Hybrid polymer fabrication process for electro-enzymatic glucose sensor

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ABSTRACT

We present a novel self-aligned and hybrid polymer fabrication process for an electro-enzymatic glucose sensor. The self-aligned fabrication process is performed using polydimethylsiloxane (PDMS) as a process substrate material, SU-8 as a sensor structural material, and gold as an electrode material. PDMS has many advantages as a process substrate over conventional substrates such as bare silicon or glass. During the fabrication process, SU-8 has good adhesion to the PDMS. However, after completion of all fabrication steps, the SU-8 based sensors can be easily peeled-off from the PDMS. The PDMS is prepared on a glass handle wafer, and is reusable for many process cycles. Such an SU-8 release technique from a PDMS substrate has never been proposed before. The novel process is employed to realize a glucose sensor with active and reference gold electrodes that are sandwiched between two SU-8 layers with contact pad openings and the active area opening to the top SU-8 layer. The enzyme glucose oxidase is immobilized within the confined active area opening to provide an active electrode sensing surface.

After successful fabrication using the hybrid process, the overall thickness of the sensors is measured between 166.15μm and 210.15 μm. The sensor area and the electrode area are 2mm x 3mm and 2mm x 2mm respectively. The resulting glucose sensors are mechanically flexible. A linear response is observed for the glucose sensors, typically between 50mg/dl and 600mg/dl glucose concentrations.

Keywords: Glucose sensor, electro-enzymatic sensor, polymer fabrication, SU-8, PDMS, gold electrode

1. INTRODUCTION

Diabetes mellitus is a disease of major global importance; with the worldwide prevalence predicted to at least double to 300 million people over the next 10 to 15 years[1]. Of major concern is the tendency for diabetic patients to develop specific complications, such as retinopathy with blindness, and kidney failure with uremia. For a detailed account of the major facts relating to diabetes the reader is referred to Pickup and Williams[2].

The need for regular glucose monitoring was highlighted in a study by the Diabetes Control and Complications Trial Research Group (1993) in the USA[3]. Although early debate centered on whether there was a need for a long-term implantable glucose sensor for diabetics[4], recent long-term studies have conclusively demonstrated that if glucose levels can be tightly regulated within the normal physiological range, then diabetic complications can be controlled[5]. Glucose levels fluctuating within the normal physiological range of 110±25mg/dL can be considered to be within an acceptable level of control.

Several new techniques have evolved for glucose analyses in clinical practice[6] as well as in biotechnology[7] and in the food industry[8]. This wide field of applications has inspired much of the glucose sensor development and diversification during the last decade. In the longer term, an implanted glucose sensor could be linked to a portable insulin delivery system thus regulating insulin delivery in response to changes in blood glucose concentration in much the same way that the pancreas of a non-diabetic delivers insulin[9,10].

Many different types of glucose sensors have been developed and can be classified by different aspects of their design. Optical glucose measurement is widely used for portable hand held glucose meters[11-14]. However, this method gives

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only rough glucose concentrations instead of the exact measure of blood glucose because it is based on visual color matching. Furthermore, this method requires a needle puncture and blood extraction which can be painful and limits the number of tests that can be performed per day. Glucose detection using the affinity principle[15-17] detects a change in the physical properties of a sensitive cantilever, usually a change in the resonant frequency due to mass loading. Concanavalin A and dextran are one of the most popular ligand and receptor pairs for affinity type glucose sensors. Mass production of these sensors is very difficult because this ligand and receptor pair requires manual assembly steps during fabrication, and the devices are not as simple as those based on electro-enzymatic principles.

The oldest electro-enzymatic detection principle, developed by Clark in the 1960's, is still of interest to many researchers because of its high selectivity to glucose. The stage of proof of concept for such a device has passed, and currently efforts are aimed at improving the performance of electro-enzymatic sensors. This method faces some problems that still need to be addressed.

First among the problems with an enzymatic glucose sensor is the unpredictability of the Ag/AgCl electrode fabrication and drift in current. Some methods to fabricate Ag/AgCl electrodes have been published previously[18-23]. These methods for Ag/AgCl fabrication have very low predictability and involve complex processing steps. Moreover, platinum is widely used as an active electrode but is quite an expensive material for large scale production of glucose sensors. In addition, incorporation of different metal electrodes (e.g. Pt and Ag/AgCl) increases the number of process steps in fabrication.

