channel separating their respective apertures.

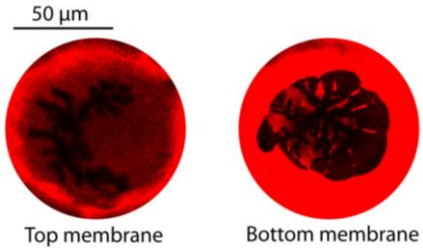

Figure 3: Two bilayer membranes formed simultaneously when a "kiss and retreat" method is performed via lipid injection in the middle channel.

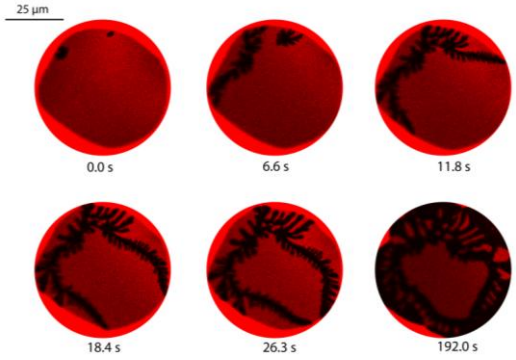

Figure 4: Real-time imaging of gel phase growth under transmembrane pressure.

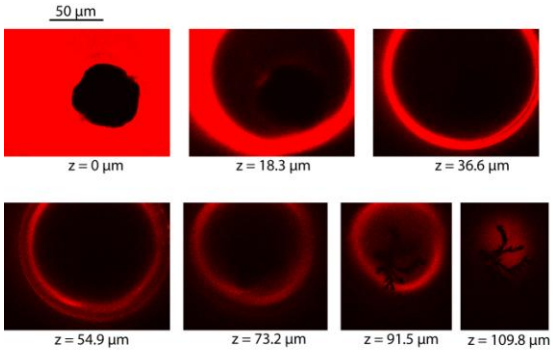

Figure 5: Bulging of membrane under applied transmembrane pressure. In this experiment, gel phase was induced within the deformed membrane.

CONCLUSION

Here we present the first effort to enable membrane interaction and fusion studies in microfluidics. With future optimization of bulging success rate and characterization of membrane contacting and fusion under different pressure condition and (bio)chemical conditions, the dBLM chips offer unique opportunities for biomembrane fusion studies using a combination of both electrical and optical characterization. Coupled measurements of membrane interaction, contact, and fusion are currently ongoing to probe membrane defects prior to fusion, with the goal of shedding light on the debate over the dominant fusion mechanism by the stalk model or defect model [1].

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REFERENCES

[1] T. Tanaka and M Yamazaki, *Membrane Fusion of Giant Unilamellar Vesicles of Neutral Phospholipid Membranes Induced by La^{3+}*, Langmuir, 20, 5160 (2000).

[2] Chenren Shao, Eric L Kendall, and Don L. DeVoe, *Electro-Optical BLM Chips Enabling Dynamic Imaging of Ordered Lipid Domains*, Accepted by Lab on a Chip, DOI: 10.1039/C2LC40077D.

[3] Chenren Shao, Bing Sun, Marco Colombini, and Don L. DeVoe. *Micro Planar Phospholipid Membrane System Enabling Kinetics Studies of Ion Channels*, Annals of Biomedical Engineering, 39, 2242 (2011).

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