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Si based Planer Type Ion-channel Biosensor and Its Applications

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1. Introduction

In the information transmission system of the life body, molecules are used as signal conveying medium, and membrane proteins, such as ion-channnel and GPCR(G protein coupled receptor) are important signal transducers which change the molecular signal to the electrical signal (ion current) or other molecular signals (second messengers), respectively. Since these membrane proteins are the key components of the signal transmission system of the life bogy, these relate to many intractable diseases and occupy over 50 % of the new drag discovery targets. Thus membrane protein biosensors which detect signal conveying molecules are extremely useful from the viewpoint of application not only to the basic research tool of the cell biology but also to the screening devices for the new drag discovery and diagnosis of intractable diseases. The pipette patch clamp is a well known and technologically established ion channel biosensor. It, however, has several technological problems such as difficulty in miniaturization and high level of skill required in operation, and a new type of device, the planer type patch clamp biosensor is proposed and several commercial base devices have bee already developed. In these planer type devices also, however, several problems still exist in several view points such as miniaturization, operation life time and speed.

Considering these situations, we are developing a noble planer type patch clamp biosensor using Si SOI substrates, aiming the ultra-small, high speed and long life operation, and in recent, we have succeeded in a ligand gated operation using TRPV1 channel transfected HEK293 cell positioned on the micro pore formed through the SOI substrate.

2. Advantage and disadvantage for using Si substrates

From the view point of miniaturization, Si has a big advantage due to easy integration with Si electronic circuits. However, from the view point of low noise, it has some disadvantage. The noise in the planer type ion channel biosensor consist of (a) current noise due to the preamplifier voltage noise, (b) thermal noise induced in the series resistance, and (c) unknown noise, which depends on the total current level. Noise due to (a) and (b) can be reduced by reducing the parasitic capacitance of the chip. From this point, there is some disadvantage in Si, for which low resistivity of Si easily gives a large capacitance. However, in recent investigations, we have shown that the parasitic capacitance can be reduced to almost equivalent to the Teflon chip, by controlling the oxide layer structure around the micro pore [1]. Concerning the noise (c), the most important challenge is to increase the seal resistance. With this point the difficulty is not different from other substrate materials.

3. Microfabrication of Si chip

The procedure of the Si chip fabrication is shown in Figs. 1 (a). The SOI wafer with a 4 μm thick Si layer and 3 ~ 4 μm thick box SiO_2 layer was used to fabricate the sensor chip. The 1 ~ 1.5 μm thick SiO_2 layer was formed on the silicon surface by thermal oxidation at 950 C using the water-saturated O_2 flow. The backside hole was made by diamond drilling. Subsequently the pyramid-shaped hole was formed by the 8% (v/v) tetramethylammonium hydroxide (TMAH) etching at 90 C for about 40 min, which reached the box SiO_2 layer. Finally the micro pore with about 1 μm diameter for the left silicon membrane was made by focused ion beam (FIB) milling from backside. The top side and the back side views around the micro pore observed by scanning electron microscope (SEM) are shown in Figs. (b) and (c).

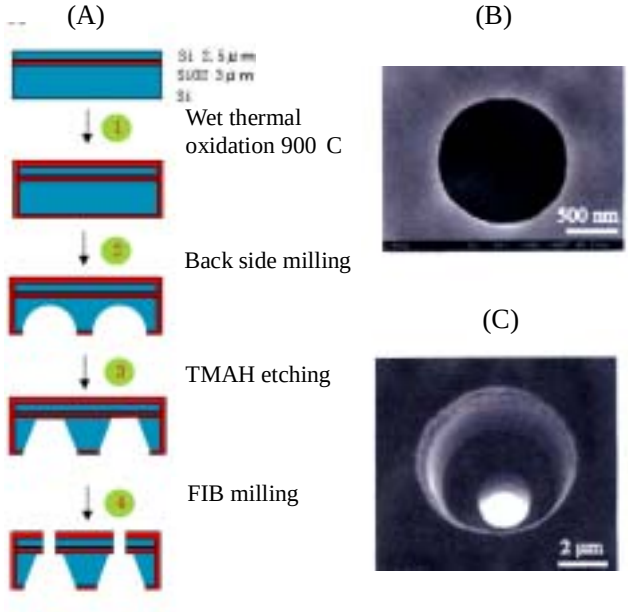

Fig. 1 Micro through hole fabrication process of the SOI substrate (A), and top side (B) and back side (C) view of the micro pore observed by scanning electron microscopy.

