International Journal of Nanoelectronics and Materials
Volume 11 (Special Issue) Mar 2019 [201-208]

Evaluation of GO and rGO on breast cancer cell line (MCF7) and normal breast cell line (MCF10a) for Cell Viability and Electrical Response

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ABSTRACT

Graphene based materials become a phenomenal in various applications including biomedical devices due to their excellent properties. Their effects towards certain diseases were broadly studies and presented. However, the work has been performed was only limited to the graphene oxide (GO) and its biocompatibility only. In this work, the interaction of GO and reduced graphene oxide (rGO) on breast cancer cell (MCF7) and normal breast cell (MCF10a) was investigated specifically on the cell viability, cell mortality and current-voltage (IV) relationship. Graphene oxide and rGO at the concentration of 100µg/mL were prepared by chemical methods. The morphology and quality of both materials were characterized using AFM and Raman Spectroscopy. The cells were treated for 24 hours and the effects of these materials on the viability and mortality of the cell were observed. The interaction between graphene-based materials and both cells significantly impact the current-voltage (IV) characteristics. The results show that GO and rGO did not affect the cell viability but only small percentage different was obtained on cell mortality. It also observed that the resistance of cell treated with rGO decreased with time for MCF7 and vice versa for MCF10a. While for GO, the resistance of cell increased with time for MCF7 and vice versa for MCF10a. These clear patterns of these interactions lead to a good input for biosensor fabrication which was aimed to be used as the early diagnosis cancer stem cell point of care (POC) device.

Keywords: Graphene Oxide, Reduced-Graphene Oxide, MCF7, MCF10a, Electronic Materials.

1. INTRODUCTION

Since its first discovery using scotch-tape method, graphene, a 2D carbon material which is the basis of many carbon material structures like carbon nanotubes (CNT), fullerenes and others become a great attraction among researchers [1]. The magnificent properties of graphene like high electrical conductivity, high surface area and its solubility in solvent especially biomedical media give a great upshot to biomedical devices. For pristine graphene, π-π stacking and hydrophobic property are the major factors that bind its structure with drugs and bio-molecules through non-covalent interactions, which make this material highly potential in many applications including biomedical devices. The use of nanomaterial like CNT in cancer disease showed side effects on health [2] but interestingly, graphene acts in different manner. However,

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the introduction of pristine graphene is quite limited, thus alternative graphene forms are preferred. Recently, the most common of graphene resulted from the oxidation process of chemical exfoliation which is known as graphene oxide (GO) becomes great interest due to the easier and cheaper process, can be produced in large scale and easy to be manipulated in many applications. The presence of certain functional groups in its structure influences the binding mechanism with the target analyte including cells. To be used in electronic devices, the conductivity of this material needs to be improved. In general, reduced-graphene oxide (rGO) can be formed either by thermal or chemical reduction process. The chemical reduction is a scalable method but it results relatively poor yield in terms of surface area and electronic conductibility. Thermal reduction normally at higher temperature creates a better surface area which closes to the pristine graphene property but sometimes can also cause imperfections at the lattice structure. This carbon form become preference in many graphene-based electronic devices [3-5]. Figure 1 below shows the common graphene forms.

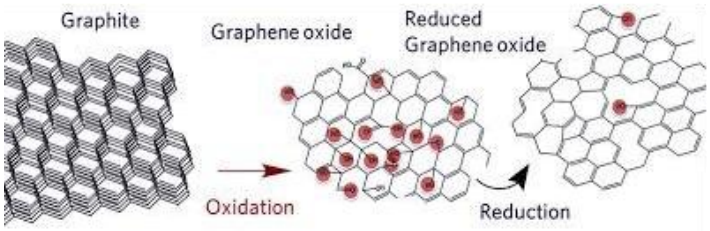

Figure 1. Common graphene forms.

Breast cancer becomes a nightmare among women all over the world. With the available diagnosis methods like mammogram, breast ultrasound, biopsy and magnetic resonance imaging, sometimes these methods can be very scary, inefficient, not accurate, time consuming and expensive. Commonly for breast cancer patients, treatments like radio and chemo therapies are given after the surgery. Unfortunately, the treatment which based on anti-cancer drug only targets the cancer cells, while the cancer stem cells (CSC) are still remained. Cancer stem cells posses the ability to differentiate tumour cell types which responsible to the spread of the cancer in the body and also responsible to the 90% of cancer deaths [2]. They also play a main role in the recurrence of tumours after treatment [6]. The effects of GO towards cancer cells were reported in [2, 6-9] where the results show that this material did not affect viability of the cell lines but has significant effects towards stem cells. GO successfully inhibited the tumor-sphere formation and reduce number of CSC which suggests that GO specifically target a global phenotypic property in stem cells that highly conserved in multiple tumour types including breast cancer. It is believed that material in flakes prevent the CSC from forming a tumour and instead force them into non-cancer stem cells [2,6].

