Integration of Aluminium Interdigitated Electrodes with Zinc Oxide as Nanocomposite for Selectively Detect Alpha-Synuclein for Parkinson’s Disease Diagnosis

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Abstract. Parkinson’s disease is associated with motor and non-motor symptoms, mostly a motor symptom such as tremor is said to be an early indication for Parkinson’s disease development. Because of higher demands for faster and more precise diagnostic methods, it has sparked trends in the development of a biosensor for the diagnosis of Parkinson’s disease. Therefore, this study has fabricated a biosensor that is capable of detecting a specific Parkinson’s disease biomarker such as aggregation of alpha synuclein and this is crucial in reducing the burden of Parkinson’s disease and to be able to detect the disease at the earlier stage. Finding the inconsistent aggregation of alpha-synuclein is a promising method for the early detection of Parkinson’s disease. Using conventional photographic process, aluminium interdigitated electrodes (ALIDEs) have been fabricated and employed with sensitive electrochemical strategy for the specific detection of the Parkinson’s disease antigen (alpha synuclein). The microelectrode was developed based on aluminium electrode sputtered on silicon substrate. Further, zinc oxide (ZnO) was deposited by sputtering on the working electrode of the ALIDEs using a spin-coating method. The ZnO nanocomposite onto aluminium microelectrode surface provides a favourable platform for efficient loading of antibody via binding with antigen alpha synuclein. The effective loading of the biomolecules (antibody and antigen) on the ZnO nanocomposite surface modified aluminium microelectrode was observed by SEM, AFM and 3D Profilometer. The current flow for each concentration of alpha synuclein was observed at 7.5×10⁻⁶ A (10 fM), 8.8×10⁻⁶ A (100 fM), and 8.5×10⁻⁶ A (1 pM) respectively.

1. Introduction
Parkinson’s disease is a neurodegenerative disease, the most secondly reported neurodegenerative disease after Alzheimer's disease [1]. More than 6.3 million people globally affected by Parkinson’s disease. Parkinson’s disease was originally labelled as a “shaking palsy.” In 1817 by Dr. James, it was recognized as a chronic neurodegenerative disease described by motor and non-motor characteristics [2]. For the early detection of Parkinson’s disease, motor symptoms manifest before the non-motor symptoms [3-4], however, motor and non-motor symptoms mostly contradict each other by manifesting at the same period of time thereby the prediction of early detection difficult to be carried out [5]. Therefore, this study has generated an alternative method of early detection of Parkinson’s disease based-on identifying abnormal accumulation of alpha synuclein in serum. Parkinson’s disease...
has a huge clinical effect on patients, families, and caregivers by affecting the muscular movements and thereby making the muscles to become rigid [6]. The motor side effects of Parkinson's disease are credited to the loss of striatal dopaminergic neurons, though the existence of non-motor symptoms supports neuronal loss in nondopaminergic areas [7]. Levodopa and Dopamine agonists are commonly being used to treat patients with Parkinson’s disease, however, they both have some complications, furthermore, psychosis, dementia, and depression are usually psychiatric problems associated with Parkinson's disease and psychosis is a drug-induced complication [8]. Currently, there are no cures, no means of slowing down the disease advancements, and no means of preventions [9]. Presently, the diagnosis of Parkinson’s disease depends on the observation of a combination of visible symptoms by a specialist (neurologist), either because of the current diagnostic procedures are poor [10]. Therefore, new technologies such as nanobiosensors are required for a specific Parkinson’s disease biomarker (alpha synuclein) detection [11].

Biosensor is an analytical tool that consists of biologically active material used in conjunction with a device that converts biochemical signal into a quantifiable signal [12,13]. A typical biosensor configuration include, bioreceptor is responsible for the selectivity of the device such as, with antibody. A transducer that translates the chemical change by recognizing the analyte, and a signal processing unit [14]. Due to the often extremely low biomarker concentration and disease selective detection, sensitivity and selectivity are important elements [15]. Common transducer elements incorporated in the biomarker biosensor platform, include electrochemical, optical, or mass-sensitive elements which can generate measurable signals [16]. When the analyte interacts with bioreceptor, a measurable signal is generated and that can be monitored by using several sensing devices [17].

