Development of Highly Sensitive Interdigitated Electrode (IDEs) Biosensor to Determine Glucose Level using Saliva as Sample

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Abstract. This research focuses on introducing a non-invasive and highly sensitive interdigitated electrodes (IDEs) biosensor to measure the glucose level in human body by using saliva as sample. The IDE was fabricated using simple conventional photolithography process and surface functionalization using MWCNT. The result of the IDE undergoes physical characterization by using HPM, SEM and AFM, beside physical characterization electrical characterization was carried out. Difference concentration of reference glucose sample (10mM-1uM) was tested before using real biomolecule sample (saliva). Blood sample measurement was taken by using blood glucose meter and saliva was using IDEs. The result was taken and been compared. The blood and saliva sample was taken from a voluntary person every two hours starting from 10 am until 10 pm.

1. Introduction

High intake of sugar in daily life might lead a person having diabetes. Diabetes will lead to some misfunction of our human body such as kidney failure, vision loss and many more [1] by 2025 more than 300 million of people will be suffering in diabetes was predicted by World Health Organization (WHO) [2]. Due to this more and more researcher are interesting in finding a faster, high sensitivity and non-invasive biosensor. Biosensor had been successfully in any areas such as disease detection [3–6], environmental monitoring [7–9] and food processing [10–12].

Current technology that commonly use to detect glucose concentration is by using blood glucose method. This method required blood as sample which is invasive and most elderly were afraid of. Due to this more and more researcher are finding a non-invasive way to determine the glucose level in human body[13]. Non-invasive of glucose detection can be obtain from urine [14], tears [15] and saliva [16]. Saliva is the best way for replacing blood, as sample due to it is easily obtainable. However there is a huge drawback on using saliva due to the low concentration of glucose compare with in blood about (100 to 200 lesser). The conventional blood glucose meter could not detect such small amount of glucose concentration.

In this work we used an aluminium based IDE as device. This device was fabricated using conventional photolithography process. The device was then undergo surface modification which enable the probe which was GOx enzyme to bind on the device. Multi wall carbon nanotube was choosen due to the high conductivity that can increase the sensitivity of the device.
IDEs was choose to become the biosensor due to it can be fabricated using convention litography process, high sensitivity and good electrochemical signal due to high surface area [17-19]. IDEs is a twin comb shape electrodes combine, as long as the electrode does not touch each other. Due to the unique combination of two comb shape electrode IDEs can provide many nanogap in one device which is much more sensity and better compare to only one nanogap devices. Because of the attractive resistance-capacitance effect, IDEs have been generally implemented in various field from agriculture, food processing, home security, bioprocessing, surrounding environmental, medical and so forth.

2. Experimental

2.1 Material
Positive photoresist (PR1-2000A) was used for pattern transfer process. Buffered Oxide Etchant (BOE), resist develop (RD6), acetone and aluminum etch were used in addition to fabricate the sensor. MWCNT was purchased from Fibermax Composites (Greece) with diameter of 10-40 nm and length in the range of 1-25 μm. Glucose oxidase (GOx) G3660-1CAP were purchased from Sigma–Aldrich Sdn. Bhd (Malaysia). Finally, freshly prepared dextrose monohydrate (DEX) C6H12O6 sample were purchased from pharmacy. From voluntary personal saliva and blood samples was collected. All other solvents were obtained from commercial sources and used without further purification.

2.2 Wafer Cleaning
P-type with resistivity about 1-10 Ωcm silicon wafer was choose as base material. Before any step or process was done on the surface of the silicon wafer the wafer need to undergo wafer cleaning process. This process is to ensure there were no impurities on the surface of the wafer such as native oxide or dust that will effect on the nest step. RCA1, RCA2 and BOE standard solutions were used to clean the surface of the wafer. After cleaning the wafer, the wafer surface was rinsed again using ethanol and distilled water and lasly blow with dry air to ensure the surface of the wafer is dry and clean.

2.3 Wafer Oxidation
After wafer cleaning process, the wafer undergoes a high temperature process called “oxidation”. This process is to grow a uniform oxide layer on the surface of the wafer. There is two oxidation process which is called dry oxidation and wet oxidation. Dry oxidation is a process of oxidation process without any help of water else wet oxidation process is a process present of water. Dry oxidation is a slower growth rate process compare to wet oxidation. This is due to the presence of water supply in the process in which water molecules will break down into hydrogen and oxygen, which will supply more oxygen to the wafer during the process. To avoid direct oxygen gas flow on the wafer surface two dummy wafer was placed in front and behind of the wafer. Nitrogen with a flow rate of about 1L/min and oxygen flow rate of about 0.5L/min was supply when the temperature reached 1000 nitrogen gas was off and oxygen gas was set at a flow rate of 1L/min. The wafer was push inside the chamber and wait for 1 and half hours. After 1 and half hours, the chamber was ramped down and the sample was taken out from the camber. The thickness of the oxide grow was been measure for 5 points using filmetrics F20-UV.