Although great findings were obtained, it is still too early to conclude the effectiveness of GO as a therapeutic agent for cancer, but the outcomes can be utilized in biosensors and diagnosis devices. Recently, an early stage of point of care (POC) device for critical diseases like cancer, HIV, diabetes is highly demanded. In order to be used in biomedical devices, the current-voltage relationship for the interaction between GO and cells are crucial. Up to our knowledge until now, there is no work has been reported so far on the current-voltage characteristics. The electrical trend of this material towards cancer cell may lead to the interesting outcomes and can be used for early diagnosis device for CSC. For this purposes, some of device structure like flexible smart conducting paper [4], interdigitated electrode [10], FET [11] and others are suggested.

Herein, GO was prepared by chemical exfoliation and chemically reduced using sodium borohydride to obtain rGO. Both materials at the concentration of 100 µg/mL were being used experimentally towards MCF7 (breast cancer cell) and MCF10a (normal breast cell).

In particular, it was recognized that graphene based materials underwent complex interaction with the cells which impact significantly on the electrical conductivity. The efficacy of these materials on the cell viability and mortality also has been investigated.

2. EXPERIMENTAL PROCEDURES

2.1 Chemical Exfoliation for Graphene Oxide

The fine powder graphite (NE Scientific) and chemicals such as sulphuric acid, potassium persulfate, phosphorus pentoxide, potassium permanganate, hydrogen peroxide and hydrogen chloride (Sigma Aldrich) were purchased and used as described. 12 mL concentrated sulphuric acid was prepared in round bottom flasks and placed in oil bath setup at the temperature of 80°C. 3 g of graphite powder was added into it and stirred at 900 rpm. The mixture was then added with 2.5 g potassium persulfate and phosphorus pentoxide respectively and left for 4.5 hours. The mixture was diluted with 0.5 L of DI water after cooled down and left overnight. The suspension was filtered using vacuum filtration using PTFE membrane. The pre-oxidized graphite powder was mixed with 120 mL sulphuric acid in a flask at ice bath setup. The prepared solution was stirred and slowly added with 15 g potassium permanganate and continued stirring for 2 hours. The mixture was then stirred at 35°C and diluted with 250 mL DI water and left for 2 hours. After that, 700 mL DI water was added followed by 20 mL hydrogen peroxide (30%). Here, the colour changed from dark brown to bright yellow as a sign of well oxidized. In order to remove metal ions and residual acids, the resulting product was filtered and rinsed with 1:10 hydrogen chloride solution (1 L) followed by 1 L DI water. The graphite oxide powder paste was collected and dispersed in DI water before underwent ultrasonication process for 30 minutes to exfoliate the graphite oxide to graphene oxide (GO). The centrifugation at 4000 rpm was applied to separate unexfoliated GO. The paste was collected at the filter membrane and dispersed in DI water with ultrasonication in order to exfoliate the GO sheets. The obtained brown dispersion was subjected to centrifugation process at 4000 rpm for 30 minutes. The solution of GO was prepared at the concentration of 100 μg/mL as it is the best concentration as referred in [6].

2.2 Chemical Reduction Process

Reduction of GO was prepared using sodium borohydride (37.83 g/mol) (Sigma Aldrich) as a reducing agent in the ratio of 1:1. The 100 mM sodium borohydride (45.4 mg) was added to 12 mL DI water and underwent magnetic stirring at 300 rpm for 2 hours. The suspension underwent vacuum filtration method and washed again in 12 mL DI water. For the experiment, the rGO solution was prepared at 100 μg/mL as to compare with the same concentration of the GO.

2.3 Cell Culture

Breast cancer cell (MCF7) and normal breast cancer cell (MCF10a) were cultured in RPMI-1640 medium (HyClone, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (HyClone, USA) and 1% penicillin. The cells were grown on 75 cm^2 attached types, filter-cap culture flasks (NunClon, Denmark) before incubated at 37°C in a 90% humidified atmosphere of 5% CO_2. Once the cell 80% confluent, the cells was washed with phosphate-buffered saline and then harvested with 0.05% trypsin and 0.025% EDTA. For cell viability and mortality, trypan blue was used and the cells were counted using hemocytometer.