Biosensors such as interdigitated electrodes are used for the detection of various biomolecules with the disease diagnosis. Interdigitated electrodes have some advantages, such as high sensitivity and shorter response times. Interdigitated electrodes mostly depend on the measurement of currents and voltages to detect the binding. Interdigitated electrodes use antibody or DNA that selectively binds to an analyte and form a complex. Using interdigitated electrodes, majority of analytes in disease diagnosis can be detected. In nanobiosensors, lower detection limits are achieved by utilizing nanomaterials such as nanorods. Therefore, interdigitated electrodes are fabricated for this project. Interdigitated electrodes are the most transducers, used in various analytical applications, due to their low cost and excellent sensitivity [18–22].

2. Material and Method
pH solutions were bought from HANNA Instruments with the accuracy. Acetone, aluminium oxide, zinc oxide, resist developer (RD6), and positive photoresist (PR1- 3000A) were bought from Futurrex, Inc. Zinc oxide nanocomposite was prepared in our lab and used to coat on the fabricated surface. Carbonyldiimidazole (CDI), Alpha-synuclein, and Phosphate buffer saline (PBS, pH 7.4) were procured from Sigma-Aldrich (USA). Mouse anti-alpha-synuclein antibody was from purchased from Promega, USA. Other chemicals used in this experiment were obtained from Mallinckrodt Baker and used without further purification. Ethanolamine from Fisher Scientific (UK) was used as a blocking agent. Human serum was purchased from Sigma Aldrich (USA) for the specificity analysis.

2.1. Device Fabrication
The mask was designed using AutoCAD with two finger electrodes for the creation of gaps (Figure 1 and Table 1). 4-inch silicon was used as the bas material for the fabrication of the interdigitated electrode. Silicon wafer was cleaned with RCA1 and RCA2 solutions. Oxide layer was developed on the wafer surface. Conventional photolithography method was used for the fabrication of aluminium interdigitated electrodes. Aluminium was deposited on the silicon wafer and spin-coated for the 30s at 2500 rpm. Subsequently, the photoresists were exposed to UV light. The substrate was developed using RD6. Next, the photoresist pattern was etched in the aluminium etchant and the photoresist was stripped off using acetone and final fabricated structure was obtained.
2.2. Deposition of ZnO on the ALIDEs

The prepared zinc oxide solution was coated on aluminium electrodes using a spin coater, next was heated at 60°C for 30 min for the formation of crystallization of zinc oxide thin films. The sample was cooled down to 50°C to avoid distortion of the structure on the thin films. Lastly, the zinc oxide thin film was annealed at 200°C for 20 min.

2.3. Characterization of IDE

After the aluminium interdigitated electrode was fabricated, characterization of the aluminium interdigitated electrode was conducted using 3D-nanoprofilometer, AFM, HPM, and SEM for both bare and after the device was coated with zinc oxide. Further, electrical characterization was conducted using I-V characterization.

2.4. Surface functionalization of ALIDEs with different pH solutions

Different pH ionic solutions which ranging from pH 1-7 were dropped on the desired surface of the ALIDEs to determine the influence of the electrolytic solutions. Picocometer/Voltage source with a probe station was used to measure I-V. Next, pH solutions were dropped on the microgaps after washing with deionized water. The current changes were determined and measured for analysing the influence of the electrolytes on the sensor using different pH solutions.

