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 (TiO₂) thin film deposition on the IDE surfaces using sol-gel method, acts as a sensing area. TiO₂ 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 TiO₂ 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, Karma, 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 (TiO₂) (Kar, Pandey, Greer, and Shankar, 2012), etc. have been used. TiO₂ 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 TiO₂ 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 TiO₂ can be enhanced due to the characteristics which analogous to the n-type semiconductors (Banerjee, 2011). There are three natural phases of TiO₂ such as anatase, rutile and brookite (Lee, Song, Jurng, and Park, 2011).

TiO₂ 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 TiO₂ 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.

**EXPERIMENTAL METHODOLOGY**

**Fabrication process**

A layer of silicon dioxide (SiO₂) is then growth on the wafer surface through wet oxidation furnace at temperature of 1000 °C for 1 hour; SiO₂ 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 TiO₂ 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 TiO₂ 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 TiO₂ is spin coated on the IDE surface, the unwanted TiO₂ 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 TiO₂. The solution consists of APTES, ethanol and H₂O with ratio of (2:50:50) respectively. APTES is dropped on top of the TiO₂ 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 TiO₂ thin film (a) P-type silicon wafer substrate (b) SiO₂ growth by using oxide furnace with 300 nm thickness (c) Deposition of aluminum by thermal deposition and patterning of IDE (d) Deposition of TiO₂ 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) Immobilization 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 TiO₂ thin film were investigated using Semiconductor Parameter Analyzer (SPA). Structural properties of TiO₂ 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 TiO₂ 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 TiO₂ 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 slightly increased with the presence of TiO₂, 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, TiO₂ thin film, APTES, antigen and antibody deposition on top of IDE with gap size of 400 μm.

Structural properties of TiO₂ as sensing materials
Aluminium and TiO₂ physical structural are characterized by using the atomic force microscope (AFM). It is clearly shown that with the presence of TiO₂, 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 TiO₂ grain size by average was around 40 nm compared to aluminium which has grain size around 100 nm.

For verification of TiO₂ composition and aluminium element, the EDX characterization is performed as shown in Figure-8. At 20 kV of electron energy, we can observe the elements SiO₂, aluminum and TiO₂ were exist. For SEM images shown in Figure-9, both aluminium and TiO₂ surfaces images was taken at 30 k magnification. It can be observed that the aluminium possess very low surface roughness. Unlike TiO₂, 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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