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TITANIUM DIOXIDE INTERDIGITATED ELECTRODE (IDE) FOR DETECTION OF CARDIAC TROPONIN BIOMARKER

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ABSTRACT

Electrical-based biosensor application in the medical application systems bring advantage in terms of fast responsive time of disease detection and with low cost production i.e. compatible with CMOS technologies. In this paper we present the interdigitated electrode (IDE) for detection of cardiac troponin biomarkers. IDE device is fabricated and followed by titanium dioxide (TiO_2) thin film deposition on the IDE surfaces using sol-gel method, acts as a sensing area. TiO_2 nanoparticle not only provide high surface to volume ratio and chemically-environment-dependent electrical properties, but it also promotes natural oxygen group bonding that is crucial for biomolecule linkage. After that, the TiO_2 surface undergo surface functionalization process to allow the reaction of antigen-antibody protein. Surface morphology is characterized by using Atomic Force Microscope (AFM) and Scanning Electron Microscope (SEM). For electrical characteristics after hybridication, the IDE is characterized using Semiconductor Parameter Analyser (SPA). Result shows that, our IDE device is capable to detect troponin-I protein biomarkers.

Keywords: biosensor, titanium dioxide sol-gel, interdigitated electrode, cardiac troponin-I.

INTRODUCTION

The term of "biosensor" is short form for "biological sensor". The biosensor is generally defined as an analytical device, which converts the biochemical responses into quantifiable electronic signal (Fathil *et al*., 2015). The device is made up of a transducer and biological receptor. The transducer surface need to be functionalized with biological receptor which can be an antibody (Bongini *et al*., 2007), an enzyme (Tseng, Wang, and Zocchi, 2010) or a nucleic acid (Gowtham, Scheicher, Pandey, Karna, and Ahuja, 2008). The biological receptor is employed to identify the specific target (i.e. analyte, biomarkers or antigen) molecule and the transducer to transform the specific interaction of the analyte into electronic signal. Figure-1 illustrates the components inside biosensor.

Most of the technique currently employed for the hybridization and biomolecules detection, use various reagents such as fluorescent, enzymatic or radiochemical label. In spite of their sensitivity and low detection limits, all these techniques suffer from the fact that it's time-consuming, expensive and complex to implement. Moreover, those techniques often require highly skilled laboratory personnel. The labeling may affect the target-receptor interactions caused by possible conformational or steric hindrance changes induced by the label. Therefore, the development of sensitive, reliable and high-throughput techniques that could distinguish specific biomolecules without the need for labeling (i.e. label-free biosensing) is of a great interest. Label-free molecular biosensors offer enormous potential as clinical diagnostic tool in comparison to other methods, particularly because of real-time and multi-target analyses, rapid diagnosis, automation and reduced costs. Intergitated electrode (IDE) is among the transducer concept of the device used for biosensors.

Incorporated nanosized particles in transducer have gained much more interest recently years due 1D nanostructure which increase surface to volume. Several materials such as gold nanoparticle (AuNP) (Delong *et al.*, 2010), zinc oxide (ZnO) (Neveling, van den Heever, Perold, and Dicks, 2014) (Fathil *et al*., 2015) silver, titanium dioxide (TiO_2) (Kar, Pandey, Greer, and Shankar, 2012), etc. have been used. TiO_2 nanoparticles provide high qualities properties such as high uniformity, high surface to volume ratio, and outstanding biocompatibility (Kar *et al*., 2012) (Conde-Gallardo *et al.*, 2005). Thin film TiO_2 can carry out an activity which is similar to n-type semiconductor which has a small band gap small for electron excitation (Vlazan *et al*., 2015). Hence, performance of the biosensor with thin film TiO_2 can be enhanced due to the characteristics which analogous to the n-type semiconductors (Banerjee, 2011). There are three natural phases of TiO_2 such as anatase, rutile and brookite (Lee, Song, Jurng, and Park, 2011).

TiO_2 thin films can be deposited by many techniques such as sputtering, spray pyrolysis (Conde-Gallardo *et al*., 2005), chemical vapor deposition (CVD) (Lee *et al.*, 2011), and sol-gel technique (Senthil, Muthukumarasamy, Agilan, Thambidurai, and Balasundaraprabhu, 2010). Sol-gel deposition technique has some advantageous in terms of low cost deposition and simple preparation of the solution with low temperature operation.

Surface functionalization of the TiO_2 surface is a key parameter in this research. The physico–chemical properties of the interface play an important role in achieving optimal recognition of the target and limit the non-specific adsorption. Stability, sensitivity and specificity of a biosensor depends on recognition receptors immobilization sustainability of the surface functionalization. Immobilization of bio-receptors through

robust and stable covalent bonds is a good means to gain stability (Shi *et al.*, 2013).