2.4 Characterizations

The morphology of GO and rGO sheets was characterized using SPI3800N Atomic Force Microscopic (AFM) (SII) and analyzed using Raman Spectroscopy. The cells before and after treated with GO and rGO at 24 hours were dropped on IDE (DropSense). The IDE contains two gold electrodes with dimension gaps of 10 µm fabricated on glass substrate. The current-voltage (I-V) relationship was tested using Keithley 4200A-SCS Parameter Analyzer, SPA.

3. RESULTS

3.1 Graphene Oxide and Reduced-Graphene Oxide

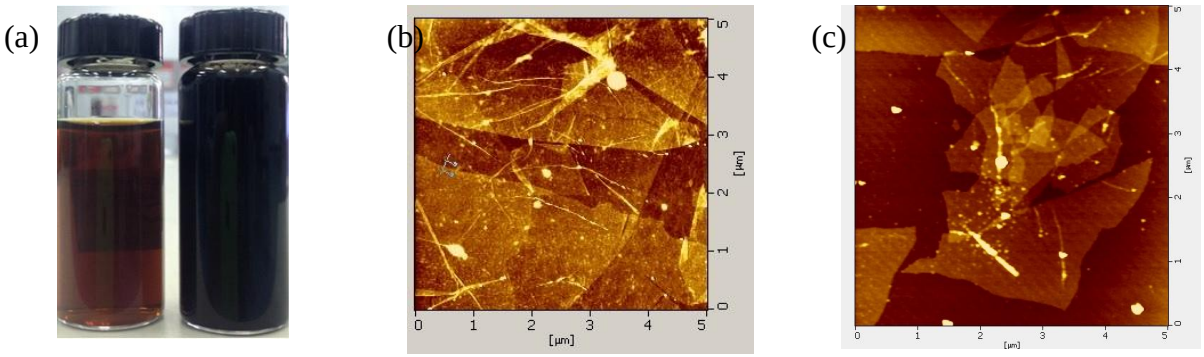

Figure 2. (a) physical state of GO and rGO; and AFM images (b) GO; (c) rGO.

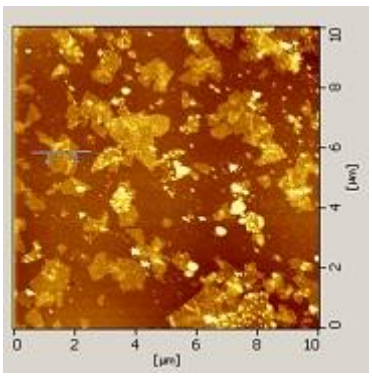

Figure 3. Small flake GO.

The physical state of GO and rGO was observed after solution preparation. The colour of brownish solution was changed into black after removal of some functional groups as shown in Figure 2(a). The structural of GO and rGO at the concentration of 100 µg/mL was examined using AFM as shown in Figure 2(b) and (c), respectively. From the results observed, the sheets of GO were heavily stacked compared to rGO due to strong van der Walls effect. The thickness of GO was ~1.33 nm while for rGO the thickness was reduced to ~0.9 nm respectively. The decrement of graphene sheets thickness due to the reduction of π-π stacking and some functional groups. The size of flakes of these materials can be controlled by centrifuge process as shown in Figure 3. The size of GO flakes showed the same potential in inhibiting the anchorage-independent proliferation of MCF7 stem cells [6]. It also reported that the size of GO especially at higher concentration give an effect towards MCF7 cells toxicity and biocompatibility [12].

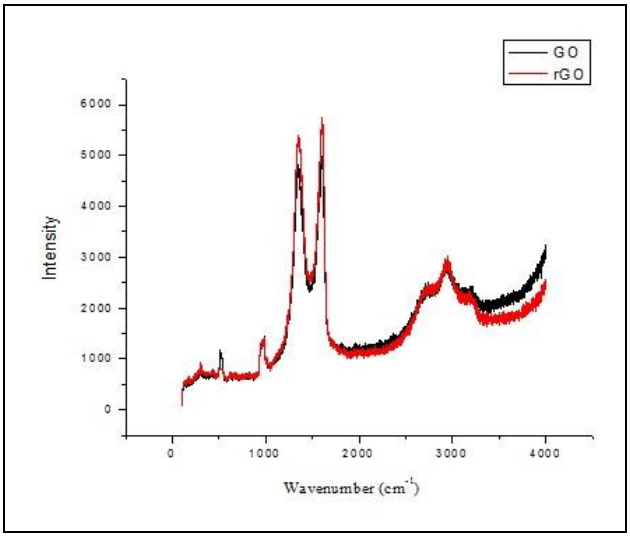

Figure 4. Raman spectra for GO and rGO.

