The effect of photoresist thin film on cyclic voltammetry measurement for DNA immobilization and hybridization of indicator-free DNA sensor

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The effect of positive photoresist thin film fabricated between electrodes on the glass slide is the focus in the present study. A 1.04 mm^2 electrode size is used and the cyclic voltammetry (CV) of 0.02M $K_3Fe(CN)_6$ in 0.1M KCl has been analyzed throughout the work for Au bare electrodes, DNA probe immobilization and DNA target hybridization. An adhesive bonding method of polydimethylsiloxane (PDMS) is implemented. The atomic force microscope (AFM) analysis on the root mean square (RMS) value is then carried out to prove that the surface roughness and electrode thickness do not influence the analysis.

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1. Introduction

The conventional DNA microarrays utilize various DNA labeling methods such as fluorescent [1] and radioactive [2]. These methods compel the target sequence to be labeled before they are correlated with the probe sequence [3-6]. Moreover, the fluorescent detection DNA chips require–specialized software and expensive laser scanners, making the technology not portable and costly.

Recently, the electrochemical DNA sensors have been expanded and utilized by researchers and many efforts have been made towards miniaturizing these biosensors [7-9].These micro-scale sensors has several advantages in ways that they are faster, more sensitive, have lower power supply and consumption, smaller scale volumes, cheaper, as well as being more portable and disposable.

A conventional electrochemical cell consists of the electroactive species dissolved in an ionic electrolyte and previous studies [10, 11] demonstrate the electrochemical quantization of DNA through the use of ferricyanide. Thus, this method will be used in this research which aims to propose the electrochemical DNA sensor by using a cost-effective fabrication technique through the use of thermal evaporator and wet etching method. This is purposefully done to pattern the electrodes in three electrode systems as to measure the current by using the cyclic voltammetry (CV) method. The purpose of the sensor is to be able to detect electrochemically various genes of various probed DNAs' immobilization and its 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 [12]. 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 the DNAs' processes mentioned.

Previous development on DNA sensors and microarrays demonstrates that the photoresist is utilized as a coating layer [13, 14] between the three electrodes: working, reference and counter electrodes. The purpose of the layer is claimed to be an insulated 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 interactions between the exposed Au line and the indicator [13]. However, in this research we fabricate PDMS to act as a coating layer between the exposed Au terminal electrodes and the sensing electrodes.

The used of photoresist as a coating layer on the silicon (Si) or silicon dioxide (SiO2) [15] is supported by the fact that semiconductor is an electrical conductivity material. However, few journals have reported the use of photoresist as a coating layer on insulator materials such as glass [13,14]. Theoretically, when insulator is used as a base material, the use of photoresist between the electrodes should not be implemented. Many biologists have reported the use of Au printed on ceramic substrate

that has not implemented the insulated materials between the electrodes as the ceramic itself acts as an insulator [16, 17]. Thus, this research is brought about to investigate the effect of insulated materials (photoresist) between electrodes that have been fabricated on the insulator (glass) as a base material. A study has been carried out by using two types of DNA sensors fabricated on the glass slide: an insulated photoresist and insulated free photoresist between the electrodes. The performance between these two types of sensors is then conducted and investigated by using the CV graphs measurement.

This paper concerns with the part of a project that aims to develop a microfluidic device that can be used as a biosensor with a standalone and portable readout circuitry. The measurement can be done with a basic concept of CV and electrochemistry redox reaction. This paper also outlines the design and fabrication method for Au/Ti sensor which fully utilizes the cost-effective equipment and methods such as the thermal evaporator, wet etching, soft lithography and PDMS-glass adhesive bonding. Thus, the proposed biosensor should be applicable to produce a current of high value so that it can be measured easily through the simple constructed potentiostat circuit which consists of the digital multimeter and battery.

2. Material and methods

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 the 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). Deionized water is used in all procedures.

CV measurements and data are compiled using ECO ChemiemicroAutolabIII (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 mMKCl) at a scan rate of 0.05 V/s from -0.2 V to 0.6 V.

A glass slide is cleaned sequentially with acetone and isopropanol and dried with nitrogen. RF sputtering (Auto 500: BOC Edwards) functions to fabricate Ti as an adhesion layer and then a thermal evaporator (Auto 306: Edwards UK) performs 1 um Au electrode layer.

Then the electrode layer is spin-coated for 15 seconds with positive photoresist (PR1-1000A: FuturrexInc, USA). A positive mask (Hapmax (M) Sdn. Bhd., Penang, Malaysia) is then put onto the coating electrode layer and being UV-exposed (OAI 150 Exposure Timer: Teltec HK) for 45 seconds. Then it is patterned, using the wet chemical etching method of aqua regia, the 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, the next step is for it to be dried under nitrogen flow.