Another problem in designing implantable glucose sensors is the biocompatibility of the sensor material. The interference from protein and other constituents has been studied by researchers for several decades[24-28]. For biocompatibility and haemocompatibility, a few polymer membranes (e.g. Nafion and polyurethane[29]) can be used to suppress platelet aggregation, although these materials are not as easily microfabricated as other polymers.

Finally, flexibility and optical transparency is crucial for sensors that may be worn or implanted, such as within contact lenses in the eyes for detecting glucose in tears. However, a fully transparent sensor must be fabricated using optically transparent electrodes (e.g. ITO) and such a transparent sensor should be designed using a flexible material such as a polymer. The only design that is both optically transparent and mechanically flexible has been proposed by Mitsubayashi et. al.[30]. However, this design uses platinum or indium tin oxide (ITO) and silver/silver chloride electrodes and metallization on the PDMS surface that is not a highly reliable process.

In order to address some of the problems discussed above, an SU-8 based flexible glucose sensor with optically transparent substrate material is presented here. Gold electrodes (both the active and the reference electrodes) are presented for the first time with a characterization of the current drift over time. In order to prepare a fully transparent glucose sensor, the gold electrodes can be replaced with a transparent electrode material such as indium tin oxide (ITO). A simple microfabrication compatible fabrication process flow without any manual interaction is also presented. This hybrid polymer fabrication process helps to easily remove flexible SU-8 based sensors from the PDMS process substrate. The method for immobilizing glucose oxidase on the biocompatible sensor (SU-8[31-33] and gold[32,34]) is described in detail.

2. SENSOR DESIGN

The flexible electro-enzymatic sensor is fabricated using SU-8 as a sensor material. SU-8 is widely used for microfluidic and biomedical applications because of its ease of fabrication and biocompatibility[31-33]. PDMS is used as a process substrate which helps to easily release the SU-8 based sensors from the process substrate. The proposed hybrid polymer fabrication process offers easy release of the SU-8 based sensors from the PDMS process substrate. Such a hybrid polymer process for SU-8 release has never been proposed before.

In addition, both the active and the reference electrodes of the flexible sensor are fabricated using only gold. Gold has long been recognized as a traditional material for microfabrication because it is easy to pattern, unlike silver/silver chloride (Ag/AgCl), and it is much cheaper than platinum. Gold, similar to platinum, is also a biocompatible material[32-34]. The motivation for further characterization of gold electrodes for the flexible sensors comes from the primary results obtained on a glass substrate[35].

The flexible and optically transparent electro-enzymatic glucose sensor is designed based on the material background discussed above. The presented hybrid polymer fabrication process can be easily carried out with the basic equipment

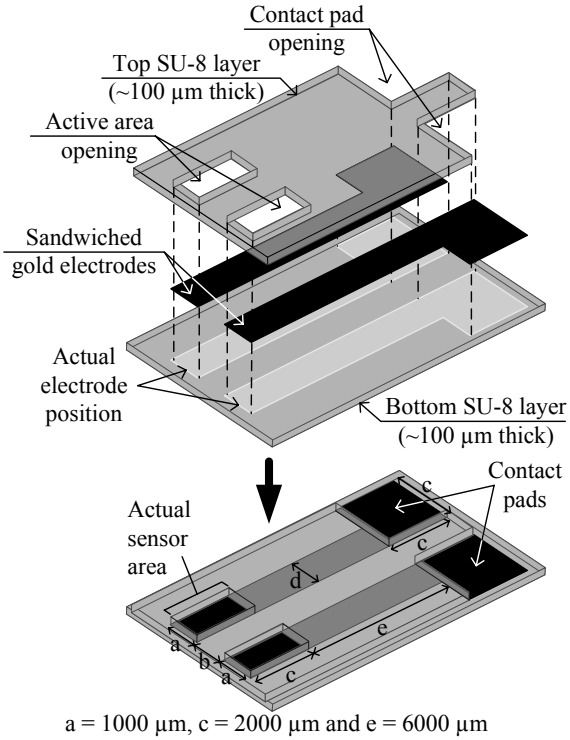
and materials available in any microfabrication facility. Furthermore, the process can be used for batch fabrication of many sensors at once, which is crucial for commercialization of any glucose sensor. The sensor design avoids any manual assembly or alignment steps and only uses a mask aligner, a metal deposition system, a spin coater, and hot plates. The presented sensors are intended to be very cost effective, simple to fabricate, and easy to release after complete fabrication.