2.5. Real time binding of Antibody

Before the real-time monitoring of the antibody, ALIDEs was washed with DI water to clean the surface and drive away any foreign matter that may exist on the ALIDEs surface. Then, 0.5 M CDI was dropped on the ALIDEs surface. Similarly, 1 M concentration of ethanolamine was prepared and dropped on the ALIDEs surface which used as a blocking agent. The real-time binding of the antibody was monitored for one hour. In order to remove any unattached antibody, phosphate-buffered saline was used to wash the ALIDEs. Then treatment with Ethanolamine was carried out for the blocking of some free surface clusters, thereby eluding any nonspecific binding. 100nm antibody was
taken in the stock and applied on the ALIDEs and left for 1 hour for the real-time monitoring of antibody. After one hour of monitoring, PBS was used to wash the ALIDEs surface 3 times. The remaining binding surface was blocked to prevent the nonspecific binding of the antibodies to any remaining sites that initially served to immobilize the proteins of interest.

2.6. Biochemical fractionation of human serum
Fibrillation of human synuclein proteins has been performed at various concentrations. For this assay, target protein concentration was kept constant. (Accordingly, 1 aM-100 pM target proteins were used for the fibrillation and non-fibrillation synuclein proteins. Assays were performed in 20 mM sodium phosphate at pH 7.5, Kinetics of fibril formation was measured using Keithley 2450. Buffer was incubated at 37 °C, 200 rpm for 72 h in an orbital shaker. After 72 h, the supernatant was discarded, and the fibrillar pellet was washed twice with phosphate buffer and then suspended in 20 mM phosphate buffer. Immobilization of different concentrations of alpha synuclein on the modified sensing surface of microelectrode was further carried out.

2.7. Biomolecular interaction analysis
For the analysis of biomolecular interaction, Surface functionalization was conducted initially with CDI followed by anti-alpha- synuclein immobilization. After blocking the remaining surfaces, different concentrations of protein alpha-synuclein were interacted on the ALIDEs surface individually. The current flow was monitored for different concentrations of alpha-synuclein, ranging from attomolar to picomolar (10 fM, 100 fM and 1 pM).

3. Results and Discussion
A biosensor is an analytical device that consists of biologically active materials used for the conversion of biochemical signals into quantifiable signals (Figure 2). A typical biosensor configuration consists of a bioreceptor, an enzyme, an antibody, a lipid, and a transducer that translates the physical and chemical modifications with the aid of recognizing the analyte, and signal processing unit. As a result of very low biomarker concentration and diseases selective detection, sensitivity and selectivity are of paramount importance for a biosensor.

![Figure 2. Biosensor configuration for biochemical analysis.](image)

3.1. Surface area characterization of bare IDE and with zinc oxide
Characterization of the devices is important after the devices are fabricated in order to inspect the physical appearances of the devices. This will enable us to see the effectiveness of the fabrication and
to observe if there are some broken finger electrodes so as to avoid current leakages later. After the device was successfully fabricated, 3D Profilometer and high-power microscope were utilized for the morphological characterizations (Figure 3). The result shows that, the fabrication was effective with no broken finger electrodes as indicated by the high-power microscope images of ALIDEs as shown in Fig. 3a. Furthermore, for the grains between ALIDEs, 3D Profilometer was used. A Profilometer was similarly used for analyzing the surface roughness of the IDE bare. Based on the images obtained from 3D Profilometer of the active surface areas of ALIDEs as shown in Fig. 3b, shows that the ALIDEs was excellently fabricated without defects as the finger electrodes can be seen clearly and also sharps and smooth finger electrodes edges were exposed and the coated zinc oxide could be seen as reddish brown on the surface of the ALIDEs. Therefore, it can be said that the etching procedure has reached the required level of the developmental processes. High power microscope and 3D images confirmed the fabrication of the ALIDEs as effective. Based on the measurement of the ALIDEs. From the image of high-power microscope of the bare ALIDEs as shown in Figure 3a, it can be said that the device has been fabricated and ready to be used without any adjustment. It can be clearly seen that the device was fabricated well without any flaw for long term use and for application as diagnostic sensors.

