

# A new monolithic microbiosensor for whole blood analysis

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## Abstract

A new microbiosensor has been proposed and fabricated for the measurement of glucose concentration in whole blood. The proposed microbiosensor has been monolithically integrated with enzymatic metal microelectrode array in a micromachined chamber attached to a microsyringe. The fabricated microbiosensor has a high sensitivity of 470 nA/cm<sup>2</sup> mM for detecting glucose levels in a wide linear range of 0–50 mM in potassium phosphate buffer solution (pH 7.0). © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** Biosensor; Blood; Glucose; Enzyme; Microelectrode; Electropolymerization

## 1. Introduction

MEMS technologies have been widely utilized to develop miniaturized biosensors having multiple functions. MEMS devices provide a number of advantages over other technologies because they are small, reliable and inexpensive. Glucose monitoring devices are one of them for disposable, inexpensive, reliable biosensors, as particularly required for diagnosis and medical treatment of diabetic patients.

Recently, several kinds of microglucose sensors have been reported for the determination of glucose levels in blood. Miniaturization of enzyme sensors is one of the key factors in these clinical diagnosis and monitoring devices, for both in vivo and in vitro measurements. This has been achieved using microelectrodes prepared by MEMS technologies [1,2]. Also, the development of glucose sensors based on enzymatic glucose oxidation has reached an advanced status [3,4]. However, most of them are focused on microelectrode fabrication and the blood sample extraction part has not been developed and integrated. Therefore, the existing blood sensors do not meet an increased need in recent years for monolithic sensors integrated with microfluidic blood sample extraction/preparation part.

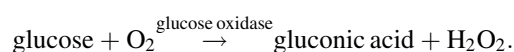
In this paper, we have proposed a new microbiosensor for blood analysis, which monolithically integrates enzymatic metal microelectrode array in a micromachined chamber with a microsyringe. Fig. 1 shows the schematic drawing of the proposed microbiosensor. The proposed sensor is composed of metallic microstructures and electrochemical

sensors. Metallic microstructures can draw blood from the body through the microsyringe by capillary force and transfer it to the reaction chamber where enzyme electrodes are located. In the reaction chamber, the glucose concentration in blood is electrochemically measured by the enzyme electrodes.

In the miniaturized microsensors, due to their small size, macroelectrodes cannot be applied. Typically, the microelectrodes fabricated in this sensor have the features of increased current density and decreased influence from stirring when compared with conventional macroelectrodes [5]. However, the current signal output is very low for microelectrodes because of their small size. The current signal of amperometric microsensors can be amplified by the design of microelectrode arrays as shown in Fig. 2.

## 2. Electrochemical measurement and immobilization of enzyme

In this work, an electrochemical transducer has been applied for the measurement of glucose concentration in whole blood. Fig. 3 shows the principle of electrochemical measurement of glucose concentration. The electrochemical system for measurement of glucose concentration is composed of two electrodes: one gold electrode (working electrode) and one silver electrode (reference electrode) [6,7]. The surface of the gold electrode has been deposited with the enzyme film which has specificity and selectivity on glucose. Glucose oxidase catalyzes the following reaction:



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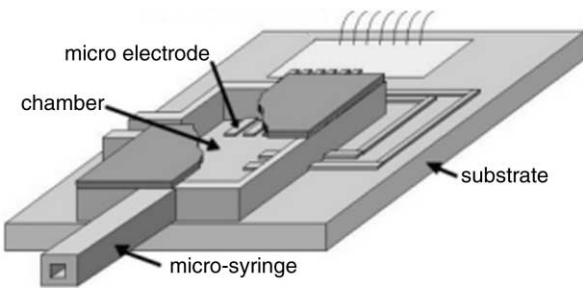


Fig. 1. Proposed microbiosensor.

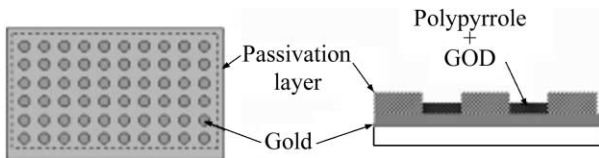
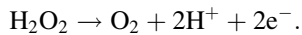


Fig. 2. Schematic diagram of microelectrode arrays.