In 2008, an estimation has been made with 17.3 million death due to cardiovascular disease only. With high death rate globally, it can be considered one of the most terrifying disease in the world. One of the alternative way to detect cardiac troponin protein is by using an qualitative measurement called enzyme-linked immunosorbent assay (ELISA). Unfortunately, the detection cannot be measured in depth due to limitation of characterization method. ELISA can only be measured only by concentration of colour changes due to antigen-antibody reaction. By approaching qualitative method in this case by electrical characterization, better sensitivity and specificity of devices can be implemented, which the use of specific biomarkers i.e. cardiac troponin I or T. Troponin complex is a form of protein which divided into 3 subtypes called troponin C, troponin I and troponin T as shown in Figure-2. But only troponin I and T protein were generally exist within cardiac muscle while troponin C only exist in skeletal muscle (Januzzi, Filippatos, Nieminen, and Gheorghiade, 2012) (Kong, Su, Zhang, Zhang, and Cheng, 2012). As the troponin I and T elevated, several cardiovascular disorder symptom occurred. (Fathil *et al.*, 2015) explain the concentration of related cardiac troponin protein was diagnosed from 0.0001 µg/l as a normal condition of cardiovascular organ until 100 µg/l that introduce severe myocardial infarction that cause cardiac malfunction.

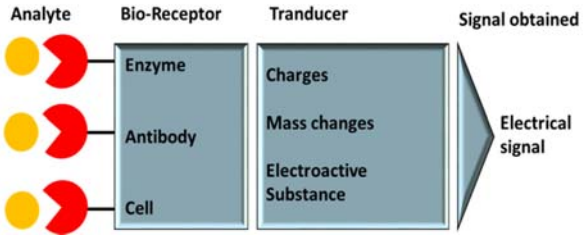

Figure-1. Component and mechanism inside a biosensor (Dugas, Elaissari, and Chevalier, 2010).

In this paper, we combine those features, i.e. IDE incorporated with TiO2 nanoparticles as transducing material for detection of cardiac troponin in diagnosis mild-heart attack. Figure-2 shows the cardiac troponin protein and Figure-3 shows the fabricated structure of IDE biosensor for this experiment.

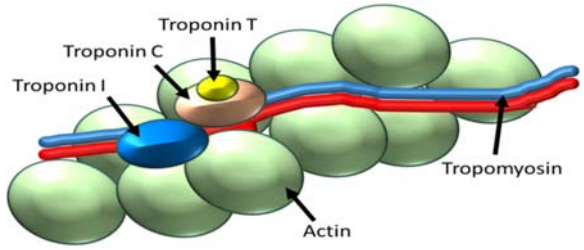

Figure-2. Composition of cardiac troponin protein complexes.

Figure-3. IDE structure with multiple non-contact finger.

EXPERIMENTAL METHODOLOGY

Fabrication process

P-type silicon wafer is used as the base of the devices. A layer of silicon dioxide (SiO_2) is then growth on the wafer surface through wet oxidation furnace at temperature of 1000 °C for 1 hour; SiO_2 thickness obtained was around 300 nm. An IDE device is fabricated with distance between fingered gap around 400 µm patterned through photolithography process and the aluminum is deposited using thermal evaporator as a contact electrode. For preparation of sol-gel TiO_2 process, the titanium isopropoxide with absolute ethanol and acetic acid is mixed with ratio of (1:9:0.1). The mixture is stirred at 600 rpm rate at controlled temperature of 80 °C. The prepared sol-gel of TiO_2 thin films are then spin coated on the IDE surface. Prior to that, the electrode contact is covered by silver. Silver is deposited by thermal evaporator. In this case, silver is used as a sacrificial layer to cover the contact electrode. Same method was implemented by (Adzhri *et al.*, 2015). After the TiO_2 is spin coated on the IDE surface, the unwanted TiO_2 which cover the electrode contact is removed through the lift-off process. The fabrication process is depicted in Figure-4.