2.4 Aluminum Deposition
After oxidation process the wafer was cleaned again to avoid any dust on the oxide layer which might effect our next step which was metal deposition. The cleaning process is just a simple step which the wafer was air-blow using nitrogen gun. A layer of approximately 200um of aluminium layer was deposited on the oxide layer as a conductive layer on the silicon substare. Physical vapour deposition was use in this process.
2.5 Fabrication of IDE
Photolithography is a process that transfers the pattern of IDE from mask to the wafer surface. In this research a layer of 1000nm-1200nm of photoresist (PR) was coated with no air bubble on the surface of the wafer using spin coater. There are two type of photoresist that was used in the conventional lithography process which is positive photoresist and negative photoresist. When exposed to UV positive photoresist will become softer else negative photoresist will become harder. As in this research the pattern of the IDE was designed to be same as the mask so positive photoresist was used. After the layer of photoresist was deposited, the sample was undergo “soft bake” which help in semiharden the photoresist. After soft bake the sample was undergo UV exposure for 10-20s. After exposure the sample was develop by immerse it in photoresist developer for 30 second. The unwanted part which was exposed to UV was remove. After that the sample was heated up 100°C for 30 second ( hard bake). This process is to remove the unwanted moisture and harden the photoresist. High power microscope was used to examine wheater the sample was fully developed. This process to ensure that there is continuous flow of current between the electrode. After the inspection the sample was immerge in aluminium etching solution to remove the unwanted part. Again the sample was inspect again under high power microscope to ensure the sample was developed as same as the design. Finally the photoresist was removed by using acetone. After the device was fully develop, current verses voltage test was taken to ensure that the device was functionalize.

2.6 Multiwall Carbon Nanotube Deposition

2.6.1 Preparation of functionalize multi wall carbon nano tube (MWCNT)
A simple sonication method with mild acid solution was used to functionalise MWCNT. By going through acid oxidation with the help of ultrasonic agitation MWCNT can produce carboxyl function group on the surface of the MWCNT. A mixture of 300ml 6M of nitric acid with 6M sulfuric acid ( 3:1 ) and 300g of MWCNT was immerse in the solution. The mixture was then undergo ultrasonic for 4 hours at around 40°C. After ultrasonic bath the mixture was diluted with DI water until it reaches ph 7. When the mixture reaches ph 7 the mixture was filtered using filter paper, the residue(fMWCNT) was grounded until it become power form and keep in a vacuum oven at 80°C overnite. The dry COOH-MWCNT(fMWCNT) was keep in dry cabinate and ready to be used. Upon usage concentration of 1mg/1ml of COOH-MWCNT was mix with dimethylformamide (DMF).

2.6.2 Deposition Process
1uL of fMWCNT was droped on the active area surface of the IDE. After dropping the fMWCNT the device was kepeed in a humidity chamber for two hours. The condition of the humidity chamber was set to 27°C with 80% humidity. After 2 hours, the device was rinsed with phosphate buffered saline and was air blown. Electrical measurement was carried out to test the performance of the device.

2.7 Enzyme (Glucose Oxidase) Immobilization
Glucose oxidase (GOx) originally came in powder form which can’t directly immobilize directly on the device. GOx was prepare by dilute the powder in potassium phosphate. The solution was keeped in well kept in freezer -20°C for long term storage. Upon usage the soluble GOx was left in room temperature until it fully melted in aqueous form which is suitable to dropped on the device. 0.5 uL of GOx was dropped on the surface on fMWCNT which was deposited on the active area of the device. After dropping the GOx the device was kepeed in a humidity chamber for two hours. The condition of the humidity chamber was set to 27°C with 80% humidity. After 2 hours, the device was rinsed with phosphate buffered saline and was air blown. Electrical measurement was carried out to test the performance of the device.
2.8 Target Sample Preparation
Before testing with real biomolecule sample (saliva), reference glucose was prepared by dissolving Dextrose Mono-hydrate in phosphate buffered saline. 5 different concentration of DEX solution was prepared starting from 10mM until 1uM. The solution was prepared using serial dilution method. After testing the performance of the device using electrical measurement, sample of blood and saliva was collected from voluntary personal every 2 hours from 10am until 10pm. The blood sample were measured using Accu-Check Performa glucometer system and the saliva samples were measured using the device.

3. Results and Discussions

3.1 IDE Physical Characterization Under High Power Microscope
Physical characterization was one of the method to visualize element of the surface of the IDE. To inspect the fabricated IDE HPM was used. Table 1 shows the comparaison image of the photoresist development after been exposed to UV for 10s, 15s and 20s. Another images of table 2 shows the contact pad, active area and wire of the IDE after etching. All these images was taken under 5X magnification. From the images from table 1 we can conclude that 20 s is the optimum time for UV exposed. The pattern was prefect and maintain the same as the designed pattern on the mask. 10 s and 15 s is too short for the unwanted part to be soften, causes there is damages when undergo photoresist development. Table 2 shows that the device was clearly develop with 50 µm gap size between the microelectrodes.

