Electro-Enzymatic Sensor for Non-Invasive Glucose Measurement

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Abstract— *In this article, a cost effective and simple electro-enzymatic sensor to measure glucose concentration non-invasively is proposed. Measurement of blood glucose concentration for early diabetes detection is one of the major health concerns. Usually, blood or other biofluid is collected by puncturing the finger or fore arm (invasive) which is a painful process. Hence, blood glucose measurement by non-invasive method is more desirable. Here, we propose a first step towards our final goal of non-invasive glucose measurement from human tears using contact lenses.*

Keywords- Glucose sensor, electro-enzymatic sensor, non-invasive sensor

I. INTRODUCTION

Blood glucose of diabetic patients is measured principally to detect hypo- and hyperglycemia and to monitor treatment in order to maintain normal glucose levels. Several new techniques have evolved for glucose analyses in clinical environments [1] as well as in both biochemical [2] and in the food based industries [3]. This far-reaching and diverse field of applications has inspired substantial development and diversification of glucose sensors. The desire to control diabetes remains a dominant force behind the research efforts of numerous investigators from different academic disciplines and industry, with significant efforts being focused on implantable short and long-term glucose sensors. 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 [4],[5]. The onset of any diabetes related complications could be more easily detected with such an implantable sensor with potential savings in long-term health care expenses.

The goal of maintaining normal physiological levels of glucose has led to the development of many glucose sensing devices suitable for measuring glucose levels both *in vivo* and *in vitro* [6],[7]. Most of these sensors are based on electrochemical principles and employ enzymes for molecular recognition. Continuous long-term monitoring *in vivo* using electrochemical biosensors is, however, still the focus of much research. Reliability in manufacturing and operating these biosensors is a significant limitation to their utilization in practice.

The first electro-enzymatic glucose sensor was developed in 1960's by Clark [8] Since then, many researchers have concentrated on designing glucose sensors for early diabetes detection. Previously, glucose concentration was only measured in a clinical environment. Necessity for continuous monitoring and patient-administered therapy has resulted in pocket-sized devices for the patient to directly measure glucose concentrations.

The oldest electro-enzymatic principle proposed by Clark is still of interest to many researchers because of its high selectivity to glucose. Devices incorporating this technology have already passed the stage of proof of concept. Efforts are now being made to improve the performance and reliability of electro-enzymatic sensors.

Recent research also focuses on the electron transfer mediator between the enzyme layer and the electrode layer [9] to improve the performance of glucose sensors. For example, conducting polymers [18]-[20] can mediate the transfer of electrons. Polypyrrole doped PDMS and Nafion membranes [20] can also increase glucose permeability and haemocompatibility.

Here, we focus on our first step towards development of non-invasive electro-enzymatic glucose sensor. The electro-enzymatic glucose sensors were designed using gold (Au) electrodes on glass substrates to reduce production cost and simplify the fabrication process. Glucose oxidase was used as a glucose sensitive enzyme layer to measure glucose concentration.

In the following section, the design of the glucose sensor will be discussed. In section III, the fabrication process for the proposed glucose sensor is described. In section IV, experimentation preparation and test setup is explained. Test results are mentioned in section V. Finally, the paper is concluded by highlighting the main results of our research.

II. PROPOSED DESIGN

We fabricated two types of sensors based on previous designs of electro-enzymatic glucose sensors (Figure 2.1 and Figure 2.2). These sensors were fabricated using gold electrodes on a glass substrate. Gold electrodes are easy to pattern using traditional micro-fabrication techniques and are less expensive than platinum electrodes. Careful consideration of materials costs is paramount if the sensors are to be mass produced.