From this reaction, the glucose concentration can be measured by measuring the amount of H<sub>2</sub>O<sub>2</sub> byproduct. Furthermore, the hydrogen peroxide generated is electrochemically oxidized at the gold electrode (working electrode), well known as amperometry [8]:



Therefore, by measuring the current in the working electrode, the glucose concentration can be determined.

Operation of electrochemical biosensors requires a conjugation of the biochemical and electrochemical reactions.

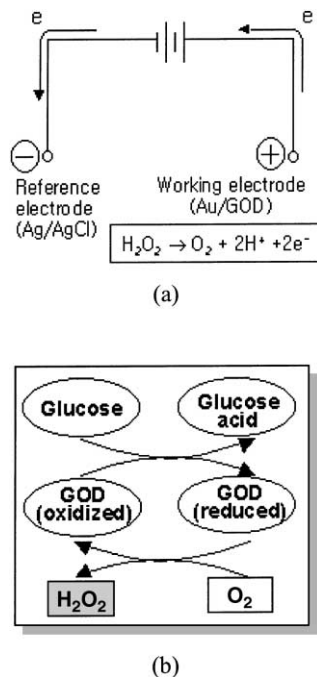


Fig. 3. Mechanism of the electrochemical measurement of glucose concentration in (a) two electrodes systems and (b) enzyme GOD reaction on the working electrode.

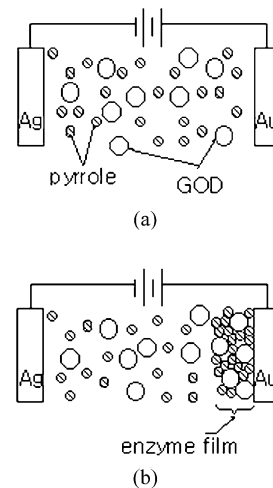


Fig. 4. Electropolymerization for enzyme immobilization (a) before the process electropolymerization and (b) in situ electropolymerization process.

For this aim, the biological recognition element should be immobilized at the electrode surface. In this work, the electropolymerization method has been applied for the immobilization of glucose oxidase (GOD) with polypyrrole (PPy) on the working electrode [9]. Fig. 4 schematically illustrates the process of GOD immobilization on the surface of working electrode during in situ electrochemical growth of PPy. PPy enables films to be grown from aqueous solutions at the relatively low oxidation potential [10].

The polymerization occurs during the electrochemical oxidation of pyrrole monomers. The electrochemical immobilization has several advantages. First, one can control the amount and spatial distribution of enzyme in the polymer by manipulating preparation parameters such as the charge transferred, as well as monomer and enzyme concentrations in the electrodeposition solution. Second, it is also possible to immobilize enzyme on the surface of the electrode by a one-step process regardless of the types and shapes of the substrates to be deposited. Finally, the suppression of interference is expected since a conducting polymer possesses the size exclusion property which is effective in eliminating the interference from electro-oxidizable compounds such as ascorbate and urate [11,12]. The electropolymerization has been performed at room temperature. PPy films have been grown at a static potential of 0.8 V with respect to the Ag reference electrode in potassium phosphate buffer solution (pH 7.0) which consists of 0.1 M KCl containing 0.1 M pyrrole and 5 mg/ml GOD. After the electropolymerization, the film has been overoxidized to the background current level for the measurement of glucose concentration.

### 3. Microstructures

The microstructures including a microsyringe and a reaction chamber have been fabricated using multi-exposure single-development (MESD) method [13] and electroplating

