Distinct Detection of Ganoderma Boninense On Metal Oxides-Gold Nanoparticle Composite Deposited Interdigitated Electrode DNA sensor

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Abstract. Oil palms suffer severe losses due to Ganoderma boninense infection that causes Basal Stem Rot (BSR). The available detection measuring the severity of BSR disease have not proved satisfactory output. Due to the influence of oil palm industry in country’s economy, effective and efficient means of diagnostic measure is mandatory. Among the available diagnostic tools, biosensors were redeemed to yield the most rapid and selective results. To overcome the current issues, herein Interdigitated Electrode (IDE) electrochemical DNA biosensor to detect Ganoderma boninense was successfully designed and fabricated by thermal deposition. Lift-off photolithography fabrication process was applied followed by the surface chemical functionalization via seed deposition. Zinc Oxide (ZnO) and Titanium Dioxide (TiO₂) were overlaid and the functionalized metal oxides IDE surfaces were used to detect DNA sequence complementation from Ganoderma boninense. Furthermore, gold nanoparticles were doped to increase the surface to volume ratio and enhance biocompatibility. Characterizations were made by validating the sensor's topology characteristics and electrical characteristics. From the results recorded, it has been justified that IDE with ZnO doped with gold nanoparticles surface serves as an excellent DNA sensor for the detection of Ganoderma boninense with a remarkable current of 290 nA and 176 nA for immobilization and hybridization respectively.

1. Introduction
In Malaysia, among the plantations that yield enormous income in the agricultural sector, oil palm has its own standalone reputation. In 1960, the areas of oil palm recorded were 54,000 hectares which drastically have increased to 5.81 million hectares in 2017, reflecting an average compound annual growth of 10.10%. Production yield was increased from 94,000 tonnes in 1960 to 17.89 million tonnes in 2017, reflects ~190 times increment within 57 years. This represents a compound annual growth of 31.22% [1], however, one of the significant factors that contribute to the economic loss in the oil palm plantations is the white rot fungal pathogen, Ganoderma boninense. The damage worsens as it occurs increasingly early from one planting cycle to the successive cycles. Losses with Fresh Fruit Bunch (FFB) was estimated to be 0.16 tonnes per hectare and it immediately showed an obvious decline in revenue for the nation in agricultural and more specifically in the oil palm sector [2]. Nanotechnology has attracted tremendous interest due to their noticeable performance in electronics, optics, and photonics.
especially with the aspect of nanostructures [3–10]. Among nanostructures, zinc oxide (ZnO) nanostructure is one of the most important nanomaterials for the current nanotechnological achievements. ZnO is a semiconductor material with a direct wide band gap energy (3.37 eV) and a large exciton binding energy (60 meV) at room temperature. ZnO is also biocompatible, biodegradable, and biosafe for the medical and environmental applications. Due to their remarkable performance in electronics, optics, and photonics, ZnO nanostructures are attractive candidates for different applications, such as UV laser, light-emitting diode, solar cell, nanogenerator, gas sensor, photodetector, and photocatalyst [11–14]. On the other hand, titanium dioxide (TiO₂) nanostructures also exhibit remarkable capabilities especially with photocatalytic activity [15]. Moreover, it is stable, cost-less and non-toxic. Due to its high photo-stability and redox selectivity, TiO₂ is known for its resistance to photochemical and chemical erosions [16]. Both ZnO and TiO₂ are the excellent metal oxides, commonly used as a conductive layer on biosensors for a wide range of applications. In this present investigation, we have developed Interdigitated Electrode (IDE) based DNA sensor functionalized by both ZnO and TiO₂ nanostructures distinctly to detect *Ganoderma boninense* in a rapid and robust fashion.

2. Materials and methods

2.1 IDE Fabrication and Surface Modification

The Interdigitated Electrode was developed using a silicon wafer. The silicon platform had undergone wet oxidation, aluminium deposition and finally photolithography process as stated in previous study [17]. Whereas the ZnO and TiO₂ deposition was conducted similar to V. Thivina *et al* and N.K.S. Nordin *et al* respectively [18,19]. For the next surface modification, gold nanoparticles were deposited onto the metal oxide deposited IDEs as stated and elaborated in past research [20].

2.2 DNA Immobilization and Hybridization Process

Both the synthetic probe and target sequences specific to *Ganoderma boninense* are obtained from Apical Scientic Sdn Bhd. There are steps needed to follow in order to investigate the probe binding (immobilization) and the target binding (hybridization). The processes started with 10 μmol/L of the probe being measured using micro pipette and dropped on the selected IDEs which are bare, doped AuNPs, ZnO, ZnO doped with AuNPs, TiO₂ and TiO₂ doped with AuNPs. All these IDEs showed different sensitivity depending on the materials are coated. Then all of the IDEs were kept warm on room temperature for 1 hour as shown in and as the last step they were rinsed using PBS followed by DI water and dry it up with a blower [21]. Hybridization process followed the abovementioned procedures with the complementary target DNA before the electrical measurement was taken [21]. The whole process of IDE development is as shown in Figure 1.

![Figure 1](image_url). Entire flow chart starting from fabrication till electrical characterization of IDE for sensing DNA sequences from *Ganoderma boninense*. 
3. Results and discussion

3.1 Surface texture performance
Stylus profile meter or profiler’s purpose is to measure the thickness of the IDE surface. After coating three layers on the active area of IDE using a spin coating method, the measurement of thickness was done using a profiler. There are three types of the graph in the provided figures to compare the thickness before and after coating ZnO and TiO2. The average measurement of aluminium recorded is 0.2 μm (Figure 2a) followed by TiO2, which is 1 μm (Figure 2b) and 1.5 μm for thickness with ZnO (Figure 2c).

