Artificial lipid bilayers in silicon chips as a model for neuronal cell membranes

Ayumi Hirano-Iwata

Graduate School of Biomedical Engineering, Tohoku University
6-6 Aoba, Aramaki, Aoba-ku, Sendai, 980-8579, Japan

Reconstitution of ion channels in free-standing bilayer lipid membranes (BLMs) can be a model system of neuronal cell membranes and also provide an excellent system for drug screening under chemically controlled condition. However, mechanical instability of BLMs hinders their wide range of applications and confines the bilayer method to laboratory use. Although extensive studies have been made to improve the stability of free-standing BLMs through the combination with microfabricated devices, they have not provided increased resistance to physical shocks, for example, mechanical shocks during exchanging aqueous solutions surrounding BLMs. In this talk, I will show you our recent approaches for improve the stability of BLMs through the combination with silicon microfabrication and BLM formation.

Microapertures (diameter: 20-30 micrometer) were fabricated in a 240 nm thick Si_3N_4 layer deposited on a FZ Si (100) wafer (>9000 ohm cm, 200 micrometer in thickness). The apertures were formed by isotropic etching in hot phosphoric acid. The edge of the aperture was smoothly tapered (Fig. 1) by the isotropic etching. BLMs were prepared across a microfabricated aperture by the monolayer-folding method and current recordings were performed with an Axopatch 200B patch-clamp amplifier (Molecular Devices).

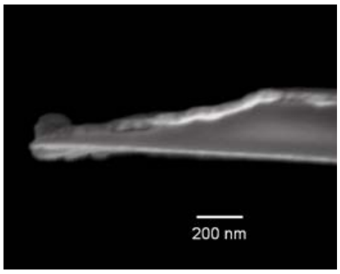

Fig. 1 SEM image of the aperture edge.

BLMs suspended in the microfabricated apertures showed improved membrane stability. The BLMs were resistant to applied voltage of ±1 V and the lifetime of the membranes was 15-43 h with and without incorporated channels. The BLM containing gramicidin channel exhibited tolerance to repetitive solution exchanges [1]. Since the edge of the aperture was smoothly tapered (Fig. 1), the stress on lipid bilayers at the contact with the septum was minimized, leading to dramatically improved membrane stability. However, the BLMs showed large background noise and slow current transients for open↔close transitions due to the large capacitance of the Si chips. We then tried to reduce the chip capacitance by coating the chip with insulator layers [2]. After coating with a Teflon layer, the peak-to-peak noise current was reduced to 3-5 pA. This change corresponds to the reduction in the total capacitance from 252±19 pF (mean±SEM, n=49) to 98±22 pF (n=21). Further reduction in the total capacitance to 40±4 pF (n=80) was observed for the Si chips coated with thermal oxide and Teflon. BLMs formed in this chip showed the noise current of 1-2 pA in peak-to-peak, which is comparable to those observed with conventional BLMs. The current transient was also reduced to < 0.5 ms after the SiO_2/Teflon coatings. The use of a SiO_2/Teflon dielectric layer worked to reduce the capacitance of the total chip, making the background noise and current transient suitable for single-channel measurements. The mechanically stable BLMs having electric properties suitable for recording activities of biological channels will open up a variety of applications including high-throughput analysis of ion-channel proteins.

References

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