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A New Planar-Type Leakage Current and Impedance Microsensor for Detection of Interaction between Electrolyte-Entrapping Liposome and Protein

P. Lorchirachoonkul[a], I. Goto[a], T. Shimanouchi[b], K. Yamashita[a], and M. Noda[a, *]

[a]*Graduate School of Science and Technology, Kyoto Institute of Technology, Kyoto 606-8585, Japan*
[b]*Graduate School of Engineering Science, Osaka University, Osaka 560-8531, Japan*

Abstract

We have developed a new leakage current microsensor by using simpler planar processes than Si-surface-bulk micromachining processes used in the previous microwell structure. This sensor fabrication and structure can easily make a target solution volume smaller than μL with excellent immobilization of the droplet and intact biomolecules as sensing elements, as a result, reduce effectively the background noise current in the microsensor and improve reproducibility of the results. The leakage current due to the biochemical interaction was successfully evaluated, dependent on the droplet protein concentration. Cole-Cole plots from the impedance analysis also show quantitative difference between with and without the interaction, depending on the charge-transfer impedance that results from the condition and structure of liposome and lipid membrane after the interaction.

Keywords: Leakage current; Impedance; Microsensor; Electrochemical; Liposome; Protein

1. Introduction

The liposomes entrapping a fluorescence probe or electrolyte can release them by an interaction with specific biomolecules such as protein through a membrane perturbation effect [1]. This characteristic of the liposome is advantageous for the design of the biomolecule sensor as a leakage current sensor on a basis of DC amperometry, whereas the protein is generally insensitive to an electrochemical detection. The released ions can contribute to the current generation, which relates directly to the interaction between liposome membranes and external proteins.

Recently, we have newly developed a highly-sensitive leakage current microsensor by using the entrapping DPPC liposome and a protein denaturant. The addition of denaturant successfully resulted in

* Corresponding author. Tel.: +81-75-724-7443; fax: +81-75-724-7400
E-mail address: noda@kit.ac.jp

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the prominent improvement in sensitivity up to 129-fold of magnitude, although target protein weakly interacts with liposome under the normal condition [2].

In this work, firstly, we have further developed a new leakage current microsensor by using simpler planar processes than Si-surface-bulk micromachining processes used in the previous microwell structure. It is expected that the sensor fabrication and structure can easily make a target solution volume smaller than μL with excellent immobilization of the droplet and intact biomolecules as sensing elements. Secondly, impedance behaviour has been evaluated other than leakage current in order to consider the effect of perturbation in surface properties and molecule structure of liposome.

2. Prepared Biochemical Solution

This time, $K_4[Fe(CN)_6]$ solution was entrapped inside of liposome of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholline (DPPC). Carbonic Anhydrase from Bovine (CAB, Mw=28.8 kDa) was used as added protein for the interaction. The CAB is selected because it was conventionally used and evaluated in the experiments with chemical laboratory instruments such as chromatography [3], so the experimental results are compared between this work and the conventional ones.

3. Leakage Current Microsensor

3.1. Device fabrication

Figure 1 illustrates a cross-sectional view of new planar-type leakage current microsensor, where the droplet of the liposome solution was immobilized on the hydrophilic SiO_2 and Pt film surface sensing area. Major fabrication processes are illustrated in Fig. 2. As a final step, a surface photoresist well (ex. OFPR-800, SU-8) was made by photolithograph processes (Fig. 3).

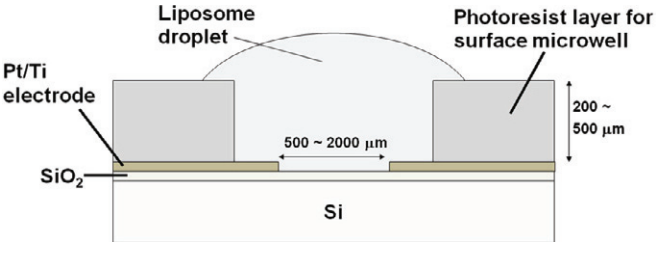

Fig. 1. Cross-sectional view of planar-type leakage current sensor.

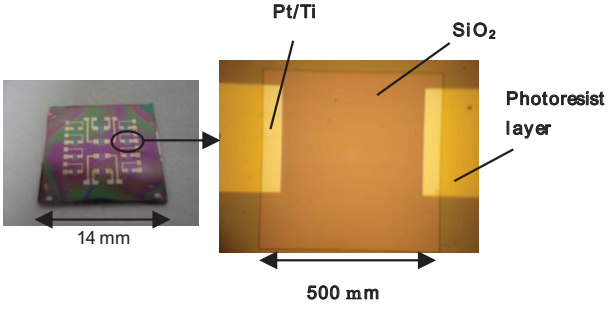

Fig. 3. Surface photographs of the fabricated sensor chip.

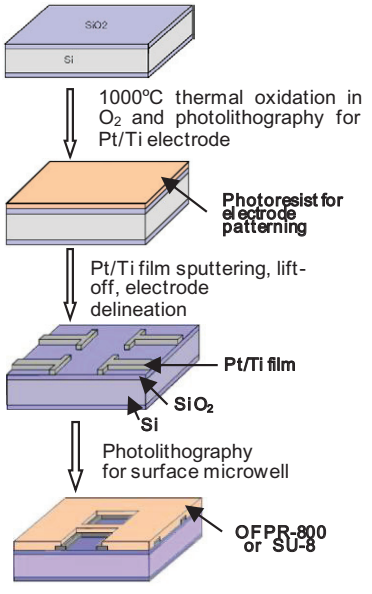

Fig. 2. Fabrication process of planar-type leakage current microsensor.