![Graph of thickness measurement using profiler after deposition of, a) aluminium deposited IDE, b) TiO2 coated onto aluminium deposited IDE and c) ZnO coated onto aluminium deposited IDE.](image)

3.2 Surface morphology by high-resolution microscopic observation
Another distinct way to find the discrete differences between bare IDEs and metal oxide coated IDEs is to observe using the aid of Energy Dispersion X-Ray diffraction (EDX) display. On the spectrum results in Figures 3a and b, it shows the existence of metals present on the surface of the IDEs and thus, it proves that the coating of ZnO and TiO2. Meanwhile Atomic Force Microscopy (AFM) is a scanning probe microscopy for the force measurement. Figures 3c and d shows the 3D images and surface roughness analysis taken by AFM. Based on the results, the grain particles of ZnO and TiO2 are visible and compact on the IDE surface.
3.3 Electrical performance on device and DNA complementation

The electrical testing was carried out by using the current-voltage (I-V) system (Keithley 6487 Picoammeter). The measurements used are to test the bare IDEs is DC voltage is set-up from 0 V to 1V and step voltage is 0.1V with 11 points of the graph.

Figure 4 shows the result of I-V graph (a) before and (b) after the immobilization process. Metal oxides such as ZnO and TiO$_2$ are selected to coat on IDEs because of their advantages such as biocompatibility and the superb kinetic movement. Each of the tested devices showed different kind of conductivity depending on materials that being coated or doped. Before immobilization in Figure 4a, ZnO-gold nanoparticle (AuNP) functionalized IDE elucidated the highest current considering the fact that ZnO being a better sensing material as well as a remarkable platform for AuNPs attachment. Similar to ZnO-AuNP based sensor, with IDE based on TiO$_2$ doped AuNPs, the current conducted was high because AuNPs naturally enhance the entrapment of DNA with any inorganic material leading to high conductivity and thus, easier electron flow [22] but still does not match that of the IDE based on ZnO doped AuNPs. The others are in decreasing order followed by with ZnO coated IDE, AuNP functionalized IDE, TiO$_2$ coated IDE and bare IDE. After immobilization, according to the observation on Figure 4b, the pattern of the current-voltage graph conforms to the pattern of graph prior to immobilization. However, the only difference is that the current reading becomes lower because of the presence of *Ganoderma boninense* DNA. The DNA probe is a negatively charged biomolecule, thus, an increment in negative electrostatic charge is naturally shown in the lower shift of the graph. The current flow for each material tested for immobilization at 1V is displayed in Figure 4b. The electrical measurements of bare TiO$_2$ IDE and immobilized TiO$_2$ IDE do not show much difference in value because of the non-existence of AuNPs to enhance the entrapment of the DNA on the biosensor. Thus, the results justify that TiO$_2$ needs an extra linker or perhaps another layer of coating added to the sensor to improve the ability of IDE for detection purposes.
Based on Figures 4b and 4c, it shows that the graph of immobilization is remarkably higher than the graph of hybridization. Hybridization is a process in which the immobilized probe binds with the complementary target via a hydrogen bond to form a double-helix structure of a complete DNA. Since there are now two strands on the surface of the sensor, there is an abundance of negatively charged ions on the sensing area, which is known as the crowding effect [23]. This phenomenon causes the current to further shift below the immobilization shift. Not unlike the immobilization graph, the after-hybridization graph shows the highest current for ZnO-AuNP functionalized IDE as can be observed in Figure 4c. The value of ZnO-AuNP is recorded higher than TiO2-AuNP since ZnO-AuNP has faster electron movement and better biocompatibility compared with TiO2 [14]. This material is well known for the best chemistry causing the high conductivity than others [24]. The second highest is TiO2-AuNP where the difference in the value of immobilization is little but instead shows a huge gap in the hybridization process. ZnO is placed on third highest followed by TiO2, AuNPs and bare IDE. Both ZnO and TiO2 are favourite materials in sensing application due to a good electrical transfer and enhancement of AuNP makes the electrical conductivity faster. The presence of AuNPs on both metal oxides, ZnO and TiO2 is to give a higher conductivity since AuNP has high biocompatibility on IDE [25]. The bare graph supposed to have the lowest current reading because of the non-existence of active materials on top of active area which indicates there is a very low electric flow occurred.

Meanwhile, Figure 5 shows the current measurement for each material for after immobilization process (a) and after the hybridization process (b). According to Figure 5, it was observed and calculated that the IDE coated with ZnO thin film and doped with AuNPs showed the highest conductivity with current readings of 290nA and 176nA for immobilization and hybridization results respectively.
4. Conclusion
In this research, apart from the comparison in current between immobilization and hybridization being the highlight, the main issue that needs to be shed light on is how different metal oxides with and without gold nanoparticles affect the immobilization and hybridization. Both zinc oxide and titanium dioxide exhibit commendable biosensing properties and excellent bonding with gold nanoparticles. However, according to our studies, zinc oxide has shown better advantages than titanium dioxide in terms of detecting the distinct current changes before and after immobilization and hybridization. Zinc oxide coupled with gold nanoparticles serves to be the best surface modification coupled with functionalization for a biosensor in the detection of \textit{Ganoderma boninense}.

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