Table 1. Comparison image of photoresist development after exposed to UV for 10s, 15d and 20s.
Table 2. Images of contact pad, active area and wire of IDE after etching process.

| Contact pad | Active area | Wire |
|-------------|-------------|------|
| ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) |

3.2 MWCNT Deposited Physical and Electrical Characterization.

After deposition of MWCNT on the active area of the IDE, physical characterization was implemented to visualize the structure of MWCNT on the active area of IDE. To visualize the structure of MWCNT, scanning electron microscopy (SEM) was used. Figure 1a and figure 1b show the different concentration of MWCNT deposited on the active area of IDE. Beside SEM images, figure 1c and figure 1d show the images of atomic force microscopy (AFM). From the figure 1a and figure 1b, we can clearly see the shape of MWCNT is still preserved as a long hollow tube and from figure 1c and figure 1d, show the existence of material on the active area hence it shows that MWCNT was successfully deposited on it. For electrical testing, Keithley 6487 Picoammeter was used to measure current versus voltage (I-V). Before testing the deposited IDE, bare IDE was tested. The test started by supplying DC at 0V and ended at 1V with a step voltage of 0.02V which provided 51 points on the graph. Figure 2 shows the I-V graph of bare IDE and two different concentrations of MWCNT (0.1mg/mL and 1mg/mL). Different concentrations of MWCNT provided different current values, the higher the concentration, the higher the current value. 0.1 mg/mL was chosen due to the optimum current. Enzyme generally is a very sensitive biomolecule and any electron pressure beyond the supply 1V might denature the characteristics of the enzyme and thus, alter the electrical measurement.
3.3 GOx enzyme immobilization Physical and Electrical Characterization

After immobilization of enzyme, images of AFM was taken for physical inspection. From figure 3 a layer of material was shown on top of the IDE hence it shows that the GOx enzyme was successfully immobilization. Electrical testing which started from 0V-1V was test on the IDE. Figure 4 shows the I-V graph of bare IDE, surface modification of MWCNT and after GOx enzyme immobilization.
3.4 Reference Glucose, Blood and Saliva Measurement and Analysis

Before any measurement on the real biomolecule sample, reference glucose sample was first been tested to ensure the IDE was functional. 5 different concentration of sample was tested starting from 10mM until 1 uM. Figure 5 shows the graph of electrical measurement of the 5 different concentration. From the graph the higher the concentration if glucose the higher the current will be. Eq. 1 shows the chemical reaction of glucose and GOx enzyme. When glucose react with GOx enzyme it will produce gluconic acid and hydrogen peroxide. Eq. 2 shows the changes of hydrogen peroxide when voltage was supplied, hydrogen will break down into H\(^+\) ion, 2 e\(^-\) and oxygen. The current value increase when concentration increases is due to 2 e\(^-\) that was produced during voltage supplied. The e\(^-\) act as a carrier and enhance the electron to flow. As concentration increase the amount of e\(^-\) increase hence the current increases.

\[
\text{H}_2\text{O} + \text{O}_2 + \text{glucose} \xrightarrow{\text{GOx}} \text{hydrogen peroxide} + \text{gluconic acid} \quad (1)
\]

\[
\text{Gluconic acid} \xrightarrow{\text{voltage supplied}} 2 \text{e}^- + 2\text{H}^+ + \text{O}_2 \quad (2)
\]
Figure 5. Graph of electrical measurement of the 5 different reference glucose concentration.

Blood measurement was carried out by using conventional glucose meter, comparison between blood reading and saliva sample was carried out. The blood and saliva sample was taken from voluntary person who was not a diabetes patient. The sample was taken every 2 hours start from 10 am until 10pm (7 times). Figure 6a show the blood sample measurement reading, figure 6b shows the electrical measurement of saliva using IDE. From the graph, at 10am the value of current is very low indicate the glucose level in the voluntary person was low there was increase in current value during the test at 12pm shows that the glucose level increase as the voluntary person had lunch. Figure 6c shows the comparison between the current obtained from the measurement of saliva sample with the measurement obtained from the conventional blood glucose meter.
Figure 6. Sample measurement using conventional glucose meter and IDE. (a) Blood glucose meter reading. (b) I-V graph of IDE using saliva as sample. (c) Comparison of blood glucose meter reading with I-V graph of IDE.
4. Conclusions
A high sensitivity glucose biosensing IDE was fabricated based on MWCNT surface modification. The IDE was fabricated by conventional photolithography and PVD deposition method. The IDE was later deposite by a layer of MWCNT and immobilize with GOx. Sample of real glucose reference and saliva were used for glucose level detection using electrical measurement. A small amount (0.5μL) of reference glucose and saliva were required for the detection In this research a highly sensitive device was suggested by only using conventional photolithography method which can detect an invasive and small amount of sample (0.5μL) biosensor which it might had a big impact on early detection of diabetes in the future.

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