3.2. Leakage current measurements

As a target protein, Carbonic anhydrase from bovine (CAB) protein solution (1 μL) was supplied onto the liposome droplet (1 μL) with a micropipette. Time course of the current with 0.1 V bias between the electrodes is monitored before and after the CAB dropping with a semiconductor parameter analyzer (Agilent 4156B).

Figure 4 shows background leakage current vs. time for both the conventional surface-bulk microwell sensor[2] and this work. The background current is significantly improved for the latter. Generally, a rough or structured surface increases the surface leakage due to generation of electric field confinement at surface edges, or due to increase in adsorption of extrinsic conductive molecules. It is found from Fig. 4 that the background current (around 1.0-1.5 nA) for the planar-type is improved by more than an order of magnitude from the conventional well-type (about 80 nA). This is intrinsically important for advancing larger array approach of leakage detection with minute current.

In Fig. 5 is plotted leakage current versus time as a parameter of target CAB concentration when the target CAB protein is spotted on the liposome droplet immobilized on the sensing area in Fig. 1, after applying DC bias of 0.1 V. Increased peak area (current x time) corresponds to the generated charge originated from the released Fe^{2+} ions. It is found that the larger the target CAB concentration, the larger the peak area and current flowing time due to the interaction. It is therefore considered that the sensor detects the increase in the concentration successfully.

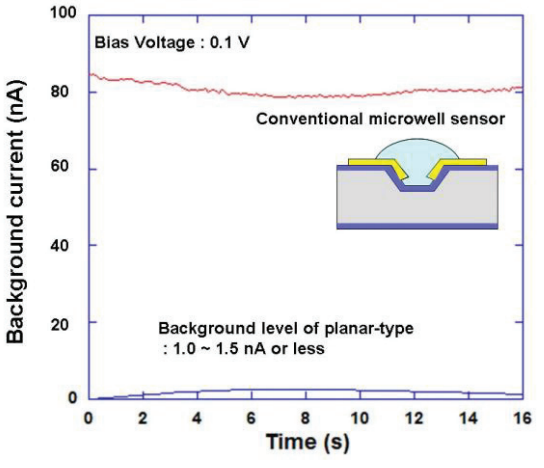

Fig. 4. Background current vs. time for both the conventional surface-bulk microwell-type[2] and planar-type (this work).

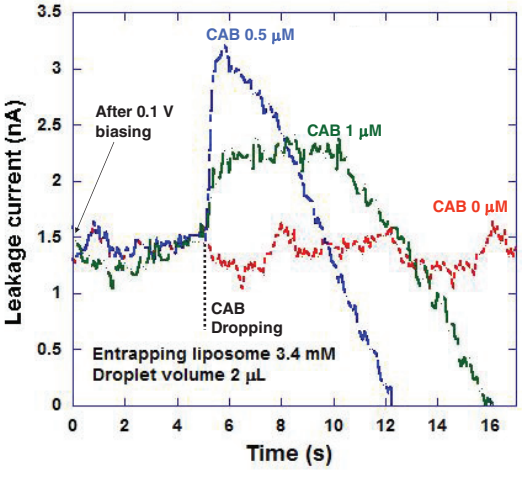

Fig. 5. Leakage current vs. time as a parameter of target CAB concentration when the target CAB protein is spotted on the liposome droplet immobilized on the sensing area.

4. Impedance Microsensor

4.1. Cole-Cole plot measurements

After the leakage phenomena were finished, we can evaluate change in surface properties and molecular structure of the lipid membrane of the liposome from impedance characteristics such as Cole-Cole plot. Figure 6 shows Cole-Cole plots measured from 100 Hz to 10 MHz for the droplets with and

without CAB on the sensor. It is observed that the two kinds of RC parallel equivalent circuits (semicircular curve), which is considered to correspond conventional interaction between the electrode and solution interface. It is also found that the semicircular curve with CAB becomes smaller than without CAB for frequency higher than 1 MHz. This indicates that the impedance change due to generation of liposome-CAB complex occurs especially for the higher frequency then reducing the charge-transfer resistance (R_{ct}), corresponding to the reduced horizontal diameter of the semicircle, which results from fusion and aggregation of the lipid membrane.

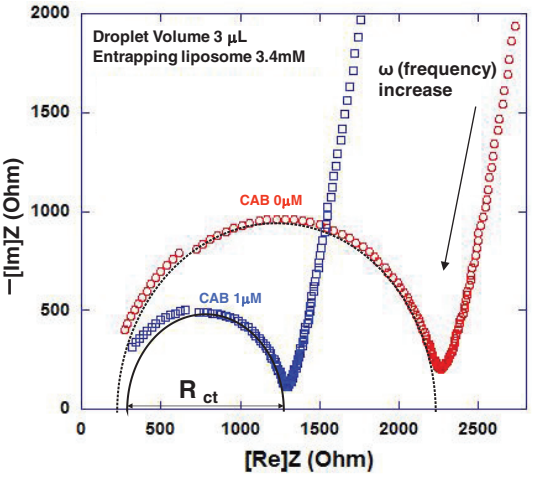

Fig. 6. Cole-Cole plot measured from 100 Hz to 10 MHz for the droplets with and without CAB on the sensor.

5. Conclusions

A planar-type leakage current microsensor was considered and developed by simpler planar processes than Si-surface-bulk micromachining processes used in the previous microwell-type. The new sensor reduced effectively the background noise current in the detection. The leakage current due to the biochemical interaction was successfully evaluated, dependent on the droplet protein concentration. Cole-Cole plots from the impedance analysis also show quantitative difference between with and without the interaction, depending on the charge-transfer impedance that results from the condition and structure of liposome and lipid membrane after the interaction.

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