Development of lipid membrane array device Using micro-flow chip

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Introduction

The basic structure of a biological membrane is a phospholipid bilayer. Because a phospholipid is an amphiphilic molecule, having both hydrophilic and hydrophobic groups, an organized such as bilayer is spontaneously formed in water. Several methods have been used to investigate biological membranes and the channels therein. The black membrane method is a technique used for constructing an artificial membrane.[1] This method can be used to reconstruct biological membrane and channel complex, or characterize synthesized channel-forming chemical compounds. One of the problems that have prevented the wide-use of the black membrane method is its low reproducibility and low satability. One the other hand, the studies that chemical analysis system integrated on a chip has been carried out using photolithographic or micro-machining technique. These are called micro total analysis system (μTAS) and had been applied in biotechnology. We also have performed the development of chemical analysis systems using micro-chamber array arranged for bimolecular. Now, we developed a new method for formation of planar lipid membranes with micro-array chip. The lithographic techniques were used to fabricate micro-array chip that was made of polydimethylsiloxane (PDMS). We found that PDMS is transformed with infiltration of hexane. We demonstrated that the transformation of PDMS is applied for formation of lipid membrane on micro-array chip. First, the transformation of PDMS is controlled with micro-orifice pattern. The micro-orifices are closed like a lattice with hexane, and opened as before structure with evaporation of hexane. Next, using lipid / hexane solution, lipid thin layer are formed on micro-orifice. And, We demonstrated this mechanism using a micro-flow channel of lipid solution.

Material and methods

Using lithographic techniques, a PDMS sheet possessing 20 μ m diameter of pore was prepared (Fig.1a). When Hexane was dropped onto the surface of micro-array chip, the orifice structure is transformed quickly like as a lattice (Fig. 2b). And this micro pattern was opened as before structure with evaporation of hexane. This motion was reversible and repeatedly. Fig.2 shows formation mechanism of lipid membrane using the transformation of PDMS orifices on a chip. At first, when solution of phospholipid in hexane is dropped, the orifices are closed quickly and phospholipid spreads on orifices. After that, hexane is evaporated and the orifices are closed like a lattice with hexane and opened as before structure. With this mechanical extension of bulk lipid film, the lipid layer becomes thinner and thinner.

Result and discussion

The lipid thin layer formed on the orifices of the chip. Lipid layers were observed like rainbow colors (Fig.3a). These were because optical interference. So, it seems that the thickness of these lipid layers are less than 1 μm. These lipid layers burst within a few minutes. Therefore, We tried the modification of PDMS surface with silane coupling reagent. Aminosilane is reported that it is used for the improvement of interaction of lipid membrane and orifice interfaces[2]. Using aminosilane- coated PDMS orifices, lipid layer was observed. These were very flat and very stable as like crystal. And a few lipid layer shows black domain at its optical interference (Fig.3b). It indicates that the lipid layer becomes thinner and thinner, finally lipid bilayer is formed on orifice. Because the thickness of the bilayer, about 10 nm, is less than wavelength of visible light, it appears black domain of lipid layer. Next, we demonstrated with micro-flow channel in these processes for the lipid membrane formation. Lipid layers were formed same as drop process system. It is possible to control micro litter solution of lipid automatically and continuously. This new method of lipid membrane formation using micro-chamber and micro-flow channel is available for artificial biomembrane model device as μTAS technology.

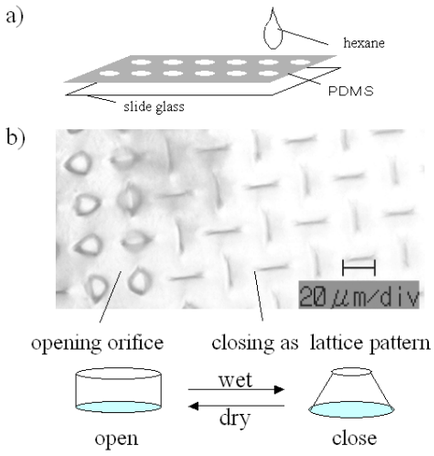

Fig.1. a) PDMS microarray chip. b) Microscopic image of PDMS transformation with hexane.

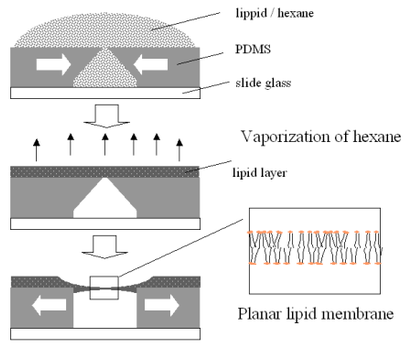

Fig.2. Schematic image of new method with PDMS taransformation.

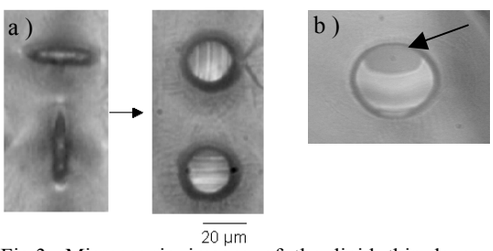

Fig.3. Microscopic images of the lipid thin layer formation. a) With non coatted PDMS. b) The growth of black domain with aminosilane coatted PDMS.

[1] Miller, C. Ed. "Ion Channel Reconstitution" , Plenum, New York (1986)

[2] M. Washizu, et al, IEICE Trans. Electron. , Vol.E78-C,No2 (1995)