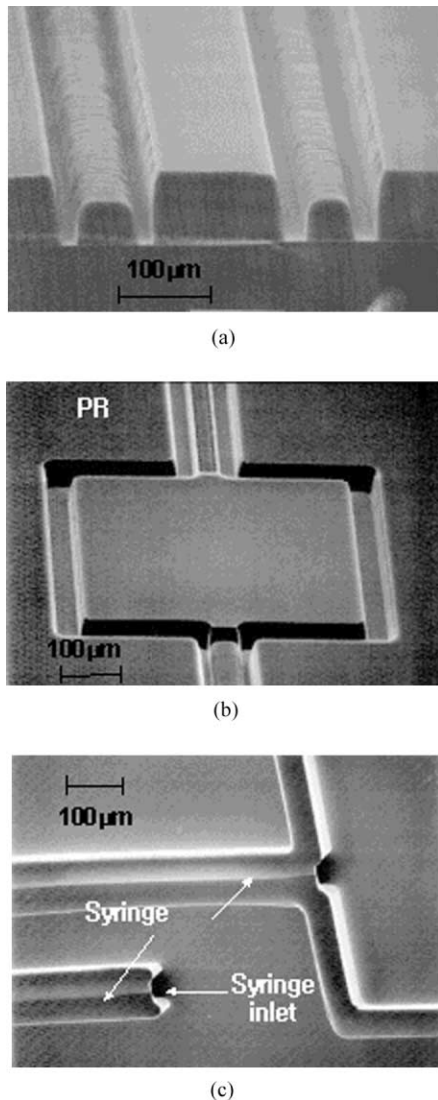


Fig. 5. (a, b) Multi-depth 3D PR mold patterned by MESD and (c) Ni microstructures were fabricated by MESD and electroplating.

technology. The MESD method, which had been developed by our group, allows one to form a multi-depth three-dimensional (3D) photoresist mold by using different exposure times in multiple photomask steps and single final development as shown in Fig. 5. For the thick photoresist, we used the AZ9260 manufactured by Hoechst. As shown in Fig. 5, the maximum thickness of photoresist mold is 90  $\mu\text{m}$ . After the mold pattern, metal electroplating is performed until the desired metallic microstructures are obtained. Electroplating conditions had been reported in our previous work [14]. The fabricated metallic microstructures are shown in Fig. 5c.

#### 4. Fabrication

The fabrication process is shown in Fig. 6. The microbiosensor is formed on a p-type (1 0 0) silicon wafer. Silicon nitride is deposited and patterned as a mask layer for the

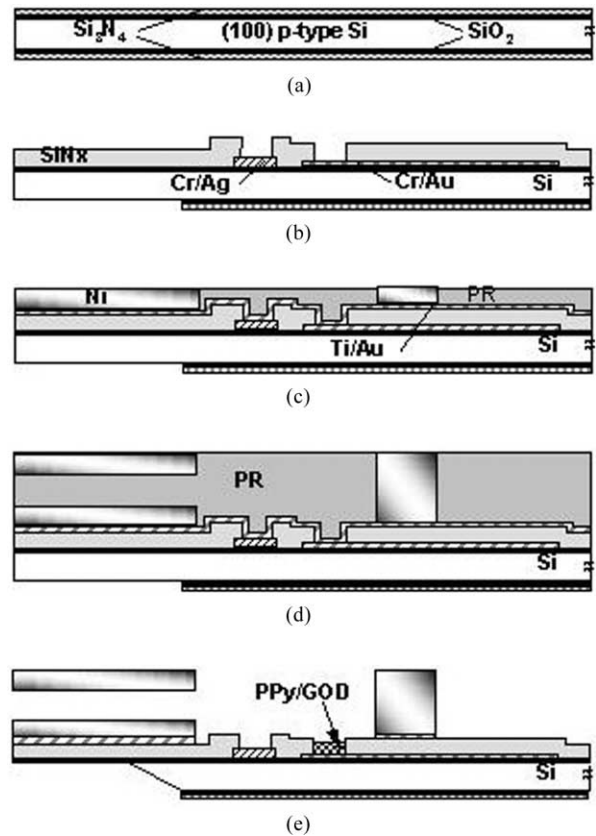


Fig. 6. Fabrication process: (a) LPCVD silicon nitride deposition (1500  $\text{\AA}$ ); (b) metal electrodes formation (Au, Ag) and passivation layer PECVD silicon nitride deposition and patterning; (c, d) Ni electroplating for making microstructures; (e) bulk Si etching and GOD immobilization.

