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Enzyme Immobilization Methods for Semiconductor Biosensor

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ISFET's advantages over a conventional electrode are as follows. First, its construction is the same as that for the MOSFET, so the small size is easily obtained. Next, this sensor can be fabricated by a semiconductor facility, so sensor mass production is possible. In addition, sensor quality control is easily obtained. This also means that a low cost sensor will be realized in the near future. On the other hand, because of its small size, achieving a miniature one chip sensor fabrication involves many problems.

A major problem preventing development of a semiconductor single chip biosensor was the ISFET substrate current leak. Usually an ISFET is fabricated on a silicon substrate. If an individual cut off device was immersed into water without any sort of encapsulation, current would flow through the cut off surface.

In 1984, the authors developed a completely monolithic ISFET biosensor using SOS/ISFET devices. This biosensor has a thin silicon layer on a sapphire dielectric substrate (*1*). The first sensor, shown in Fig. 1, has two ISFETs and a gold layer back surface. One of the two ISFET ion sensitive regions has a urea immobilized membrane. The excess area was covered by an inactivated enzyme membrane. A gold layer was spread over the entire back surface.

The authors' first work showed the possibility of fabricating a single chip biosensor. The disadvantage of the developed method is that only one enzyme can be immobilized on one device. To solve this problem and to realize a multienzyme immobilized sensor, various methods were used to adopt a very small enzyme membrane at a certain position on the device.

To realize a thick and characteristic-controllable membrane, the authors developed a novel enzyme membrane patterning method (*2–4*). In this method, a BSA-based enzyme membrane was employed that has better

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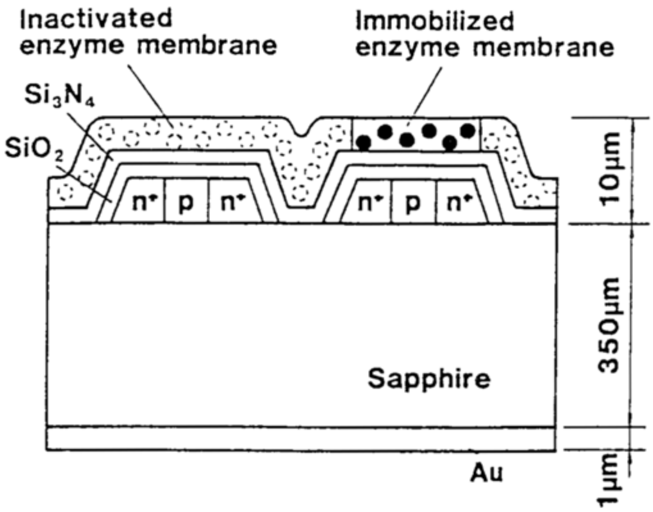

Fig. 1. Single chip SOS/ISFET bio-sensor structure.

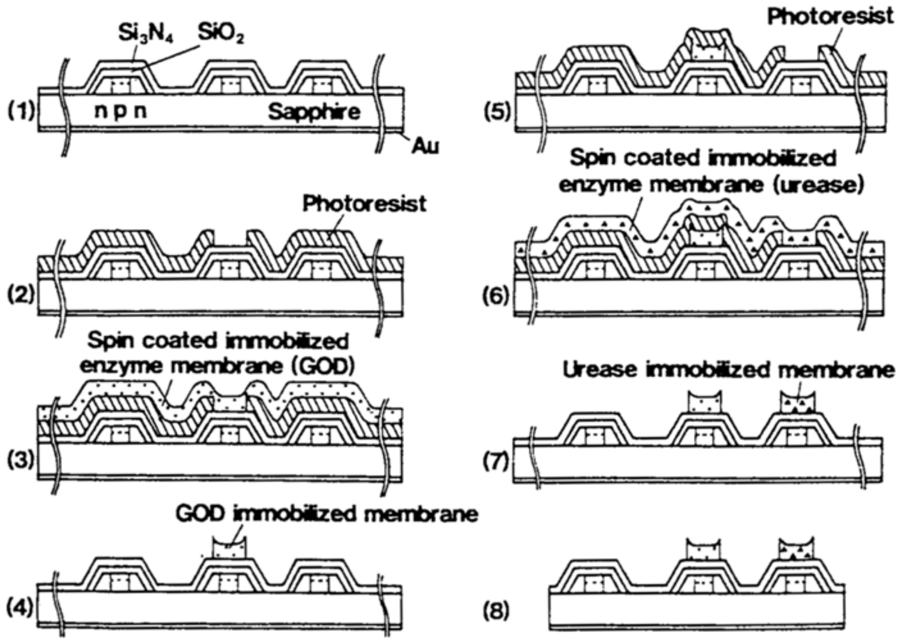

Fig. 2. Enzyme-immobilized membrane fabrication process.

adhesion between the membrane and ISFET surface than that of artificial polymers. This membrane has no light sensitivity, so that photolithography must be carried out by another photosensitive membrane. Actually, a positive-photoresist was used, which consisted of phenol resin and a photosensitive reagent. After a prepared SOS/ISFET wafer was covered by positive photoresist, holes were created where immobilized enzyme membrane had to be fabricated by photolithography technology (Fig. 2-2) involving UV light irradiation and development in an alkaline solution. Next, the entire wafer surface was covered by a BSA crosslinking membrane (Fig. 2-3). Finally, the excess membrane was removed by ultrasonic vibration in acetone, then small patterned enzyme membranes were precisely patterned (Fig. 2-4). By repeating these processes, plural enzyme patterned small biosensors were obtained (Fig. 2-7).

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