ELECTRO-ENZYMATIC GLUCOSE SENSOR USING HYBRID POLYMER FABRICATION PROCESS

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Abstract

In this article, we present an electro-enzymatic glucose sensor fabricated using a novel self-aligned and hybrid polymer fabrication process. The self-aligned fabrication process was carried out using polydimethylsiloxane (PDMS) as a process substrate material, SU-8 (a negative, epoxy based photoresist) as a sensor substrate material and gold as an electrode material. The electro-enzymatic glucose sensor was assembled from microfabricated components using a self-registration step. The sensor substrate is optically transparent and flexible. Utilizing the process, a wide range of bio-sensors for different constituents (e.g. lactate, pO_2 etc.) can be fabricated.

The glucose sensor was successfully fabricated using our new multilayer SU-8 process on PDMS substrate. The sensor thickness was measured between 117.15 µm and 140.15 µm. The current response of the multi-layer electroenzymatic sensor using different glucose concentrations is also measured. Besides, the current response for long term stability is also presented.

I. INTRODUCTION

It has been well established that diabetes is major disease throughout the world. The abnormality of blood glucose concentration may lead to serious physical conditions like retinopathy with blindness and kidney failure with uremia [1]. Hence, maintaining a desirable blood glucose level is very important.

Different types of glucose sensors have been designed utilizing different principles. Glucose sensors using optical detection [2]-[5] and affinity binding [6]-[8] has been designed previously. However, electro-enzymatic principle [9]-[16] for glucose detection is most popular among all the techniques. Since their invention in 1960's, electro-enzymatic glucose sensors have remained a major focus for glucose sensors research.

Previously, a flexible and optically transparent electro-enzymatic glucose sensor using polymer membranes was proposed [10]. However, the fabrication required many steps of manual alignment and assembly of different components which is not always easy or reliable.

Usually, glucose sensors are designed using platinum (Pt) as an active electrode metal and silver/silver chloride (Ag/AgCl) as a reference electrode metal. Platinum is an expensive metal and the fabrication process for silver/silver chloride is not easy or reliable. Because of the reliability issues with the silver/silver chloride electrodes, the sensor response usually drifts over a longer period of time.

In order to improve some aspects of the electro-enzymatic glucose sensor, we propose a novel and simple method to fabricate an electro-enzymatic glucose sensor providing a cost effective solution for higher process reliability. The proposed sensors are designed using SU-8 and gold. Both the active and reference electrodes are fabricated with only gold. These gold electrodes are sandwiched between the two SU-8 layers which is an optically transparent material.

The gold electrodes are easy to fabricate using conventional microfabrication facility and gold is not as expensive as platinum. Similarly, SU-8 can also be processed without any special equipment. The sandwiched sensors fabricated using gold and SU-8 are fairly flexible which make them ideal for curved surfaces.

II. ELECTRO-ENZYMATIC SENSOR DESIGN

The electro-enzymatic sensor is designed using multilayer SU-8 and gold process. The exploded view of the sensor (Figure 1) indicates the sandwiched gold electrodes between two SU-8 layers.

The bottom SU-8 layer is a supporting base for the electrodes and it also separates each sensor on the same substrate. The top SU-8 layer is designed to enclose the gold electrodes. The electrodes in the actual sensing (active) area are left uncovered to interact with the glucose solution as well as to confine the glucose oxidase (enzyme) on one of the electrodes. The contact pad openings are made to connect the sensor to the external world for the electrical testing.

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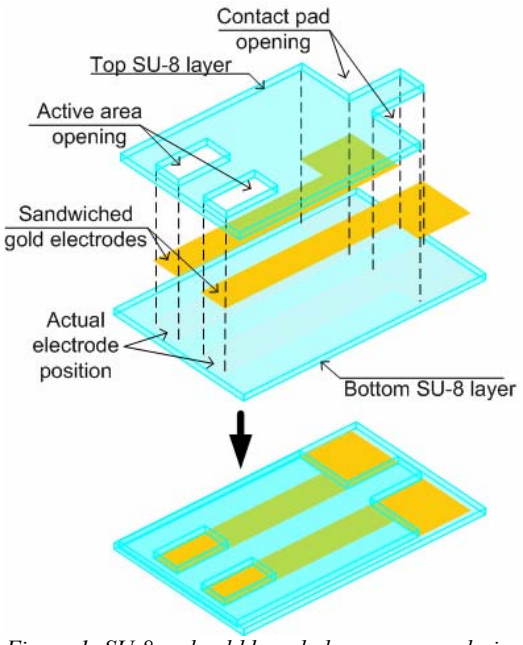