In order to test and characterize different properties of the flexible electro-enzymatic glucose sensor, we designed a flexible sensor with sandwiched gold electrodes between two SU-8 layers (Fig. 1). The bottom layer of the sensor is a 100µm thick rectangular SU-8 layer. This bottom layer is little larger than the area occupied by the electrodes.

The top sensor layer is designed using a 100µm thick SU-8 layer with contact pad openings and electrode openings (Fig. 1). The electrode opening is provided only in the sensing area. Two separate openings are designed so that any of the gold electrodes can be used as an active electrode by immobilizing the enzyme on them. The area of the top SU-8 layer is a little smaller than the bottom layer area and the contact pad opening is not fully enclosed. And, as mentioned previously, both gold electrodes are sandwiched between the two SU-8 layers.

Three designs of electrodes are used to fabricate different types of sensors (see Fig. 1). All three designs consist of three sections: a sensing area, a connecting conductor, and a contact pad. Each sensor die is 5 mm x 10mm in size and is fabricated with gold electrodes sandwiched between two layers of SU-8 (Fig. 1). In a single step metal patterning process, the active and the reference electrodes, the connecting conductors, and the contact pads were fabricated using only gold. It should also be noted that the sensor dimensions, including bond pad dimensions, were chosen for easy handling and testing; the batch fabrication process could be utilized for both larger and much smaller sensors, with scaling effects currently under investigation.

The actual sensing area, in all three designs, is clearly labeled in Fig. 1. The sensing area in the first design is 2 mm x 3 mm (Fig. 1) and 2mm x 2.5mm in the other two designs (Fig. 1). However, the active and reference electrode areas in



a = 1000 µm, c = 2000 µm and e = 6000 µm

	Design 1	Design 2	Design 3
b	1000 µm	500 µm	500 µm
d	1000 µm	1000 µm	500 µm

Fig. 1: Design of the flexible polymer based electro-enzymatic glucose sensor showing dimensions for three different designs.

all three designs are 2mm x 2mm in size.

Large contact pads are designed for each electrode to make wire connections easier. Each 2mm x 2mm contact pad is connected with a 6mm long gold conductor. The openings in the top SU-8 layer for the contact pads and the sensing electrode (Fig. 1) are made for the physical connection with wires and for contact with the enzyme respectively. The gold conducting wire is covered with SU-8 to avoid direct external contact (Fig. 1).

3. FABRICATION PROCESS

The hybrid polymer fabrication process of the flexible electro-enzymatic glucose sensor is carried out using PDMS as the process substrate. The PDMS substrate is used to facilitate release of the dual layer sandwiched SU-8 sensor. Moreover, the PDMS process substrate helps to achieve the desired surface profile from the SU-8 process without any process related issues such as cracking in the SU-8 layer. A handle glass wafer or glass slide is used as a support for the PDMS process substrate, and can be reused for many process cycles. In order to obtain different flexibilities, the thickness of both SU-8 layers is varied. A trade-off exists between the flexibility and the breaking strength of the SU-8 in order to design glucose sensors that are both reliable and flexible.

The fabrication of the SU-8 based flexible glucose sensors is divided into two parts. The first part is the fabrication process of the gold electrodes sandwiched between the two SU-8 layers and the second part is the preparation of the glucose oxidase solution and the enzyme immobilization of the solution on the active gold electrode.

3.1 Fabrication of sandwiched gold electrodes between two SU-8 layers

A hybrid polymer fabrication flow for the gold electrodes sandwiched between the two SU-8 layers is presented in Fig. 2. Each step of the hybrid polymer fabrication flow is explained below.

