CANCER SENSORS BASED ON GRAPHENE AND GRAPHENE COMPOSITES

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ABSTRACT

A comparison of performance of three different types of graphene biosensors for the detection of prostate specific antigen was reported. Three different graphene structures were synthesized by mechanical exfoliation, microfluidic and self assembly respectively, representing graphene composites from pure to hybrid. Various parameters of different types of graphene biosensors were compared, such as detection limits, sensitivity, stability, etc. Hybridized materials in the graphene composites will change the electrical stability. However, hybrid or decorated materials enhance the absorption of target molecule, which introduce much higher sensitivity and detection limits. As were expected, self assembled graphene biosensor demonstrated the highest detection limits and sensitivity, but showed poor stability. On the other hand, pure graphene and microfluidic induced graphene presented lower detection limits and sensitivity, but better stability due to the absence of hybrid polymer in the graphene composites. The results and discussion demonstrated here can provide a guidance for the design of graphene based biosensors.

KEYWORDS: Graphene; Hybrid Nanocomposite; Self Assembly; Microfluidic; Cancer Sensor.

INTRODUCTION

Cancer markers are specific molecules existing in blood or tissue, whose measurement or identification is elucidated very critical and efficient in cancer prediction, diagnosis, and monitoring [1]. The prevailing biosensor protocols for detection of cancer markers include the enzyme linked immunosorbent assay (ELISA) [2], surface plasmon resonance (SPR) [3], nano-material sensor array, etc. Clinical utility of cancer biomarker to diagnose disease requires the capability to measure extremely low concentration of proteins, which is also important to understand cellular processes and to search for new protein biomarkers. However, the detection limits of these methods have lagged far behind the requirements for clinical utility and research. Recently, to overcome the hurdles of the previous detection methods, graphene, a two dimensional monolayer of sp2-bonded carbon atoms, is attracting tremendous interests due to its unique structural, electrical, chemical, and mechanical properties. Graphene-based biosensors are proposed for sensitive and label-free detection of many types of biomarkers with rational physical and/or chemical modification. Several graphene synthesis approaches were developed, including lithographic patterning, unzipping of carbon nanotubes, epitaxial growth on SiC or metals, chemical exfoliation of graphite oxide, and chemical vapour deposition (CVD) growth on metal substrates. In order to extend the good properties of graphene to achieve specific applications, pure graphene sheets have been extensively integrated with other materials such as polymers and nanoparticles into various hybrid structures to enhance the performance of biosensors.

At present, some researchers have been dedicated to the construction of graphene nanocomposites with various strategies. Among different types of pure or hybridized graphene structures, three typical structures are the most popular. Pure graphene is believed to be the most direct and simple structure (i.e., single or several layers of graphene sheets generated by exfoliation method or CVD method), and has been successfully used to prepare pH and DNA biosensors [4]. The second type of structure is graphene nanoplatelets assembled composites (i.e., after synthesizing stable dispersion of graphene nanoplatelets solutions, the substrate was introduced for the assembly of graphene nanoplatelets without any other materials hybrid) [5]. Several biosensors were developed based on the graphene composites. The third type of structure is hybridized graphene composites (i.e., nanoparticles or polymer were hybridized with graphene platelets to synthesize hybrid structure) [6]. Numerous biosensors were derived from this type of graphene structure with enhanced performance and specific functions. Despite the greatly important application of graphene-based nanocomposites in biosensors, only few researchers focused on the comparison of the performance among different graphene structures.

Therefore, in this paper, three types of graphene based composites from pure to hybrid were synthesized for the construction of prostate specific antigen (PSA) biosensors, which are widely used for the early detection of prostate cancer. Herein, pure graphene was generated by mechanical exfoliation method developed by our group. assembled graphene composites were fabricated by microfluidic method without introducing other hybrid material. Hybridized graphene composites were formed by self assembly technique. The performance of different types of PSA biosensors were investigated, such as sensitivity, detection limits, stability, etc. These results and discussion demonstrated here can provide a guidance for the design of graphene based biosensors.

EXPERIMENTS

Fabrication procedures of the three types of PSA biosensors were illustrated in Figure 1. To fabricate pure graphene biosensor, a photoresist layer (Shipley S1818) was used as a transfer stamp to exfoliate graphene patterns from highly oriented pyrolytic graphite (HOPG, MikroMasch Inc., ZYA grade), and transferred patterns onto a SiO_2/Si substrate. After that, chromium/gold layers 50/200 nm thick were deposited on the substrate by an AJA sputter system (Model ATC