Figure 3. Characterization of interdigitated electrode using higher power microscope and 3D Profilometer. (a), image of bare interdigitated electrode captured using higher power microscope, (b), image of interdigitated electrode coated with zinc oxide captured using 3D Profilometer.

3.2 Atomic Force Microscope (AFM) Images
Characterization of the devices is important so as to let us know the information of the fabricated surfaces of the device. Atomic force microscope was used to image IDE at the nanometer scale after the device was coated with zinc oxide and bare device, using cantilever with a sharp probe scanned the surface of the ALIDEs (Figure 4). After the fabrication of ALIDEs was conducted using photolithography process, bare ALIDEs and ALIDEs coated with zinc oxide were imaged by AFM. Based on the images produced by AFM, it can be seen that the device was well fabricated and modified as the images of the ALIDEs coated with zinc oxide can be clearly seen as precipitated formation as shown in Figure 4 a and image of the bare ALIDEs can also be seen clearly with revealed finger electrodes as shown in Figure 4 b.
Figure 4. Characterization of interdigitated electrode using atomic force microscope. (a) Image of interdigitated electrode coated with zinc oxide captured using atomic force microscope. (b) Image of bare interdigitated electrode captured using atomic force microscope.
3.3 Scanning Electron Microscope characterization of ALIDEs
Scanning electron microscope (SEM) was used to image the surface of the ALIDEs to extract the information of the surface topography. As indicated by SEM images shown in Figure 5a. The SEM revealed the sharp edges between finger electrodes of the ALIDEs. Based on the images obtained from SEM, it has been confirmed that the surfaces of the ALIDEs was successfully fabricated and ready to be used. SEM measurements revealed a gap size and a finger electrode length as shown in Figure 5b. This validates the best characterizations and fabrication of the ALIDEs. Finger electrodes of the ALIDEs in micron scales with nice sharps and patterns show the gap of 20 and finger electrode length of 178 (Figure 5b). It is crucial to reveal the surface topography so as to confirm the effectiveness of the fabrication technique. Scanning electron microscope is useful in imaging the aluminium interdigitated electrodes there by revealing the surface topography of the fabricated microelectrons. Scanning electron microscope also could able reveal the zinc oxide coated on the ALIDEs as whitish spots as shown in Figure 5b.

![Figure 5](image_url)

**Figure 5.** Characterization of interdigitated electrode using scanning electron microscope. (a), image bare of interdigitated electrode captured using scanning electron microscope. (b), image of interdigitated electrode coated with zinc oxide captured using scanning electron microscope.

3.4 Electrolytic analysis of IDE
After the electrical characterization of bare ALIDEs as shown in Fig. 6a, different pH concentrations ranging from 1-7 were dropped on ALIDEs surface. The IV characterization of ALIDEs curves are presented for acidic and alkaline media. The pH scouting was performed based on mechanism of electrolysis and observed the effects of each concentration as IV characteristic curves after the devices was deposited with zinc oxide. We analysed and observed the responses of the device to different pH concentrations with zinc oxide. The effects of each pH concentration were observed for the device surface modified with zinc oxide. We can conclude that, the deposition of zinc oxide brought about the stability of the device as the changes were observed from the influences of the acidic and alkaline regions as shown in Fig. 6b. The generation of voltages in the course of current-voltage analysis have proven that the ALIDEs was designed with similar parameters and dimensions.
Figure 6. Characterization of interdigitated electrode using IV for pH scouting and bare. (a), image of interdigitated electrode with pH scouting generated using IV. (b), image of bare interdigitated electrode generated using IV.