Surface modification

Surface functionalization starts with coupling agent of 3-Aminopropyltriethoxysilane (APTES) which act as linker to link protein with surface of TiO_2. The solution consists of APTES, ethanol and H_2O with ratio of (2:50:50) respectively. APTES is dropped on top of the TiO_2 and dried out for about 30 minutes. Glutaraldehydes that act as a secondary linker is attached to promote carboxyl group to allow the immobilization process (antibody probing). Finally, antigen-antibody hybridization process is conducted as the troponin-I antigen was dropped and bovine serum albumin (BSA) is used to block the unattached probe to prevent unspecific binding that reduce the sensitivity of the device. Both antigen and antibody has a concentration of 6400 µg/L. Each of the process is necessarily washed by using phosphate buffer saline (PBS) to remove any loosely bonded biomolecule and contamination. Surface modification process is explained in Figure-5.

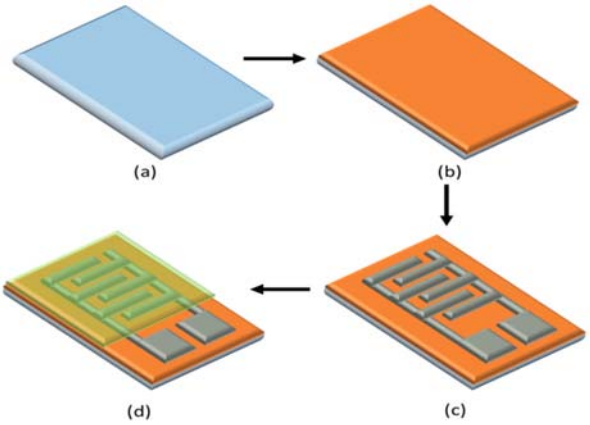

Figure-4. Fabrication process of IDE and TiO_2 thin film (a) P-type silicon wafer substrate (b) SiO_2 growth by using oxide furnace with 300 nm thickness (c) Deposition of aluminum by thermal deposition and patterning of IDE (d) Deposition of TiO_2 thin film and patterning process by using lift-off method with silver as a sacrificial layer.

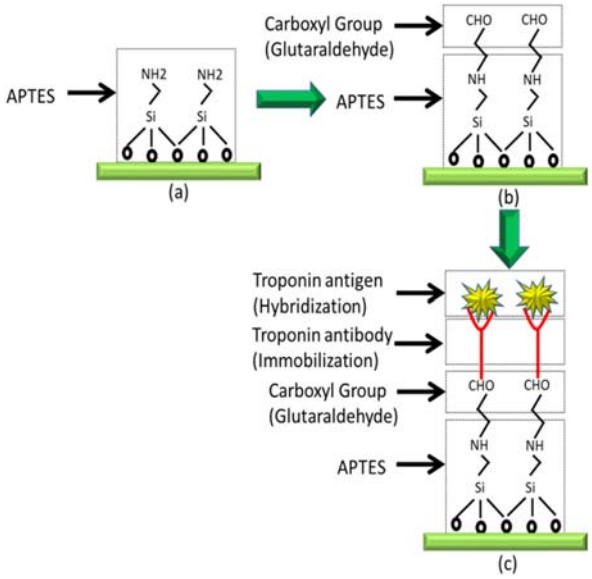

Figure-5. Surface functionalization for detection of troponin-I protein biomarkers (a) first linker binding by using APTES (b) secondary linker binding by using glutaraldehyde (b) Immoblization of troponin-I antibody and hybridization between troponin-I antibody and antigen.

Characterization of electrical and structural properties

Electrical properties of every surface functionalization steps on TiO_2 thin film were investigated using Semiconductor Parameter Analyzer (SPA). Structural properties of TiO_2 before annealing and after annealing were performed using atomic force microscopy (AFM) and scanning electron microscopy (SEM) with Energy-dispersive X-ray spectroscopy (EDX) to determine the element composition of the device.

RESULTS AND DISCUSSIONS

I-V characteristics of TiO_2 thin film IDE surface functionalization

Figure-6 shows the current-voltage (I-V) characteristics for each surface functionalization steps for our IDE devices with gap size of 400 µm. One can see the current changes with the changes in surface. Initially, the current for the bare aluminium electrode and with TiO_2 on the IDE finger is quite low. This is due bare IDE behave as open-circuit at the gap between the IDE fingers. The current is slight increased with the presence of TiO_2, which connecting the IDE fingers. After the surface treated with APTES, the current increase significantly and slightly reduced with immobilization of antibody. Similar trend can be seen i.e. current is slightly reduced with the binding between antigen and antibody.

