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Urine glucose analysis with functionalized graphene oxide as a material for amperometric sensor

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Abstract— **New functionalized graphene oxide (FGO) was systematically coated on the fabricated Au-PCB for the detection of glucose in urine. The electrical response of FGO-Au-PCB exhibited a wide linearity of 1.7 ~ 44.4 mM of glucose levels and a constant variables was less than 3% of the previously performed multiple measurements. The practical application has been demonstrated by measuring the electrical response against glucose in urine samples. In addition, our findings show similar improvement in urine glucose; within each current level, there were significant improvements in urine glucose. Comparison between the urine glucose and blood glucose showed no significant different level from the same subjects.**

I. Introduction

Metabolic disorders such as diabetes have been widely studied and a number of effective biosensors have been developed for detection and monitoring of such diseases [1, 2]. Worldwide glucose monitoring device market is expected to be more than 16 billion US dollars by 2014 [3]. Therefore, there is a constant growing need of commercially available glucose monitoring devices with improved analytical performance, robustness against interfering materials, and possible multiplexing analysis. Among several biosensor methods, electrochemical analysis has inherent advantages of simplicity, sensitivity, and selectivity, and easy integration with low cost. For the electrochemical or amperometric biosensors, it is highly required to have functional modification on electrodes to improve analytical performance [4-7]. In this paper, we have developed nanoscale composite material composed of graphene oxide and silver silica and applied them on the electrode substrate to enhance electron transfer between glucose oxidase and electrode substrate. Graphene oxide (GO) have gained significant attention for its enlarged surface area, solubility in water, biocompatibility and low manufacturing cost [8, 9]. We have fabricated metalloid-polymer hybrid nanoparticles (MPHs) for functionalization of GO. As we could see in previous studies, FGO could provide unique nanostructure and chemical properties making a good candidate for biosensor or glucose monitoring sensor [10, 11]. Therefore, we demonstrated the feasibility of a functionalized graphene oxide (FGO) based amperometric biosensor for glucose detection in this study.

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II. Experimental

Materials

A Precursor materials required for the synthesis of metalloid polymer hybrid nanoparticles (MPHs) such as silver nitrate ($AgNO_3$), tetraethoxyorthosilica (TEOS) ($Si(OC_2H_5)_4$), sodium borohydride ($NaBH_4$), ammonium hydroxide (NH_4OH), and silane chemical 3-aminopropyltriethoxysilane (3-APTES) need for the functionalization of graphene oxide (GO) were purchased from Sigma. Poly (ethylene glycol) (M_n= 10000 g/mol) (PEG) was obtained from Aldrich. Expandable graphite powder was purchased from Sigma–Aldrich, USA. Sulfuric acid (H_2SO_4), potassium permanganate ($KMNO_4$), hydrogen peroxide (H_2O_2) and hydrochloric acid (HCl) were obtained from Daejung Chemicals and Metal Ltd, South Korea. D(+)glucose and glucose oxidase from *Aspergillus niger* were purchased from Sigma. Milli-Q water with a resistance greater than 18 MΩ was used in all our experiments. All chemicals were of analytical grade and were used as received without any further purification.

Diabetic patient's urine and serum samples utilized in the biosensor study are kindly provided by Seoul National University Bundang Hospital, South Korea. All experiments were performed under an approved protocol of the Institutional Review Committee at Seoul National University Bundang Hospital. Before exposure to any study-related procedures, all patients gave their written informed consent.

Functionalization of GO

The brownish colloidal suspensions of GO nanosheets utilized in the current experiment was prepared by harsh oxidation of graphite powder, according to the modified Hummer's method [2]. Then functionalization of MPHs on the surface of GO was achieved by two step process, following the recent report [7, 8]. General protocol for functionalization of MPHs on the surface of GO can be explained from its two important surface chemical reaction such as amination process (carboxylic group of GO and silanized MPHs). Briefly, 200 µL of above prepared MPHs (5 mg/mL concentration) and 40 µL of 3-APTES (3% in C_2H_5OH) were added to a vial containing 3 mL of anhydrous ethanol and kept under magnetic stirring (800 rpm) at room temperature for 10 h. Followed by an aqueous solution of 200 µL GO (2.5 mg/mL concentration) was added and allowed to

stir (800 rpm) for another 10 h to make sure the covalent reaction between silanized MPHs and GO. After completion of reaction process, the MPHs functionalized GO sheets were separated by centrifugation at 12,000 rpm for 30 min, washed thrice with ethanol and utilized for construction of biosensor platform.

