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Performance Characteristics Study of Fabricated Au/Ti Sensor by Using Ferricyanide Redox Reaction for DNA Hybridization Detection

A prototype of DNA indicator-free sensor has been fabricated in-house in the present work using a cost-effective technique and equipment: the latter include thermal evaporator, wet etching, soft lithography, and assembled with adhesive bonding method of polydimethylsiloxane (PDMS). Evaporated gold (Au) of 1 μm thickness acts as the electrode material and Titanium (Ti) of 30 nm thickness serves as an adhesive layer. The Au/Ti layer is designed in a three-electrode system which consists of counter electrode (CE), working electrode (WE) and reference electrode (RE). The output reading, measured by its current and voltage for the three-electrode system, has been verified by customized potentiostat microAutolabIII (μAUT III) in combination with the powerful GPES software. Cyclic voltammetry (CV) of 0.02 M $K_3Fe(CN)_6$ in 0.1 M KCl is analyzed throughout the work for Au bare electrodes, DNA probe immobilization and DNA target hybridization. Two characteristics have been investigated for the DNA sensor prototype, which are the surface roughness and thickness. The root mean square (RMS) value for the surface roughness from atomic force microscope (AFM) image analysis produces values which range from 5.5 to 6.2 nm throughout all Au/Ti fabricated surface. CV results confirmed that the in-house fabricated Au/Ti sensor can be used for DNA detection as it produced sharp and gradient CV curve, the same criteria as compared to the outsourced screen-printed Au sensor.

Key words: Indicator-free DNA sensor; PDMS; Cyclic voltammetry (CV); Electrochemical; Three electrode systems; Redox

INTRODUCTION

Recently, the development of DNA sensors has been aimed to produce devices which are smaller, faster, safer, more accurate and cost-effective. Despite the use of bulky, lab-based, expensive equipment and complicated procedures such as fluorescent [1] and radioactive [2], researchers have reported various DNA detection methods which utilize multidisciplinary applications, such as the electrical readability as in Ion-Sensitive Field Effect Transistor (ISFET) reported by Bergveld (2003) [3] and Bandiera et al. (2007) [4]. The use of semiconductor materials in ISFET which is capable of producing electron and ion movement under selectable threshold voltage will generate the output current that is measurable. Other alternative methods include physical shifts monitoring in the scheme of Surface Plasma Resonance (SPR) [5], Surface Acoustic Wave (SAW) [6] and Quartz Crystal Microbalance (QCM) [7]. These sensors are able to manipulate the heavier size of double-stranded DNA (dsDNA) or hybridized DNA as compared to the single-stranded DNA (ssDNA). It can detect the shift of optical wave at the surface of dsDNA as compared to the ssDNA through the use of a visible infrared light beam. Finally, the knowledge and importance of having a large number of exposed surface area for the DNA immobilization and hybridization have been the motivation behind the development of nanotubes [8, 9], nanowires [10] and gold nanoparticles [11].

Electrochemical methods offer unique advantages over other transduction methods such as those based on the physical interaction, for example SPR, SAW and QCM. The principal advantage is that electrochemical events are kinetic in origin. The resulting electrical signal is demonstrated in a function of the rate of a charge transfer or partitioning process [12]. The electrochemical method of cyclic voltammetry (CV) in ferricyanide is used in this research to determine the electron transfer rate constants of the redox couple reactions that is of interest in the design and improvement of electrochemical biosensors [13]. This research aims to propose the design of electrochemical DNA sensor by using a cost-effective fabrication technique through the use of thermal evaporator and wet etching method to pattern the electrodes in a three-electrode system. The purpose of the sensor is to detect various genes of various probe DNAs immobilization electrochemically and their label-free target DNAs hybridization on the electrodes simultaneously. Thiol group at the 5'-end of probe DNAs is used to link the DNA to the Au electrode [14]. Indicator-free target DNAs are used to be hybridized with the immobilized probe DNAs on the Au electrode. The output current reading from probe DNAs immobilization and target DNAs hybridization are measured electrochemically by the CV method. The anodic peak current from the redox reversible ferricyanide has shown a difference between DNAs immobilization and hybridization.

