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Si-Based Planer Type Ion-channel Biosensors

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1. Introduction

It is known that more than 50 % of the target molecules of the drag discovery based on the genome information is the membrane protein. It is, however, a serious problem, that huge amount of the cost (~$5x10^{11}$ yen/1 target) is required to develop a new drag for the membrane protein target.

The main reason for this huge development cost is in the fact that there is no suitable high through put biosensor for membrane proteins, which can be used for the screening test essentially necessary in the drag discovery.

Although pipette patch clamp technique is an unique practical ion channel biosensor, it is unfortunately unsuitable for the screening applications for drag discovery due to that it requires a high level of skills and the measurement is not stable.

To overcome this weak point of the pipette patch clamp technique, many researchers including our group are studying a new planer type patch clamp technique, in which lipid bilayer/ion-channel protein system is put on the micro-pore made on the planer solid substrate surface [1].

We are especially interested in using Si substrates, which have significant advantage in integration of the biosensor and electric circuits on the same chip, although it has not been generally considered in Europe and USA that Si is the best as a substrate material due to its low resistivity causing the large noise.

Recently in our group, we have succeeded in realizing a quite low noise using Si substrates comparable to the Teflon substrate in the gramicidin single ion channel recordings by carefully designing the pore structure. .

2. Substrate fabrication and measurements

A spheroidal hole with depth of about 550 μm was made by a diamond grinder on the back side surface of the Si(100) SOI substrate with about 600 μm thickness, then after etching to the SiO_2 layer by TMAH etching, the micro pore with diameter of 1 to 100 μm penetrating the left 2-3 μm SiO_2/Si(100) layer was made by focusing ion beam (FIB) (Fig. 1).

The Teflon two chambers were divided by this Si chip (7x7 mm^2) and lipid bilayer was formed by painting the decane solution (10mg/ml) of diphytanoyl-phosphatidylcholine (DϕPC) on the micropore after filling the Teflon chamber by 1 M/l KCl solution.

After adding the gramicidin A solution to the both Teflon chambers (Fig 2), the single ion channel current was observed using the AgCl/Ag electrodes and patch calmp amplifier (CEZ-2400, Nihon Koden, Japan). The observed back ground noise in the single ion channel current measurement was 1.21 pA rms, which was almost equal to that of Teflon substrates (Fig 3).

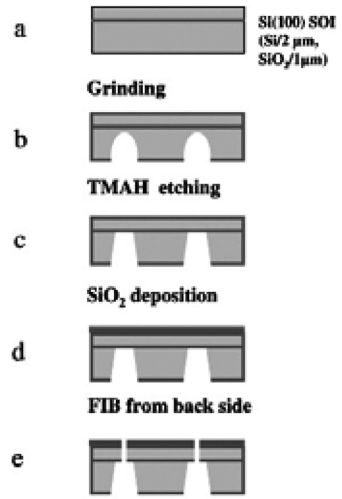

Fig. 1 Schematics of the substrate fabrication process. (4 dvice elements on a 14x14mm^2 Si wafer)

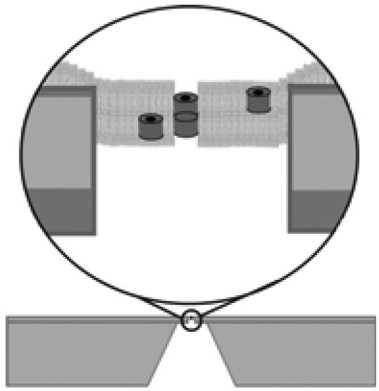

Fig. 2 Schematics of the chip. The inset shows the gramicidin A reconstructed in a lipid bilayer structure.