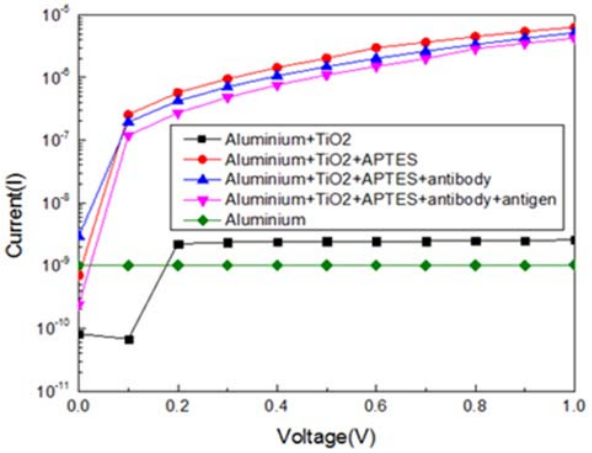

Figure-6. Current-voltage characteristics of aluminium, TiO_2 thin film, APTES, antigen and antibody deposition on top of IDE with gap size of 400 µm.

Structural properties of TiO_2 as sensing materials

Aluminium and TiO_2 physical structural are characterized by using the atomic force microscope (AFM). It is clearly shown that with the presence of TiO_2, the surface becomes rough as to bare aluminium IDE as shown in Figure-7. The surface roughness improves the sensitivity of biomolecule detection as greater number of surface area was obtained. Higher surface-to-volume ratio allows higher sensitivity detection of troponin-I antigen. In addition, smaller size of grain allow better molecule interaction as the surface has been functionalized TiO_2 grain size by average was around 40 nm compared to aluminum which has grain size around 100 nm.

For verification of TiO_2 composition and aluminum element, the EDX characterization is performed as shown in Figure-8. At 20 kV of electron energy, we can observe the elements SiO_2, aluminum and TiO_2 were exist. For SEM images shown in Figure-9, both aluminum and TiO_2 surfaces images was taken at 30 k magnification. It can be observed that the aluminum possess very low surface roughness. Unlike TiO_2, harsh surface with high

porosity surface pattern formed. Basically the rough surface provides higher surface to volume ratio that allows better sensitivity towards the biosensor devices.

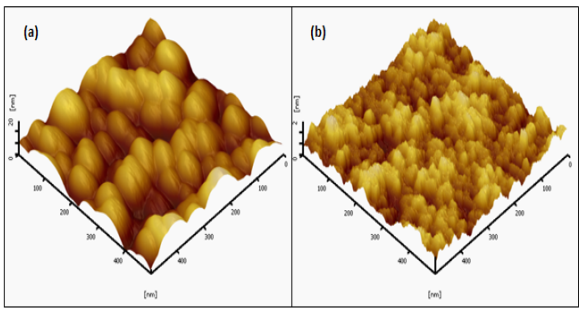

Figure-7. 3D AFM structure of (a) Aluminium (b) TiO_2 thin film.

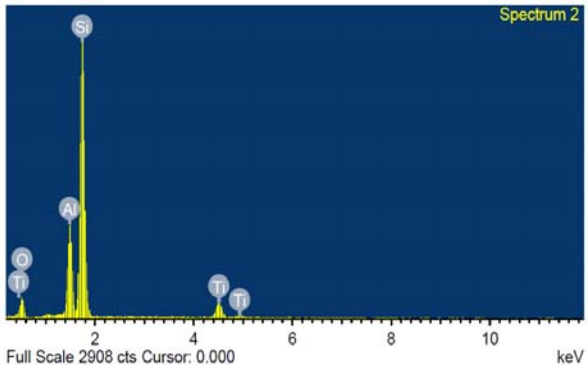

Figure-8. EDX Spectrum of TiO_2 on IDE.

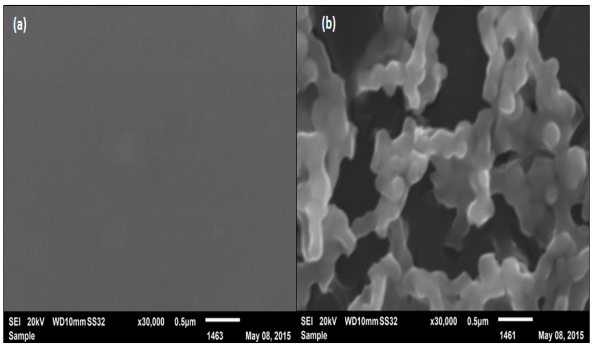

Figure-9. SEM images for structure of (a) Aluminium (b) TiO_2 at 30000 times magnification

CONCLUSIONS

From this experiment and analysis, it can be concluded that TiO_2 thin film IDE biosensor devices was successfully synthesized for the detection of cardiac troponin-I. Electrical properties and structural properties of the surface functionalization of thin film TiO_2 IDE showed devices has biosensing detection capabilities for cardiac troponin I-antigen.

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