- To fabricate the PDMS process substrate, Sylgard® 184 elastomer with 10% curing agent is mixed and is poured on a glass substrate. The mixture is cured at 85° C for 2 hours and 30 minutes (Fig. 2(a)).
- 100μm thick SU-8 2035 is spun on the PDMS and is soft baked by ramping the temperature. The SU-8 layer is exposed with the first mask to realize the bottom rectangular layer. The 365nm (i-line) UV source is used to expose the SU-8 layer. The SU-8 is baked again for a post exposure bake (to cross-link the polymer) by ramping the temperature. Finally, the unexposed SU-8 layer is developed using SU-8 developer until the unexposed SU-8 is completely dissolved (Fig. 2(b)).
- To make gold electrode patterns, 50nm chrome and 100nm gold layer are sputtered using the Corona sputtering system (Fig. 2(c)).
- Positive photoresist is patterned using the electrode mask, and gold and chrome are etched using Transene® gold and chrome etchant. After stripping the photoresist, the gold electrodes are ready for further processing (Fig. 2(d)).
- A second 100μm thick layer of SU-8 2035 is spun. This layer is soft baked and exposed with the top layer SU-8 mask, which is patterned with the electrode and the contact pad openings (Fig. 2(e)).
- After the post exposure bake, the second SU-8 layer is developed with SU-8 developer. The gold electrodes sandwiched between the SU-8 layers are released from the PDMS process substrate. Before releasing the sensors, all the SU-8 layers are hard baked on a hot plate (Fig. 2(f)).
- All the sensors spontaneously are detached from the PDMS while the PDMS is peeled off from the glass substrate.

Sensors are marked with identification numbers, and are connected with wires using a conductive silver epoxy for testing. At this point, after optical verification, each sensor is ready for the enzyme immobilization and electrical test (Fig. 2(g)).

3.2 Immobilizing glucose oxidase

The step-by-step process to prepare the glucose oxidase solution and to immobilize the solution on the active gold electrode is as follows:

- Glucose oxidase powder (Sigma-Aldrich Canada Ltd.) is mixed with a 7.4pH phosphate buffer (VWR, stock # CA34170-115).
- After the addition of glutaraldehyde (Sigma-Aldrich Canada Ltd.) and bovine serum albumin (BSA) (Sigma-Aldrich Canada Ltd.) the solution is stored in a 4ºC fridge until needed.
- Meanwhile, the electrodes are 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, serves for selective deposition of the enzyme.
- Holes in the top SU-8 layer over the gold electrode are filled with the chilled enzyme solution and are left at room temperature for 30min to immobilize the enzyme. The gold electrode on which this solution is immobilized works as the active electrode.
- Sensors with the immobilized enzyme are again stored at 4ºC until needed for either further processing or analysis.

4. EXPERIMENTATION

Characterization of our gold electrodes included analysis for consistency between each of the final sensors. We used a combination of optical microscopy and profilometry to monitor uniformity in dimensions and thickness of our flexible sensor array. Each sensor was tested for electrical conductivity before immobilizing the enzyme on them.

Before testing the sensors, calibrated solutions of glucose (50, 200, 400 and 600mg/dl) were prepared by mixing glucose powder (VWR, stock# CADX0145-1) in the phosphate buffer (VWR, stock # CA34170-115). The glucose concentration in each solution was verified using a commercially available digital blood glucose meter (LifeScan OneTouch Ultra blood glucose monitoring system).

The circuitry used to measure and log the current response of each sensor is shown in Fig. 3. We prepared a test setup using Labview® (National Instruments). As seen in Fig. 3, the supply voltage (0.5V) was also supplied by the PC based

