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The Effect of Aptamer Concetration towards Reduced Graphene Oxide-Field Effect Transistor Surface Channel for Biosensor Application

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Abstract. Aptamer are artificially produce bioreceptor that has been developed to bind with various target biomolecules such as ion, cells, protein and small molecules. In this research, an aptamer concentration of 0.5 nM, 1 nM, 5 nM, 10 nM, and 50 nM were immobilized on reduced graphene oxide (rGO) integrated with field effect transistor (FET) respectively to study the effect of aptamer concentration toward rGO surface for stable biosensing platform. The 0.5 nM concentration of aptamer shows the highest current result of 84.3 µA at 1 V applied through the source and drain. After immobilized with aminated aptamer, the conductivity shows significant reduction due to the formation of amide bond on rGO surface between aminated aptamer and carboxyl group on rGO.The electrical performance of FET integrated with rGO shows stable electrical performance suitable to be used in the biosensing application.

1. Introduction

The previous technology of detecting biomolecules has faces various challenges. The detection of biomolecules usually involves complexity of device structure, long response time and low sensitivity [1].For example, the chromatographic techniques have issues relating to the formation of assay, complexity of the system, and multi-step purification limits [2]. To solve these issues, biosensor technology is introduced in the arena. Aptasensor and immunosensor are the biosensor that commonly used in detection of biomolecules. The immunosensor technology such as enzyme linked immunorsorbent assay (ELISA) technology has an advantage of lower detection limit than aptasensor but it faces problem of long time-consuming labeling process and complicated features [3]. Besides, immunosensor is being replaced by aptasensor due to the cost constrained and undesirable immune response [4]. Thus, an aptasensor based reduced graphene oxide field effect transducer (rGO-FET). to detect biomolecules has been established.

Aptamers are single stranded DNA or RNA molecules that made of from artificially in vitro evolution process called systematic evolution of ligands by exponential enrichment (SELEX) [5]. In the SELEX process, the binding targets are first to immobilize, then the aptamers are applied in the pool and the binding process will carry on. After that, the weak binders are washed away while the

bound aptamers are eluted and magnified. It was then reapplied to the binding target again and repeated several times to get a strong stringency. Aptamer has properties that could bind to various target from small organic and inorganic molecules to cells with high sensitivity. This is due to their tendency to form single stranded and helices loop. The advantage of using aptamer instead of antibody is aptamer has a smaller size compare to antibody and will result in a higher sensitivity and lower limits of detection in biosensor [6]. It can be developed to detect almost any analyte or biomolecule. Up until now, there are no report studies on aptamer concentration influence the biosensor performance.

In this research, we presented a solid platform for the aptamer as bioreceptor immobilized on rGO-FET. Different aptamer concentration were used to investigate the response of the aptamer towards rGO-FET. The optimum aptamer concentration are needed to be applied to get reliable biosensor output. In FET biosensor technology history, graphene field effect transistor (GFET) has been commonly developed and researched [4] but then, graphene is transferred during the micromechanical cleavage cause complicated and limited in implantation [7-8]. Hence, rGO that are prepared by reducing graphene oxide was introduced to the FET system to detect the biomolecules [8]. rGO can be developed with low-cost technique and high yield production can be archieved and can be easily applied to wide range of substrates [9]. Besides, rGO has a better conductivity compare to graphene oxide due to the reduction of the oxygen group which makes the ratio of carbon higher. However, it also has shortcoming compare to graphene oxide which is it has a lower binding rate than graphene oxide during addition of the linker. Nevertheless, this type of FET has advantages of detecting biomolecules with ultra-sensitivity, label-free, and real-time monitoring system [11-12].

2. Methodology

2.1. Fabrication of FET

A thick oxide layer with 3000 Å above was developed on a <111> dimension wafer to isolate the device by acting as a dielectric layer and insulator in bio-FET. Aluminum was then deposited on the SiO_2 by using Physical Vapor Deposition. The wafer was put in the spin-coater and a positive photoresist was dropped on it. 3 steps of spin rpm parameter were used which are 700 rpm for 10 s, 3000 rpm for 25 s and 0 rpm for 5 s. Then, the wafer was soft bake by heating it with 100℃ for 90 s before photolithography process. A mask with 200 μm channel was used to pattern the transducing channel of the wafer. Resist developer (RD6) was mix with diluted water in ratio of 1:1 to etch the exposed photoresist area. After that, the aluminum was etched by using aluminum etchant. Figure 1 shows the fabrication process of FET.

