PRECISE ENZYME IMMOBILIZATION AT THE BOTTOM OF A MICRO FLOW CHANNEL AND ITS APPLICATION TO A SENSING SYSTEM

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In a trend of the μTAS or Lab-on-a-Chip research, biosensors have been incorporated in various microfluidic systems. Considering the efficiency of detection and easiness for handling of the microfluidic system, it might not be the best choice to immobilize an enzyme on a working electrode as found in ordinary biosensors. In this study, an enzyme was not immobilized on the electrode surface but was immobilized at the bottom of a flow channel by using a newly developed method. The method enabled precise batch-immobilization of enzymes in a small area. Although the sensor output is affected by various structural and conditional parameters, the influence has not been examined systematically. Therefore, we examined the behavior of the sensor output by changing the device parameters.

In the method of immobilization, a precursor solution containing 3-aminopropyltriethoxysilane (35 μl), 2-(3,4-epoxycyclohexyl)ethyltrimethoxysilane (10 μl) and 0.1 M HCl (5 μl) along with glucose oxidase (4 mg) was used to form the enzyme-immobilized layer. The solution was stamped onto a protruding structure of a silicone rubber template covered with a gold layer (Fig.1 (b)). After complete gelation of the solution, a precursor solution of silicone rubber was applied to the template and was cured (Fig.1 (d)). Then, the cured silicone rubber layer was peeled off. As a result, the enzyme was precisely immobilized at the bottom of the micro flow channel (Fig.1 (e)).

The substrate with the flow channel was placed on a substrate with a thin-film three-electrode system (Fig.2). The working and auxiliary electrodes were formed with platinum, whereas the reference electrode was formed with Ag/AgCl. The active area of the working electrode was 500 μm × 500 μm. A glucose standard solution was introduced into the micro flow channel using a micro syringe pump. During a series of measurements, swelling of the enzyme-immobilized layer was negligible because of its relatively rigid structure.

In the flow channel of this system, the flow is supposed to be laminar. However, since the enzyme-immobilized layer is separated from the working electrode and is placed in the upper stream, hydrogen peroxide produced by the enzymatic reaction diffuses to the direction normal to the flow. Therefore, it is assumed that the profile of transport changes and the output current is affected by the structural or conditional parameters.

Dependence of the output current on flow rate was small at higher glucose concentrations. However, a significant increase in current was observed as the flow rate decreased to less than 1 μl/min (Fig.3). As the height of the flow channel decreased, the output current increased. Similarly, as the immobilized enzyme was placed farther in the upstream, the output current increased. These tendencies are due to the increase in the flux of hydrogen peroxide which reaches the working electrode surface in the downstream. No noticeable dependence of the response time on flow rate, channel height, enzyme position, and concentration was observed.

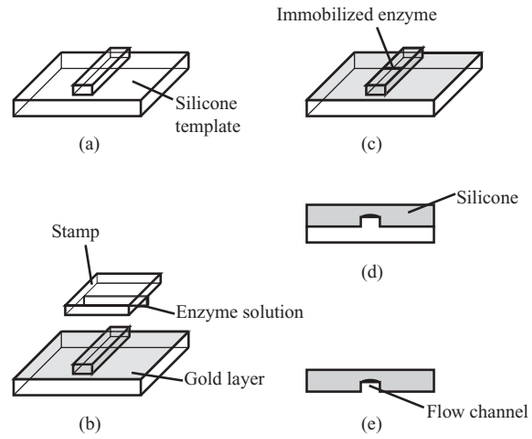

Fig.1 Immobilization of an enzyme at the bottom of a micro flow channel. (a): a silicone rubber template is made by casting a precursor solution on SU-8 structures formed on a glass substrate. (b), (c): the template is coated with gold and a precursor solution containing the enzyme is printed at the top of the protruding structure as indicated. (d): another precursor solution of silicone rubber is applied and cured. (e): the cured silicone rubber is removed from the template. The enzyme layer is transferred to the bottom of the flow channel.

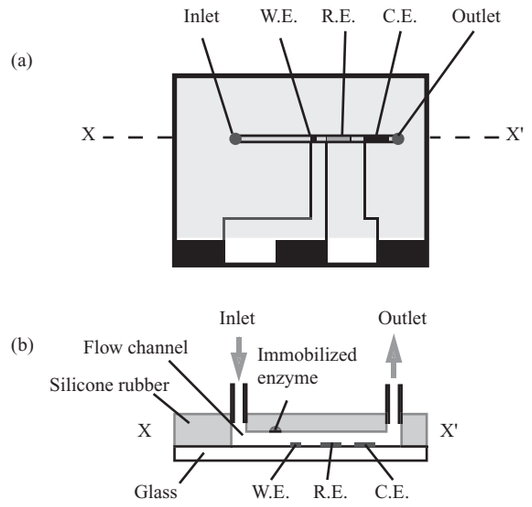

Fig.2 Construction of the system. (a): plan view, (b): cross section along the line X-X'.

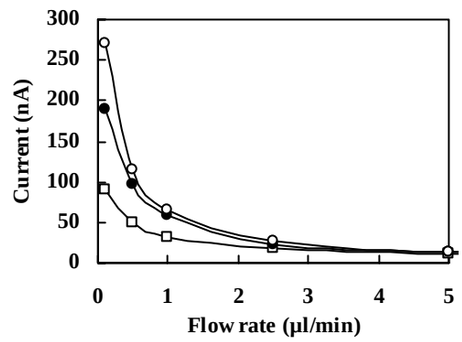

Fig.3 Dependence of the output current on flow rate. The immobilized enzyme was placed over the working electrode. Channel height: 100 μm. Glucose concentration: ○, 10 mM; ●, 6 mM; □; 2 mM.