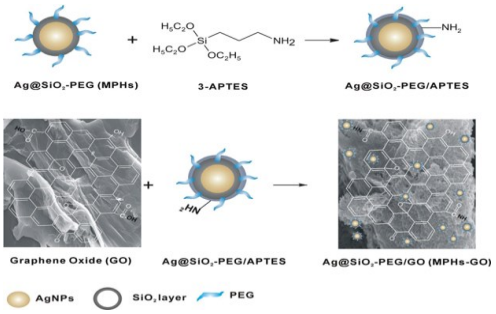


Figure 1.
Schematic of synthesis of functionalized graphene oxide. Metalloid polymer hybrids (MPHs) were systematically integrated with graphene oxide.

Preparation of FGO-Au-PCB

Glass based Au chip is fabricated by conventional MEMS process composed of photoresist deposition and gold evaporation followed by passivation. Fig. 1 shows electrodes formation process, electrodes on 4 inch glass wafer and integration of glass chip on PCB. Each glass wafer contains over 400 chips.

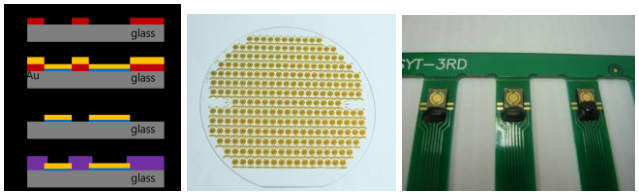


Figure 2.
Formation of electrodes on glass substrate (left), electrodes on 4 inch wafer (middle), and electrodes on PCB (right)

Aqueous dispersion of FGO (4 µL) was spin coated onto the oxygen plasma cleaned Au electrodes and the solvent was allowed to evaporate at ambient temperature for 1 hour. The resulting substrate was then coated with nafion substrate and mixture of Glucose oxidase (GOX), BSA, and glutaraldehyde (GA). FGO-Au-PCB was then kept under refrigeration at 4 ^{o}C for one hour and then utilized for electrochemical characterization.

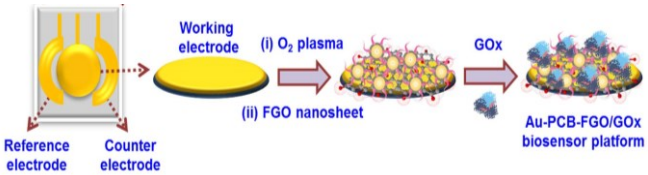
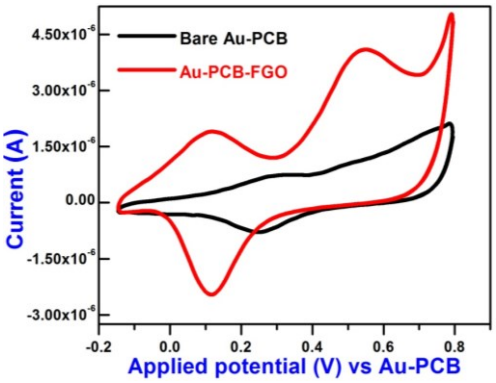


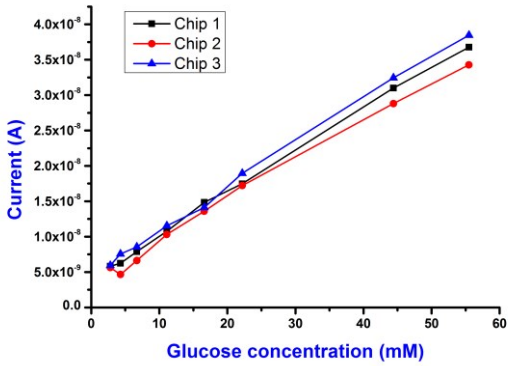
Figure 3.
Schematic of the formation of FGO-Au-PCB. The solution of combination of GOx, BSA, GA was coated on each working electrode.

