LIPID BILAYER AT VERTICALLY ALIGNED NANOLITER DROPLETS GENERATED BY TWO-LAYERED MICROFLUIDIC CHANNELS

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ABSTRACT

We propose a microfluidic device for observable lipid bilayer formation between a pair of vertically aligned aqueous droplets. The device feature includes visibility of the bilayer and variable volume of the droplets, together with sub-microliter volumes of droplets and time-course observation. Referring the metering and storage modules that we recently reported, a pair of droplets was generated in symmetrical two-layered (top and bottom) channels; the droplets then moved into the storage well and contacted with each other from the top and the bottom layers. We demonstrated a bilayer formation using the device and observed crystallization of potassium ferricyanide, which was probably attributed to water permeation through the bilayer.

INTRODUCTION

Phospholipids are amphiphilic molecules consisting of a polar head group and hydrophobic hydrocarbon chains at tails. Phospholipids self-organize bilayer forms in aqueous media, based on hydrophobic, noncovalent interaction. Its mechanical and physicochemical characteristics attract much attention of researchers in various fields, from material engineering to life science.

Conventionally, such lipid bilayers were formed by using the Montal-Mueller method and painting methods, which were laborious and time consuming. Over the last decades, bilayer formation methods have been advanced using MEMS technologies. In 2006, we developed a methodology that enabled the formation of a planar lipid bilayer between a pair of aqueous droplets with simple, rapid, and reproducible manner. As shown in Figure 1a (left), the method took advantage of the self-assembly of phospholipid molecules between an aqueous phase and an oil phase. This conceptual design was implemented in a double-well device, where the bilayer was formed between the two wells [1]. Figure 1a (right) represents a different geometry of a lipid bilayer using the contact method; a lipid bilayer was formed at the mouth of a chamber, by using sequential flows of aqueous, lipid/oil, and aqueous solutions [2]. These devices have been used for numbers of artificial cell membrane studies [3].

In this work, we attempt to develop a visible lipid bilayer system for microscopy by using vertically aligned two droplets (Figure 1b). The features of the system will be: (i) visualization of a bilayer (area), (ii) variable droplet volumes, (iii) sub-microliter size of droplets, and iv) time-course observation. The target applications are, for example, water permeability tests for lipid bilayers and (bio)molecular crystallization [4, 5].

DESIGN

In previous works, we demonstrated passive micro-modules for manipulation of droplets [6, 7]. Here, we took advantage of these modules. Briefly, at the metering trap, an aqueous solution is metered by the barrier, in which the excessive volume of the aqueous liquid is pushed away from the trap by the stream of continuous phase bypassing the droplet through the shallow channel, so called “bypass”. Once the flow is stopped, the metered droplet in the trap minimizes the surface energy by entering to the wider-and-deeper storage well. Refer the detailed principle in

