

GIANT VESICLE FORMATION THROUGH THE ASSEMBLY OF 2D SUPPORTED LIPID BILAYERS

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ABSTRACT

We report vesicle formation through the assembly of patterned supported lipid bilayers (SLBs) aiming at easy production of giant vesicles with the properties of (i) unilamellar, (ii) organic solvent free, and (iii) fine size control. We prepared SLBs which were formed by a conventional vesicle fusion method using small vesicles of ~ 100 nm in diameter on patterned SiO₂ surface and attempted to produce giant unilamellar vesicles from the SLBs by electroformation. The SLBs containing fluorescently-labeled lipid were investigated with fluorescence recovery after photobleaching (FRAP) and atomic force microscopy (AFM). Results of the FRAP and the AFM observations showed that the lipid membranes were single-layered patterned homogeneous SLBs. After the electroformation, we obtained images of vesicles which implied that they were derived from the patterned planar SLBs. Furthermore, the result of vesicle size distributions seemed to indicate that the formed vesicle size depended on the pattern of 2-dimensional SLBs.

KEYWORDS

Liposome, Vesicle fusion, Supported lipid bilayer, Fluorescence recovery after photobleaching, Electroformation.

INTRODUCTION

Lipid membranes are used for a model system of plasma membranes. Many researchers have been focused on producing of cell-sized and monodisperse liposomes or vesicles from lipid membranes [1, 2] and using them as mimic cells or applying them to carriers for drug delivery systems (DDS). In these days, it is reported that vesicles are actually utilized for compartments of self-reproducible cell-like artifacts [3, 4]. And also, a recent research has shown that vesicle size has an effect on the drug efficacy in DDS [5]. Thus, there is an increasing demand for producing size controlled unilamellar vesicles in large quantity with uniform quality. To fulfill the requirements of producing such vesicles, some microfluidic devices (e.g. T-junction, and flow-focusing devices, etc.) are very usable. In those kinds of approaches, however, it is often a concern that the formed membranes are unilamellar or not. In addition, there is an anxiety about residues of biologically destructive organic solvents that have to be used in processes of lipid membrane preparations. On the other hand, it is noted that single-layered supported lipid bilayers (SLBs) can be easily formed through the vesicle fusion or SLB self-spreading without any concern for residues of organic solvents [6, 7]. Here, we attempted to produce unilamellar vesicles from the unfolded 2-dimensional pattern SLBs as schematically shown in Figure 1.

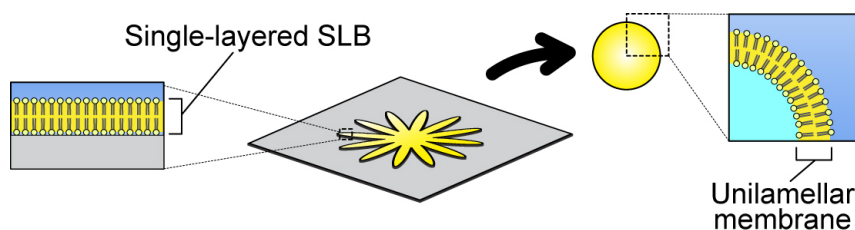


Figure 1: Concept image of vesicle formation through the patterned single-layered supported lipid bilayer (SLB).

EXPERIMENT

We prepared the patterned SLBs on silicon substrates using photolithography techniques and vesicle fusion methods as shown in Figure 2 and described below.

4-inch silicon substrates (P-type, Boron doped, Si(100), 525±25 µm thickness, 11.4–11.8 Ω·cm resistance, Ryoko Sangyo Corp.) were preliminarily cleaned with three chemical solutions in the order of (i) boiling piranha solution (H₂SO₄/H₂O₂, 4:1, v/v) for 10 min, (ii) NH₃/H₂O₂/H₂O (1:4:20, v/v) mixture for 10 min at room temperature (RT), and (iii) HF solution (5%, v/v) for 10 min at RT. The substrates were then rinsed with distilled water and subsequently dried by blowing with dry N₂ gas. Finally, the cleaned substrates were oxidized with thermal oxidation to form SiO₂ surface layer whose thickness was about 100 nm. We prepared the SiO₂ layer for fluorescent observation particularly since it was known that the intensity of fluorescence we used could be enhanced in appearance on the about 100-nm-thick SiO₂ [8]. As shown in Figure 2, Ti and Au were sputtered using sputtering equipment (C-7250, Canon ANELVA Corp.) on the substrates surface after photoresist patterning and reactive ion etching processes. Ti was used for adhesion layer between Au and Si. And Au was used for preventing vesicles' rupture and forming SLBs outside of SiO₂ areas.

