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A layered graphene oxide/graphene electrode for electrochemical biosensor applications

Po-Yu Chien[1], Chi-Hsien Huang[1,2*], Ying Li[2]

[1]Department of Material Engineering, Ming Chi University of Technology, Taiwan
[2]Center for Thin Films Technologies and Applications, Ming Chi University of Technology, Taiwan

Abstract

Recently, many researchers have indicated that microRNA-21(miRNA-21) has a close relationship with cancer and can be found in blood stream. Abnormal expression of miRNA-21 is associated with cancer. It has considerable potential to be used as a biomarker. A graphene oxide-graphene layered electrode was developed for the detection of the carcinogenic sequence miRNA-21. The preparation of graphene oxide was achieved by subjecting the stacked double-layer graphene to a low-damage plasma treatment (LDPT). In our previous study, we confirmed that adding hydrogen during oxygen plasma treatment (O/H-LDPT) can increase the carboxyl group content using X-ray photoelectron spectroscopic analysis. We also compare the immobilization results of two different plasma conditions (O-LDPT and O/H LDPT) obtained using chronocoulometric analysis. A probe (ssRNA) was further immobilized onto the surface of the graphene oxide-graphene double-layer electrode via a covalent bond and then hybridized with its target miRNA-21. The change of peak current due to the hybridized target miRNA-21 could be used for the quantitative sensing of miRNA-21 concentration.

1. Introduction

Graphene and graphene-based materials have been attracting great research interest because of their unique structural features; high surface area, thermal conductivity, good electron transfer and flexibility, and chemical stability. Due to its fast electron mobility, high specific surface area, quantum, Hall effect, and upstanding electric conductivity, graphene is becoming an ideal material for the construction of biosensors for nucleic acid hybridization, protein electrochemistry, and small-molecule detection. As we know, graphene oxide shows good hydrophilic and biocompatibility due to oxygen functional groups, it can be used in super capacitors, flexible solar cell and biosensors. In here, graphene oxide as a biological receive surface. In our double layer graphene,only top layer graphene was oxidized and bottom layer graphene still keep the natural as a transmission layer.Until now, such electrode structure-based electrochemical sensor is not yet developed to detect miRNA-21.

miRNA-21 has been shown to play important role in development of heart disease. It is one of the microRNAs whose expression is increased in failing murine and human hearts.

2. Experimental

2.1. Sample preparation

Chemical vapor deposition (CVD) has been successfully used to synthesize graphene films on copper foil in a tubular quartz furnace. The as-grown graphene was transferred from the Cu foil to an indium tin oxide (ITO) glass substrate (thickness is 0.7 mm and sheet resistance is 7 Ω/□) using the wet transfer. Then, we use the oven thermal annealing process for annealing, In the wet process we used a lot of photoresist, the acetone could not break it down completely. So we used oven to remove the Residual photoresist and allow a better coupling. In order to achieve a double layer graphene (DLG), the previous steps were repeated again.

2.2. Graphene oxide/graphene preparation

Graphene oxide was prepared by applying LDPT [1] on the stacked DLG through the effective control of oxidation. We used O-LDPT and O/H to oxidize DLG grown by CVD to obtain Graphene oxide/graphene structure. In our DLG, only the top graphene layer was oxidized; the bottom graphene layer was kept as the natural electrical transmission layer.

2.3. RNA immobilization and hybridization

The immobilization of the probe was achieved by adding 100 mL of a probe solution, with a concentration of 10^{-7} M, onto the surface of the graphene oxide-graphene electrodes and the mixture was kept at room temperature for 16 hours. Then, the probe-modified electrodes were rinsed with phosphate-buffered saline(PBS) water for 10 times to remove those probe that were weakly bonded onto the electrode surface or free-standing in the electrolyte solution. Different concentrations target miRNA-21 were dripped onto the samples at 90 ℃ for 5 minutes to hybridize with probe.

2.4. Electrochemical measurements

Chrono coulometry(CC), Cyclic voltammetry (CV), and Different pulse voltammetry (DPV) were conducted using a CHI 6111e electrochemical analyzer/workstation (CH Instruments, Austin, TX). CC measurements were carried out with 50 μM $Ru(NH_3)_6^{3+}$ and the measurement potential ranged from 0 V to –0.3 V. The pulse width was 0.25 s, sensitivity (A/V) was 0.001, and quiet time was 2s. Both CV and DPV measurements were carried out in a 10 mM ferricyanide aqueous solution (1 M KCl as the supporting electrolyte) at room temperature. A CV scan rate of 0.1 V/s was chosen for the rest of this work. The scan potential ranged from 0.7 V to -0.3 V. The pulse period for the DPV measurement was 0.5 s, pulse width was 0.05 s, pulse amplitude was 50 mV and quiet time was 2s.

3. Result & discussion

3.1. LDPT

Raman spectroscopy is a fast, non-destructive, and powerful instrument for characterizing graphene. Fig. 1 shows the Raman spectral changes of DLGs before and after O/H-LDPT. After a treatment time of 3 and 8 minutes the intensity of the D band (ca,1335 cm^{-1}) increases, while that of the D' (1615 cm^{-1}), G (ca,1575 cm^{-1}) and 2D (ca,2680 cm^{-1}) bands decrease. The results indicated the successful graphene transfer and oxidation.