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

The PCB mold is placed in a petri dish onto which PDMS (Dow Corning Sylgard 184) is poured. The latter is then mixed with the catalyst at a rate of PDMS:catalyst=10:1. Then it is defoamed in a vacuum desiccator for 24 hours, before it is peeled off from the petri dish.

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, baking the electrode in the oven (E28: Memmert GmbH & Co. KG) at 110°C for another 5 minutes. The PDMS relief is immersed into ethanol and sonicated for 5 minutes before it is fully dried with nitrogen. Then the liquid PDMS with a ratio of PDMS:catalyst=10:1 is spin-coated on an electrode fabricated on the glass slide, brought into contact with the PDMS relief that has been prepared earlier. Then the bonding contact is dried at room temperature of 25°C for six (6) hours. The described process is as demonstrated in Fig. 1(a)-(d). Fig. 1(e) shows the close-up photo on the final structure of the sensor after PDMS-glass bonding.

Firstly, Au electrodes are cleaned and are wetted with deionized water. Then they are reversibly cycled in a 0.05 M of sulphuric acid (H_2SO_4) from 0.00 to 1.25 V at the scan rate of 0.1 V/s. Then deionized water is used again to rinse off the acid. A 10 μL of 1 μM DNA capture probe solution is deposited onto the Au electrodes for 1.5 hours to allow the immobilization between the Au surface and the thiol group to take place. 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 are not adsorbed. After washing the Au electrodes, the immobilization of the DNA capture probe is confirmed by the CV measurement. Finally, it is rinsed off, again, using the phosphate buffer.

The hybridization procedure starts with a 10 μL of 4 μM DNA target solution being pipetted onto the same Au electrodes used for DNA probe immobilization and is then left for 1 hour. The next stage is to wash the electrodes with phosphate buffer to remove any excess of unhybridized DNA target, before the CV measurement follows.

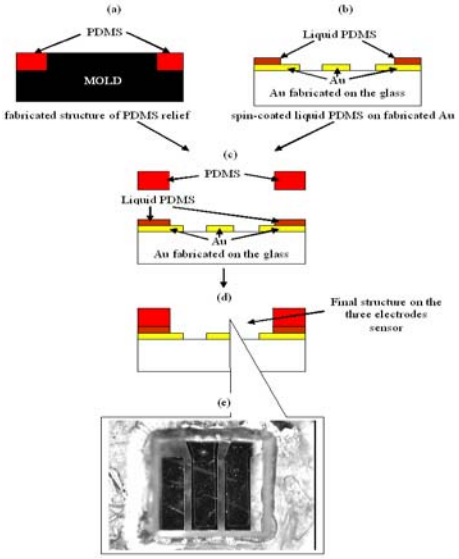

Fig. 1. (a) PDMS fabricated on the PCB mold; (b) Liquid PDMS is spin-coated on the glass slide; (c) PDMS relief brought into contact; (d) Final structure for fabricated three electrodes sensor after the PDMS-glass sealing process; (e) Close-up photo on the final PDMS fabricated structure of three electrodes sensor.

3. Results and discussion

Two types of Au electrode sensors have been tested in the research: these are namely the insulated photoresist (PR1-1000A: FuturrexInc, USA) electrodes and insulated-free photoresist electrodes. Fig. 2 shows both of the CV graphs obtained from these two different sensors for the bare Au electrodes.

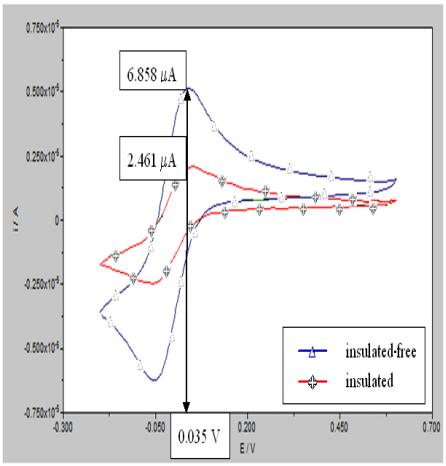

Fig. 2. CV graphs of insulated and insulated-free photoresist electrodes.

The graph reveals that insulated free photoresist electrodes produce more gradient and sharper curve, thus producing a higher peak anodic current value, 6.858 µA as compared to the insulated electrodes, 2.461 µA at 0.035V. The big gap between this anodic current value reveals that the insulated free photoresist electrodes are more suitable to be used as a biosensor. Fig. 3(a) and (b) reflect the close-up photo for the insulated and insulated free photoresist electrodes respectively. The width measurement for each electrode in both insulated and insulated free photoresists is 0.581mm.

