

# MICRO-SCALE DROPLET CONTACT METHOD BY MECHANICAL MOTION: REPRODUCIBLE AND ROBUST LIPID BILAYER FORMATION

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## ABSTRACT

We prepare a micro-scale droplet contact method by mechanical motion which applying the Split-and-Contact Device (SCD) theory. The interval distance of well opening is designed for limiting the surface area of interface of water droplets. When the droplets are split and joined together, the lipid monolayer surrounding them at the interval combine to form a robust lipid bilayer. The  $\alpha$ -hemolysin that acts as pores, incorporate into the lipid bilayer and allows the ionic currents to be flow across lipid bilayer and measured. We present three different interval distance of well opening.

**KEYWORDS:** Split-and-Contact Device (SCD), Micro-scale droplet, Bilayer lipid membrane (BLM)

## INTRODUCTION

Bilayer Lipid Membrane (BLM) and membrane proteins are the essential components of cell membrane systems. The representative functions of cell membranes are a barrier to the external environment, transducers of chemical signals, and transports of ions/molecules in and out of cells. Since membrane proteins play the major part of the functions, the malfunctions of membrane proteins are the common targets of drug screening research [1-3]. On the other hand, there have been numbers of biosensor systems reported, integrating their high functionalities [4].

Artificial BLM systems have long been studied in order to examine/utilize the characteristics of these membrane proteins *in vitro*; because the membrane protein functionalities are usually preserved when the proteins are reconstituted in BLMs. Previously, we proposed a simple approach for formation of a planar BLM, called the Droplet Contact Method (DCM) [1]. The method takes advantage of a pair of aqueous droplets submerged in lipid-dispersed organic solvent. The key is a lipid monolayers on the droplet surfaces, formed by self-assembly of amphiphilic lipid molecules at the interface between the aqueous droplet and the solvent. By contacting the droplet pair, the monolayers become a planar BLM at the contacted interface. The Double Well Chip (DWC) is the typical device that implements DCM, and allows formation of a stable, robust, and reproducible planar BLM [4]. DWC consists of two circular wells connected with a narrow slit, and helps holding the droplets at the desired positions; this design improved the characteristics of DCM on BLM formation and became applicable for various membrane protein studies [5].

Recently, we further developed the Split-and-Contact Device (SCD) that enables repetitive BLM formation based on DCM [6]. As shown in Fig. 1, BLM formation with SCD starts with one aqueous droplet. Additionally, the wells are designed as movable. By the mechanical motion of the wells, the one droplet can be split into two, and lipid monolayers are spontaneously formed at the split surfaces. After putting the droplets back, a planar BLM is formed at the interface of the two droplets. Repetition of the motion easily reproduces a BLM. Although SCD incredibly increased the number

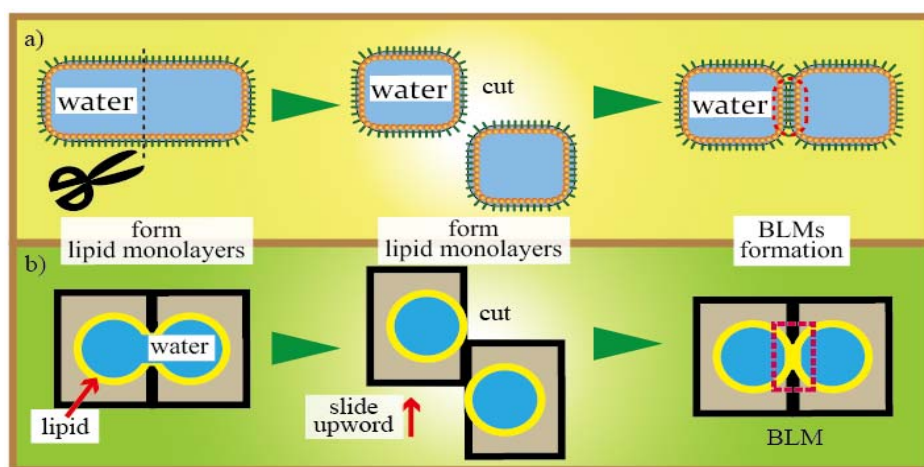


Figure 1: A schematic diagram of the Split-and-Contact Method. a) Concept of Split-and-Contact Method to form a planar BLM: A water droplet is mechanically cut and produced two droplets covered with lipid monolayers. Putting the droplets back in place gives a planar BLM at the interface of two droplets. b) The device implemented the split-and contact method.

of experiments with one device in a short period of time, the motion of the droplets deteriorated the stability of BLM. One solution to this problem is to reduce the BLM area by using a separator at the droplet interface as previously reported [7-10]. Another approach, which we describe in this paper, is to minimize the droplet size. Here, we propose a device for micro-scale BLM formation based on the miniaturized SCD.