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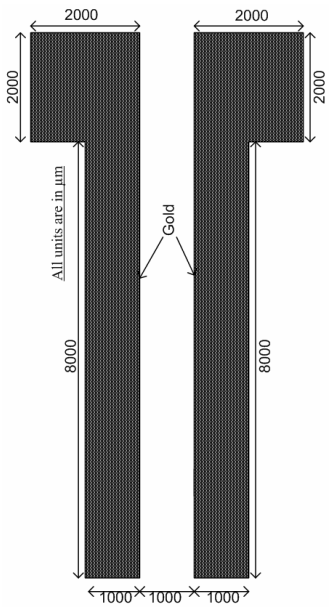

Figure 2.1: First design

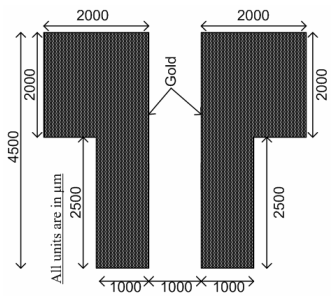

Figure 2.2: Second design

Our active electrode is the gold electrode on which the glucose oxidase enzyme is immobilized. The other gold electrode is used as a reference electrode and to establish a conductive link between the active area and the contact pad. Length of the connecting conductor in the second design is longer for ease of testing and wiring. The larger dimension of the contact pads are designed to connect wires using conducting epoxy.

After finalizing the glucose sensor design, the mask layout of both types of sensors was made using Cadence tools. Only one mask is required for the gold and chromium pattern.

III. Fabrication Process

The fabrication of the glucose sensors consists of two main parts: The fabrication process of the electrodes on glass slides and the immobilization of the glucose oxidase on the gold electrodes.

A. Fabrication of electrodes

The micro-fabrication process for the glucose sensors (Figure 3.1) is performed on glass substrates and is very straight-forward.

First, 0.1 µm thick chromium and gold are sputtered by a Corona sputter process (step 1). Chromium is used as an adhesion layer between the glass and gold. In the next step, photoresist is spun, exposed and patterned (step 2) followed by etching of exposed gold and chromium (step 3). Finally, photoresist is stripped using acetone to reveal the final glass supported gold-on-chromium structures (step 3).

B. Immobilizing glucose oxidase

Before immobilizing glucose oxidase on the electrodes, a thin PDMS mask with rectangular access holes was created to confine the enzymes to the gold electrode. Holes in the PDMS

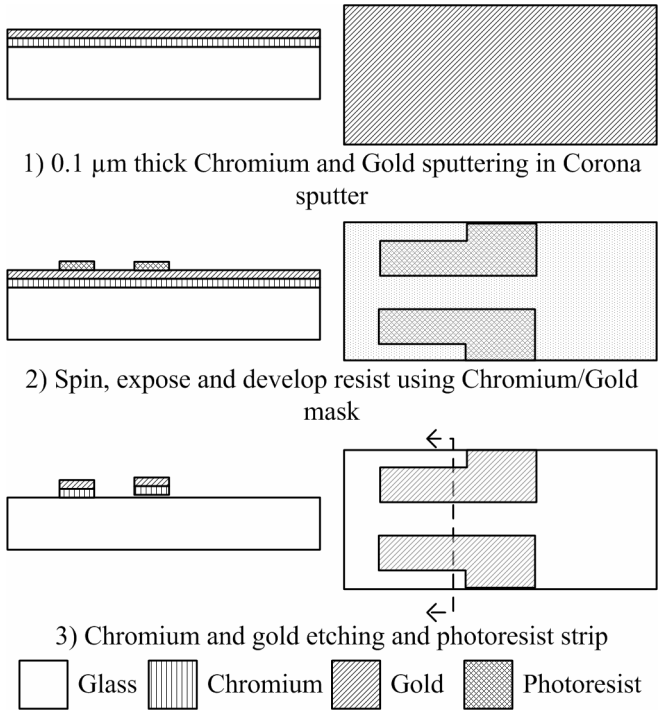

Figure 3.1: Process flow of glucose sensors

mask aligned over the gold electrodes were filled with the chilled solution of enzyme to immobilize the enzyme. Sensors with immobilized enzyme were stored at 4ºC until needed for further processing or analysis. Individual sensors were prepared by cutting the array of electrodes using a dicing saw.

IV. 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 arrays. Further optical microscopy characterization after immobilization of the glucose oxidase confirmed the presence of enzyme on the gold electrodes.

