An Enzyme Sensor Fabricated On A Transparency Film By Applying Line Patterning Method To Prepare Basal Electrodes

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Disposable biosensors are now indispensable tools for individual health monitoring. In the case of electrode based disposable sensors, screen-printing is a popular fabrication method. It is simple, fast, and cost benefit for mass production. However, it requires a patterned screen that is not easy to prepare without special equipments. Therefore, it is inconvenient during the development cycle, especially in the small laboratories. Here we report an enzyme sensor fabricated using an alternative simple method called "Line Patterning Method". We would like to show how an excellent glucose sensor, as an example, is easily fabricated by this fabrication technique.

The "Line Patterning Method", a kind of lift-off method originally invented by the group of MacDiarmid,[1,2)] is a method to make a patterned layer of conducting polymer on a transparency film. They obtained conducting polymer pattern by removing the polymer together with the underlying toner from the film where the polymer was allover coated. The crucial point of this method is that the printer toner but the conductive polymer dissolves into some organic solvents.

In our case, we fabricated metal electrodes on a transparency film. Because laser printers utilize heating to fix toner on transparency films, the films are resistant in the evaporation chamber where the temperature sometimes increases over one hundred degrees centigrade.

The photo in Fig. 1 shows an example of our glucose sensor, which has three electrodes. The rightmost one is an enzyme immobilized electrode, the middle one is a Ag/AgCl electrode, and the leftmost one is a counter electrode. These electrodes were fabricated on a transparency film by the following procedure.

The film (poly(ethyleneterephtalate), 297 x 210 mm^2, thickness = 100μm) was a usual one for overhead projection. The electrode pattern was printed negatively on the transparency film with a laser printer. Eighty same sensors were designed on a single transparency film. The film was then transferred into the chamber of an electron beam evaporator, and Cr, Au, Pt, Ag were evaporated in this order. The thickness of these layers were 5, 40, 30, 200nm, respectively. After the formation of metal layers, the film was cut into the individual chips, dipped into acetone and sonicated for several minutes to remove toner and metal layers from it. At this stage, the surface of all three electrodes was Ag. The surface Ag layer of two electrodes was removed by applying 1000 mV (vs. Ag/AgCl) in 18% HNO_3 solution for 5 sec. The surface of the remaining silver electrode was oxidized to form AgCl by applying constant current (100μA) for 10sec in 100mM phosphate buffer solution (pH 6.9) containing 200mM NaCl.

The sensor was accomplished by immobilizing glucose oxidase (GOx) on the working electrode of the chip. However, before the immobilization step, the surface of the working electrode (Pt) was made inert to suppress nonspecific response to reducing sugars by applying 1200mV (vs. Ag/AgCl) for 15 min in 100mM phosphate buffer solution (pH 6.9). Then a droplet of glucose oxidase (GOx) solution (5μl) was put on the Ag/AgCl electrode and incubated to adsorb GOx onto the electrode for 30 min. The remaining GOx was rinsed away, and then 1% glutaraldehyde solution (5μl) was put on the electrode and incubated 30 min again. After rinsing to remove glutaraldehyde, the sensor chip was dried. Finally, another transparency film was put on the sensor with a double-sided adhesive tape to form a tiny measurement cell.

Figure 2 shows the response curve of this sensor. In this case, a small amount of sample solution (containing various concentrations of glucose with 200mM NaCl and 100mM phosphate buffer (pH 6.9)) was introduced onto the cell. The current was measured by a kind of pulse voltammetry similar to the method in the reference 3. We obtained a Michaelis-Menten-type response.

This measurement requires only a very little amount of sample solution (25μl), and it takes only about 40 sec to measure the glucose concentration. Furthermore, it has very little response to fructose, a kind of reducing sugar, which is sometimes nonspecifically oxidized at the electrode.

References

1) A. G. MacDiarmid, D. Hohnholz, H. Okuzaki, PCT/US01/15773 (2001).
2) D. Hohnholz, A. G. MacDiarmid, Synthetic Metals, 121, 1327 (2001).
3) Y. Ikariyama, et al., Anal. Lett., 21, 953 (1988).

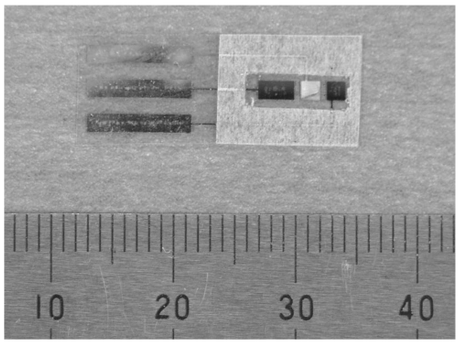

Fig. 1. An example of glucose sensor.

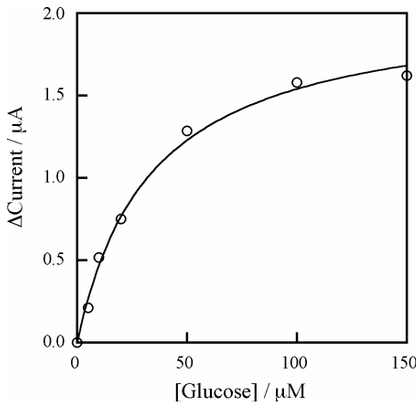

Fig. 2. A response curve of glucose sensor.