Figure 1: SU-8 and gold based glucose sensor design

The actual sensing area is 2 mm long and up to 3 mm wide (Figure 2). However, the electrode area in the sensing region is 2 mm x 2 mm in all the designs.

The overall sensor is 10 mm long and 5 mm wide (Figure 2). The thickness of the sensor can be varied to achieve desired flexibility and strength. Three different electrode designs (Figure 2) using the hybrid polymer process enable determination of electrode separation as well as conductor dimension on the sensor response.

PDMS is used as a process substrate material for the electro-enzymatic glucose sensor. The advantages of PDMS as a process substrate become apparent during the fabrication process. For example, during the fabrication process the SU-8 has good enough adhesion on to the PDMS substrate. However, after completion of all the fabrication steps, including hardbake of the SU-8, the fully processed SU-8 based sensors easily peel-off from PDMS because SU-8 and PDMS has different flexibility and not excellent adhesion. The glass/silicon substrate is reusable for more process cycles. Such an SU-8 release technique from a PDMS substrate has never been proposed before.

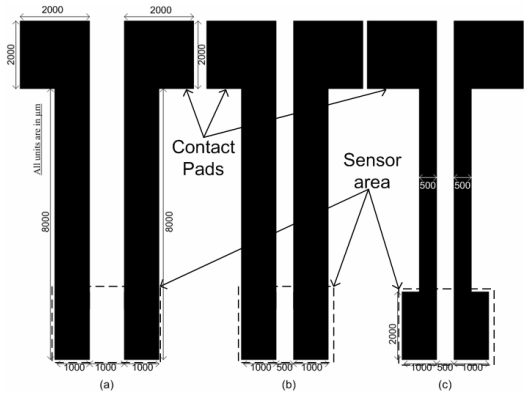


Figure 2: Three designs of gold electrodes for the glucose sensors

III. HYBRID FABRICATION PROCESS

The electro-enzymatic sensors fabrication was done in two different steps. First, the gold electrodes sandwiched between the SU-8 layers were fabricated using the hybrid polymer fabrication process. Finally, the glucose oxidase solution (enzyme) was immobilized on one of the gold electrodes.

A. Fabrication of the sandwiched gold electrodes

In the hybrid polymer fabrication process, two polymers, PDMS and SU-8 were used. The hybrid fabrication process was carried out using simple self registration fabrication process (Figure 3).

The fabrication steps for the sandwiched gold electrodes are given below:

a. To fabricate the PDMS process substrate, Sylgard® 184 elastomer with 10% curing agent was mixed and poured on a glass substrate. The mixture was cured at 85° C for 2 hours and 30 minutes (Figure 3(a)).
b. 100 µm thick SU-8 2035 was spun on to the PDMS and soft baked by ramping up the temperature. The SU-8 layer was exposed with the first mask to make the bottom rectangular layer. The 365 nm (i-line) UV source was used to expose the SU-8 layer. The SU-8 was baked again for a post exposure bake by ramping up the temperature and finally, the unexposed SU-8 layer was developed using the SU-8 developer until the development was complete (Figure 3(b)).
c. To make gold electrode patterns, 50 nm chromium and 100 nm gold layer was sputtered using the Corona sputtering system (Figure 3(c)).
d. Positive photoresist was patterned using the electrode mask, and gold and chromium were etched sequentially using Transene® gold and chromium etchant. After stripping the photoresist, the gold electrodes were ready for further processing (Figure 3(d)).
e. Again, a 100 µm thick layer of SU-8 2035 was spun. This layer was soft baked and exposed with the top layer SU-8 mask which was patterned with the electrode and the contact pad openings (Figure 3(e)).

