Fabrication of Silicon-Based Planar Ion-Channel Biosensors and Integration of Functional Cell Membrane Model Systems on Solid Substrates

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To combining the human brain and the supercomputer is a dream of the scientist. To realize this dream we must develop several interface devices which can pick up several signals from neural cells such as electrical, optical and molecular signals. We run two main projects targeting the reactions on cell membranes. One is the fabrication of Si-based ion-channel biosensor, which is one of the above interface device to detect the neurotransmitter molecules. The other is the fundamental understanding of bilayer membrane properties using the artificial lipid bilayers on solid substrates, which is called supported bilayers, by means of atomic force microscope and fluorescence microscope-based techniques.

1. Supported Planar Lipid Bilayers on Step-and-Terrace TiO_2 Surfaces

We studied the influence of substrate surface properties on supported planar bilayer (SPB) using atomic force microscope and fluorescence microscope. Effects of surface hydrophilicity and atomic structures on the SPB formation process, morphology, and phase-separation were investigated on single-step-and-terrace rutile-TiO_2 low index surfaces. Step-and-terrace surfaces of rutile-TiO_2 (100), (110) and (001) were formed by etching in 10% HF *aq*. and annealing at O_2 flow (1.0 L min^{-1}) at 700–850 °C. Figure 1a shows the step-and-terrace TiO_2(100) surface. The height of each step was 0.25 nm, that corresponded to the height of the TiO_2(100) unit cell. Flat and continuous SPB was formed on both TiO_2 (100) and (001) surfaces by the vesicle fusion method. The dipalmitoleoylphosphatidylcholine (DPoPC)-SPB on the single- and double-step TiO_2(100) had a ratchet-like structure following the step-and-terrace structure of the substrate, but that on the half-step TiO_2(001) did not have the morphology reflecting the substrate structure. The atomic steps on the TiO_2(100) substrate affected the domain shapes in the binary bilayer of DPoPC and dipalmitoylphosphatidylcholine (DPPC). Some of the gel-phase domain (DPPC-rich) edges on the step-and-terrace TiO_2(100) surface run along the atomic step on the substrate (Figure 1b). This results shows the only 0.25 nm atomic structure on the substrate definitely affects to the lateral lipid assembly in several hundreds nanometer scale.

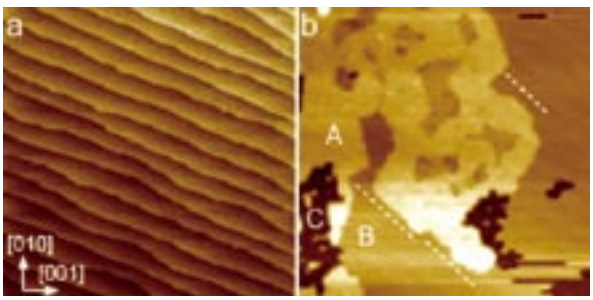

Figure 1. (a) AFM image (2.0×2.0 μm^2) of the step-and-terrace TiO_2(100) surface. The image was obtained in air. (b) AFM image (2.0×2.0 μm^2) of the DPoPC+DPPC binary bilayer on the TiO_2(100). The regions with the brightest (A), middle (B) and darkest (C) contrast are gel-phase domain, liquid crystal domain and defects (bare TiO_2), respectively. The dotted line represent the direction of the substrate atomic step. The image was obtained in a buffer solution.

2. Analysis of Alzheimer's Disease Pathogenesis by *in situ* AFM

Alzheimer's disease (AD) is one of the most common age-associated pathologies, which inevitably leads to dementia and death. Amyloid plaques and neurofibrillary tangles containing beta-amyloid (Aβ) are two of the pathological hallmarks of AD. A key event in AD pathogenesis is the conversion of Aβ peptide from soluble to toxic aggregation in the brain. In particular, how to aggregate and interact with lipid membranes is one of the most important researches because the membrane

surface might be responsible for both neurotoxicity and senile plaque formation.

Supported planar bilayers (SPBs) are useful *in vitro* mimicking system for natural biological membranes. Recently, several reports showed that these inert substrates affected to the SPB fluidity, phase-transition, formation rate, and domain structures. Detailed substrate effects of such systems are of paramount importance for SPBs to mimic cell membranes successfully and for the design of new applications in biosensors and surface bio-functionalization.

In this study, we investigated the interaction of Aβ40-GM1 on the ternary-SPB of Ganglioside (GM1), sphingomyelin (SM) and cholesterol (Chol), which is used as the natural membrane raft, by AFM, fluorescence microscopy, and CTX-B and Thioflavin T assay. The novel phase- separations with triangle domains are observed on mica surface by AFM (Figure 2a), whereas the second bilayers were formed on SiO_2 substrate at the same conditions (Figure 2b).