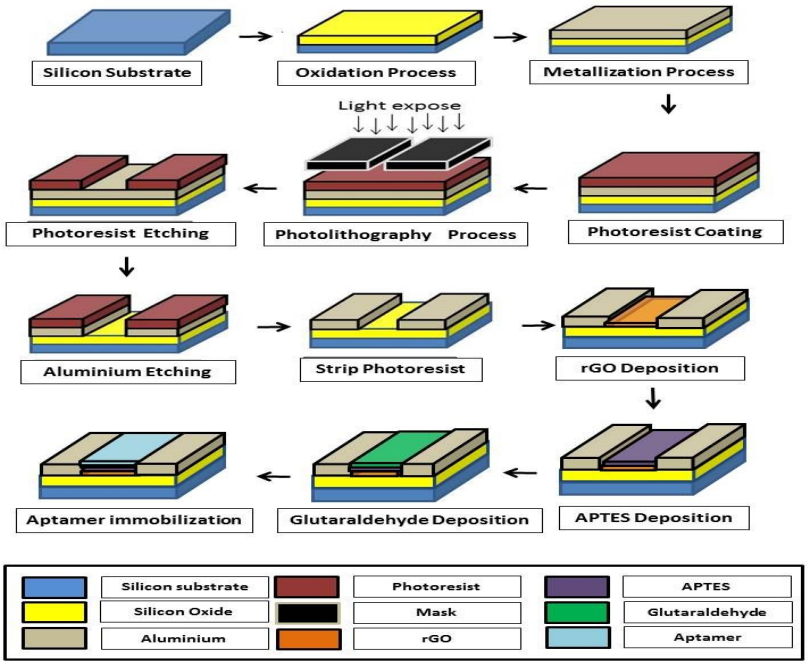

Figure 1. Overview of fabrication process aptamer integrated rGO-FET.

2.2. Deposition of rGO

Before deposit rGO on the transducing channel surface of FET, the rGO solution was sonicated for 1 hour. Next, by using a micropipette, a total volume of 30 µL of rGO solution is drop-casting onto the transducing channel of FET device. It is then was dry at 70℃ for 3 minutes on a hot plate to dry up the rGO.

2.3. Surface functionalization

3-aminopropyltriethoxysilane (APTES) was mixed with ethanol and diluted in deionized water in ratio of 2:50:50 to increase the surface tension. 30 µL of APTES was then dropped onto rGO surface with micropipette and incubated at room temperature inside a dry chamber for 1 hour. APTES was attached to the hydroxyl group of rGO and provide amine functional group for further binding process. After that, glutaraldehyde (GA) was mixed with diluted water in a ratio of 1:39 and drop onto the rGO by micropipette with a volume of 30 µL to make sure the GA cover the whole surface area. It was incubated in a dry chamber at room temperature for 1 hour.

2.4. Aptamer immobilization

5 different concentration of aptamer was used to detect the effect of the aptamer towards the FET surface. The concentration used are 50 nM, 10 nM, 5 nM, 1 nM and 0.5 nM. The aptamers are first mix with phosphate buffer saline (PBS). Then 30 µl of the mixture was deposited on the FET transducing channel and incubated at 38℃ with 85% humidity for 1 hour. The surface was then washed three times with 50 µL of PBS to clean out the unbound aptamer. The process was repeated for different concentration by using same procedure. Figure 2 shows the schematic diagram of surface functionalization on the transducing channel.

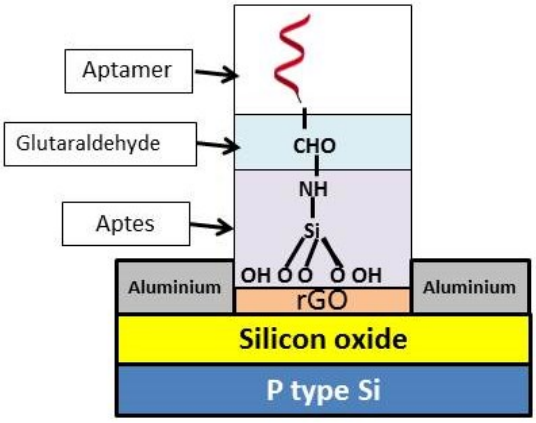

Figure 2. Schematic diagram of linkage on the transducing channel surface

3. Result and discussion

3.1. Scanning electron microscope (SEM)

Figure 3 and Figure 4 shows image of rGO and GO in a working distance of 20 kV with spot size of 10 mm and magnification of 50k, respectively. By comparing the image, we can clearly see that rGO in Figure 3 has wrinkle structure with corrugation and scrolling that are typical structure in graphene. Besides, it is also has a more separated flake compare to the GO structure in Figure 4. GO has a sponge-like structure and multilayer disorder sheet morphology. This is due to rGO has a lower ratio of hydroxyl group and hence shows the properties of graphene clearer than GO.