Previous development on DNA sensors and microarrays has been seen to have utilized photoresist as a coating layer [15, 16] between the three electrodes: working, reference and counter electrodes. The purpose of the layer is claimed to be as an insulating layer, yet it could eliminate the generation of the non-specific redox current from the Au line between the sensing electrodes and the terminal electrodes, due to direct interaction between the exposed Au line and the indicator [15]. However, in this research we have fabricated PDMS to act as a coating layer between the exposed Au terminal electrodes and the sensing electrodes.

The research gives an emphasis on two types of DNA sensors: the outsource screen printed Au sensor which has been commercially used in the Microbiology and Parasitology Lab, School of Medical Sciences, USM Kubang Kerian and the fabricated Au/Ti sensor which has been fully fabricated in the Nano Optoelectronics Research and Technology Laboratory, School of Physics, USM Minden. A study has been done to investigate the performance of the proposed fabricated sensor in comparison to the outsource

screen printed Au sensor. The performance of these two types of sensor is then compared and investigated using the CV graphs measurement.

The fabrication process aims to make use of cost-effective methods and techniques such as soft lithography, thermal evaporation, wet etching and adhesive bonding method for PDMS-glass. Despite using these inexpensive methods and apparatus, the fabricated Au/Ti sensor has proven to be suitable to be used as a sensor device. This paper serves as a part of a project that aims to develop a microfluidic device as a label-free DNA detection sensor interfacing with a hand-held readout circuitry, which consists of a digital multi meter and battery.

MATERIALS AND METHODS

DNAs and apparatus

The DNA capture probe modified with thiol group at the 3' end (5'-GGG GCA GAG CCT CAC AAC CT-$(CH_2)_3$-SH-3') and its complementary DNA target (5'-AGG TTG TGA GGC TCT GCC CC-3') are synthesized by Integrated DNA Technologies (Coralville, IA, USA). Before use, the thiol modified DNA capture probe is treated with DTT and purified by elution through a NAP 10 column of Sephadex G-25 (Amersham Pharmacia Biotech, Uppsala, Sweden) to cleave the disulphide linkage of the thiolated DNA capture probe.

Potassium ferricyanide ($K_3Fe(CN)_6$), potassium ferrocyanide ($K_4Fe(CN)_6$) and potassium chloride (KCl) are purchased from Sigma Aldrich Sdn Bhd (Malaysia), while deionized water is used in all the procedures. CV measurements and data are carried out via ECO Chemie microAutolabIII (Metrohm, KM Utrecht, The Netherlands) using the software package GPES 4.9. Unless otherwise stated, all CV experiments are performed at room temperature using $Fe(CN_6)^{3-}/Fe(CN_6)^{4-}$ redox couple (2 mM each in10 mM KCl) at a scan rate of 0.05 V/s from -0.2 V to 0.6 V.

Electrode fabrication

A glass slide is cleaned sequentially with acetone and isopropanol and dried with nitrogen. RF sputtering (Auto 500: BOC Edwards) of 100% Ar, RF power at 210 W and 2 x 10^{-2} mbar vacuum pressure has been used for Ti fabrication. Then a thermal evaporator (Auto 306: Edwards UK) of 6.0 x 10^{-5} mbar at 68A has been used for Au electrode layer.

Then the electrode layer has to go through the spin-coating process for 15 seconds with positive photoresist (PR1-1000A: Futurrex Inc, USA). A positive mask (Hapmax (M) Sdn. Bhd., Penang, Malaysia) is then placed on the coating electrode layer prior to it being UV-exposed (OAI 150 Exposure Timer: Teltec HK) for 45 seconds. Then it is patterned using wet-etched technique of aqua regia, a mixture of nitric acid and hydrochloric acid in a volumetric ratio of 1:3 respectively. Similarly, the Ti layer is removed using a mixture of H_2O, H_2O_2 and HF in a volumetric ratio of 20:1:1 respectively. Then it is placed to dry under nitrogen flow.

