Fabrication of electrolyte-gate nanocrystalline diamond-based field effect transistor (NCD-EGFET) for HIV-1 Tat protein detection

Nurul Atiqah Ahmad¹, Ruslinda A Rahim¹*, Nur Syakimah Ismail², Bohuslav Rezek³

¹Institute of Nano Electronic Engineering, Universiti Malaysia Perlis, 01000 Seriab Perlis, Malaysia.
²School of Microelectronic Engineering, Universiti Malaysia Perlis, 02600 Arau Perlis, Malaysia.
³Institute of Physics, Academy of Science, Na Slovance 1999/2, 18200 Prague, Czech Republic.

Email: *ruslinda@unimap.edu.my

Abstract. In this paper, we reported on the fabrication process of electrolyte-gate field effect transistor using nanocrystalline diamond as a sensing transducer. The fabrication procedure was begin with the growth of nanocrystalline diamond thin film on silicon/silicon dioxide (Si/SiO₂) substrate using microwave plasma-enhanced chemical vapour deposition (CVD). Then the photolithography process was performed in order to design and pattern the field effect transistor device with the active gate channel of 60 µm length and 20 µm width. Each device consists of three active gate channel which connecting to three different pairs of source and drain contact. The surface morphology of fabricated NCD-EGFET was characterized using Scanning Electron Microscope to clarify the active gate channel of the device and the grain size of nanocrystalline diamond. The current-voltage (I-V) measurement of the device were carried out to study the electrical behaviour for HIV-1 Tat protein detection via RNA aptamer as sensing probe.

1. Introduction

Biosensor is defined as a device that uses a specific biochemical reaction facilitated by isolated organelles, enzymes, tissues, or the whole cell to detect chemical compounds usually by optical, electrical or thermal signals. It is the combination between the component that recognizes the analyte (bioreceptor) and produces a signal, a signal transducer, and a signal reader.

![Figure 1. Components of biosensor.](image)

There are several types of biosensor have been developed by researchers such as potentiometric, amperometric, impedimetric, and voltammetric biosensor [1]–[3]. Potentiometric biosensor including field effect transistor (FET) is one of a well-known sensing device for biological detection. Compared to other types of biosensor, FET is more favourable as it needs low production cost, high possibility of...
miniaturation, flexible configuration for simple measurement, and most importantly biocompatible especially for bioanalytical applications [4], [5].

Several types of FET have been reported previously such as electrolyte-gate FET (EGFET)[6]–[9], back-gate FET (BGFET) [10]–[12], and top-gate FET [13], [14]. EGFET is a type of field effect transistor that work in wet condition. The working principle of EGFET is based on the charge modulation from the biomolecules on the active gate area. The EGFET is different from BGFET in terms of the gate development. For BGFET, metal gate is used as gate while for the EGFET, metal gate is replaced by electrolyte and Ag/AgCl reference electrode. For biosensing application, it is better to used EGFET as biomolecules will be more stable in wet condition.

Herein, we demonstrated the fabrication of electrolyte-gate field effect transistor on silicon substrate with nanocrystalline diamond as transducing material. Nanocrystalline diamond was chose as transducer as it promises stability, surface-wettability, non-toxicity, large volume to surface ratio, electron affinity, and high electrical conductivity.

2. Materials and Methods

2.1. Growth of nanocrystalline diamond thin film

The nanocrystalline diamond thin films were grown on silicon substrates. Silicon substrates in size of 10 mm × 10 mm × 1 mm were primarily oxidized in oxidation furnace to gain the oxide layer on top of the substrates. Next, all the substrates were ultrasonically cleaned in isopropyl alcohol for 10 min and deionized water (DIW) was used to rinse the substrates. Subsequently, substrates are immersed for 30-40 min into an ultrasonic bath with a colloidal suspension of diamond nanopowder for nucleation process. For the nucleation solution, 200 ml of DIW was mixed with 5 ml of NanoAmendo solution. NanoAmendo solution consists of 5 nm dispersed buckydiamond with concentration of 5.0 w/v%/ and 4.8 nm ± 0.6 nm diamond size. From this process, the formation of 5-25 nm thin layer of diamond will occur necessary to initiate the diamond growth in a thin film.

Once the nucleation process done, nanocrystalline diamond (NCD) thin films were grown in a microwave ellipsoidal cavity reactor by chemical vapor deposition (CVD) process. The process takes 4.5 h with the gas pressure of 30 mbar, gas mixture 1% methane (CH₄) in hydrogen gas (H₂) and microwave power of 1000W. The deposition temperature was in the range of 550-600 °C. These deposition parameters led to the growth of ~450 nm thick diamond film with the grain sizes ~250 nm. Nanocrystalline diamond thin films were further hydrogenated in the same microwave plasma reactor at 600°C in hydrogen plasma (H₂) for 10 min to induce the surface conductivity.

