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Self-spreading Behavior of Supported Lipid Bilayer through Single Sub-100-nm Gap

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1. Introduction

Lipid bilayer membranes supported on solid substrates retain the dynamic properties of cell membranes such as lateral fluidity and are regarded as a good structural motif for cell membranes. Recently such supported lipid bilayers have attracted a lot of attention in the field of biocompatible coating and biosensing. Lipid bilayers are natural hosts for transmembrane proteins and good electrical insulators. Hence, supported lipid bilayers are potentially of great interest as bioelectronics platforms.

Supported lipid bilayers are typically prepared by one of two methods: vesicle fusion and the Langmuir-Blodgett technique. Various dynamic properties of lipid bilayers have been investigated using these methods. In addition to these studies, work has been undertaken related to self-spreading, which is also an interesting dynamic characteristic of lipid bilayers [1-3]. Recently, the self-spreading behavior of supported lipid bilayers on patterned surfaces has been attracting considerable interest [4-6].

In this study, we investigated the way in which a sub-100-nm scale nanogap affects the self-spreading of a lipid bilayer. For this purpose, we designed and fabricated patterned structures with a nanogap in a microchannel. Using these devices, we observed the self-spreading behavior of lipid bilayers passing through a nanogap.

2. Experimental

Figure 1 shows the device structures used in this study. Nanostructures forming nanogaps were fabricated by electron beam lithography and the liftoff technique using Au / Ti (30 nm / 5 nm) on a silicon wafer with a 300-nm SiO_2 layer. The gap distances were 15-100 nm. Microchannels that were either 5 or 10 μm wide and that had wells at both ends were fabricated on these nanogap structures by using a conventional photolithography technique with an organic photoresist.

A chloroform solution of dye-conjugated lipid (fluorescein-DHPE) was mixed with L-α-Phosphatidylcholine (L-α-PC) to prepare a solution of L-α-PC containing 5 mol% of fluorescein-DHPE. The chloroform was evaporated with a nitrogen gas stream and the residue was dried in vacuo overnight to yield a sticky solid. A small amount of the solid was attached to the tip of a glass capillary and transferred inside the well. The self-spreading of the lipid bilayer was initiated by immersing the device in a buffer solution (100 mM NaCl + 10 mM Tris-HCl (pH = 7.6)).

An Olympus BX51-FV300 confocal laser scanning microscope with a 488 nm laser for excitation was used for obtaining fluorescent images. All the observations were performed in a buffer solution at room temperature.

3. Results and Discussion

A single lipid bilayer develops from a lipid source by self-assembly. When a self-spreading lipid bilayer is introduced into the microchannel, its front edge will eventually reach the nanogap. Figure 2 shows the typical time evolution of a self-spreading lipid bilayer before and after it passes through a nanogap, where $t = t_0$ is the time at which the advancing lipid bilayer reaches the nanogap. A lipid bilayer forms a semicircular structure when passing through a nanogap. It is confirmed that the lipid bilayer does not develop on the photoresist and gold patterns but only on the hydrophilic SiO_2 surface. It is clear that a lipid bilayer will self-spread through a nanogap even when its width is less than 100 nm.

To provide a more quantitative understanding of the self-spreading behavior through a nanogap, Figure 3 shows a double logarithmic plot of the velocity of the advancing lipid bilayer as a function of time. Throughout the measurement, the observed velocities were well fitted by a line with a slope of -1/2, which agrees well with a previous report [1]. Although this fact is surprising, it should be mentioned that there is no significant difference in the front edge velocity before and after the nanogap, and all the experiments have similar results under the present conditions.

The time evolution of the fluorescence intensity profile as a function of distance is shown in Figure 4, where the position at the nanogap is set at 0. A noteworthy feature is that the fluorescence intensity decreases discontinuously in the vicinity of the nanogap. The rate of this decrease is about 10%. This result implies that the dye-conjugated lipid experiences interference when it passes through a sub-100-nm nanogap. This phenomenon was also observed using other devices. However, it is difficult to find a direct correlation between the rate of decrease and the nanogap size as it was about 10% for each sample in this study.