## EXPERIMENTAL

### Device design

In the previous work, we designed SCD with the well diameter of 4 mm and the well opening length of 2 mm [6]. Here, we introduced micro SCDs ( $\mu$ SCDs) with the well opening lengths of 500  $\mu$ m, 300  $\mu$ m, and 150  $\mu$ m (see Fig. 2). The well diameter was fixed at 2 mm. As shown in Fig. 2d,  $\mu$ SCD consists of a movable well and a fixed well adjacent to a pool. The movable well was designed to slide upward and backward to produce BLM, while the pool allows to buffer the extra amount of lipid-dispersed organic solvent during the motion.

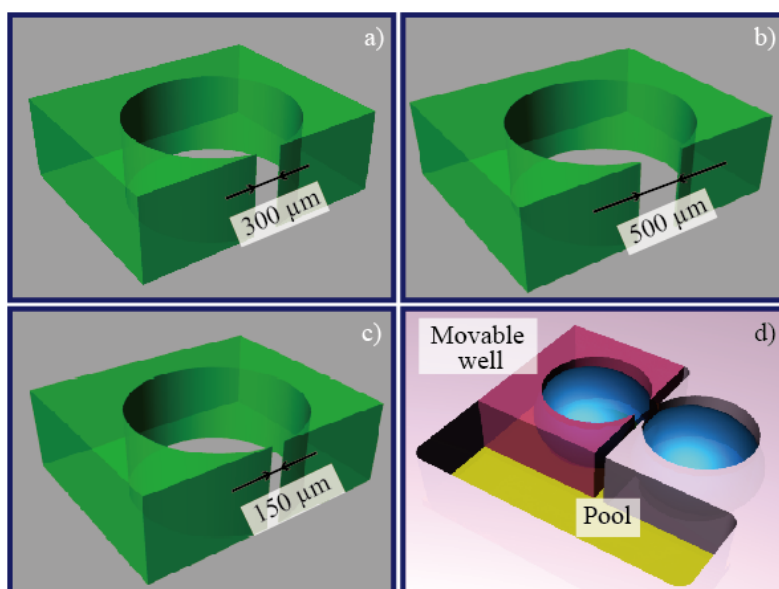


Figure 2: a-c) Schematic diagrams of the well opening with length of 500  $\mu$ m, 300  $\mu$ m, and 150  $\mu$ m. d) A schematic diagram for the  $\mu$ SCD which consists of a movable well, a fixed well, and a pool.

### Device fabrication

Both parts, the movable well and the fixed well with the pool, were fabricated by using a micromachining process with a poly(methyl methacrylate) (PMMA) plate. The Ag/AgCl was deposited at the bottom of each well, which was connected to the electrode via a through-hole, for electrical recordings. The fabricated movable part was simply mounted on the other part.

### Planar BLM formation and current recordings

With the  $\mu$ SCD, a planar BLMs was formed as follows; *n*-decane containing 20 mg/ml of EggPC was injected to the pool. Two droplets of a buffer solution, containing 1 M KCl, 10 mM PBS and 30 nM  $\alpha$ HL, pH 7.4, were dropped into each well. By sliding the movable well up and down, a planar BLM was formed at the well opening in a few minutes. Then, we observed current signals from  $\alpha$ HL nanopores at 120 mV with a patch-clamp amplifier connected to the electrodes.

## RESULTS AND DISCUSSION

### Relationship between the well opening and the droplet contact area

Here, we fabricated  $\mu$ SCDs with three different sizes of the well opening to confirm the applicability of DCM with such micro-scale droplets. First, the droplet contact area at the well opening was observed by a microscope as shown in Fig. 3. The droplet contact lengths estimated from the microscopic images were shown in Table 1. Here, we assumed that the dark line between the two droplets were the droplets interface. The two aqueous droplets were contacted well even at the minimized sizes of the well opening.