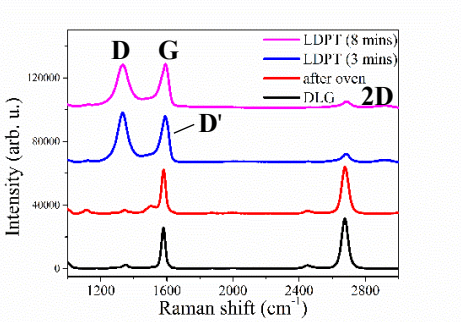

Fig. 1. Raman spectra of the DLGs before and after LDPT.

3.2. CC measurements

Due to the adsorption of the $Ru(NH_3)_6^{3+}$ on the surface of the electrode and its reduction boosting ability. The redox charge of $Ru(NH_3)_6^{3+}$ can be used to determine the probe's surface density [2] by electrostatic interaction with the anionic RNA backbone. The probe's density of probe of O-LDPT and O/H-LDPT are 1.00×10^{12} and 1.42×10^{12} molecules/cm^2 with the addition of 100 nM probe (Fig.2). The electrodes surface probe density of O/H-LDPT is much higher than O-LDPT

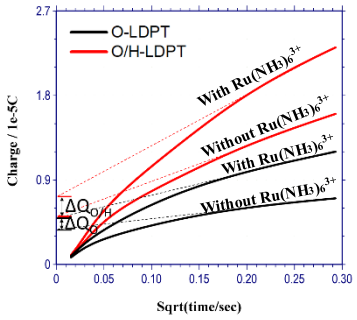

Fig. 2. Chronocoulometric measurement result shows amount of $\Delta Q_{O/H}$ is bigger than ΔQ_O, is attributed to more carboxyl groups content can react with more probes. Leads to increases the amount of $Ru(NH_3)_6^{3+}$ adsorption on electrode surface.

3.3. CV measurements

A pair of well-defined redox peaks was observed for different target miRNA-21 concentration. These redox peaks correspond to the $K_3Fe(CN)_6$ electron transfer process. The redox capacity of the electrode is not significantly different due to the difference in scanning rate in the range of 0.01 to 2 volts (Fig. 3(a)) indicating that our electrode has a very good stability. Fig. 3(b) shows the comparison of CV curves characteristic for four different target concentrations (0, 1, 10, and 100 nM) of miRNA-21 on the graphene oxide-graphene electrode. The results show that when the target concentration is higher, a lower current and a large potential peak-peak separation are obtained.

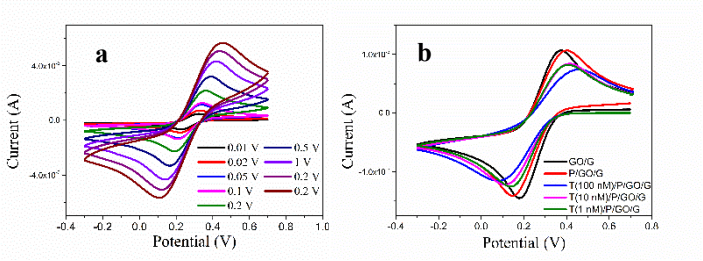

Fig. 3. CV characteristics of samples after hybridization under different miRNA-21 concentration.

3.4. DPV measurements

DPV shows high current sensitivity compared to cyclic voltammetry. This is due to the lower concentration detection, magnified resolution, lower background noise and well defined peak current. DPV measurements have been carried out to evaluate the sensitivity of this DNA biosensor in the ferricyanide system by varying the concentration of target miRNA-21 from 10^{-7} M to 10^{-12} M as shown in Fig. 4. The R^2 value for this equation is 0.9687 and limit of detection calculated from the best set of results is found to be 2.36 pM.

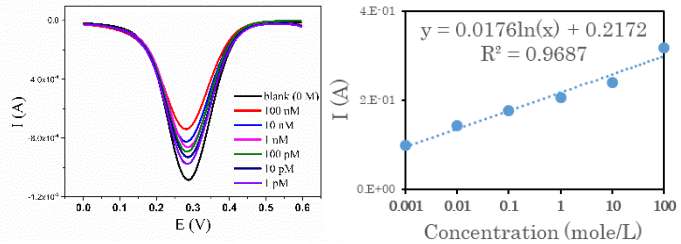

Fig. 4. DPV recorded after hybridization with different concentrations of target miRNA-21 varying from 10^{-7} to 10^{-12} M.

4. Conclusion

In this work, we have presented an electrochemical biosensor based on a layered graphene oxide/graphene structure electrode miRNA-21 detection. We used CC to confirm that O/H-LDPT can effectively increase the carboxyl content. The graphene oxide/graphene based sensor exhibits a wide linear range and limit of detection of 2.36 pM.

Acknowledgements

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References

1. Cheng, H.E., et al., Surface & Coatings Technology, 2016. 303: p. 170-175.
2. Liao, W.C. and J.A.A. Ho, Analytical Chemistry, 2009. 81(7): p. 2470-2476.