Extended Abstracts of the 2006 International Conference on Solid State Devices and Materials, Yokohama, 2006, pp. 786-787

P-11-3

Effect of Chemical Modification of the Substrate Surface on Lipid Bilayer Formation

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1. Introduction

Bio-compatible surfaces and interfaces are required in the functional analysis of living cells and biological molecules as well as in their application to various biotechnological devices because those cells and molecules have to be immobilized on the solid surfaces without danaturation of the bioactivities. To fabricate bio-compatible interfaces, control of the initial solid surfaces is crucial.

Cell surfaces consist of a lipid bilayer and membrane proteins. The reserch on membrane proteins is important in application to biochips and biosensors. Artifical lipid bilayers are often used as a model system of the cells in *in vitro* studies of the fundamental properties of the membrane proteins..

For fomation of lipid bilayers, we used vesicle fusion method illustrated in Fig. 1. Lipid bilayers, which consist of two amphiphilic phospholipid layers formed in polar solution, are formed by fusion and expansion of the vesicles. In the formation process, hydrophilic and/or hydrophobic interactions between the vesicles and the substrate surface are essential. Generally the surface control includes structural and chemical approach. In the formation of uniform low-defect bilayers, the chemical state control plays more important role. We focused on the chemical controls of the substrate surfaces, in particular control of hydrophilicity and/or hydrophobicity of the surfaces. We also examined the effect of surface charge by modifying the surfaces with various self-assemble monolayers (SAMs).

2. Experiments

The materials to form the lipid bilayers are 1,2-Dimyristoyl-*sn*-Glycero-3-Phosphocholine (DMPC) and florescently labeled 1-Myristoyl-2-[12-{(7-nitro-2-1,3-benzoxadiazol-4-yl)amino}dodecanoyl]-*sn*-Glycero-3-Phosphocholine (NBD PC). The mixture of the lipids (DMPC : NBD PC = 100 : 1 w/w) was dissolved in chloroform. The lipid solusion was dried and added to the buffer solusion (150mM KCl, 1.0mM $CaCl_2$, 10mM HEPES/NaOH, pH 7.4) warmed up above ger-liquid crystal transition temperature (23.9 ºC). The vesicles were formed by stirring this lipid solusion. Si wafers were used as substrates. The Si surfaces were cleaned by a mixture of H_2SO_4 and H_2O_2, and then the surface oxides were removed by diluted HF solusion. To examine the effect of the oxide formation process, the surfaces were oxidized chemically using a mixture of HCl and H_2O_2 or thermally by oxidation at 800 ºC for 30 min in air. SiO_2 surfaces are hydrophilic. To make SiO_2 surfaces hydrophobic, octadecyltrichlorosilane (OTS) was deposited on SiO_2 surfaces [1]. To form cationic surfaces, the SiO_2 surfaces were modified with 3-aminopropyl- triethoxysilane (APTES). To form the anionic surfaces, the SiO_2 surfaces were modified with 2-(carbmethoxy)-ethyltrichlorosilane (CMETS) and then the CMETS surfaces were treated by HNO_3 for carboxylation [2]. All SAMs were deposited by the dipping method. The lipid bilayers were deposited by the vesicles fusion method on the oxizied surfaces and the chemically modified surfaces. To facilitate this expansion, the lipid solution temperature was kept above the phase transition temperature during the incubation. The formed lipid bilayers were observed by a fluorescence microscopy in a buffer solution.

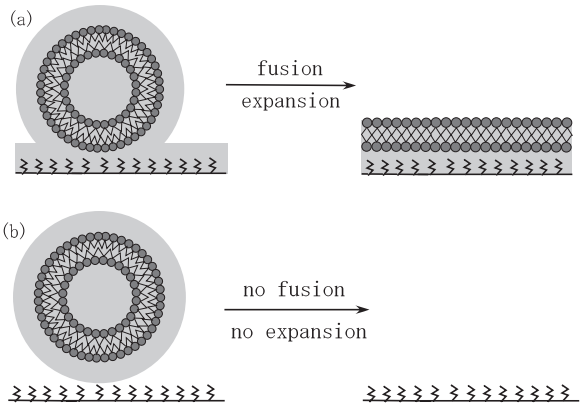

Fig. 1 Schematics of lipid bilayer formation process by the vesicle fusion method: (a) on the hydrophilic surface, (b) on the hydrophobic surface.