I. Results

Fig. 4 (a) shows the cyclic voltammogram of Au-PCB and FGO-Au-PCB in 100 mM TES buffer (pH 7.0). As can be seen in Fig. 4 (a), GOx modified Au-PCB did not show any obvious redox peak. On the other hand, redox peaks clearly appeared as FGO-Au-PCB is used. The applied voltage of 0.6 V was selected based on cyclic voltammogram where peak current is appeared at that voltage. Fig. 4 (b) displays glucose concentration vs. current with using three different FGO-Au-PCB chips. The linear response with respect to various concentrations of glucose in TES buffer indicates that enzyme immobilized on the surface of electrodes provide the suitable environment for electron transfer between the glucose and sensor surface which is attributable to the detection of hydrogen peroxide obtained during the enzymatic reaction (GOx: glucose + O_2 → gluconolactone + H_2O_2). Fig. 5 shows the current level versus the addition of ascorbic acid to measure the current stability in the existence of interfering material. As can be seen in Fig. 5 there were no current level changes observable with increase of concentration of ascorbic acid from 5 mg/dL to 20 mg/dL. The nafion layer under GOX layer could prevent ascorbic acid from passing through the FGO coating.



(a)



(b)

Figure 4.
(a) Cyclic voltammogram of bare Au-PCB, (b) amperometry against the various concentration of glucose with using FGO-Au-PCB.

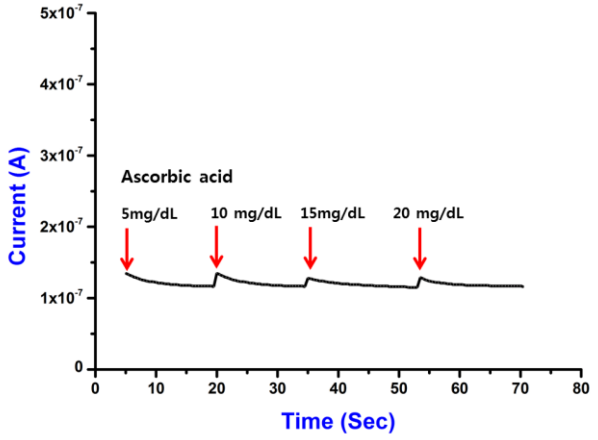


Figure 5.

Current stability in the existence of ascorbic acid. As ascorbic acid increases, no current level changes observable.

In order to evaluate the amperometric response of FGO-Au-PCB electrode against urine glucose, we tested nine patients' serum samples. Their blood glucose level was measured to be 83, 85, 110, 121, 133, 158, 174, 190, and 276 mg/dL. Measurements of blood glucose were performed using commercial glucose sensor Accu-CHECK (ROCHE). Figure 6 shows the comparison between urine glucose measured by our FGO-Au-PCB and blood glucose level. Although sera and urines were collected from only nine subjects, we could see similar patterns from both FGO-Au PCB and Accu-CHECK indicating that those who have high blood glucose level they also showed high levels of urine glucose.

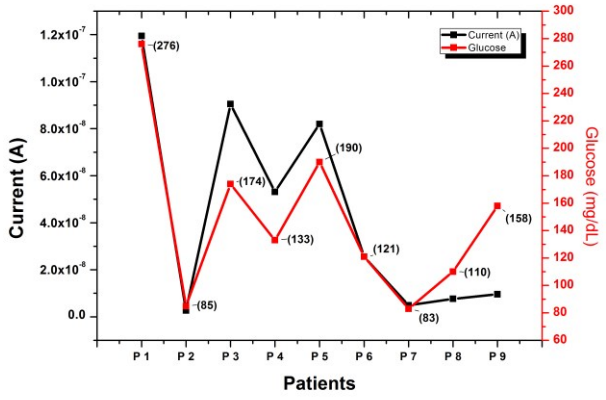


Figure 6.
Comparsion between urine glucose measured by FGO-Au-PCB and blood glucose measured by Accu-CHECK. Nine serum and urine samples were collected. Black line indicate measured currents (Left label) and red line indicates blood glucose (Right label).

II. Conclusions

In summary, we have fabricated metalloid polymer hybrids (MPHs) to functionalize graphene oxide (GO). The functionalization affects the structure as well as provides electron transfer enhancements. These results were well explained in previous studies [10, 11]. Urine glucose electrode was then developed with layers of FGO, nafion, and glucose oxidase (GOx). The amperometry investigation demonstrate that functionalized GO can provide a suitable platform for glucose detection, which facilitated the electron-exchange between products and electrode and increase detection ability at multiple composition range. In this study, we have compared measured current signal response to urine glucose level with blood glucose level. Although it is required to analyze more serum and urine samples to find out their correlations, our findings show intriguing results on their positive correlation. Our ongoing researches regarding the urine glucose monitoring include the integration of point of care system based on FGO electrodes and data analysis of both urine glucose and blood glucose from samples collected from same subjects using that system.

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