### Current recordings with a nanopore protein

The electrodes at the bottom of each well allow to confirm the BLM formation with the developed  $\mu$ SCDs.. In this work, we used a nanopore forming membrane protein,  $\alpha$ HL, by mixing it in a buffer solution. As shown in Fig. 3, step-like current signals were observed at all  $\mu$ SCDs. Although the formation of BLMs was not clear only by observing the droplet contact length above, the current recordings of nanopore signals confirmed the successful BLM formation. The typical step-like signals represent the reconstitution of nanopores in a planar BLM one by one.. The conductance of the single  $\alpha$ HL nanopores in the BLMs was shown in Table 1 and Fig. 3.

Table 1: Relationship between the well opening and the droplet contact length

Well opening [ $\mu$ m]	Droplets contact length at well opening [ $\mu$ m]	Conductance of the single $\alpha$ HL [nS]
500	317	0.74 $\pm$ 0.05
300	287	1.10 $\pm$ 0.03
150	107	0.91 $\pm$ 0.02

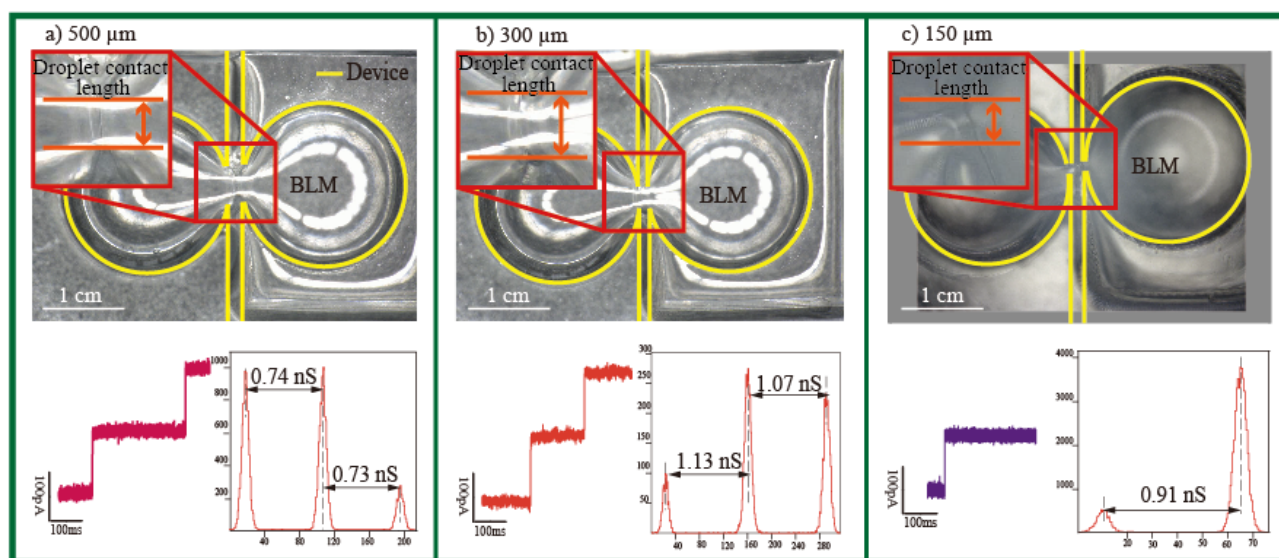


Figure 3: Planar BLM formation with the  $\mu$ SCDs. Three devices with different sizes of the well opening, 500  $\mu\text{m}$  (a), 300  $\mu\text{m}$  (b), and 150  $\mu\text{m}$  (c), were prepared. (top) Microscopic images of the droplet contact area. (bottom) Current-time trace and its current histogram to estimate the conductance of  $\alpha$ HL nanopores in BLMs. See also Table 1.

## CONCLUSION

In this paper, we proposed the Micro-Split-and-Contact Device ( $\mu$ SCD) for preparing a micro-scale droplet interface bilayer (DIB) with a mechanical motion, which is expected to improve the stability and reproducibility of BLM formation compared with the previously developed SCD. Here, we confirmed that planar BLMs were formed at the well opening even though the droplet interface area became a micrometer size.

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