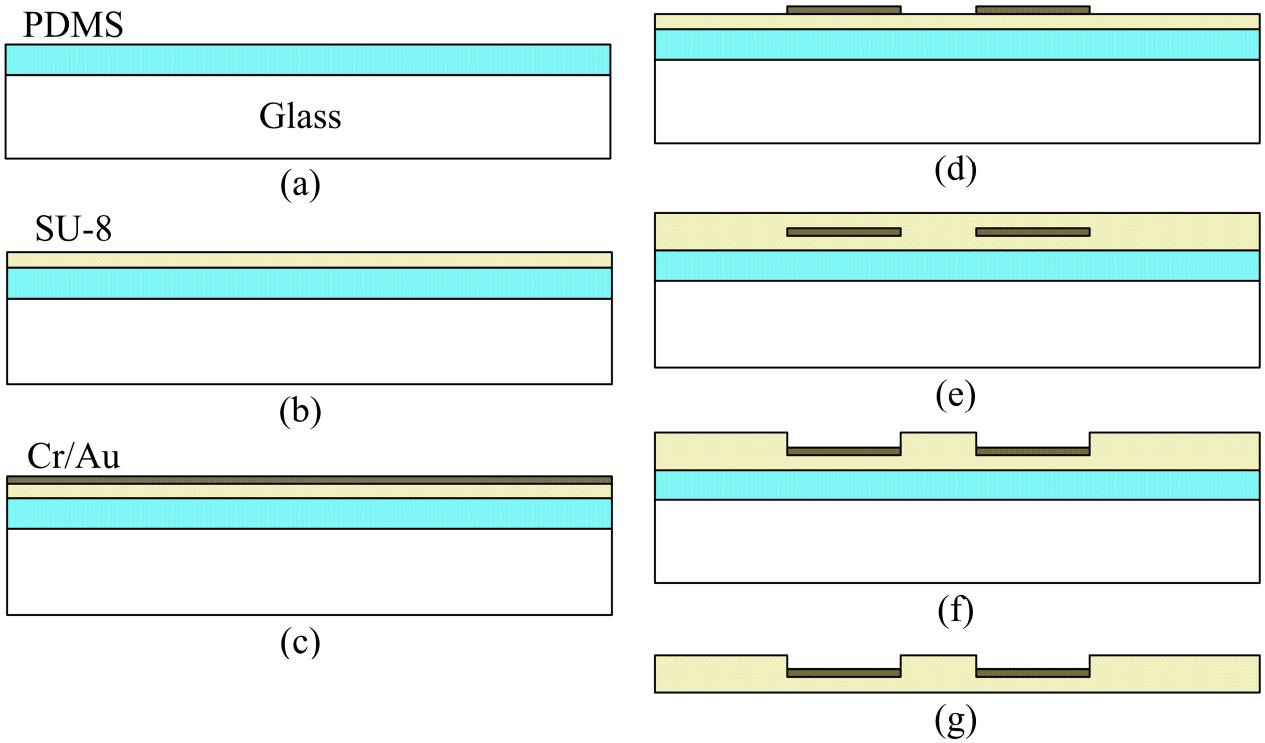


Fig. 2: Hybrid polymer fabrication process for the sandwiched gold electrodes between the two SU-8 layers: (a) Pour PDMS on a glass slide/wafer and cure it to obtain PDMS-on-glass process substrate; (b) Spin, soft bake, expose, hard bake and develop the first (bottom) SU-8 layer; (c) Sputter chrome and gold on the bottom SU-8 layer; (d) Pattern gold and chrome to obtain the gold electrodes; (e) Spin and soft bake the second (top) SU-8 layer; (f) Expose, hard bake and develop the second (top) SU-8 layer (this will open the contact pad openings and the active area openings, but leave the gold covered everywhere else); and (g) release each sensor die by peeling off the PDMS process substrate from the glass slide/wafer

Labview system and readings were measured using the same system. The initial measurement reflected that the actual current generated was very low and was very hard to measure. Hence, to reliably measure the low current, a 1MΩ resistance was connected in series. To quantify the actual current value, the voltage drop across the resistance was measured. The picture of the actual test setup is shown in Fig. 4.

5. RESULTS AND DISCUSSION

The wafer with sensors was optically checked several times during the hybrid polymer fabrication process. After the electrodes were patterned on the first layer of SU-8, electrical conductivity of all the electrodes were satisfactorily checked before further fabrication. The results obtained from each test during the fabrication process and testing using actual glucose solution of different concentrations, as well as sensor drift characterization, are discussed below.

5.1 Results after fabrication process

After successful fabrication of each sensor on the PDMS process substrate, all the sensors were released from the PDMS substrate without difficulty. The electrical conductivity of each sensor was checked and the sensors with lost conductivity were discarded. In total, 102 sensors were fabricated in three process runs. The process yield for usable sensors was ~79%. A collection of dual layer sandwiched SU-8 sensors are shown in Fig. 5. All of the sensors shown in this picture are working devices from the same process run. The microscopic view of the sensing area of one sensor is shown in Fig. 6. The active and reference electrode openings (active area opening) are identified in the figure. The active area opening is useful in immobilizing the enzyme to a confined space on the active electrode. The image shows uniform and defect-free gold electrodes and SU-8 layers.