3.5. Analysis of α-Synuclein-spiked serum Detection

After the ALIDEs was fabricated, zinc oxide was coated and followed by antibody and later blocked the regions that were unattached by using ethanolamine as shown in Fig. 6a. With the purpose of detecting disease-specific α-synuclein in the human serum, we used antibody against alpha synuclein, and we found out that our protein makers provided more reliable results with mostly low background, probably due to the combination of the higher sample of volume and intensive washing of the microelectrode surface. We used a commercially bought antibody that does not bind to the physiological monomeric form of α-synuclein but is highly specific for the disease-associated forms. Using serum concentration, we could detect some amount of α-synuclein aggregates as serum does not
have enough amount of alpha synuclein. Different α-synuclein concentrations of 1 fM, 10 fM, 100 fM and 1 pM) respectively were used for the detection of the aggregation of the alpha synuclein. Similarly, the immobilization of the α-synuclein concentrations was carried out from the lowest concentration to the highest concentration on the zinc oxide modified aluminium microelectrode sensing surface, and the current flow for each concentration was observed for each immobilization of alpha synuclein concentration. The current flow was observed at 7.5×10^{-6} A (10 fM), 8.8×10^{-6} A (100 fM), and 8.5×10^{-6} A (1 pM) respectively as shown in Fig. 7b. These measurements show that zinc oxide nanocomposite improved the sensing surface performance of the ALIDEs. More active interaction occurs between the α-synuclein and probe on the modified ALIDEs surface as the current increases from concentration 10 fM to 1 pM. Furthermore, the current versus voltage response among the concentrations reached the sensitivity of 1 pM. We can conclude that surface modified ALIDEs is effective in detection the aggregation of alpha synuclein in human serum and as an electrochemical biosensor, it has a great potential for real time applications. The advantage of using ALIDEs is that it allows the precise orientation of immobilized biomolecules on its surface. The immobilization of alpha-synuclein on the ALIDEs surface after the injection of ethanolamine permits the formation of a strong affinity for the specific analyte attachment. PBS buffer was altered with sodium and applied for the rinsing of the ALIDEs surface for each measurement conducted. The graphs of the current against voltage plotted in this paper were based on origin and excel. Stability analysis demonstrates that the ALIDEs sensor used this in work is highly stable as a newly existing high-performance sensor as shown in Fig. 6b. α-synuclein was detectable in the serum, which mimics a subject with alpha synuclein pathology in the brain. Detection of disease-associated alpha synuclein combined with the total levels of α-synuclein is a promising tool for the diagnosis of α-synucleinopathies, including Parkinson’s disease. In these studies, the properties of antibodies that selectively detect aggregated alpha synuclein in pathological inclusions were characterized. The selectivity of this antibody in detecting aggregated alpha synuclein cannot be solely because of their reactivity to the N-terminus, an antibody that also reacts with the extreme N-terminus, does not share this selectivity. The conformational anti-alpha synuclein an antibody characterized in this paper is vital tool to study alpha synuclein pathology and has given more understandings into the effect of alpha synuclein aggregation in Parkinson’s disease and associated diseases. Moreover, the use of unique tools such as an antibody characterization has confirmed that the abundance of alpha synuclein pathology has been grossly under-estimated by conventional alpha synuclein immunohistochemistry. The antibody characterized in this paper has a unique capability to better understanding of the structural changes that occur during protein aggregation.
4. Conclusion

Aluminium Interdigitated electrode was integrated with zinc oxide as nanocomposite for sensitivity detection of antibody to identify antigen alpha synuclein to be a biomarker for Parkinson’s disease identification. The coating of zinc oxide nanocomposite on the ALIDEs surface has several advantages, such as, electrochemically active surface area and the better accessibility of the analyte to
the surface on the surface of the electrodes. The photolithographic method has a better advantage over the other methods. The bond of the coating is better and zinc oxide nanocomposite was already adhered to the ALIDEs surface without the application of any supplementary chemical that might inhibit the subsequent sensing materials. The method developed in this work to detect alpha-synuclein aggregates based on antibody characterization is highly recommendable for early diagnosis of abnormal aggregation of alpha-synuclein in human serum for Parkinson’s disease identification. Sites for site-specific immobilization and oriented immobilization can be achieved by using zinc oxide surface-modified aluminium IDE and aggregated alpha synuclein could be detected successfully in human serum.

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