4. Device assembly and channel current measurements

Fig. 2a shows the schematic of the microfluidic

device integrated with the biochip. This system is composed of two compartments: upper chamber and lower chamber. The biochip is sandwiched between the two compartments. The fluidic circuit from the upper chamber to the lower chamber resulting from suction in the lower chamber is for the single cell positioning on the pore. The fluidic circuit in the upper chamber is for the injection and washout of capsaicin. The TRPV1-transfected HEK-293 cell was positioned on the micro-pore. Detail of the HEK293 cell incubation and preparation of the cell suspension processes are described in ref. [2].

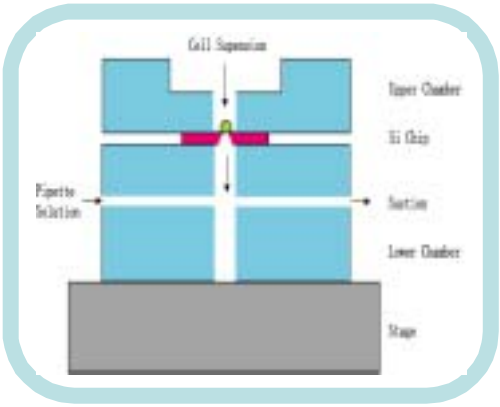

Fig. 2 Schematic illustration of the biosensor device.

For the cell experiments, the lower chamber was injected with intracellular solutions containing (in mM): 140 KCl, 5 EGTA, 10 HEPES, pH 7.4 (with NaOH). The upper chamber was filled with extracellular solution conaining (in mM): 140 NaCl, 5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 Glucose, 10 HEPES, pH 7.4 (with NaOH). The experiments were executed as follows. Firstly, the lower chamber was filled with intracellular solution and upper chamber with a droplet (30-50 μl) extracellular solution. Then the access resistance (Ra) was measured by applying a square wave voltage (10 mV amplitude and 250 ms duration) across the chambers with Ag/AgCl electrodes. After that TRPV1-transfected mammalian HEK-293 cells suspension was injected in the upper chamber and, at the same time, a gentle suction was applied in the lower chamber to position the cell on the pore while simultaneous monitoring the patch resistance with the amplifier and the flow process with the microscope. The seal resistance was measured after the formation of a higher resistance seal, in which the negative pressure in the lower chamber was kept to promote interaction between the cell and micro-pore walls. The whole cell configuration was obtained by applying a short but strong suction in the lower chamber, or by applying the ionophore such as nystatin and amphotericin B to the lower chamber. Finally, capsaicin solution was injected into the upper chamber and washed out, and the whole cell current was recorded as shown in Fig. 3.

5. Prospect for the Si integrated circuit for ion channel biosensors.

If we consider the application of the planer type ion-channel biosensors to some multi-channel devices such as screening devices, it is extremely attractive target to make the channel current amplification system by the Si integrated circuit. We are now considering the application

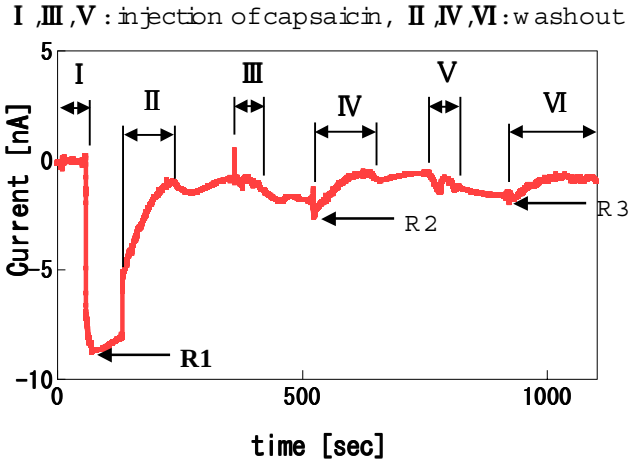

Fig.3: Observed whole-cell current recording of TRPV1 channel stimulated by capsaicin.

of our sensor to the neural cell functional analysis. In this application also, the amplification system using Si integrated circuit is very attractive. These future plan will be also mentioned briefly in the presentation.

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References

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