The resulting CV data and measurement for bare Au electrodes, DNA probe immobilization and DNA hybridization for electrodes insulated with photoresist are concluded in Fig. 4, whereas for the insulated free electrodes, it is highlighted in Fig. 5. From Fig. 5, the anodic current value for insulated free electrodes after immobilization and hybridization shows a clear, rather prominent gap as compared to Fig. 4, indicating that the electrodes tend to be more robust when being used as a biosensor. The analysis from Fig. 5 has resulted in the anodic current value to show 1.009 µA and 0.107 µA after the processes of immobilization and hybridization respectively. The data for both is measured at the potential of 0.035V.

Further analysis has been carried out to investigate the surface structure on the Au electrodes for both insulated and insulated free photoresist electrodes. The atomic force microscope (AFM) analysis establishes the data as inferred in Table 1.

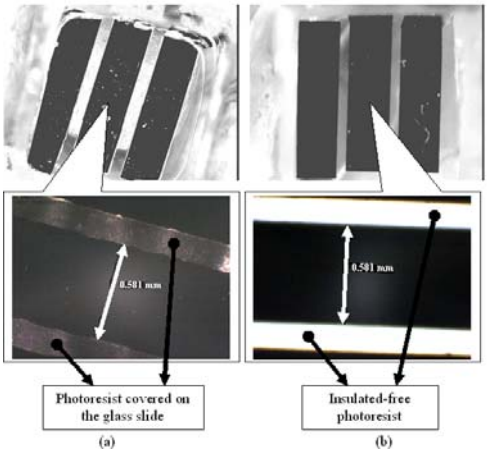

Fig. 3. (a) Close-up photo for insulated photoresist between electrodes; (b) Close-up photo for insulated-free photoresist between electrodes.

Table 1. Root mean square value for surface roughness measurement in 5 µm dimension at the Au electrodes surface.

	Insulated electrodes	Insulated free 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

The analysis is conducted to prove that the data as revealed in the CV measurement for Figs. 4 and 5 serves to be the perfect outcome of the effect of insulated photoresist (PR1-1000A: FuturrexInc, USA), and not due to the structure of the surface [18-21]. The result reveals that the surface roughness is almost the same for these two electrodes, and therefore does not influence the CV measurement.

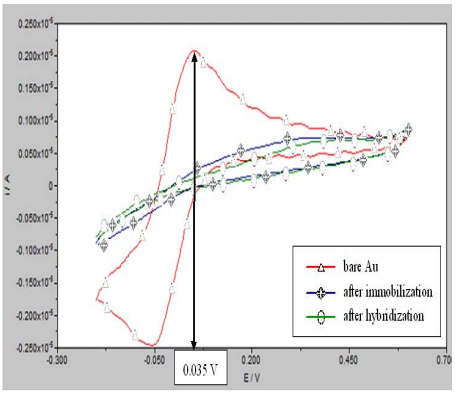

Fig. 4. CV measurement for bare Au, DNA immobilization and DNA hybridization for electrodes insulated with photoresist.

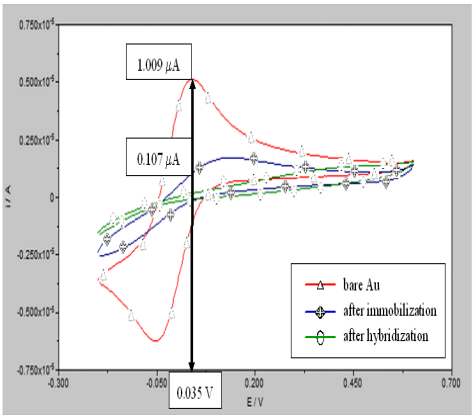

Fig. 5. CV measurement for bare Au, DNA immobilization and DNA hybridization for insulated-free electrodes.

4. Conclusions

The PDMS in this research is manipulated, to act as a coating layer and simultaneously to eliminate the occurrence of the non-specific redox current from the Au line between the sensing electrodes and the terminal electrodes. The CV analysis has successfully proven that photoresist is not needed due to the fact that it does not generate a gradient and sharp curve, automatically suggesting that it does not produce a higher peak anodic current value. An insulated photoresist between electrodes implies that the current value at 0.035V is almost the same for both DNA probe immobilization and DNA target hybridization. The difference between these two current values is too small, making it unsuitable to be developed as a biosensor and to be used in the standalone and portable potentiostat circuitry.

The outcome of this research proves that the photoresist is unnecessary whenever the insulator material such as glass or ceramic substrate is used as a base. The analysis by atomic force microscope (AFM) ensures that the surface roughness for the two sensors is identical. The measurement in root mean square (RMS) value is done on 5 µm x 5 µm dimension on the fabricated Au/Ti surface for all the three electrodes of the two sensors. The recorded value ranges from 5.40 nm to 6.15 nm. These values therefore, confirm that the CV analysis on the sensors is not affected by the surface roughness and structure.

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