The microscopic image (Fig. 6) shows no significant defects in the SU-8 layers and the gold layer. The thicknesses of all the layers were measured using a profilometer throughout the hybrid polymer fabrication process. The thickness of the first SU-8 layer was measured to be between 88µm to 108µm. The thickness of the chrome and the gold layer was measured to be 0.15±0.02µm and the thickness of the top SU-8 layer was measured to be between 78µm to 102µm.

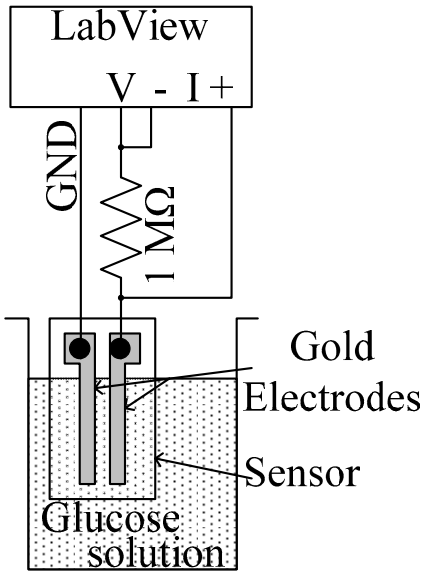


Fig. 3: Test setup using Labview® to measure and log the current drift and the linearity response

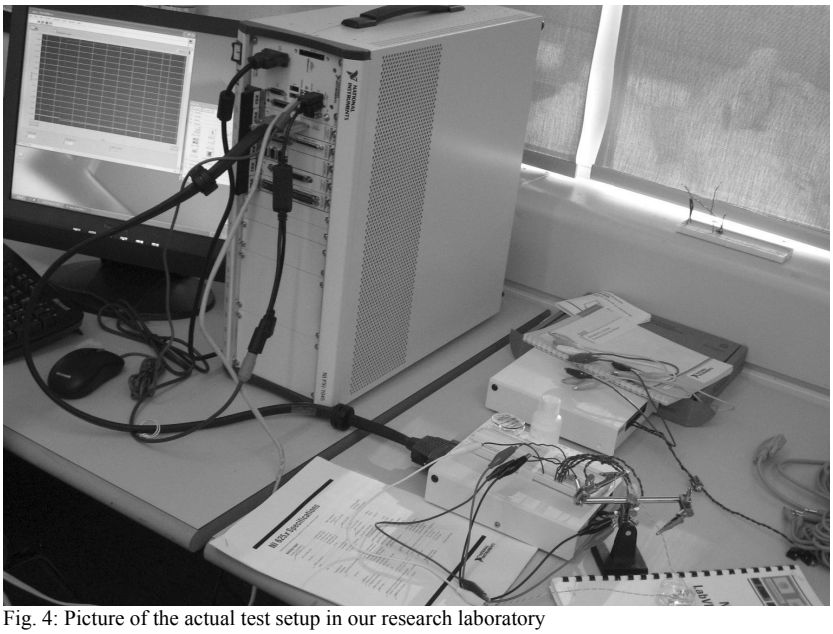


Fig. 4: Picture of the actual test setup in our research laboratory

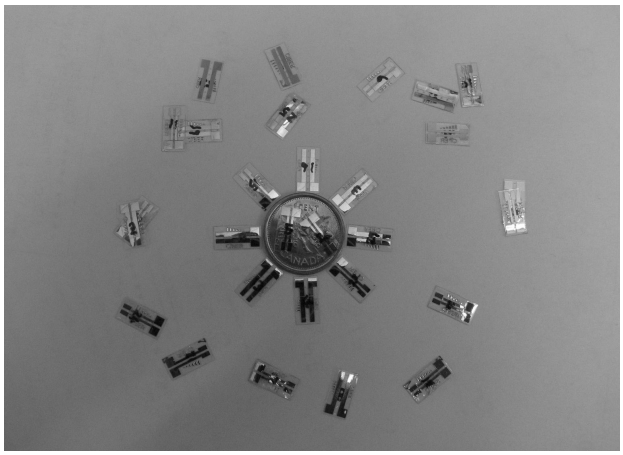


Fig. 5: Successfully released flexible electro-enzymatic glucose sensors from the PDMS substrate