KOH etching of the bulk silicon in the later process step. Next, working electrodes (Cr/Au) are defined. These electrodes will be used for electrochemical measurement of glucose concentration in blood. In the similar way, reference electrodes (Cr/Ag) are formed by lift-off process. A PECVD silicon nitride layer is deposited and patterned for passivation. A seed layer (Ti/Au) is evaporated followed by the definition of the first photoresist mold of 20  $\mu\text{m}$  in thickness and nickel is electroplated. The first photoresist mold is removed and the second photoresist mold of 60  $\mu\text{m}$  in thickness is patterned using the MESD method and nickel is electroplated again for the structural material. After photoresist mold is removed, bulk silicon is etched in KOH. Finally, enzyme GOD is immobilized on the gold electrodes by electropolymerization method.

#### 5. Results and discussion

The fabricated microbiosensor is shown in Fig. 7. Total chip size is 6 mm  $\times$  2.5 mm. The length of syringe is 4 mm, while both the width and height are maintained at 40  $\mu\text{m}$ . The thickness of electroplated walls is 20  $\mu\text{m}$ . We have tested the fabricated microbiosensor by making it draw ink

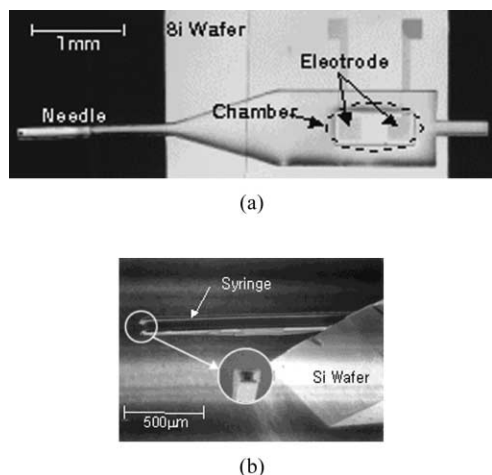


Fig. 7. (a) Microphotograph and (b) SEM picture of the fabricated microbiosensor.

by capillary force and have measured that it takes <15 s to transfer ink from the inlet of syringe channel to the reaction chamber. For the mechanical stability, metallic microstructure has been made in a triangular shape. We have designed another type microsyringe in order that its tip can be smoothly inserted into human body. The shape of the thick photoresist mold for the microsyringe tip have been sharpened as shown in Fig. 8a. Fig. 8b shows the fabricated microsyringe tip using nickel electroplating technique and the sharpened photoresist mold.

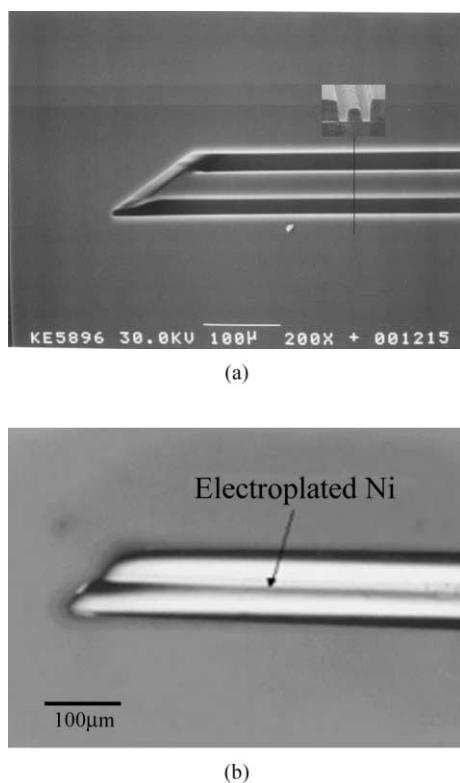


Fig. 8. (a) SEM picture of the thick PR mold to fabricate the microsyringe with the sharp tip and (b) microphotograph of the fabricated microsyringe tip.

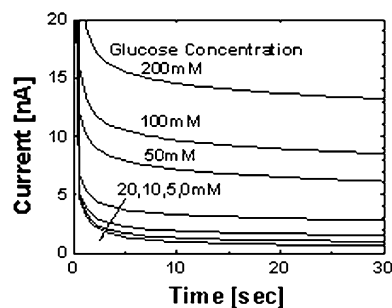


Fig. 9. Response characteristics of the microbiosensor. The microelectrode diameter is 6 µm in a 30 × 30 array.