2000), and patterned to electrodes. To fabricate assembled graphene composites without other material hybrid, microfluidic method was introduced to deposit the graphene nanoplatelets. Prepatterned substrate was immersed into HF buffer solution (10:1) to obtain the microchannel 250 nm deep in the silicon dioxide layer. Graphene suspension solution were introduced into the reservoir. Due to the hydrophilic property of silicon dioxide, the capillarity induced the graphene suspension solution into the microchannel. Next, the electrodes were fabricated as described before. To fabricate hybridized graphene composites, multiple graphene/PDDA nanocomposite layers were layer-by-layer self assembled on a clean silicon wafer with SiO_2 300 nm thick by immersing the substrate into the charged suspensions with a sequence of [PDDA (10 min) + PSS (10 min)]$_2$ + [PDDA (10 min) + graphene suspension (20 min)]$_5$. Next, the electrodes were fabricated as described before. After manufacture, the biosensor was functionalized by immobilization of capture antibody on the surface. A graphene sensor was first immersed into a 0.1% poly-l-lysine (PLL) aqueous solution positive charged for 1 hour. Next, the graphene sensor was incubated for overnight at 4 ºC in PSA capture antibody solution at a concentration of 10 µg/ml. Next, the sensor was incubated in 3% BSA blocking solution at room temperature for 5 hours to block nonspecific binding sites. After repeating the rinsing step, the label free sensor was ready for testing. The detection principles of the graphene biosensor are demonstrated in Figure 2. Given that the conductance of graphene is proportional to the product of charge carrier density and mobility, changes in density and/or mobility of charge carriers must be responsive when molecules or ions are absorbed by graphene. The conductance of the graphene based biosensor modified with the PSA capture antibody shifts as the concentration change of PSA solutions. The shifts of graphene devices were monitored using Agilent Data Logger (34970A, Agilent Inc.).

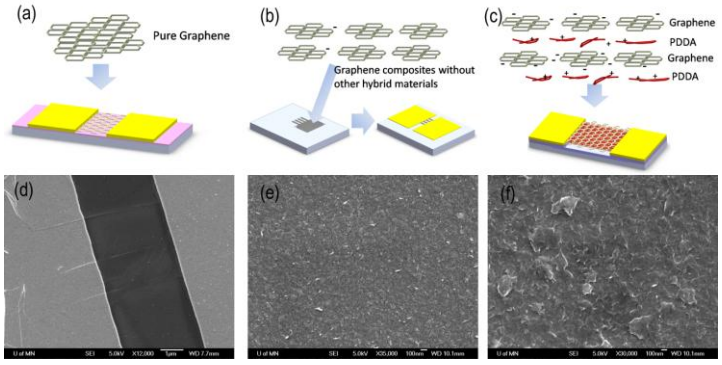

Fig.1. Sketches of three different types of graphene based biosensors from pure to hybridized graphene composites: (a) mechanical exfoliation method derived graphene biosensor structures; (b) microfluidic method induced graphene composites without introducing other hybrid material; (c) self-assembled graphene hybridized with polymer. (d-f) SEM images of surface profile of pure graphene, microfluidic induced graphene composites, and self-assembled hybridized graphene composites, respectively.

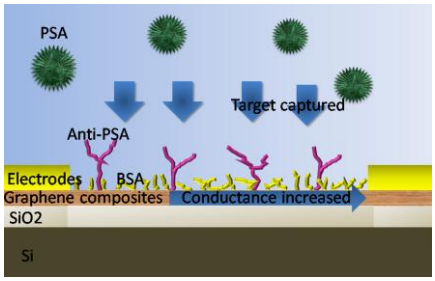

Fig. 2. Schematic illustration of detection principles of the graphene biosensors. After graphene biosensor modified by bioreceiptor, the immunoreation between PSA capture antibodies and target protein PSA will take place when different concentration PSA solutions introduced, and the resistance of graphene changes according to the absorption of PSA. Blocking solution BSA is introduced to enhance the specificity of the biosensor.

RESULTS AND DISCUSSION

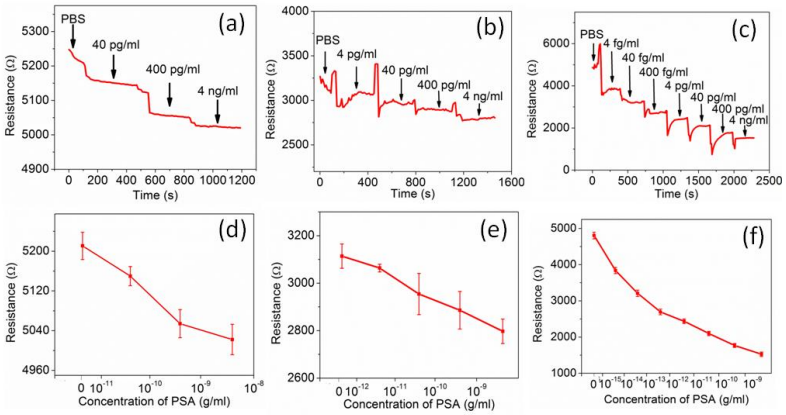

Fig. 3. (a, d) Change of resistance versus PSA concentration for mechanical exfoliation graphene biosensors. The detection limit is 40 pg/ml. (b, e) Shifts of resistance versus PSA concentration for microfluidic induced graphene biosensors. The detection limit is 4 pg/ml. (c, f) Shifts of resistance versus PSA concentration for self-assembled graphene biosensors. The detection limit is 4 fg/ml. The data for figures in the right was extracted from the stable region of the real time conductance measurement curve in the left. After delivering the PSA solution each time, it takes several minutes for the resistance to get stable, allowing sufficient immunoreaction and avoiding the disturbance generated by the delivery of PSA solutions.