2.2. Fabrication of nanocrystalline diamond-based electrolyte-gate field effect transistor (NCD-EGFET)

Fabrication of NCD-EGFET were divided into four steps; mark aligning, channel aligning, contact aligning, and final cover. In the primary step, the samples were cleaned using nitrogen gas (N₂) to remove the dust from the samples. Next, the sample was put into the spin coater and dropped with Hexamethyldisaline (HDMS) 1:4 which work as the adherent layer. The sample was spin coated for 10 sec at 3000 rpm. Subsequently, the sample was dropped with positive photoresist MA 15 and spin coated at 3000 rpm for 30 sec. The sample was then soft baked at 100 °C for 2 min. Further, the sample was aligned under the mask aligner and exposed under UV for 6 sec to build the crosses on the sample. The sample was developed in the developer for 45 sec and rinsed with distilled water. The sample was then exposed to the oxygen gas in the oxygen plasma for 1 min. The next process was 50 nm gold deposition on the sample with the adherent layer of 10 nm titanium (Ti). After the metal deposition process, the gold was removed by lift off procedure in acetone so that the gold only remained on the crosses of the sample.

The channel aligning step was started with putting the sample into the spin coater and dropped with HDMS 1:4 with condition of 3000 rpm. The sample was then dropped with positive photoresist MA 15 and spin coated at 3000 rpm for 30 sec followed by soft baked at 100 °C for 2 min. Next, the sample was aligned under the mask aligner for 10 sec. The sample was exposed under UV light for 6 sec to
build three active gate channels with size 20 µm x 60 µm on the sample substrate. After the aligning process, the sample was developed in the developer for 45 sec and rinsed with distilled water. Subsequently, the sample was then exposed to the oxygen gas in the oxygen plasma for 1 minute followed by removing the photoresist.

For the contact aligning procedure, the same process as channel aligning were done starting from spin coating the sample with HDMS 1:4 up to developing the contact pads for 45 sec in the developer. The next process was metal coating with 50 nm gold on the sample with 10 nm Ti as adherent layer. After the deposition process, the gold was lifted off in acetone so that the gold only remained on the arm and contact pad of the sample. The sample was then checked its conductivity which should be good at $10^{-8}$ A.

As the conductivity of the sample was fine, the final cover of the sample was done. The sample was put into the spin coater and dropped with HDMS 1:4 and spin coated for 15 sec at 4000 rpm. The sample was then dropped with positive photoresist AR-P-3220 and spin coated at 4000 rpm for 60 seconds followed by soft baked at 95 °C for 150 seconds. Next, the sample was aligned under the mask aligner with UV for 13 sec to build the final cover on the sample so that only contact pads and active gate channel are free from photoresist. After the aligning process, the sample was developed in the developer for 6 min and rinsed with distilled water. The sample was then post-bake in the oven at 100 °C for 45 min. The final photolithographic step created openings of 60 µm × 20 µm to define the active gate area.

$$\text{Figure 2. Steps for EGFET fabrication.}$$

2.3. HIV-1 Tat detection

Primarily, RNA aptamer was immobilized on the active gate channel of the NCD-EGFET. Every sample was treated with a mixture of 0.4 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 0.1 M N-hydroxysuccinimide (NHS) for 1 h to trigger the carboxylic functional groups of the sample. Then, the first strand of RNA aptamer was manually drop-casted on the surface of the active gate channel and incubated at 38 °C for 2 hours in a humidified chamber, followed by rinsing with PBS. The binding of 100 pM HIV-1 Tat with the probe RNA aptamer was done at room temperature for 1 h. The concentration of the aptamer derived second strand used was also 100 pM. After that, Tris–HCl buffer solution was used as rinsing agent to avoid the nonspecific binding. An 8.3 M urea solution was used to remove the tested HIV-1 Tat from the active gate channel to study the repeatability of the measurement.
3. Results and Discussion

3.1. Surface morphological studies

Nanocrystalline diamond thin film was analysed using scanning electron microscopy (SEM). Fig 3 shows the image of a grain particle of nanocrystalline diamond with an average size of ~250 nm, observed at 100,000× magnification. Fig 4 shows the arrangement of active gate channel of the fabricated device. The active gate channels showed are light coloured determining the conductivity of the channels. The gap that joining the electrodes were 60 µm in length and 20 µm width. All the gaps were terminated with hydrogen bonding.