Before testing the sensors, standard solutions of glucose (50, 200, 400 and 600 mg/dl) were prepared by mixing glucose powder in phosphate buffer. Glucose concentration in each solution was verified using a commercially available digital blood glucose meter (LifeScan OneTouch Ultra blood glucose monitoring system). For the test, we have only used glucose solution with 600 mg/dl concentration.

The circuitry to measure current from the sensor is shown in Figure 4.1. After testing using this circuitry, we observed that a more sensitive measuring scheme would benefit our experiments due to the very low current generated by the sensor. It was necessary to modify the measuring circuit to accurately measure very low current (on the order of nano amps) using a multimeter (Figure 4.2). Figure 4.2 indicates that we replaced the multimeter with a 1 MΩ resistor in parallel with a multimeter to measure voltage drop across this resistor. The resistor multiplies the current with 10^6 which converts nA current to mV voltage which can be easily measured using

multimeter. One small problem faced with this new circuit is that the voltage across the sensor is not constant because of the variable current generated from the sensor.

V. Results and Discussion

In this section, test results obtained during different stages of fabrication and testing are mentioned first. The obtained final results follow.

A. Test results

Glass slides with gold electrodes before dicing and immobilization of the enzyme are shown in Figure 5.1. A higher magnification optical microscopy image shows the defects on some of the gold electrode (Figure 5.2).

Small cracks in some of the gold electrodes were observed. The thickness of the gold was measured as 0.1 ± 0.006 µm.

Optical characterization of the individual sensors clearly indicates the presence of immobilized glucose oxidase. In Figure 5.3 the immobilized enzyme is confined with a piece of PDMS.

The sensors were tested using glucose standards. The test measurements were performed with the 600 mg/dl standard, for 45 minutes to observe the drift of the gold electrodes with time.