We used two kinds of synthetic phospholipid molecules which were 2-(4,4-difluoro-5,7-diphenyl-4-bora-3a, 4a-diaza-s-indacene-3-pentanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (β-BODIPY[®] 530/550 C₅-HPC,

Life Technologies Corp.) and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC, Avanti Polar Lipids, Inc.) These two kinds of lipids were dissolved with chloroform and mixed with the molar ratio of 1:100 (β -BODIPY : DOPC) and the mixture was subsequently spread promptly on inner wall surface of clean glass tubes at RT. After the chloroform was volatilized completely, we added a buffer solution (100 mM KCl, 25 mM HEPES/NaOH pH 7.4) and violently agitated to make multilamellar vesicles. The vesicles were broken up by freeze-thaw cycles and extrusion. And we eventually obtained suspensions containing small vesicles of ~ 100 nm in diameter. We dropped the vesicle suspension on the above-mentioned substrates and left the samples for 1 hr at 45 °C. The supernatant containing redundant vesicles was substituted thoroughly with the above-mentioned buffer solution at RT after the SLB formation. The formed SLBs existed in aqueous solutions through the whole process.

The SLBs were investigated with fluorescence recovery after photobleaching (FRAP) and an atomic force microscope (AFM) (NanoWizard® II, JPK Inst. AG) before electroformation. Electroformation was performed using a function generator (WF1974, NF Corp.) that gave sinusoidal AC field of 10 V with 10 Hz at RT for 1 hr. After the electroformation, we measured the particle size distributions with Coulter counter (Multisizer™ 4, Beckman Coulter, Inc.).

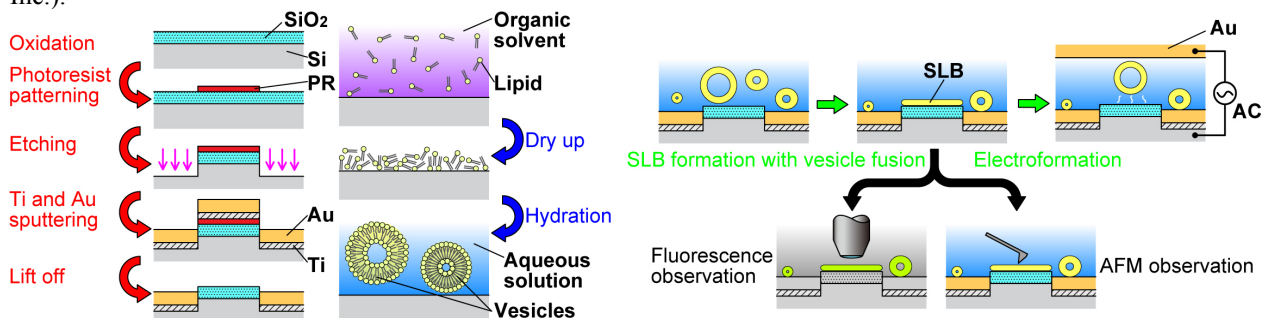


Figure 2: Process flows of the substrate fabrication, vesicles preparation, SLB formation, and electroformation. The SLBs were investigated through fluorescence and AFM observations.

RESULTS AND DISCUSSION

As shown in Figure 3A and 3B, we successfully fabricated the substrates which were patterned with SiO₂ and Au. Then, we succeeded in forming patterned lipid membranes selectively on the SiO₂ area through the vesicle fusion method as shown in Figure 3C and 3D. The fluorescence micrographs had good contrast between lipid membrane area and the outside due to the about 100-nm-thick SiO₂ underlayer.