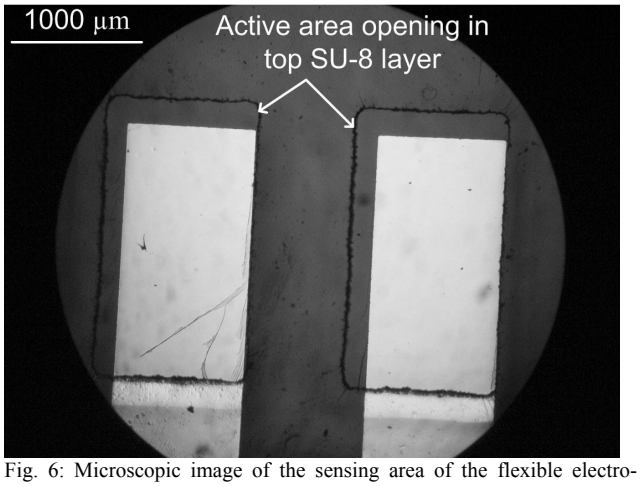


Fig. 6: Microscopic image of the sensing area of the flexible electro-enzymatic glucose sensor

5.2 Results for the current response

After successful fabrication, the current response of the glucose sensor was tested. The current response of the fabricated sensors were measured by submerging the sensors in a pre-calibrated glucose solution. The current response (Fig. 7 and Fig. 8) was measured and logged using the LabView® system (Fig. 3). Current drift at 600mg/dl glucose concentration is plotted in Fig. 7 and response of the glucose sensor with different glucose concentrations is shown in Fig. 8. The current drift (Fig. 7) during the test period (about 90min.) was measured and linear approximation was drawn. 0.1µA current was measured in the beginning (Fig. 7). Steadily, this current kept rising and finally reached 0.2µA after the 90min period.

The linearity of the flexible sensor with different glucose concentration was also measured. The current response for the fabricated sensors using different glucose solutions (0mg/dl, 200mg/dl, 400mg/dl and 600mg/dl) is shown in Fig. 8. As

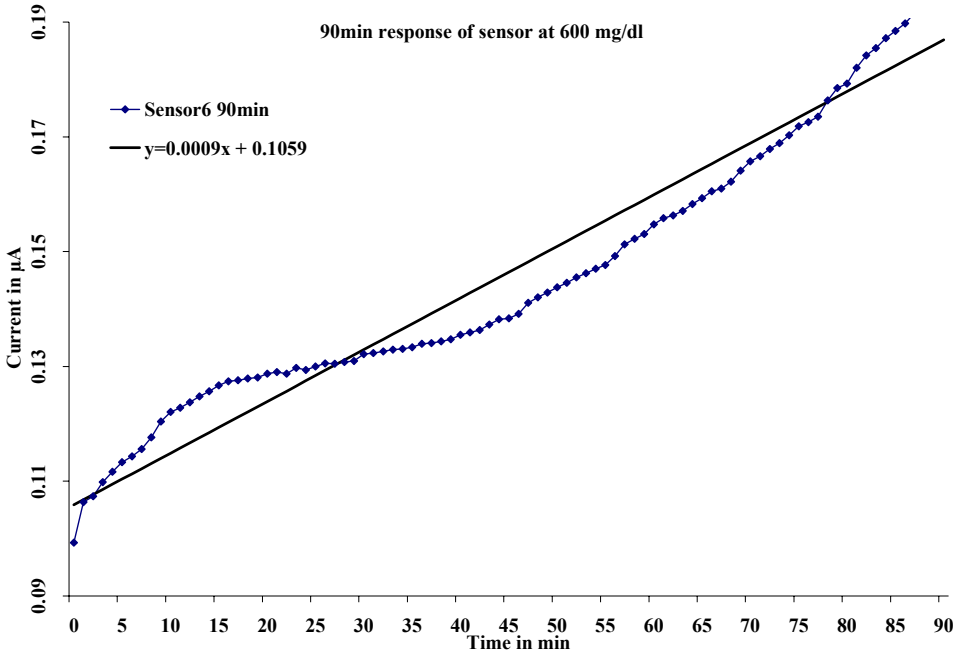


Fig. 7: Current drift response of the flexible sensor (with enzyme) in the glucose solution of 600 mg/dl