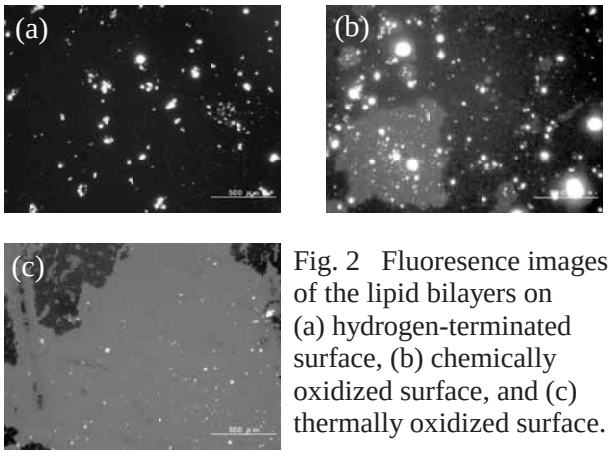

Fig. 2 Fluoresence images of the lipid bilayers on (a) hydrogen-terminated surface, (b) chemically oxidized surface, and (c) thermally oxidized surface.

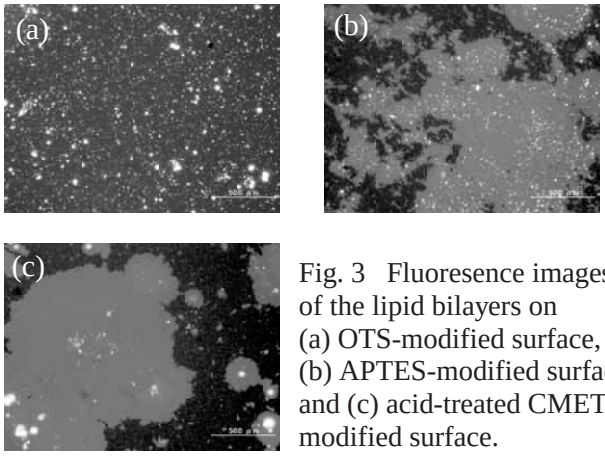

Fig. 3 Fluoresence images of the lipid bilayers on (a) OTS-modified surface, (b) APTES-modified surface, and (c) acid-treated CMETS-modified surface.

3. Results and Discussion

Figure 2 shows fluorescence microscope images of the lipid bilayers (a) on the hydrogen-terminated surface, （b） chemically oxidized surface, and (3) thermally oxdized surface. No lipid bilayer fromed on the hydrogen-terminated surface. Lipid bilayer islands, on the other hand, were formed on the oxidized surfaces, and the island size is larger on the thermally oxidized surface than the chemically oxidized one.

Fig. 3 shows fluorescence microscope images of the lipid bilayers on the OTS-, APTES-, and acid-treated CMETS–modified surfaces. No lipid bilayer fromed on the OTS surface. On the APTES and acid-treated CMETS surfaces, lipid bilayers were formed. However, the difference between the chemically oxidized and thermally oxidized surfaces was found to be small.

The above results show that uniform lipid bilayers can be formed on hydrophilic surfaces but not on hydrophobic surfaces as illustrated in Fig. 1. The vesicles are hydrophilic and accompanied with the low-mobility water layer on their surfaces. Therefore, the vesicles can approach the hydrophilic substrate surface and expand. On the other hand, the water layer around the vesicle prevents approach to the hydrophobic substrate surface resulting in a small rate of fusion.

The effect of the surface charge was found to be small. Because the phospholipid used in the experiments is neutral, the interaction through surface charge is less important.

The bilayer islands on the thermally oxidized surface were more uniform than on the chemically oxidized surface. One possible explanation for this difference is that the mobility of the water layer on the chemically oxidized surface is smaller than that on the thermally oxidized surface, and such low-mobility water layer suppresses the vesicle fusion. The difference on the oxide formation process is experimentally small when the surface is modified with the SAMs.

4. Summary

We studied the effect of the surface modification of the subatrate on the formation of lipid bilayers using vesicle fusion method. The most important factor for the uniform lipid bilayer formation is the control of hydrophilic / hydrophobic state of the surface. The surface charge is less important in the case of a nentral lipid. When the vesicles are deposited on the Si oxide surface directly, the oxide formation process influences the efficiency of the lipid bilayer formation.

Acknowledgement

This work was partly supported by CREST/JST ant Grant-in-Air for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology. The authors would like to thank Prof. Urisu and Dr. Tero for their support.

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