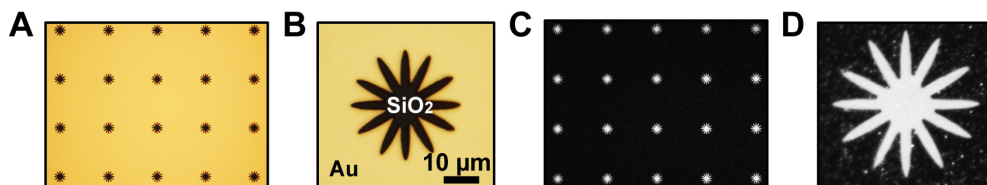


Figure 3: (A) Bright field micrograph of the fabricated substrate and (B) the magnified image before SLBs formation. (C) Fluorescence micrograph and (D) the magnified image after SLBs formation.

We observed FRAP and AFM topographies to confirm that the formed membrane was uniform, and “single” or “multi” layered. According to the results of FRAP observations, the patterned lipid membranes showed the property of uniform and homogeneous SLBs as shown in Figure 4. Fluorescence recoveries were observed in the SLB area. Meanwhile, the recoveries did not occur outside of the SLB. Hence, we found that the patterned SLBs were fluid and continuous, and there were no membranes on Au area.

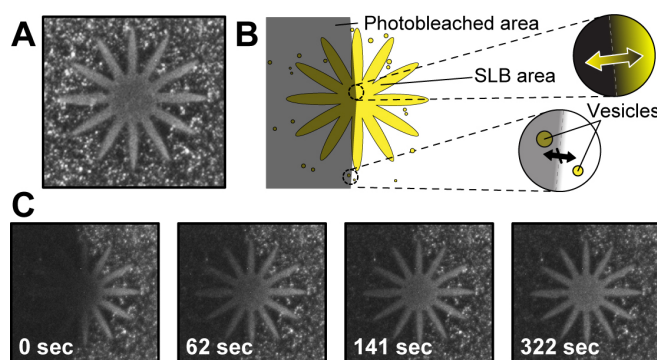


Figure 4: FRAP experiment. (A) Fluorescence micrograph of SLB before photobleaching. (B) A schematic view of FRAP for the SLB. (C) Time sequence images of the FRAP.

Figure 5A, 5B, and 5C show that topographies and section profiles of the sample before and after the SLB formation. After the SLB formation, the height of the patterned SiO₂ regions increased by 5-10 nm. These results indicate that single-layered SLBs are selectively formed on SiO₂ area. After the electroformation, we obtained images of about 10 μ m in diameter vesicles which implied that they were derived from the patterned planar SLBs (Figure 5D and 5E). And the observation results almost agree with the particle size distributions. These results mean that we successfully fabricated patterned SLBs which are potentially useful for vesicle electroformation from the assembly of 2-dimensional patterned SLBs.

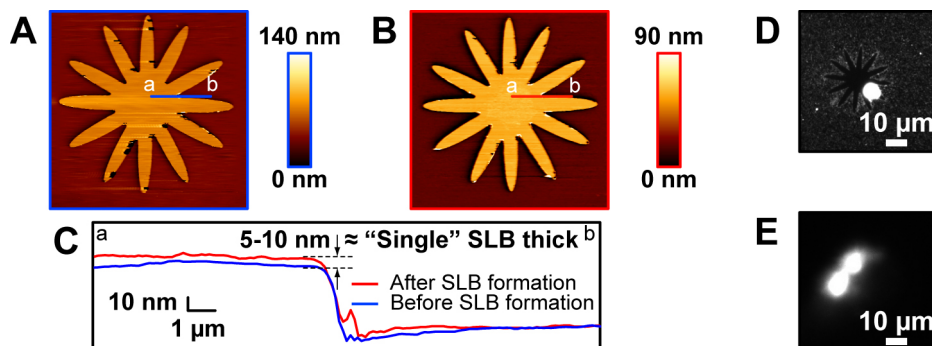


Figure 5: Topographies ($35 \mu\text{m} \times 35 \mu\text{m}$) of the sample substrate (A) before and (B) after SLB formation. (C) The cross-section profiles which correspond to (A) blue and (B) red bars a–b respectively. Fluorescence images of vesicles, (D) an adsorbed vesicle, and (E) floating vesicles, after the electroformation..

CONCLUSION

We fabricated single-layered patterned homogeneous SLBs and found that vesicles could be formed from the SLBs through electroformation. The diameter of formed vesicle agreed with the size which was expected from patterned SLB area. We expect that this approach can be used for size controlled unilamellar vesicle formation.

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