3. Noise analysis.

The main noise sources in planar bilayer recordings are; (1) I_h the current noise resulting from the interaction of the head-stage input voltage noise (e_n) and the input capacitance (C_t), (2) I_{Ra} the current noise due to the thermal voltage noise of the

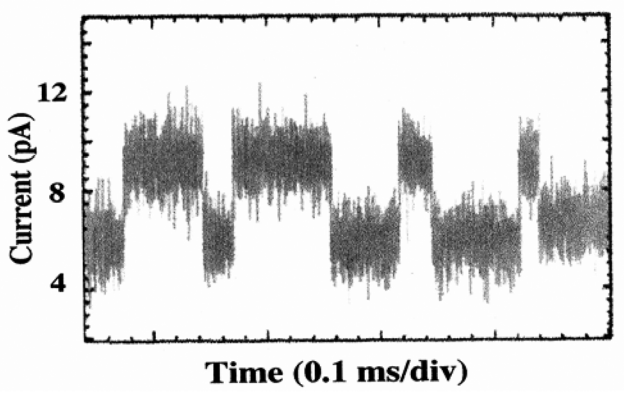

Fig3. Micro pore of the Si chip and the gramicidin single ion channel recording. Bias voltage is 150 mV

access resistance R_a in series with the bilayer capacitance C_m, and (3) I_d: the dielectric noise, where each noise variance (I^2 in A^2) is expressed as follows.

$$I_h^2 = (4/3)e_n^2\pi^2C_t^2B^3 \quad (1)$$

$$I_{Ra}^2 = (4/3)kTR_a(2\pi C_m)^2B^3 \quad (2)$$

$$I_d^2 = 4kT\pi D C_t B^2 \quad (3)$$

$$C_t = C_m + C_{sub} + C_{others} \quad (4)$$

Where B is the frequency band width, C_{sub} is the capacitance of the substrate, C_{others} is the sum of other capacitances contributing to noise. The observed noise current is given by,

$$I^2 = I_h^2 + I_{Ra}^2 + I_d^2 \quad (5)$$

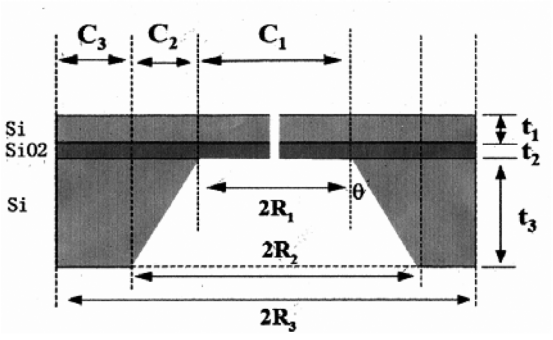

Fig. 4 Substrate structure and the dimension parameters.

The substrate structure which we used in the experiments is shown in Fig. 4.

The calculated values in the present experiments using B=5 kHz are: Cm=76 pF, Csub = 2 pF, Cothers = 1.2 pF, thus Ct = 80 pF. $I_h^2 = 5.5 \times 10^{-26}\ A^2$ (using $e_n = 2.3\times10^{-9}\ VHz^{-0.5}$ ref. 6). Ra = 1.7 kΩ (for pore diameter 100 μm), and $I_{Ra}^2 = 2.7 \times 10^{-25}\ A^2$. Thus the calculated noise rms current I = 0.57 pA is obtained. This value is in quite good agreement with the observed value 1.2 pA. Observed noise values depending on the pore diameter are compared in Fig. 5 together with other reported values.

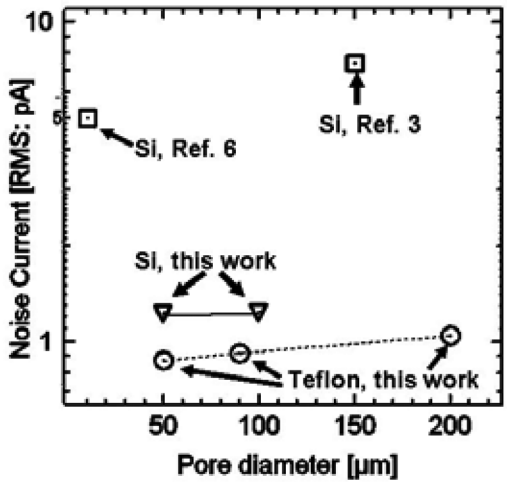

Fig. 5 Observed current noise in the Si planer ion-cahnnel biosensor structure. B = 5 kHz in the present work, 2 kHz in ref. 3, and 1 kHz in ref. 6.

4. Conclusion

The Si based planer type patch clamp biosensor experiments were carried out. The observed noise current was almost equal to the results obtained by using the Teflon substrates. The calculated value of the current noise was in good agreement with the observed one.

Acknowledgements

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