Electronic Supplementary Material (ESI) for Lab on a Chip

Supporting Infromation

Microfluidic Lipid Membrane Formation on Microchamber Arrays

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<Estimation of kinetics of the nanopore formation in the lipid membranes>

We modeled our fluidic system as a set of small micro-compartments separated from a main channel by a lipid bilayer membrane containing α-Hemolysin. Due to the constant flow of buffer, the concentration of calcein particles in the main channel, C_{ch}, can be assumed to be zero. These compartments are comparably small such that calcein particles are dissipated rapidly by diffusion and the concentration inside these chambers C_{com} is a function of time only. When the membrane is thin enough and the equilibration of membrane transport is very fast, the flux of particles from the compartments to the channels per unit membrane area (mol/μm^2/s) is quasistationary at any time and can be expressed as [1-2]:

$$\Phi(t) = P(C_{ch} - C_{com}(t)) = -P \cdot C_{com}(t), \qquad (1)$$

where P is the permeability coefficient (μm/s). This total flux Φ is the sum of the unitary fluxes through individual nanopores formed by α-Hemolysin, each having the average single-pore permeability P_{sp} (μm^3/s). For one bilayer area S, containing n pores at an average density σ (nanopores/μm^2)

$$P = \sigma C_{com}(t) = n \cdot P_{sp} / S . \qquad (2)$$

Meanwhile, from the definition of total flux, we also express Φ as:

$$\Phi(t) = [d(C_{com}(t) V_{com})/dt]/S , \qquad (3)$$

where V_{com} is the volume of a micro compartment. Finally, by substituting Equation (2) to Equation (3), we arrive at:

$$dC_{com}(t)/dt = -(PS/V_{com}) C_{com}(t) = -kC_{com}(t), \qquad (4)$$

wherein, k is transport rate across the membrane expressed by:

$$k = P \cdot S / V_{com} = n \cdot P_{sp} / V_{com}. \qquad (5)$$

[1] Kiskin, N. I.; Siebrasse, J. P.; Peters, R. *Biophys*., **2003,** *85*, 2311-2322.
[2] Weiss, T. F. *Cellular Biophysics*, Vol. 1. The MIT Press, Cambridge, MA, **1996** 119-137.

<Materials and Methods>

Solutions preparation

Three kinds of solutions were prepared to make lipid bilayer arrays on microchambers in our microfluidic method. The first buffer solution becomes the contents of microchambers, containing calcein fluorescent dyes and nanopore-forming membrane proteins, α-hemolysin. Both proteins and dyes were purchased from Sigma-Aldrich. The second solution is an organic solvent (hexadecane) in which lipid molecules (L-α-phosphatidycholine purified from egg, Avanti polar lipids Inc.). When adding fluorescent labels in the lipid membranes, we mixed 1 mol% of Texas Red 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt (Texas Red® DHPE, Invitrogen) with the lipid solution. Original lipids in chloroform were dried in vacuum overnight and dissolved in hexadecane at concentration of 10 mg/ml. Hexadecane was chosen as the solvent because it does not swell PDMS significantly and it is immiscible with water. The third solution is just a buffer. In the experiment, 1x PBS solution at ph 7.4 was used as a buffer, wherein 0.5 to 1 mg/ml bovine serum albumin, BSA, was added to prevent non-specific adsorption of molecules on the PDMS walls.

Solution loading in a silicone tube

Droplets of the prepared solutions (proteins and dyes in buffer, lipids in oil, pure buffer) were put on different cover slips respectively. A micro−syringe was connected to a silicon tube, in advance. A tip of the silicone tube was sharply cut to expose clean edge. These syringe and tube were fully filled with fluorocarbon oil (C_6F_{14}) to prevent uncontrollable fluctuation of flows when pumping solutions inside the microfluidic device. When sequentially loading the solutions in the tube, the tip of the tube was carefully aligned close and parallel to the surface of the droplet on the glasses. This alignment is critically important to prevent air-bubble contamination which destructs clean phase separation of water and oil in the device, and eventually lipid bilayer formation process.

Microfluidic device fabrication

The PDMS microfluidic device was fabricated using standard soft-lithography techniques. SU-8 negative photoresist was used as a mold of PDMS replications. The fabricated PDMS structures were then exposed to oxygen plasma to be activated, and then permanently bonded to a clean cover slip of glass. The device was heated at 95c for 60mins to complete the bonding. Then, this device was immersed in water and vacuumed overnight to fully saturate the PDMS porous device with water. This saturation prevents the absorption of water in the microchambers into PDMS walls. Maintaining the volume of solutions in microchambers sealed by lipid membranes is critically important in our system for quantitatively analyzing dye concentrations in chambers. In addition, for longer experiments such as checking membrane stability overnight, fully surrounding the PDMS device with water using a brief box could effectively prevents water evaporation from the system. Moreover, this saturation step removes away most air-bubbles from the PDMS device. Since the remaining air-bubbles make it difficult to cleanly form lipid membranes using an interface of immiscible water and oil, this saturation step is also helpful for efficient formation of lipid membranes.

Operation of the microfluidic device and formation of the lipid membranes

The silicone tube sequentially containing the solutions was connected to the PDMS microfluidic device. At this time, it is again important to avoid the air-bubble contamination since maintaining water-oil phase separation is critical. During infusion of the solutions into the channels by a syringe pump (KD

Scientific Inc., MA), we found that optimization was achieved when we maintained the flow rate at relatively high value, 1 nL/min in our set-up, over the whole procedures. When the flow rate was too small, highly viscous solvent, hexadecane with lipids, got clogged in the thin microfluidic device. Also, at relatively high flow rate, remaining air-bubbles confined in chambers are pushed out through porous PDMS walls. When the rate was too high, few of the free-standing lipid membranes could survive at last. Fluorescent recordings are taken with Olympus IX71 microscope (20× objective lens) equipped with an Hg lamp, filter sets (ND6 and ND12, and UMNIBA, Omega optical) and a CCD (300RC, MTI) coupled with Image Intensifier Unit (C-9016, Videoscope, Hamamatsu). Fluorescent data are processed and analyzed with a concentration displacement measurement software, Gray Val (Library).