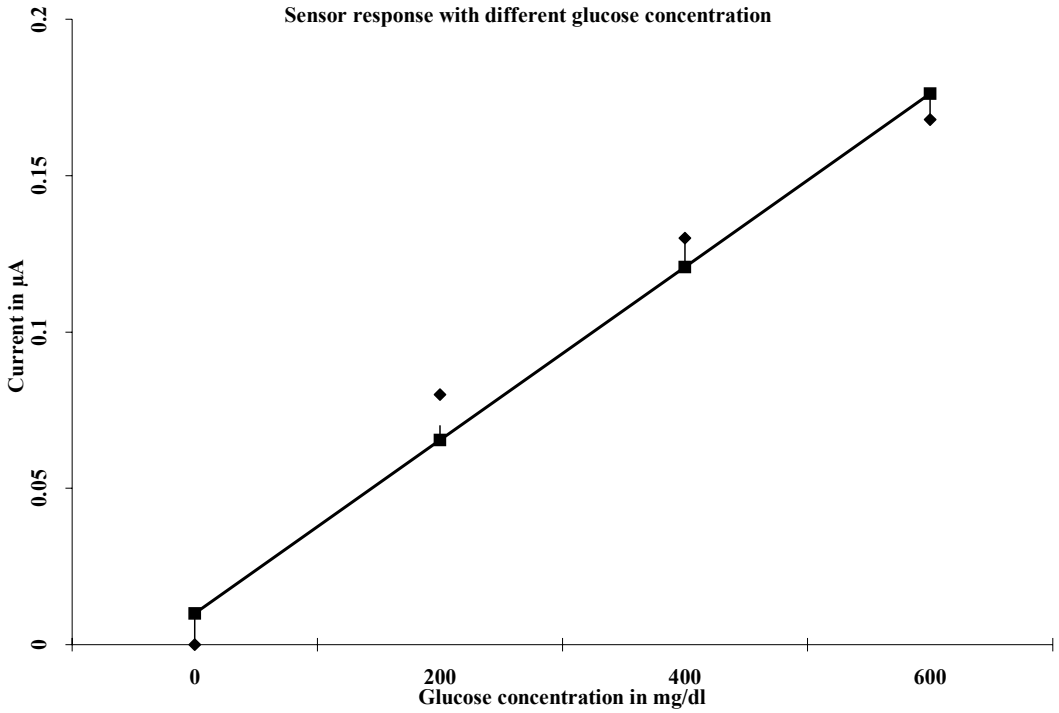


Fig. 8: Current response of the presented glucose sensor for different glucose concentrations.

presented in Fig. 8, the flexible glucose sensor response was linear in the 0 to 600mg/dl range.

The linear current response with different glucose solutions showed successful fabrication of the glucose sensor using presented novel fabrication process (Fig. 8).

6. CONCLUSIONS

A flexible polymer based electro-enzymatic glucose sensor using a new two layer SU-8 fabrication process, PDMS releasing substrate, and gold electrodes has been presented for the first time. The new fabrication process for flexible sensors with transparent hybrid polymer fabrication technique using PDMS and SU-8 was successfully demonstrated and used to fabricate 102 sensors in 3 process runs, with the process yield of 79%. The total thickness of the fabricated sensors is measured between 166.15µm and 210.15µm. The actual sensor size in all three designs is either 2mm x 3mm or 2mm x 2.5mm. Both active and reference gold electrodes were successfully tested. The SU-8 polymer based glucose sensor is economical to fabricate, with the use of gold electrodes making the fabrication process very simple to perform in any basic micro-fabrication facility.

Post fabrication tests such as electrical conductivity, profilometry, and glucose concentration response were utilized to characterize the process and working sensors. Optical imaging and electrical conductivity tests show for the first time successful fabrication of the electro-enzymatic glucose sensor using the novel materials in the dual layer SU-8 process with sandwiched gold electrodes. A linearity measurement was successfully done on working glucose sensors, showing a linear response for sensors in the 0 to 600mg/dl range. Sensor drift was also characterized, and found to be approximately linear with a 0.1 µA drift over a 90min period.

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