4. Conclusions

We investigated the self-spreading of a lipid bilayer using devices incorporating a fabricated microchannel and a single nanogap. We confirmed that the self-spreading lipid bilayers passed through a sub-100-nm gap. On the other hand, the nanogap affects the fluorescence intensity of the self-spreading lipid bilayers. This result indicates that dye-conjugated lipid molecules experience interference as

they pass through a nanogap despite the dye molecules being smaller than the nanogap. It should be mentioned that the fabricated device used in this study have a single nanogap connected to metal pads, which make it possible to perform electrical measurements such as molecular trapping for further applications.

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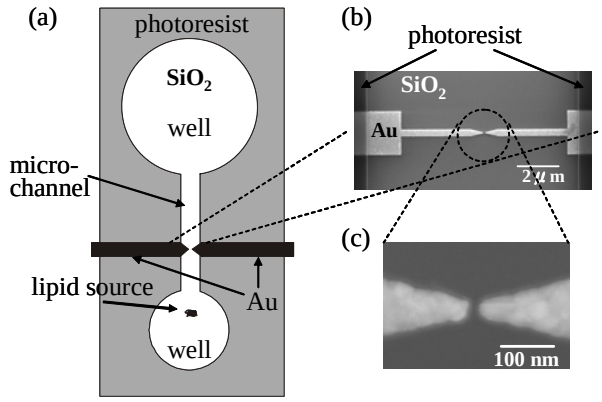

Figure 1. (a) Schematic drawing of the device. The nanogap structure is made of gold. The microchannel and wells are formed on this structure by using a photoresist. At the beginning of the experiments, a lipid source is mounted inside the well. (b) Magnified view of the device around nanogap. (c) SEM image of a 15 nm nanogap.

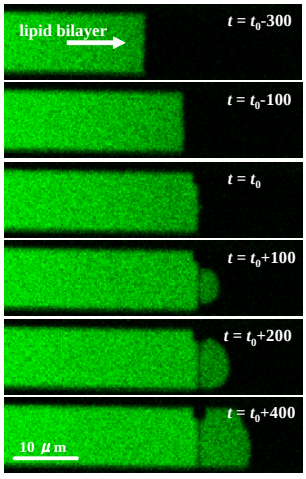

Figure 2. Time evolution of a self-spreading lipid bilayer on the device before and after passing through a nanogap. The gray areas are the fluorescence from fluorescein-DHPE. The lipid bilayer grows from left to right along the microchannel. The time at which the advancing lipid bilayer reaches the nanogap is set at $t = t_0$.

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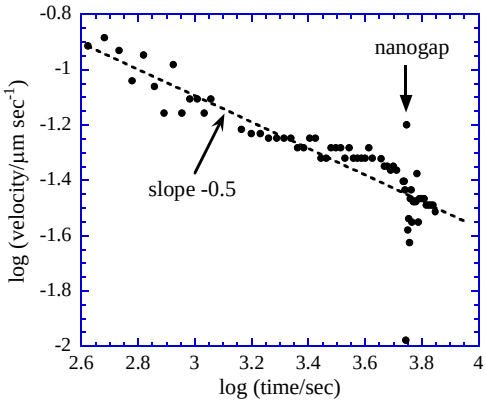

Figure 3. Double logarithmic plot of the velocity of the advancing lipid bilayer as a function of time throughout the measurement. At about log(t) = 3.7, the front edge of the lipid bilayer passes through the nanogap. The dotted line has a slope of -1/2.

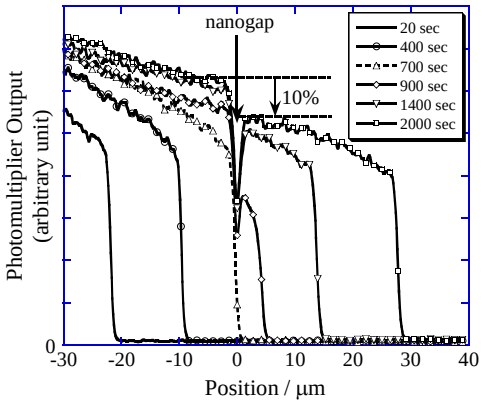

Figure 4. Time evolution of the fluorescence intensity profile as a function of distance. The position at a nanogap is set at 0.