Mold preparation

A printed circuit board (PCB) in 1mm of thickness is cut into a square size of 2 mm x 2 mm in measurement using a computer numerical controlled (CNC) machine (CCD 2: Bungard Elektronik GmbH & Co. KG). All the PCB cuttings are cleaned with isopropyl alcohol (IPA) and deionized (DI) water. Then it is also dried under nitrogen flow.

Fabrication of PDMS container

The PCB mold is placed on a petri dish onto which PDMS (Dow Corning Sylgard 184) is poured. PDMS is mixed with the curing agent at the ratio of 10:1. Then PDMS is defoamed in a vacuum box for 24 hours. Finally, it is peeled off from the petri dish.

Sealing process

Before the bonding process, an electrode fabricated on a glass slide is cleaned by immersing it in heptane solvent for 5 minutes, having it rinsed with ethanol, dried with nitrogen and finally, baked in the electrode in the oven (E28: Memmert GmbH & Co. KG) at 110°C for 5 minutes. The PDMS relief is immersed in ethanol and later, sonicated for 5 minutes and fully dried with nitrogen. Then the liquid PDMS with a ratio of PDMS:catalyst equals to 10:1 is spin coated on an electrode fabricated on the glass slide and brought into contact with the ready PDMS relief. The described process is as demonstrated in Fig. 1(a)-(d).

The described method is a straightforward process to be implemented, as well as it avoids the use of hotplate or oven for the heating process of PDMS-glass bonding which was once introduced by Samel et al. [17].

DNA capture probe immobilization and hybridization

The gold electrodes are first cleaned and wetted by de-ionized water. Then it is reversibly cycled in a 0.05 M sulphuric acid (H_2SO_4) from 0.00 to 1.25 V at scan rate of 0.1 V/s. Then it is rinsed off, again with the deionized water. A 10 μL of 1 μM DNA capture probe solution is deposited onto the gold electrodes for 1.5 hours to allow the immobilization between the gold surface and the thiol group. Later, it is rinsed off with phosphate buffer (0.03 M K_2HPO_4, 0.02 M KH_2PO_4 and 0.3 M KCl) to remove excess probes, which have not been adsorbed. After washing the gold electrodes, immobilization of the DNA capture probe is confirmed by the CV measurement. Finally, it is rinsed off with the phosphate buffer.

The hybridization procedure starts with a 10 μL of 4 μM DNA target solution being pipetted onto the same gold electrodes used for DNA probe immobilization and left for 1 hour. It is then washed with phosphate buffer to remove excess unhybridized DNA target and the CV measurement is then noted. An outsourced screen-printed Au sensor is tested as a benchmark in this research and its performance is compared to our fabricated Au/Ti sensor. The results between these two sensors are revealed in Section *Electrochemical response* of Results and Discussion.

RESULTS AND DISCUSSION

Electrode Thickness

The thickness on the fabricated electrodes have been measured by surface electron microscope (SEM) and produced the Au thickness of 1 μm and the Ti of 30 nm by using parameters of RF sputtering (Auto 500: BOC Edwards) and thermal evaporator (Auto 306: Edwards UK) as being stated in the *Electrode fabrication* of Materials and Methods section. Fig. 2 indicates the thickness on the fabricated Ti and Au.

Electrode Dimension

Fig. 3 reflects the close-up photo for the electrode dimension which has been patterned and developed by using a positive mask (Hapmax (M) Sdn. Bhd., Penang,

Malaysia) and photolithography process as being described previously. The width measurement for each electrode is 0.581 mm.

Electrochemical response

Two types of gold electrode sensors have been tested in the research: an outsourced screen-printed Au sensor and the fabricated Au/Ti sensor as described in Section 2. Fig. 4(a) and (b) show a photo image of a screen-printed Au sensor and the fabricated Au/Ti sensor respectively.

