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

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We are interested in the investigation of cell membrane surface reactions and the pathogen mechanism of the neuro-degenerative diseases, based on the molecular science. We are advancing two subjects, aiming the creation and development of new molecular science field, “medical molecular science.” One is the development of ion channel biosensor and its application to the neural network analyzer device. 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. Development of Cell-Culture-Type Planar Ion Channel Biosensor[1)]

We have developed a new planar-type ion channel biosensor with a silicon-on-insulator (SOI) substrate and a cell culture function. Fibronectin is coated on the substrate surface to promote cell growth in this sensor. A transient receptor potential vanilloid type 1 (TRPV1) channel-expressing HEK293 cell is positioned on the micropore of the SOI sensor chip and incubated. Although the seal resistance was quite small, 10–20 MΩ, compared with that of the conventional pipette patch-clamp method, the signal-to-noise level was sufficiently high. However, a much lower noise level is required for observing the opening and closing of fewer than 30 channels.

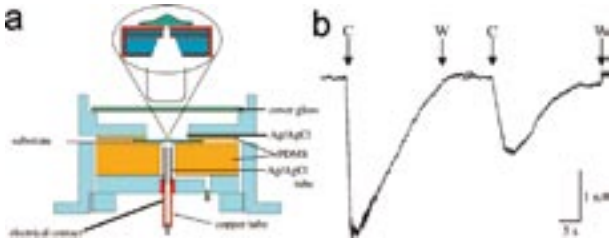

Figure 1. (a) Schematic drawing of the incubation-type planar ion channel biosensor. The sensor chip is fabricated using Si-SOI substrates (7×7 mm^2) with micropores at the center. (b) Whole-cell channel current recording of TRPV1-transfected HEK-293; current was activated by repeated capsaicin stimulation (c) and wash-out (w) cycles.

2. Noise Properties of Incubation-Type Planar Ion Channel Biosensor[2)]

Noise properties are the most important issues in the planar-type ion channel biosensors, as well as in the pipette patch-clamp and black membrane biosensors. Therefore the current noise and its power spectrum appearing in the incubation-type planar ion channel biosensor (Figure 1) were

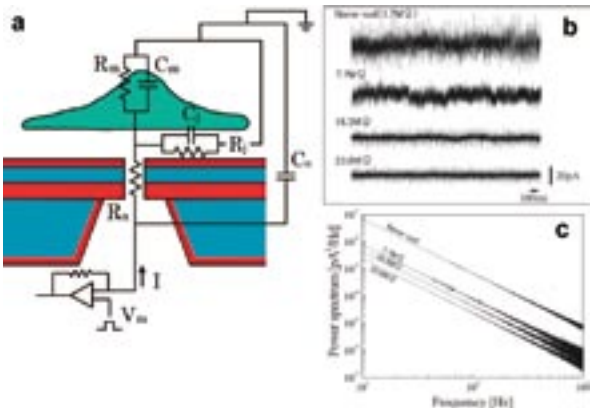

Figure 2. (a) Equivalent circuit of the incubation-type planar ion channel biosensor. The oxide layer of SOI and the ~1-μm-thick surface oxide layer are important for reducing substrate capacitance C_s. (b, c) Observed noise properties of the incubation -type planar ion channel biosensor: (b) noise current and (c) its spectral density for various cleft resistances (R_j).

measured and analyzed in detail to detect the main origin of the noise. The dominant noise sources are classified into (i) current noise induced by head stage preamplifier input voltage noise, (ii) thermal noise, and (iii) excess noise. The spectral density and variance of these noises are formulated in the general form using an equivalent circuit shown in Figure 2a. The baseline currents at various cleft resistance (R_j in Figure 2a) were measured (Figure 2b) and their power spectra were calculated (Figure 2c). We concluded that the main source of the noise in the device is the excess noise, which depends on $1/f$ and originates primarily from the current passing through the cleft between the cell membrane and the substrate surface. The measured noise level ($1.0–2.4 \times 10^{-11}$ A) corresponds to channel current through 5–10 membrane proteins, thus sufficiently small to measure the signal of whole-cell current ($\sim 10^{-9}$ A) (Figure 1b).

3. Synchrotron-Radiation-Stimulated Etching of Polydimethylsiloxane (PDMS) Using XeF_2 as a Reaction Gas[3)]

The synchrotron radiation (SR) stimulated etching of silicon elastomer polydimethylsiloxane (PDMS) using XeF_2 as an etching gas has been demonstrated. The etching system with differential pumps and two parabolic focussing mirrors was constructed to perform the etching. The PDMS was found to be effectively etched by the SR irradiation under the XeF_2 gas flow, and the etching process was area-selective and anisotropic. Extremely high etching rate of 40–50 μm was easily obtained at the XeF_2 gas pressure of 0.2–0.4 Torr. This suggests that SR etching using XeF_2 gas provides a new microfabrication technology for thick PDMS membranes, which can open new applications such as the formation of three dimensional microfluidic circuits.

4. Surface-Induced Phase Separation of Sphingomyelin/Cholesterol/Ganglioside GM1-Planar Bilayer on Mica Surfaces and Molecular Conformation that Accelerates Aβ Oligomerization

Lipid bilayers containing ganglioside GM1 (GM1) are used in the development of new therapies for Alzheimer's disease (AD), because GM1 mediates the amyloid beta (Aβ) aggregation that is the hallmark of AD. To investigate how ganglioside-containing lipid bilayers interact with Aβ, we examined the interaction between Aβ40 and supported planar lipid bilayers (SPLBs) on mica and SiO_2 substrates using atomic force microscopy, fluorescence microscopy, and molecular dynamics computer simulations. These SPLBs contained several compositions of sphingomyelin, cholesterol, and GM1 which covers compositions commonly seen in eukaryotic biomembranes and were treated at physiological salt concentrations. Surprisingly high speed Aβ aggregations of fibril formation were induced for all GM1 concentrations examined on the mica surface, but only globular agglomerates are formed slowly on the SiO_2 surfaces. Especially for the 20 mol% GM1 concentration on the mica surface, unique triangular domains were formed and the high speed Aβ aggregations were observed only outside of the triangular domains. The speed of Aβ40 aggregation and the shape of the agglomerates depend on the molecular conformation of GM1, which varies depending on the substrate materials.

5. Shape Transformation of Adsorbed Vesicles on Oxide Surfaces: Effect of Substrate Material and Photo-Irradiation[4)]

Shape transformation of phospholipid vesicles on oxide surfaces was investigated by a fluorescence microscope. The transformation of spherical vesicles to a planar lipid bilayer membrane spontaneously proceeded on mica and glass, while the intact vesicular layer formed on TiO_2. Interaction energy between the substrate and the bilayer, which was evaluated using the rigorously calculated Hamaker constant, was ~10 times larger on TiO_2 than on mica and SiO_2. The results seems inconsistent with the conventionally proposed adhesion induced tension model, in which stronger adsorption leads to easier planar membrane formation from vesicles, thus indicate that the shape transformation from vesicles to a planar membrane is dominated by the kinetic processes and the dynamics of the vesicles, rather than the adsorption state of individual vesicle. Area-selective SPLB formation of adsorbed vesicles was induced by the irradiation of strong excitation light, which was assisted by the photo-induced expansion of SPLB containing dye-labeled lipid molecules.

References

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Awards

ASANO, Toshifumi; The best poster award in 2nd International Symposium on Nanomedicine.
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