Figure 4 shows the spectrum of GO and rGO under the Raman Spectroscopy investigation. It shows the presence of both G and D peaks which indicates the presence of graphitic nature of the samples as well as the defect associated at the basal plane of graphene crystal structure. The G-peaks for GO and rGO were observed at 1580 cm^{-1} while the D-peaks were observed at 1350 cm^{-1}. It was observed that the intensity of G and D peaks for rGO was higher compared to GO. The I_D/I_G ratios of GO and rGO were 0.96 and 0.89 respectively. The lower I_D/I_G ratio of rGO was attributed by lower defects. It also shows the presence of 2D-peaks at around 3000 cm^{-1} which indicates the number of layers produced by our method.

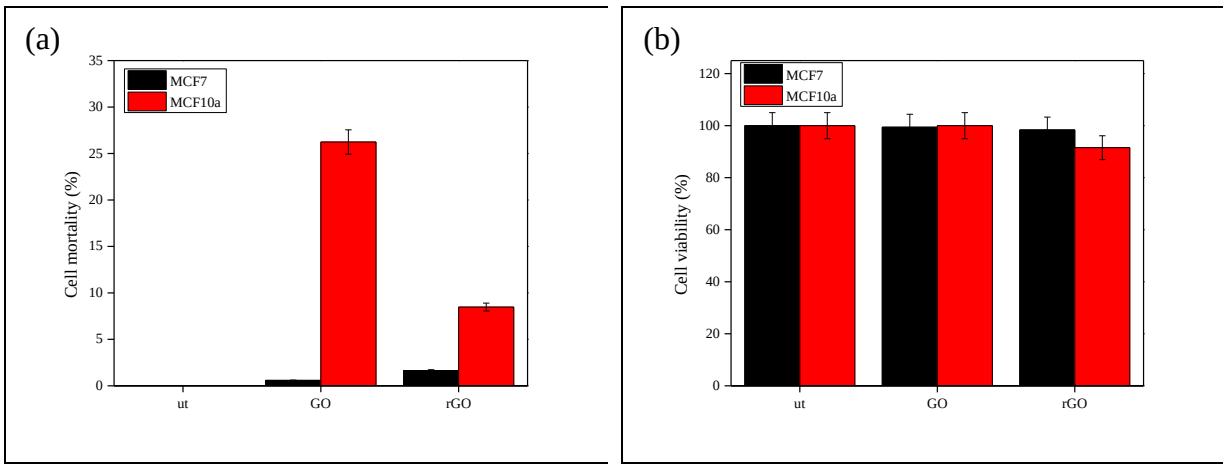

Figure 5. (a) Cell mortality; (b) Cell viability.

The ability of GO and rGO towards mortality and viability cells was summarized in Figure 5. The cell mortality was monitored by trypan blue exclusion assay where the dead cells are stained into blue while the live ones remain unchanged [13]. The accuracy of the cell mortality and viability were confirmed by taking the average from the three times repetition measurement. The mortality percentage of MCF10a was quite high compared to MCF7 after exposure for GO and rGO at higher concentration, but still in small percentage. Meanwhile, from the cell viability graph, it shows that only small percentage difference on number of cells obtained for MCF7 and MCF10a after treated with GO and rGO at the concentration of 100 µg/mL for 24 hours. This

result was comparable to the interaction towards A549 cell as reported in [2] where GO slightly decrease of the cell viability at higher concentration. This result is comparable to the similar work done in [6] where the population of MCF7 cells did not affected by the GO indicates specificity and selectivity towards cancer stem cells. It also has been reported that GO did not affect the viability of normal skin fibroblast cell line which indicates that this carbonaceous material was non toxic for normal body cells [6,14]. However, the toxicity of rGO was still unreported.

3.2 Current-Voltage Relationship

In order to develop a POC device, a clear trend of sensing material and target analyte need to be performed. It is expected that these sensing material will interact differently with both cells (MCF7 and MCF10a). This is important in a sensor fabrication as these results would describe how sensitive and selective the device to the analyte. 1 µg/mL of MCF7 and MCF10a were treated with GO and rGO at the same concentration and time. As expected, the rGO is more conducting compared to GO as the initial resistance of GO and rGO was obtained at 36 MΩ and 3.95 kΩ, respectively.