Fig. 5(a) and (b) illustrate the CV graphs obtained from the screen-printed Au sensor and the fabricated Au/Ti sensor respectively. The summarized value for all types of the three (3) measurements (output current readings: y-axis): which are at the bare Au phase, as well as after immobilization and after hybridization phases are exhibited in Table I. The measurement in the table reveals that the fabricated Au/Ti sensor shows a large scale difference for bare Au, after immobilization and after hybridization compared to the screen-printed Au sensor. The big gap of these three (3) measurement readings for the current (y-axis) at the bare Au, after immobilization and after hybridization phases suggest that the fabricated Au/Ti sensor is possible to be developed as a biosensor. However, as compared to the screen-printed Au sensor which has been commercially used in the Microbiology and Parasitology Lab, School of Medical Sciences, USM Kubang Kerian, the fabricated Au/Ti sensor recognizes an output current reading in a value of a smaller scale as opposed to the screen-printed Au sensor highlighted in Table I.

Effect of heating on sealing process

PDMS-glass adhesive bonding method as being described in Fig. 1 has been analyzed in various environment and the quality of the bonding process is summarized in Table II. It proved that the cooling down method either by the room temperature of 25°C or exposed to dry under sun of 35°C produced the best quality on the PDMS-glass adhesive bonding. On the contrary, immediate heating method either by oven or hotplate produced some trapped air bubbles. These air bubbles trapped inside the PDMS-glass bonding will caused the possibility of leakage and seal reversal.

Surface structure analysis

An analysis then follows, to investigate the surface structure of the fabricated Au/Ti sensor. The atomic force microscope (AFM) analysis establishes the data as referred in Table III.

CONCLUSION

The use of PDMS in this research is to act as a coating layer and simultaneously eliminate the occurrence of the non-specific redox current from the Au line between the sensing electrodes and the terminal electrodes. As this project has the purpose of developing a label-free DNA detection biosensor device, the output current reading should provide a large gap measurement at all the three stages of bare Au, after immobilization and after hybridization. Thus, this characteristic has been successfully fulfilled by the Au/Ti sensor fabricated in this project. An additional characteristic which is surface roughness investigated using the atomic force microscope (AFM) has recorded a root mean square (RMS) value on the 5 μm x 5 μm dimension, ranging from 5.40 nm to 6.15 nm.

These results have validated the fact that inexpensive techniques such as soft lithography, thermal evaporation, wet etching and adhesive bonding method for PDMS-glass which have been presented in this paper, may have led to a promising future. These techniques, in effect, may be able to be further developed as a label-free micro-fluidic DNA sensor that is inexpensive, disposable and functional.

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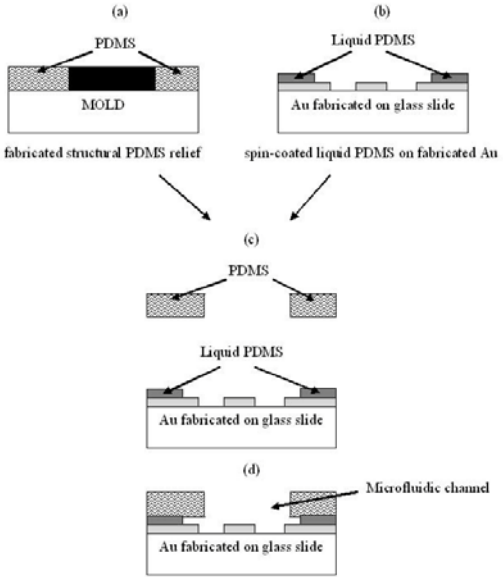


Fig.1. Fabrication procedure for PDMS-glass adhesive bonding.