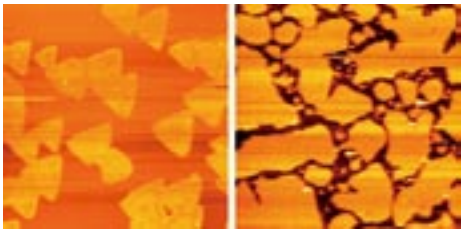

Figure 2. (a, b) AFM images of GM1/SM/Chol (20:40:40 molar ratio)-SPBs on mica (a: 10×10 μm^2) and SiO_2 (b: 5.0×5.0 μm^2) substrates.

3. Fabrication of Si-Based Planar Type Patch Clamp Biosensor Using Silicon on Insulator Substrate[1)]

The aim of this study is to fabricate the planar type patch clamp ion-channel biosensor, which can detect the neurotransmitter molecules, using silicon-on-insulator (SOI) substrate. The micropore with 1.2 μm diameter was formed through the top Si layer and the SiO_2 box layer of the SOI substrate by focused ion beam (FIB). Then the substrate is assembled into the microfluidic circuit. The human embryonic kidney 293 (HEK-293) cell transfected with transient receptor potential vanilloid type 1 (TRPV1) was positioned on the micropore and the whole-cell configuration was formed by the suction. We succeeded to measure the capsaicin-induced Ca^{2+} cannel current of the TRPV1 (Figure 3), when we added capsaicin to the extracellular solution as a ligand molecule. The channel current showed the desensitization unique to the TRPV1.

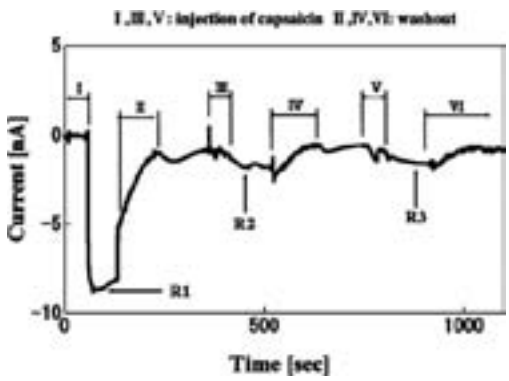

Figure 3. Whole-cell current of TRPV1-transfected HEK-293 cell activated by repeated capsaicin (7.1 μM) applications, which shows desensitization in the extracellular solution containing Ca^{2+}. The holding voltage was –30 mV.

4. Synchrotron Radiation Stimulated XeF_2 Etching Beam-Line with Focusing to Differential Pumping Pinhole

In the synchrotron radiation (SR) etching using XeF_2 gas, a high etching rate in addition to the unique characteristics of anisotropic etching and material selectivity is expected. We constructed a system for the SR-induced dry etching of Si using XeF_2 as etching gas in UVSOR. However, in the previous XeF_2 SR etching experiments, LiF_2 windows have been used at the beam entrance to the etching chamber to protect the upper stream part of the beam line from the corrosive XeF_2 gas. Due to the significant absorption of irradiation beam by this LiF_2 window and its rapid radiation damage, it was difficult to obtain a practical etching rate. Based on these our experiences, we have constructed a new XeF_2 etching beam line with expected high etching rate at UVSOR beam line 4A (Figure 4). The second focusing deflecting mirror (f = 2 mm) was set at 406cm down stream position from the first bent cylindrical pre-mirror. The pinhole (1 mm in diameter, 10 mm in length) is set at this second mirror focusing point. The sample surface position is 5 mm down-stream from this pinhole. The second mirror chamber is pumped by turbo-molecular pump (0.48 m^3 s^{-1}). And the sample chamber is pumped (4 × 10^{-4} m^3 s^{-1}) through XeF_2 gas line by rotary pump. According to this pinhole, sufficiently high vacuum (~10^{-4} Torr) in the second mirror chamber to protect the mirror from the contamination damage is kept with realizing a quite high XeF_2 gas pressure and high photon flux in the reaction chamber.

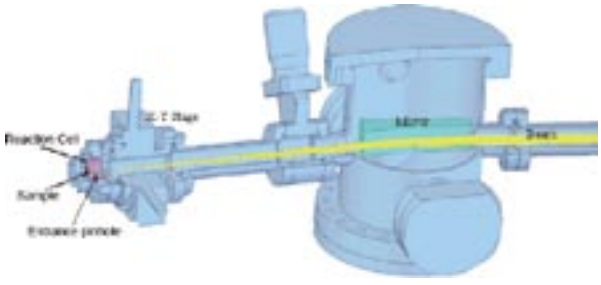

Figure 4. Schematic view of the etching chamber, pinhole and second focusing mirror.

Reference

1) Z. L. Zhang, T. Asano, H. Uno, R. Tero, M. Suzui, S. Nakao, T. Kaito, K. Shibasaki, M. Tominaga, Y. Utsumi, Y. L. Gao and T. Urisu, *Thin Solid Films* in press.