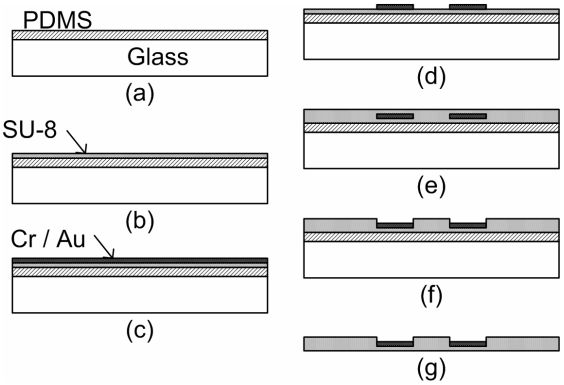


Figure 3: Fabrication process: (a) Pour PDMS on glass slides, (b) Spin SU-8, (c) Sputter Chromium and Gold, (d) Pattern Chromium and Gold using electrode mask, (e) Spin second layer of SU-8, (f) Pattern SU-8 for contact and active area opening, and (g) Remove sensors from PDMS substrate

f. After the post exposure bake, the second SU-8 layer was developed with the SU-8 developer until all the sensors were fully developed. The gold electrodes sandwiched between the SU-8 layers were released from the PDMS process substrate. Before releasing the sensors, all the SU-8 layers were hard baked on a hot plate (Figure 3(f)).

g. All the sensors were automatically detached from the PDMS while the PDMS was peeled off from the glass substrate.

After successful fabrication of the sandwiched gold electrodes, the glucose oxidase solution was prepared and immobilized as explained in the next sub-section.

B. Enzyme immobilization method

The enzyme immobilization process on the gold electrode is as follows:

- Glucose oxidase powder was mixed with a 7.4 pH phosphate buffer.
- After the addition of glutaraldehyde and bovine serum albumin (BSA) the solution was stored in a 4 ºC fridge until needed.
- Meanwhile, the electrodes were cleaned with oxygen plasma, washed with high purity DI water, and dried under a filtered stream of nitrogen gas.
- The opening in the top SU-8 layer, over the gold electrode, served for selective deposition of enzyme.
- Holes in the top SU-8 layer over the gold electrode was filled with the chilled enzyme solution and left at room temperature for 30 min to immobilize the enzyme. The gold electrode on which this solution was immobilized worked as the active electrode.
- Sensors with the immobilized enzyme were again stored at 4 ºC until needed for further processing or analysis.

IV. RESULTS AND DISCUSSION

After successful fabrication using the hybrid process, the thickness of each layer was measured using either profilometer or optical microscope. The results are tabulated in Table 1, with overall thickness of the sensors ranging between 117.15 µm and 140.15 µm (Table 1).

A magnified view of a sensor after fabrication depicts the active regions (Figure 4). The resulting glucose sensors are flexible (Figure 5) and can withstand a deflection of ~33° prior to breaking, increasing their reliability in situations where the sensors are located in instrumentation worn on the body.

The electrical testing of our completed glucose sensors was carried out using the circuit shown in Figure 6. The active electrode was connected to -0.5 V with respect to the reference electrode. LabView® 8 from National Instruments Co. was used to measure and log the current response of the sensor. As a test glucose solution, pre-calibrated glucose solutions (50 mg/dl, 200 mg/dl, 400 mg/dl, and 600 mg/dl) were prepared by mixing the glucose powder in the phosphate buffer. The concentration of the glucose solution was calibrated using a commercially available portable glucose monitors (LifeScan OneTouch Ultra ®). Typical test results are shown in Figure 7.