After immobilization of PSA capture antibody, the detection limits of three types of graphene biosensors were characterized by measuring resistance with alternate different concentrations of PSA solutions prepared by PBS. As is shown in Figure 3a and 3d, the resistance of pure graphene based biosensor decreased with the increasing of PSA concentration from 40 pg/ml to 4 ng/ml. In comparison, resistance-versus-time measurements recorded for the other two types of graphene biosensors manufactured and tested under the same conditions. As is shown in Figure 3b and 3e, microfluidic induced graphene biosensors were capable of detecting the shifts from 4 pg/ml to 4 ng/ml. And self assembled hybrid graphene biosensors had the best detection limits from the "step" response of real-time measurements. As is shown in Figure 3c and 3f, the device presented response down to 4 fg/ml PSA solutions. Although the self assembled graphene biosensors show the highest noise level, the surface profile of this type of graphene composites have great porous topography, which is more suitable to decorate capture proteins, providing the greatest sensing surface area per unit volume. This factor contributes to the detection limits more. On the other hand, the pure graphene biosensors have the smoothest surface, which demonstrates the lowest detection limits

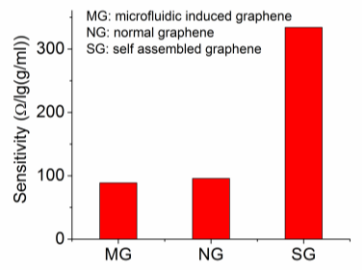

Fig. 4. Sensitivity analysis of different graphene biosensors.

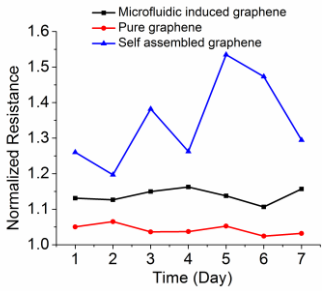

Figure 5. Long-term stability analysis of different graphene biosensors.

In addtion, the sensitivities of different PSA graphene sensors were compared. The sensitivity is defined as the input parameter change required to produce a standardized output change. Herein, the slopes of calibration lines present the sensitivities of different sensors. As is shown in Figure 4, the self assembled graphene demonstrates the largest sensitivity. This can be explained by the porous surface profile of self assembled graphene, which can enhance the absorption of molecules or ions. The long-term stability of different graphene biosensors was also investigated, by measuring the response of pH 9 solutions in continuous 7 days. During the non-measurement time, the ISFETs were kept in PBS water (pH 7) to prevent contamination and disturbance. Resistance for the PBS (pH 7) measurement was used as an initial resistance R_0. Normalized resistance was represented as the ratio of pH 9 resistance to the initial resistance. As is shown in Figure 5, the pure graphene biosensor was most stable, but self assembled graphene biosensor produced much more fluctuation results. The absence of hybrid polymer in the graphene composites results less disturbance compared with the hybridized graphene structures.

CONCLUSION

In this paper, three different types of graphene biosensors were synthesized and compared. All of them can be used for the detection of PSA. The self assembled graphene biosensors demonstrated the best detection limits and sensitivity, and the pure graphene and microfluidic induced graphene biosensors showed the better accuracy and stability.

ACKNOWLEDGEMENTS

Authors acknowledge the assistance of fabrication and characterization from Nanofabrication Center.

REFERENCES

[1] Z. Zhong, W. Wu, D. Wang, D. Wang, J. Shan, Y. Qing, Z. Zhang, "Nanogold-Enwrapped Graphene Nanocomposites as Trace Labels for Sensitivity Enhancement of Electrochemical Immunosensors in Clinical Immunoassays: Carcinoembryonic Antigen as A Model", *Biosens. Bioelectron.* vol. 25, pp. 2379-2383, 2010.

[2] M. Lu, D. Lee, W. Xue, T. Cui, "Flexible and Disposable Immunosensor Based on Layer-by-Layer Self-Assembled Carbon Nanotubes and Biomolecules", *Sensors and Actuators A: Physical,* vol. 150, pp. 280-285, 2009.

[3] J. Lin, H. Ju, "Electrochemical And Chemiluminescent Immunosensors for Tumor Markers", *Biosens. Bioelectron.* Vol. 20, pp. 1461-1470, 2005.

[4] Y. Ohno, K. Maehashi, Y. Yamashiro, K. Matsumoto, "Electrolyte-Gated Graphene Field-Effect Transistors for Detecting pH and Protein Adsorption", *Nano Lett*., vol. 9, pp. 3318-3322, 2009.

[5] S. Liu, J. Tian, L. Wang, Y. Luo, W. Lu, X. Sun, "Self-Assembled Graphene Platelet-Glucose Oxidase Nanostructures for Glucose Biosensing", *Biosens. Bioelectron.* vol. 26, pp. 4491-4496, 2011.

[6] W. Song, D. Li, Y. Li, Y. Li, Y. Long, "Disposable Biosensor based on Graphene Oxide Conjugated with Tyrosinase Assembled Gold Nanoparticles", *Biosens. Bioelectron.* vol. 26, pp. 3181-3186, 2011.

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