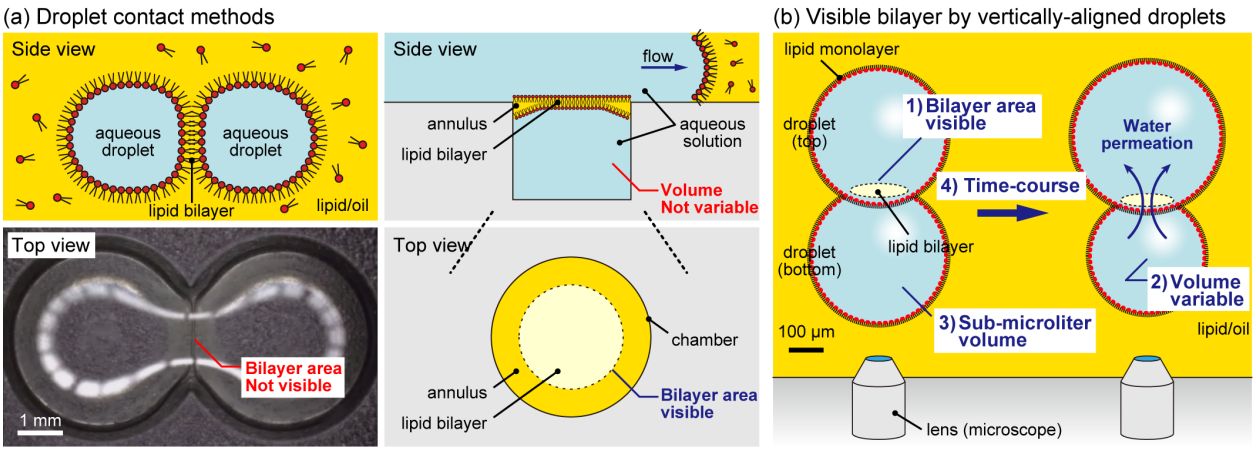

Figure 1: Conceptual diagram of a lipid bilayer formation between vertically aligned nanoliter droplets. (a) Examples of the common droplet contact method. Left: A lipid bilayer is formed between two aqueous droplets in lipid-dispersed oil. The bilayer area is not clear from top. Right: A lipid bilayer is formed at the mouth of a chamber. Although the bilayer is visible, the volume of the solution can not be changed. (b) A droplet contact system using a pair of vertically aligned aqueous droplets allows to characterize phenomena associating with flux through the lipid bilayer, e.g. water permeability and crystallization.

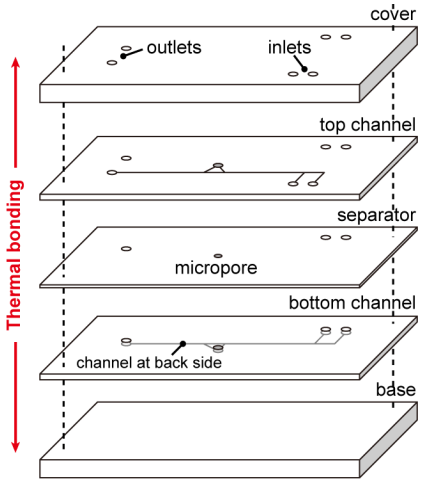

Figure 2: Overview of the developed device. Each part was fabricated with a micromilling machine on PMMA film and plates, aligned, and integrated by thermal bonding.

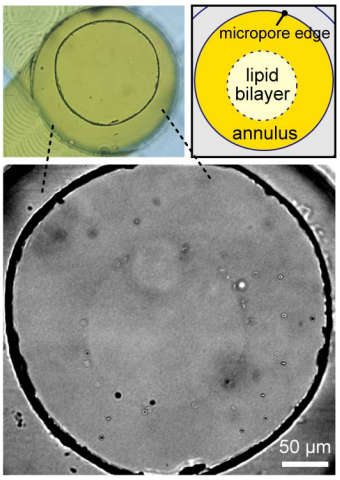

Figure 3: A lipid bilayer formation at the micropore. The contrast was adjusted in grayscale (bottom). The boundary between the bilayer and the annulus was visible; schematic shown in top-right.

References 6 and 7.

We designed two microfluidic channels at different levels and aligned their storage wells to be vertically overlapped. The wells were divided by a thin film with a micropore, where an observable bilayer is formed with contact of two droplets. The droplet size at the well was set at about 100 nL in volume, but also can be interchanged between the contacted droplets.

EXPERIMENTAL

The device was fabricated with a micromachined on an acrylic film and four acrylic plates as shown in Figure 2. A pair of symmetrical channels was milled at the middle two layers, sandwiching a separator film with a micropore. Together with the cover plate with inlets/outlets and the bottom support, all five layers were aligned and bonded using a heat press machine.

DPhPC lipid was dispersed in *n*-decane, and applied for the feeding lipid/oil. $K_3[Fe(CN)_6]$ and $CaCl_2$ were used for the aqueous droplets at the top and bottom channels.

RESULTS

First, the channels were filled with lipid/oil, and a little amount of aqueous solutions was infused to the top and bottom channels. Then, the solutions were transported to each module by the subsequent lipid/oil flow. At the module parts, the aqueous solutions were metered, moved to the storage well by stopping the flow, and became a vertically aligned droplet pair. The droplets formed a lipid bilayer at the interface (Figure 3). Followed by the bilayer formation, crystallization of $K_3[Fe(CN)_6]$ was observed probably due to water permeation by osmotic gradient.

CONCLUSIONS

We proposed a visible bilayer system with a vertically aligned droplet pairs by utilizing the metering and storage modules previously reported. By further development of the device, we envision to use the device for fundamental studies of lipid membrane phenomena associating with a large volume of flux through the bilayer.

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