The current for 600 mg/dl glucose concentration gave a real-time response of 241±49 nA over a period of 165 minutes. The sensor was subsequently immersed in a solution of 50 mg/dl glucose and the current dropped

Table 1. Thickness of different layers after fabrication

Layer Name	Thickness (in µm)	Measured with
PDMS	475 – 525	Microscope
Bottom SU-8	44 – 52	Profilometer
Chrome-Gold	0.15	Profilometer
Top SU-8	73 – 88	Profilometer
TOTAL	117.15 – 140.15	

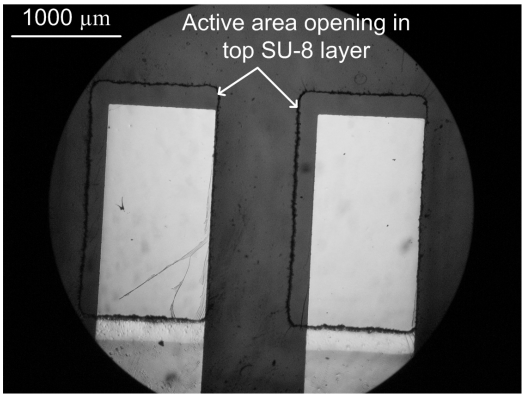


Figure 4: Microscopic image of the active area of the sensor

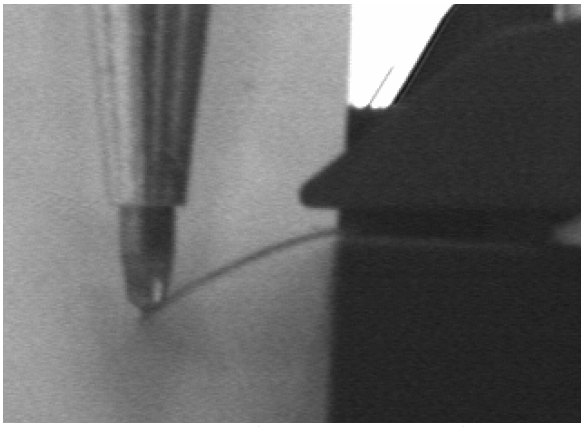
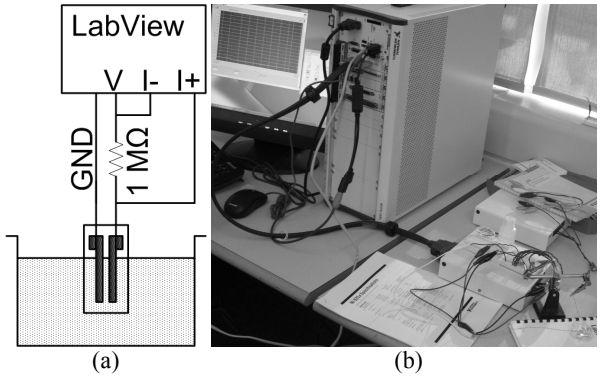


Figure 5: Flexibility test of our sensor (≈33° deflection)



(a) (b)

Figure 6: (a) Test circuit (b) View of test setup

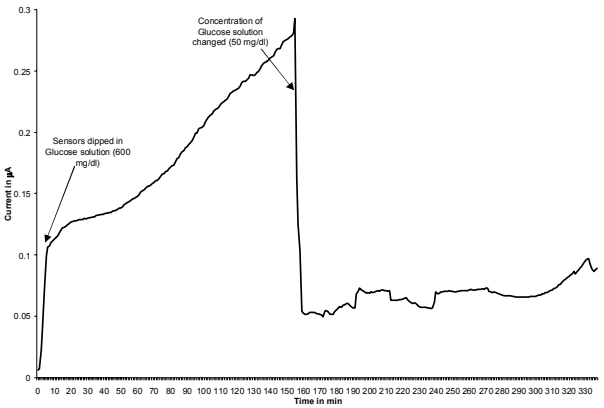


Figure 7: Measured current response of a fabricated glucose sensor

to 74±24 nA over 165 minutes. Although drift is observed in the measured current, the sensor clearly distinguishes between different glucose concentrations. Future efforts will concentrate on improving sensitivity and minimizing drift.

V. CONCLUSION

An electro-enzymatic glucose sensor design using the sandwiched gold electrodes has been presented here. The novelty of the design was described using the exploded view of the electro-enzymatic glucose sensor. The hybrid polymer fabrication process utilizing simple equipments and process steps for microfabrication facility was explained. The enzyme immobilization method has also been described.

The post fabrication results obtained using optical microscopy and profilometry demonstrated successful fabrication of the sensors using hybrid fabrication process. The electrical tests revealed desired functionality of the sandwiched electro-enzymatic glucose sensor.

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