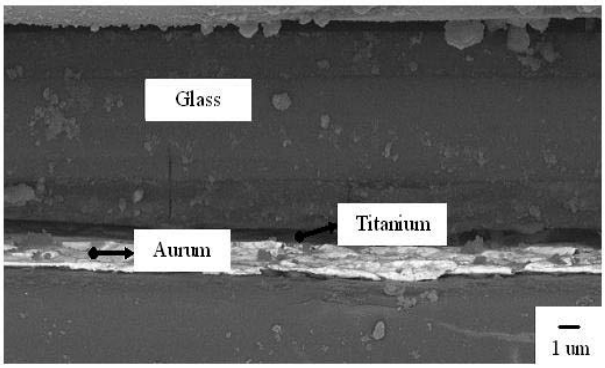


Fig. 2. SEM image on Au and Ti thickness fabricated on the glass slide

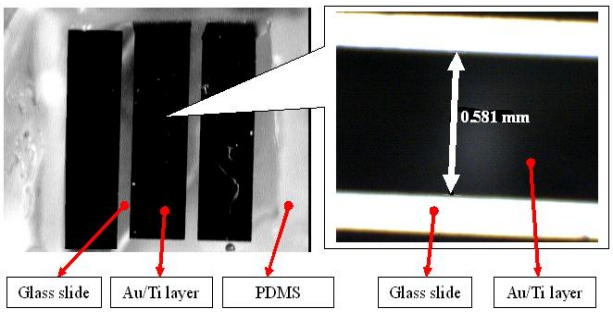


Fig. 3. Close-up photo for three electrodes sensor (a) and close-up photo for one of the sensors (b)

Table I: Comparison on the Sensor Performance

	Screen Printed Au (in μA)	Fabricated Au/Ti (in μA)
Bare Au	53.3	1.850
After Immobilization	43.8	1.009
After Hybridization	40.0	0.107

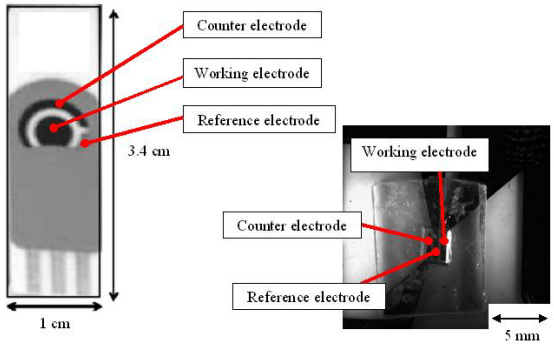
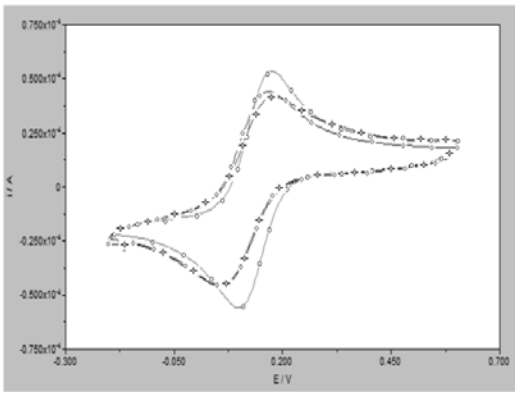
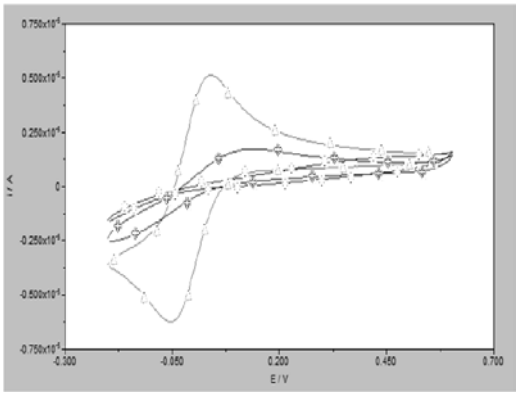


Fig. 4. The specification of screen-printed Au sensor (a) and fabricated Au/Ti sensor (b).



(a)



(b)

Fig. 5. CV measurement for screen-printed Au sensor (a) and fabricated Au/Ti sensor (b).

Table II: Summarized result on PDMS-glass adhesive bonding

	Environment	Quality Result
1	Oven 60°C	Air bubbles trapped inside the bonding
2	Oven 70°C	Air bubbles trapped inside the bonding
3	Oven 80°C	Air bubbles trapped inside the bonding
4	Room temperature 25°C	No air bubbles. Smooth and even surface
5	Exposed to dry under sun 35°C	No air bubbles. Smooth and even surface

Table III: Root mean square value for the measurement of the surface roughness measurement in 5 μm dimension at the fabricated Au/Ti sensor

	Insulating electrodes	Non-insulating electrodes
Counter Electrode (CE)	5.40 nm	6.15 nm
Reference Electrode (RE)	5.79 nm	5.53 nm
Working Electrode (WE)	5.81 nm	5.57 nm