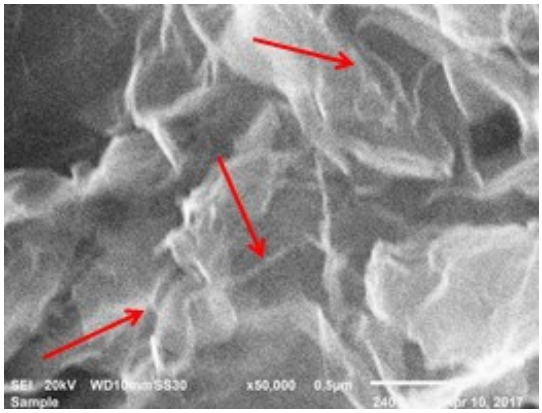

Figure 3. SEM image of rGO

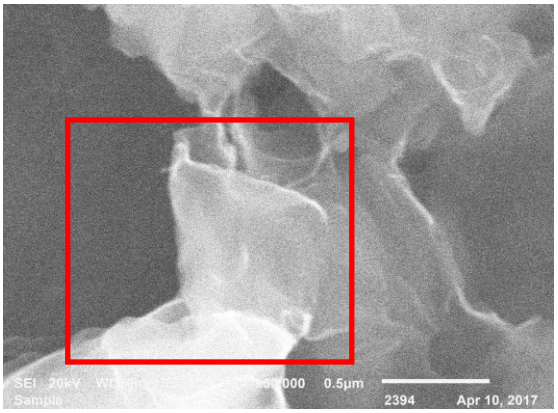

Figure 4. SEM image of graphene oxide

3.2. Fourier transform infrared spectroscopy

Figure 5 shows FTIR results of rGO and it shows several peaks which represent the functional group contained in the solution. rGO contains hydroxyl group and carbonyl group in its chemical structure. The peak shows at 3290cm^{-1} represent hydroxyl group exists in the rGO solution used. Whereas the carbon double bond oxygen has a small peak at 1720cm^{-1} reveals the carbonyl group in the solution.

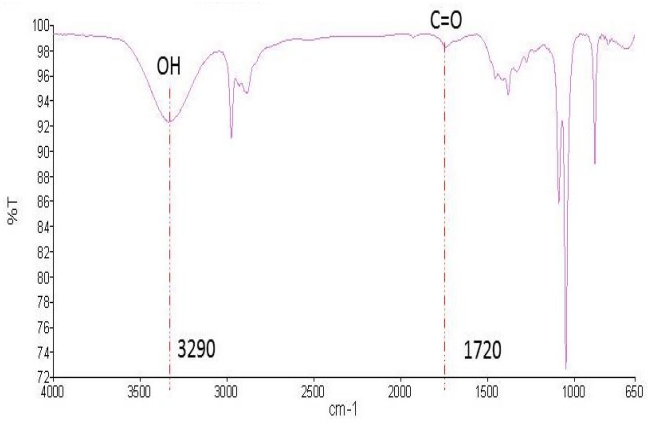

Figure 5. FTIR results of rGO

3.3. Electrical characterization

Figure 6 shows the conductivity performance of the device. After deposition of rGO, steep increase of conductivity can be seen. Before the rGO deposition, electron could not pass through the transducing channel area since the oxide layer act as dielectric layer. rGO is a conductive material hence when it is deposited on the transducing channel, the conductivity of the device will increase. The conductivity of the device decrease when the biochemical linker APTES and GA were added. This is due to the cross linker layer which formed on the surface of the transducing channel. The conductivity after immobilization of aptamer, the electrical reading decrease due to DNA aptamer contains negative charge ion thus inhibit the electrical flow from drain to source.