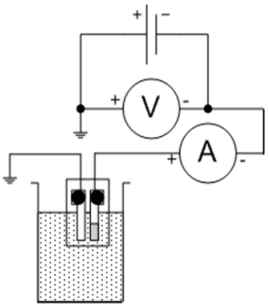

Figure 4.1: First testing circuit

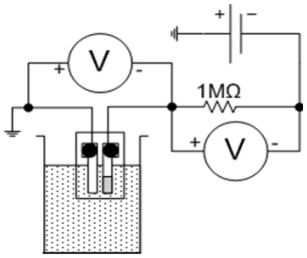

Figure 4.2: Second testing circuit

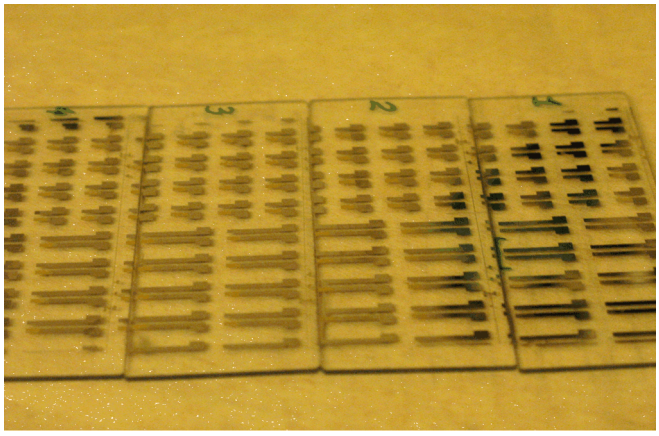

Figure 5.1: Gold Electrodes on Glass Slides

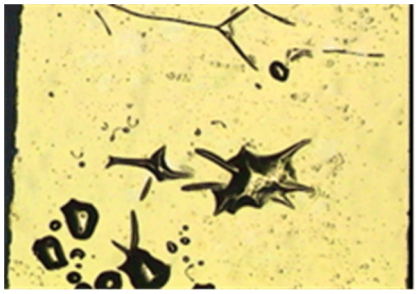

Figure 5.2: Defects in Gold electrode

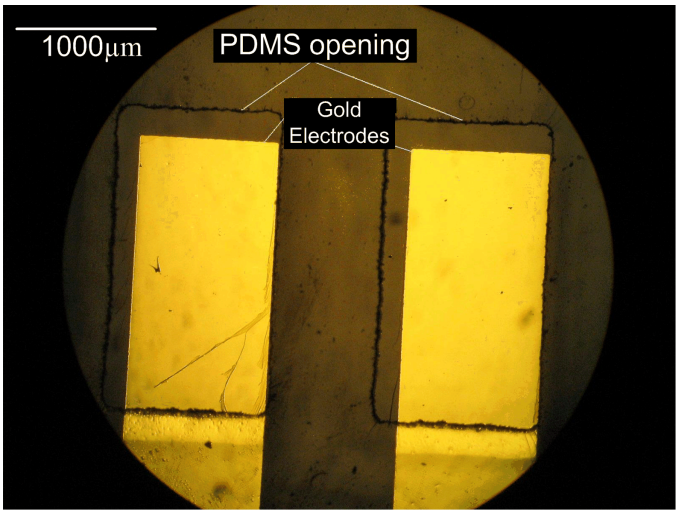

Figure 5.3: Sensor with the piece of PDMS

The current response of the fabricated glucose sensor with respect to the time in minutes is shown in Figure 5.4. The measured current at the beginning is 250 nA. In the first 5 minutes, current decreases quickly and reaches 196 nA. Then current starts to increase again rapidly yet slows down its increase after 20 minutes. The response starts to settle-down after 310 nA. As is clearly shown in Figure 5.4, the rate of increase in current slows down continuously.

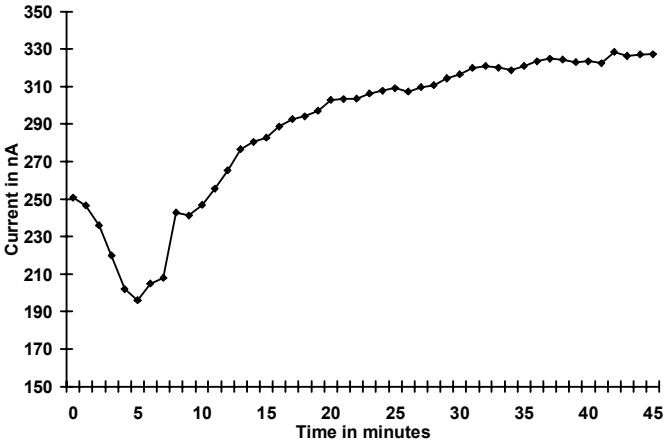

Figure 5.4: Current response of glucose sensor for 45 minutes

B. Discussion

From the test results shown in Figure 5.1 to Figure 5.4 defects in the gold electrodes were observed after the fabrication process. Some gold films lifted off from the substrate and some were badly cracked. However, the defects appear to be a minor component and only scattered amongst a few places over the gold electrode.

The current drift because of the gold electrodes is observed from Figure 5.4. We can see that the test response has changed over the time. Interestingly, results depicted in Figure 5.4 clearly indicate larger change of current initially which stabilizes afterwards.

It is clear that current is generated by the electro-enzymatic reaction at the active electrode of the proposed glucose sensor. In order to achieve more reliable and stable output from the glucose sensor, a detailed analysis of the electro-enzymatic reaction, as well as more robust test setup, is required.

VI. CONCLUSION

In this article, a first step towards realization of a cost effective and simple electro-enzymatic sensor to measure glucose concentration non-invasively is proposed. The proposed glucose sensors are fabricated using gold electrodes on glass substrate. Gold electrodes are easier to microfabricate than silver/silver chloride electrodes. Furthermore, gold is cheaper than platinum if mass production of the sensors is considered. The fabrication process steps of the proposed design are also discussed in detail.

The sensors were tested with -0.5V potential at the active electrode with respect to the reference electrode. The sensors were tested for 45 minutes continuously to observe the drift in generated current. The results show that the current generated from the electrodes decreases initially from 250 nA to 196 nA and then again increases from 196 nA. Initially, a noticeable change in current is observed which decrease continuously. These results indicate successful fabrication of the glucose sensor using only gold electrodes, with further research required to optimize the sensor and stabilize the output.

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