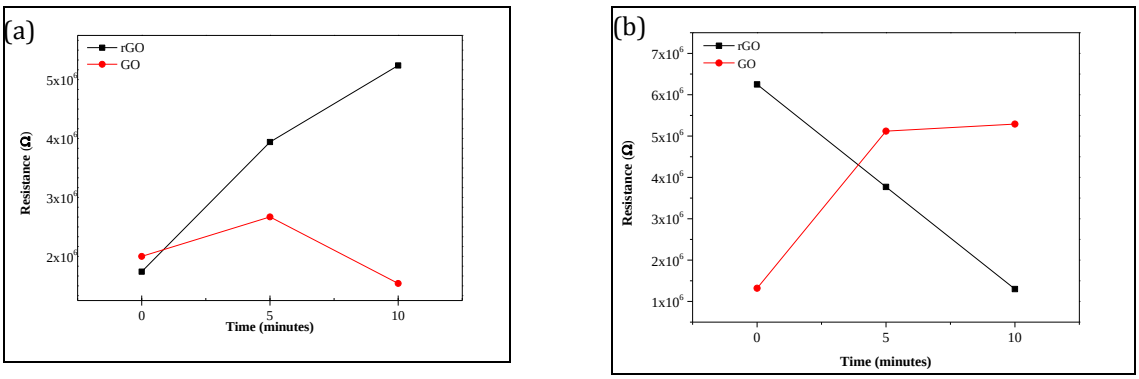

Figure 6. Resistance of (a) MCF7 and (b) MCF10A towards GO and rGO.

The interaction of sensing material (GO and rGO) and the analytes (cells) were tested immediate, after 5 and 10 minutes. The results were collected from three batches of samples and averaged from five sets of data. Based on the results obtained in Figure 6, for MCF7, the resistance of rGO increased up to 5.24 MΩ at 10 minutes. For GO, the resistance was increased after 5 minutes tested before dropped to 1.54 MΩ after 10 minutes. For normal breast cell MCF10a, different trend was observed. For rGO, the resistance for MCF10a was decreased with the tested time. While for GO, the resistance increased before slightly dropped at 10 minutes tested time. From the results, it can say that an obvious trend for both MCF7 and MCF10a was obtained after treated with GO and rGO.

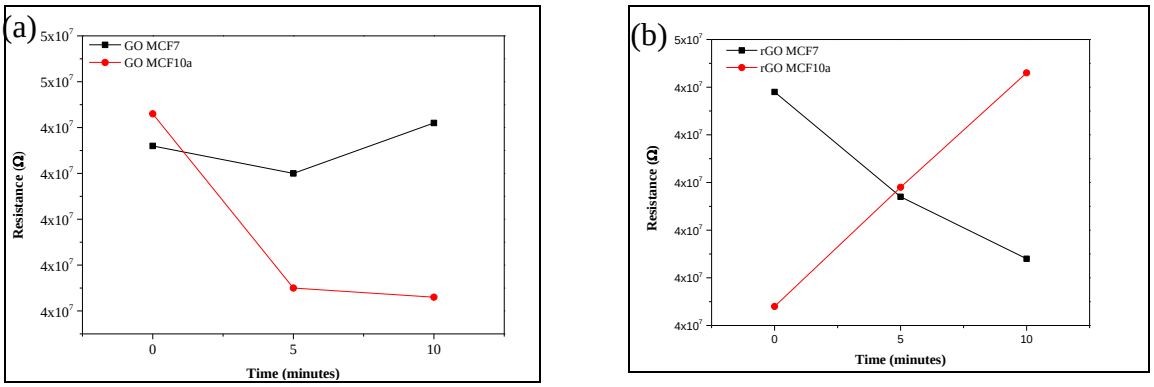

Figure 7. Summary of cell resistance due to (a) GO; (b) rGO.

The resistances of cells (by eliminating the resistance of total complete medium and GO/rGO) were summarized in Figure 7. Here, it can be concluded that GO and rGO interact differently with both MCF7 and MCF10a.

4. CONCLUSION

Graphene oxide (GO) was prepared using chemical method before chemically reduced to form reduced-graphene oxide (rGO). From the results obtained, GO and rGO showed different resistance pattern towards MCF7 and MCF10a. In order to obtain more accurate result in future, PrestoBlue cell viability assay and electrictrical characterization by using impedance analyzer will be used. However, these preliminary results become a promising outcome where these carbonaceous materials are potential to be applied in biomedical devices.

ACKNOWLEDGMENTS

This work was supported by Fundamental Research Grant (FRGS) (No.: 9003-00628) and Short Term Grant (STG) (No.: 9001-00496) funded by Ministry of Education Malaysia.

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