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

Department of Life and Coordination-Complex Molecular Science
Division of Biomolecular Sensing

URISU, Tsuneo	Professor*
TERO, Ryugo	Assistant Professor†
WANG, Zhihong	Post-Doctoral Fellow
RAHMAN, Mashiur	Post-Doctoral Fellow
ASANO, Toshifumi	Post-Doctoral Fellow
NAGAHIRO, Takeshi	Post-Doctoral Fellow
NAGAOKA, Yasutaka	Research Fellow
UNO, Hidetaka	Graduate Student
NAKAI, Naohito	Graduate Student
SHANG, Zhi-Guo	Graduate Student
FUJIWARA, Kuniyo	Graduate Student
SHIMIZU, Atsuko	Secretary

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 Neural Network Device and Precise Microfabrications

Although the patch-clamp method using the pipette is now in practical use, it is not suitable for miniaturization and high throughput screening applications, since the measurement system is large and requires high level of skills for operations. It is expected that the breakthrough for these technical problems can be realized by the planarization of the device. For the planar typed ion channel biosensor, glass (Fertig, 2002), Si (Sordel, 2006, Matthews, 2006, Pantoja, 2004), quartz (Sett, 2003) and a silicon elastomer PDMS (Li, 2006), *etc.* have been reported as the substrate materials. And for Si, it has been considered that the background noise current is large due to the free charge carrier density in the substrate. However, we have recently demonstrated that the noise current can be significantly reduced by using silicon-on-insulator (SOI) or polymethyl methacrylate (PMMA) substrate.

Commercialized planer patch clamp devices, however, can not be used in a system that requires long incubation periods. New functional analysis and/or screening devices could be realized by adding an incubation function to the planar patch clamp method, and these would be especially useful in applications such as *in vitro* systems of neurons and neural networks using dissociated cultured neurons (Tao, 2000, Taylor, 2010, Reska, 2008, Erickson, 2008). Moreover, the planar patch clamp method enables simultaneous measurement of multi-point ion channel currents and advanced 2-D bio-imaging. We have developed an incubation type of planar patch clamp device and demonstrated its operation using TRPV1-expressing HEK293 cells and capsaicin as a ligand molecule. Detailed investigation about the basic properties have not yet been done.

The recently developed light-gated ion-channel method is extremely suitable for the investigation of neural cell and/or neural network functional analysis due to its excellent time and space resolutions (Petreanu, 2007). Concerning the application of light-gated ion-channel in the planar patch clamp method, however, no investigation has been done, in spite of its extreme importance.

In this work, ion channel biosensor based on the incu-

bation type planar patch clamp method was developed and the basic properties were investigated. Due to the existence of ECM protein at the cleft between the cell membrane and the substrate surface near the miropore, it is not easy to realize the high seal resistance (giga-ohm seal). In the present case using collagen 4 as ECM, the seal resistance was usually about 10 MΩ, and the noise level was 7 pA with the 1 kHz low pass filter (Figure 1). The main noise sources were excess current noise and the thermal noise generated at micro pore resistance (R_a) and the seal resistance (R_j). All these noises can be reduced by increasing the seal resistance. Operation of the

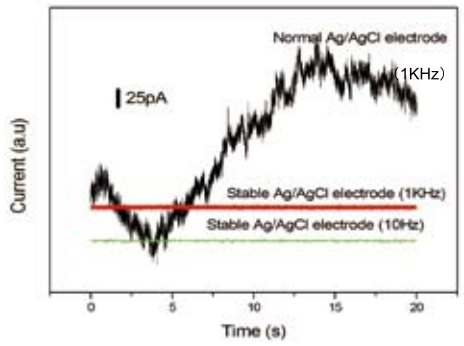

Figure 1. Observed current noise in the biosensor system.

light-gated ion channel, ChRWR, was investigated by the incubation type planar patch clamp method using laser (λ = 473 nm) stimulations (Figure 2). The channel current profile and its membrane potential dependence well agreed to the

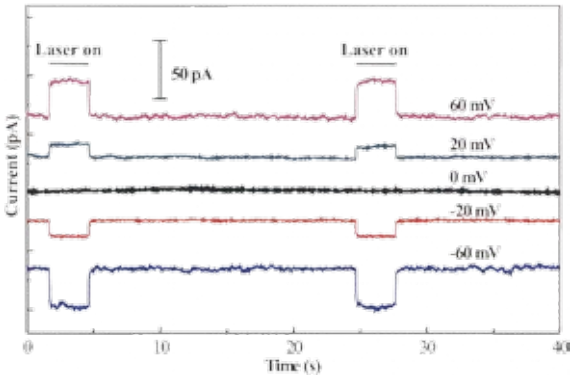

Figure 2. Observed ion channel current under voltage clamp of 473-nm laser irradiation with ChRWR-expressing HEK293. Ion channel current wave forms depend on the applied membrane potentials.

reported data measured by pipette patch clamp method. So we think that light-gated method is also useful in the neural network function analysis and high throughput screening application based on the incubation type planar patch clamp method, and also useful in the simple performance check of these devices. The biosensor operation was examined, using TRPV1-expressing HEK293 cells. Quite high sensitivity was confirmed. But for the single channel recordings, several times improvement of the seal resistance is required.

2. Extracellular Matrix Patterning for Cell Alignment by Atmospheric Pressure Plasma

Low-temperature atmospheric-pressure plasma (APP) jets and a metal stencil mask have been used for the patterning of fibronectins deposited on a silicon (Si) wafer. Fibronectins typically constitute the extracellular matrix (ECM) and a micro-patterned ECM may be used for arranging living cells in a desired pattern on the substrate surface. Such a technique can be used for the fabrication of cell chips. In this study, patterning of 100 mm wide lines of fibronectin layers has been demonstrated. Desorption of fibronectins from the surface by plasma application has been confirmed by atomic force microscopy (AFM) (Figure 3), and Fourier transform infrared spectroscopy (FT-IR).

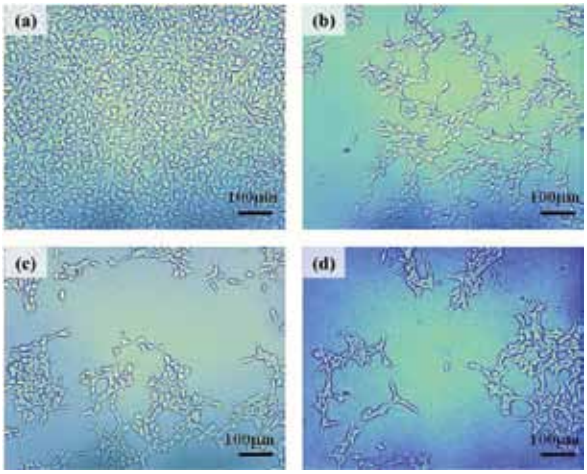

Figure 3. Photomicrographs of the sample substrate surface after plasma application of (a) 0 s (b) 10 s, (c) 20 s and (d) is 30 s. The photographs were taken after 72 hours cell cultivation.

* Present Address: Nagoya University

† Present Address: Electronics-Inspired Interdisciplinary Research Institute, Toyohashi University of Technology