3.2. Electrical characterization

Once the fabrication process was done, the device was electrically measured using Keithley 2400 Source Meter and 6487 Picoammeter. The \( I_{ds} - V_{gs} \) measurement was conducted using an Ag/AgCl as reference electrode in 1 mM PBS at pH 7.4 for HIV-1 Tat protein detection. Based on fig 5, the drain-source current, \( I_{ds} \) reduced after the introduction of HIV-1 Tat to the RNA aptamer. This is due to the reduction of electron transfer from source to drain [15]. Moreover, the reaction between positively charge ions (HIV-1 Tat) the positive charge holes from the NCD surfaces also affect the current reduction. Based on previous study, it has been discovered that HIV-1 Tat rich in positively charge ions with the isoelectric point (pI) of 9.88 [16]. Since, the binding of HIV-1 Tat was done at pH 7.4, it is confirmed that HIV-1 Tat is positively charged. The interaction between the RNA aptamer with HIV-1 Tat causing the gate potential to shift towards the negative direction by 174.8 mV for the \( I_{ds} - V_{gs} \) transfer characteristic, with respect to the potential charge changes created by the biomolecular interaction. This result validates that the NCD-EGFET responses were only identified when specific interaction between recognition molecules (RNA aptamer and HIV-1 Tat) occurred on the active gate channel. To confirm the binding of HIV-1 Tat on RNA aptamer, the experiment was repeat on the same device. From fig 6, the shifting in gate potential were recorded at 174.3 mV and 173.9 mV, subsequently. From this result, we can estimate that the binding of 100 pM HIV-1 on RNA aptamer will cause the shifting in gate potential by approximately 174 mV. This can be the reference line for further studies of HIV-1 Tat detection on NCD-EGFET via RNA aptamer as sensing probe.
Figure 5. The $I_{ds}$–$V_{gs}$ measurement of NCD-EGFET during the detection of 100 pM HIV-1 Tat, conducted in phosphate buffer solution at pH 7.4. The drain-source voltage, $V_{ds}$ was biased at -0.1 V and the gate potential, $V_{gs}$ was swept from 0 to -0.6 V.

Figure 6. The repeatability measurement of 100 pM HIV-1 Tat detection performed on the same device.

4. Conclusion

In summary, we have successfully fabricated nanocrystalline diamond-based electrolyte-gate field effect transistor for biosensing application. The grain size of the NCD were successfully proved to be in nanometres range. Later, the detection of HIV-1 Tat on NCD-EGFET was successfully conducted with the RNA aptamer as sensing probe. In the future, studies on the HIV-1 Tat detection will be conducted on NCD-EGFET in the terms of sensitivity, specificity, and selectivity in order to develop a highly precise biosensor for biological diagnosis.

References

[1] R. Monošik, M. Stredoansky, and E. Šturdík 2012 Acta Chim. Slovaca, vol. 5 109–120.
[2] A. P. F. Turner 2013 Chem. Soc. Rev. 42 3184–3196.
[3] V. S. P. K. S. A. Jayanthi, A. B. Das, and U. Saxena 2017 Biosens. Bioelectron. 91 15–23.
[4] E. Stelmach et al. 2019 Electrochim. Acta 309 65–73.
[5] A. Rahim Ruslinda, K. Tanabe, S. Ibori, X. Wang, and H. Kawarada 2013 Biosens. Bioelectron. 40 277–282.
[6] V. Procházka, M. Cifra, P. Kulha, T. Ižák, B. Rezek, and A. Kromka 2017 Appl. Surf. Sci. 395 214–219.
[7] B. Rezek, H. Watanabe, D. Shin, T. Yamamoto, and C. E. Nebel 2006 Diam. Relat. Mater. 15 673–677.
[8] K. Song, T. Hiraki, H. Umezawa, and H. Kawarada 2012 Applied Physics Letter 90 22–25.
[9] G. D. Tabi, B. Nketia-Yawson, S. H. Kang, C. Yang, and Y. Y. Noh 2018 Org. Electron. physics, Mater. Appl. 54 255–260.
[10] R. Adzhri et al. 2016 Anal. Chim. Acta 917 1–18.
[11] M. F. M. Fathil et al. 2016 Anal. Chim. Acta 935 30–43.
[12] A. R. et al. 2017 Sensors and Actuators A: Physical 259 57–67.
[13] K. Takagi, T. Nagase, T. Kobayashi, and H. Naito 2016 Org. Electron. physics, Mater. Appl. 32 65–69.
[14] P. Bolshakov, P. Zhao, A. Azcatl, P. K. Hurley, R. M. Wallace, and C. D. Young 2017 Microelectron. Eng. 178 190–193.
[15] F. M. F. et al. 2019 Process Biochem. 8 55.
[16] S. Shojania and J. D. O’Neil 2006 J. Biol. Chem. 281 8347–8356.