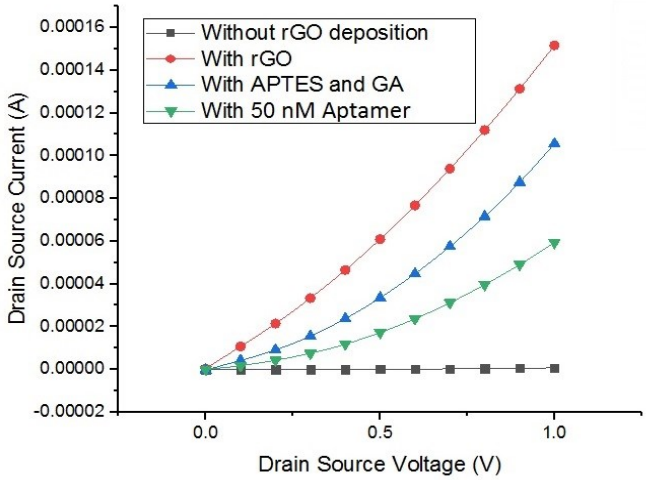

Figure 6. Electrical measurement of aptamer-rGO FET

3.4. Stability of rGO deposition

rGO material was deposited on the surface of FET by dropping a constant amount of volume towards the transducing channel which is SiO_2 layer. rGO will act as a conducting layer and immobilization site for the probe which is aptamer. Figure 7 shows a slight decrease of conductivity each time rGO surface being washed and thus proves that rGO can be deposited on the SiO_2 of FET channel without need any modification.

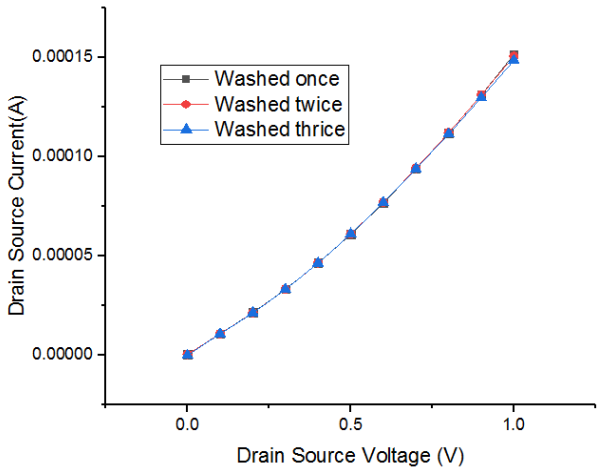

Figure 7. Stability of rGO deposition

3.5. Response of aptamer concentration toward rGO-FET

Figure 8 shows the effect of aptamer concentration on rGO-FET in electrical measurement. The voltage-current (I-V) was measured from 0 V to 1 V. The lowest concentration of aptamer shows highest electrical conductivity which is at 84.3 µA by using 0.5 nM of aptamer. When 0.5 nM, 1 nM and 5 nM of aptamer were immobilized, the device shows decrease of conductivity but the decrease level are small. However, during 10 nM and 50 nM concentration of aptamer were immobilized on the rGO-FET, the conductivity of the device shows a significant reduction in conductivity. Aptamer used are modified with amine, decreased in current was observed due to the formation of amide bond on rGO surface between aminated aptamer and carboxyl group on rGO. Higher concentration of aptamer will have more negative charge[13] that binds to the linker thus, prove the properties of aptamer binding with linker on rGO surface.

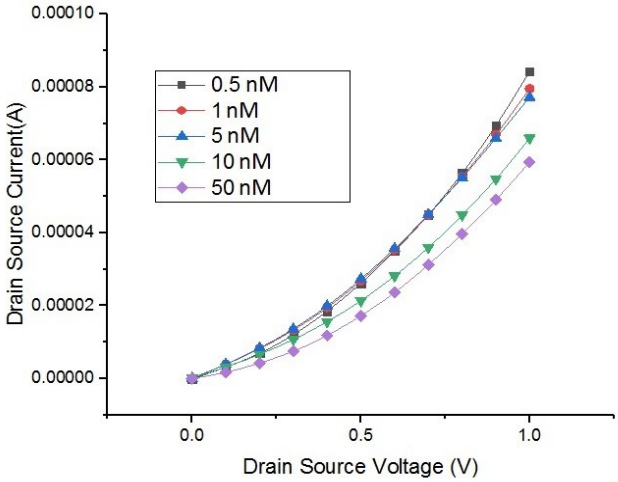

Figure 8. Electrical measurement of Aptamer concentration towards FET surface channel

4. Conclusion

In a nutshell, the fabricated device shows high sensitivity in application for detection of biomolecule. rGO-FET can be a promising detection system with integration of aptamer with concentration range from 0.5 nM to 50 nM. Among these concentrations, 0.5 nM concentration of aptamer shows highest

drain-source current at 1V whereas 50 nM concentration of aptamer has lowest current pass through source-drain at 1 V. This is due to rGO contains negative charge that formed by oxygen group in their structure repels with the electron contain in aptamer and creates depletion on the FET surface. Higher concentration of aptamer will have a higher amount of electron and thus decrease the rate of current flow through the gate. Hence, lower concentration ranging from 0.5 nM to 5 nM of aptamer suitable to be integrated with rGO-FET to get excellent conductivity between source and drain. The stability of rGO verify that rGO suitable to be used as a transducing material and immobilization site for aptamer probe.

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