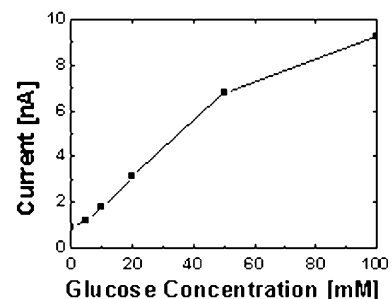


Fig. 10. Calibration curve of the fabricated microbiosensor.

Fig. 9 shows response characteristics of the microbiosensor for various glucose concentrations from 0 to 200 mM (from 0 to 360 mg/dl) in potassium phosphate buffer solution with the working electrode voltage fixed at 0.8 V. Working electrodes consist of 30 × 30 arrays of microelectrodes in 6 µm diameter.

Fig. 10 shows the measured characteristics of the fabricated microbiosensor with respect to the calibration sample. Current signal value has been measured at 15 s after the voltage was applied. It shows linear response of the fabricated microbiosensor for the glucose concentrations from 0 to 50 mM, which is the very important range to monitor diabetic patients whose glucose concentration in the whole blood is higher than 8 mM.

The glucose sensitivity may be influenced by electropolymerization conditions, especially the total charge of electropolymerization. We have measured the sensitivity as a function of total charge of electropolymerization as shown in Fig. 11. The electrodeposited PPy–GOD film thickness is

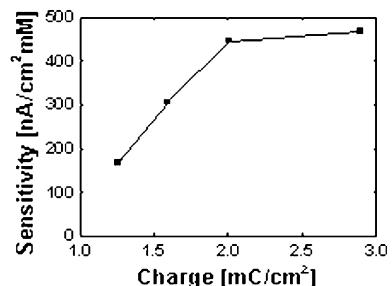


Fig. 11. Effect of electropolymerization charge.

dependent on the total charge of electropolymerization. The response increases as the film thickness increases, mainly due to the elevated amount of enzyme incorporated in the film, and then saturates beyond  $2.0 \text{ mC/cm}^2$ . The saturation in the response observed on the film thicker than  $2.0 \text{ mC/cm}^2$  can be explained by the internal diffusion of  $\text{H}_2\text{O}_2$  rather than the internal diffusion of glucose. The diffusion of  $\text{H}_2\text{O}_2$  within the film is bidirectional: (i) the one contributes to the electrochemical response on the surface of Au electrode; (ii) the other diffuses out into the bulk phase, resulting in a signal loss. As glucose diffuses into the inner part of the polymer film from the film/solution interface, a local and progressive consumption of glucose influx occurs by the enzymatic reaction of glucose oxidase, assuming that glucose oxidase is eventually distributed throughout the film. As the thickness of the film increases beyond the critical value, most of glucose would be consumed by glucose oxidase before it reaches the surface of the electrode. We have achieved the maximum sensitivity of  $470 \text{ nA/cm}^2 \text{ mM}$  for the electropolymerization charge higher than  $2.0 \text{ mC/cm}^2$ .

## 6. Conclusions

We have proposed a new microbiosensor which monolithically integrates enzymatic metal microelectrode array in a micromachined chamber with a microsyringe for blood analysis. The calibration of the microbiosensor has confirmed its response characteristics in the glucose concentration range from 0 to 200 mM with the maximum sensitivity of  $470 \text{ nA/cm}^2 \text{ mM}$ . These characteristics have demonstrated that the fabricated microbiosensors are suitable for the diagnosis of diabetic patients whose glucose level is higher than 8 mM.

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## Biographies

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*Chul-Hi Han* received his BS degree in electrical engineering from SNU, in 1977 and MS and PhD degrees in electrical engineering from the KAIST, Taejon, South Korea, in 1979 and 1983, respectively. In 1983, he joined the Gold Star Central Research Lab., Gold Star Co., Seoul, South Korea, where he worked on the development of bipolar IC's as a principal Research Engineer. In 1987, he joined the Department of Electrical Engineering at KAIST, Taejon, Korea, as an Assistant Professor. From 1998 to 2001, he was a Professor. He died in 1 May 2001. He had been researched polysilicon TFT technology, crystalline or poly-Si active-matrix liquid crystal displays (AMLCD's) and MEMS including inkjet print heads and biosensors.