Electrochemical Impedance Spectroscopy Study of Tethered Bilayer Lipid Membranes with Artificial Nanopores

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It is fundamentally critical but highly challenging to study quantitatively and reliably the electrical properties of cell membranes of living cells due to the intrinsic complexity of the cell membrane system and their deformable shapes and dimensions. Recently, tethered bilayer lipid membranes (tBLMs) have been used as a simpler model system for study of cellular membrane processes, such as mimicking protein ion channel transfer and interactions of integral and peripheral proteins[1,2]. In this paper, we report electrochemical impedance spectroscopy (EIS) study of tBLMs without and with artificial nanoholes that are immobilized on a conductive gold substrate to assess the behavior of electroporation process of cell membranes.

Devices on gold/SiO2/Si substrate with or without nanoholes are fabricated and characterized. For the nanohole devices, arrays of nanoholes with a diameter of 150 nm are patterned by e-beam lithography and? then transferred to the gold layer by dry etching. The first step of the formation of tBLMS gives a mixed self-assembly monolayer (SAM) of 20-tetradecyloxy-3,6,9,12,15,18,22-heptaoxahexatricontane-1-thiol (WC14) and β-mercapto-ethanol. The second step is the addition of lipids and solution exchange from ethanol to water[1]. The test area is defined by a circular PDMS reservoir, containing around 10,000 nanoholes. The schematic of device and measurement configuration is shown in Fig. 1. The Cole-Cole EIS spectra and fitting curves to the equivalent circuit models of devices with and without nan oholes with SAMs and tBLMs are shown in Fig. 2, respectively. In the equivalent circuits shown in Fig. 2 (a) and (c), constant phase element 1 (CPE1) models SAM or tBLM layer and CPE2 models the nanopore defect effects. The extracted parameters from fitting are shown in Table 1. The difference of CPE1-T between SAM and tBLM measurements are due to the second lipid layer molecules[2]. The CPE1-α values being close to 1.0, suggests that a nearly ideal capacitor behavior of the electrically insulating dielectric SAM and tBLM. For the nanohole SAM devices, the top gold surface and the sidewalls of nanoholes are modified with SAMs, hence it is essentially defect free. For the nanohole tBLM device, the lipid bilayers cannot form on the sidewalls of the nanoholes. As a result, artificial defects of nanopores form over the nanoholes. The extracted CPE2-T value of the nanohole device is greater than the CPE2-T value of the device with no nanopores. The CPE2-α value of nanohole device is smaller, suggesting that the surface deviates from ideal capacitance-resembled surfaces with the presence of artificial defects. The parameter dependences on nanohole sizes and electric field will be presented in detail at the conference. This study suggests that EIS is a reliable and sensitive method for characterization of tBLM properties for the study of the electroporation processes.

［1］G. Valincius, etc, *J. Phys. Chem. B*, **110**, 10213 (2006)
［2］D. McGillivray, etc, *Biointerphases*, **2**, 21 (2007)

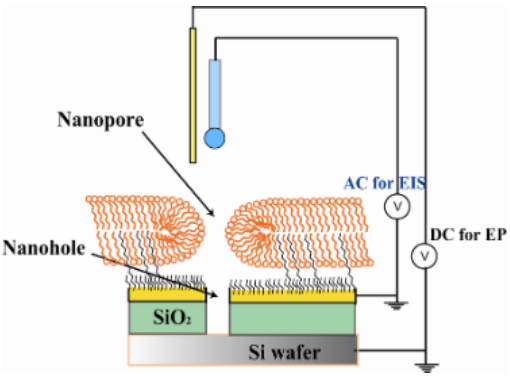

Fig. 1. Device structure and EIS test setup.

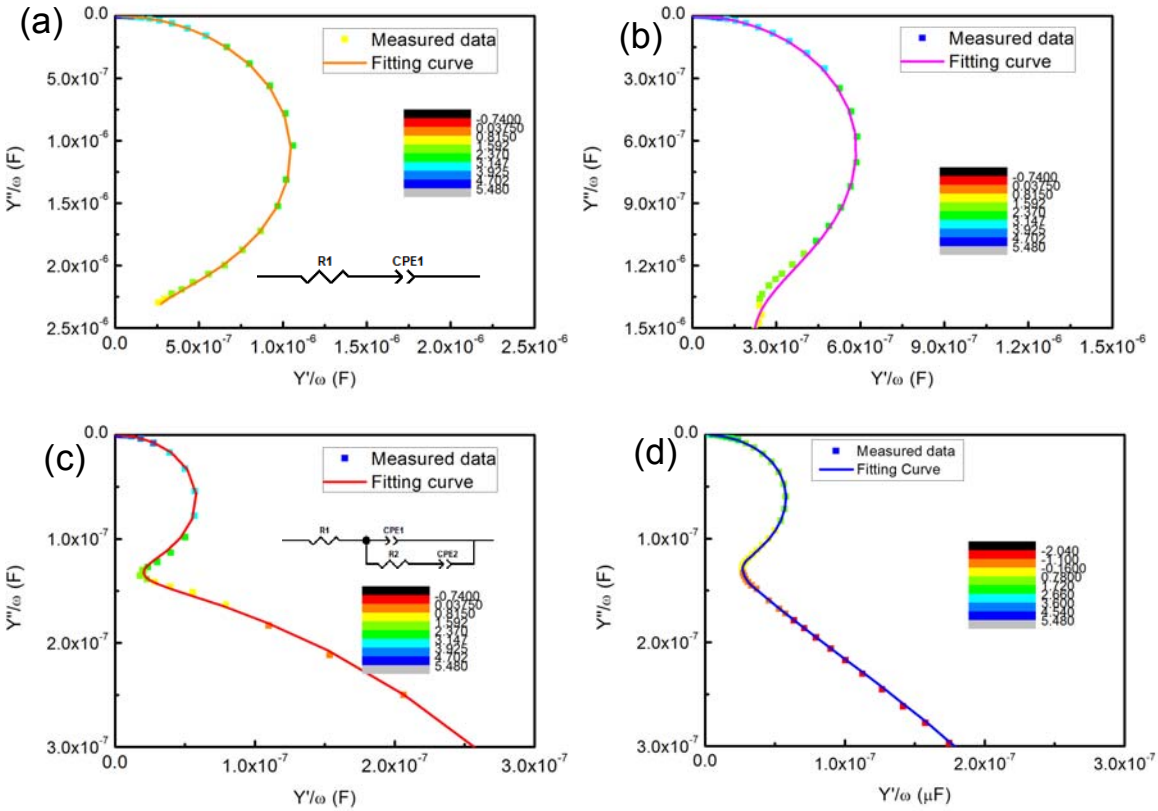

Fig. 2. The measured and fitted Cole-Cole curves. The color bars in the figures are $\log_{10}f$, where f is the frequency (Hz) for the measurements (a) plain gold device with SAMs. The inset is the equivalent circuit used to model SAM structures. (b) Nanohole device with SAMs. (c) Plain gold device with tBLMs. The inset is the equivalent circuit to model tBLMs structures. (d) Nanohole devices with tBLMs.

Table 1. Extracted parameters.

	Au SAM	Nanopore SAM	Au tBLMs	Nanopore tBLMs
CPE1-T ($F \cdot s^{(\alpha-1)}$)	2.65e-6	2.13×10^{-6}	1.82e-7	1.83×10^{-7}
CPE1-α	0.98	0.96	0.95	0.96
CPE2-T ($F \cdot s^{(\alpha-1)}$)			9.73e-7	1.30×10^{-6}
CPE2-α			0.85	0.50