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Needle-Type Glucose Sensor Based on Functionalized Graphene

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Abstract

We demonstrate a novel, highly efficient glucose sensor based on functionalized graphene. Glucose oxidase (GOD) immobilization has been apprehendedbythe direct interaction between carboxyl acid groups of the reduced graphene oxide (RGO) and amines of GOD together with the electrostatic interactions existing between the positively charged polymeric ionic liquid (PIL) and GOD. This combined system can provide a favorable microenvironment for the GOD to retain its good bioactivity. The enzyme-coated graphene biosensor exhibited glucose-dependent electrochemical measurements against an Ag/AgCl reference electrode. The resulting sensor show broad range detection, up to 100 mM glucose concentration, with a sensitivity of 5.59 μA/ decade. It was found that glucose biosensor based on functionalized graphene can be seen as an effective candidate for the detection of sugar concentration in clinical diagnoses.

Introduction

Graphene has drawn significant attention since its discovery in 2004, not only in the field of basic research but also in technological applications due to its unique physicochemical dimensions, high sensitivity and excellent mechanical, thermal and electrical properties [1-5]. Development of the biosensors can potentially be an interesting application of graphene in order to utilize its tremendously large surface area to volume ratio as a dominating and promising parameter [6,7].There are many analytical procedures for the detection of bio-molecules but enzymatic electrochemical biosensing technique is being hailed as an alternative to the non-enzymatic biosensing. Preparation of enzymatic biosensors could be achieved through the immobilization of various types of enzymes onto the biocompatible matrices depending on the target molecules. The immobilization of enzymes onto the surfaces of suitably modified electrodes can significantly improve the bioactivity due to the biocompatibility of the surface modifier materials. The dramatic decrease in the hydrogen peroxide over potential and the direct electron transfer of glucose oxidase (GOD) observed at carbon nanotube (CNT) modified electrodes have already shown great promise for the biosensing of glucose [8,9]. Graphene based chemical sensors can potentially have a much higher sensitivity because graphene is a 2-dimentional single atomic layer of graphite which can maximize the interaction between the surface dopants and adsorbates. It has much lower Johnson noise [10-12] as compared to CNT, therefore, a minute variation of carrier concentration can cause a notable variation of electrical conductivity [13]. Moreover, graphene can be obtained easily by chemical conversion of inexpensive graphite [14].

GOD is widely employed in most of the glucose biosensors due to its high selectivity to glucose. The various methods such as covalent bonding [15], embedding method [16] and cross linking method [17-19] have been used to immobilize the GOD onto different supporting materials.

One popular route of graphene synthesis is through graphite oxide [20] which can be exfoliated to graphene oxide (GO) [21]. GO contains large amounts of oxygen groups, which can be favorable to the functionalization through the action of the biomolecules for bio-recognition events during biosensing [22,23]. Graphene oxide can be reduced chemically or electrochemically and this reduced graphene oxide (RGO) has a partly restored sp^2 lattice but still holds some fraction of oxygen-containing groups.

The GO aqueous dispersion was reduced with hydrazine in the presence of a polymeric ionic liquid (PIL) [24]. PILs are low molecular weight imidazolium salts with a low melting point. We use dpolyquaternium 16 (BASF Ludwigshafen, Germany) which is a copolymer with 95% molar of imidazolium chloride and 5% molar of vinylimidazole. Ionic liquids (ILs) have many advantageous properties such as non-flammability, a wide electrochemical window, high thermal stability, wide liquid range, and small vapor pressure [25]. PILs are also known to interact strongly with the basal plane of graphite and graphene, resulting in a stable aqueous dispersion.

We prepare the biosensor working electrode on a metal wire contrary to the conventional glassy carbon flat substrates reported previously for grapheme [14,23,26-28]. Main motive behind this is to take the biosensing to the intracellular environment with this needle like graphene biosensor. Glucose biosensors based on PIL functionalized graphene have been reported recently [28] by using common glassy carbon substrates. We utilize commercial polyquaterniums which are not reported previously in graphene functionalization and biosensing. These polyquaterniumsare specifically used in conditioners, shampoos, hair mousse, hair sprays, hair dye and contact lens solutions because of being positively charged [29]. They neutralize the negative charges of most shampoos and hair proteins and help the hair lie flat. This property can be extremely advantageous for enhancing GOD immobilization a step further as GOD has a negative charge in neutral pH solutions. This positively charged ionic liquid is well capable of the electrostatic incorporation of a negatively charged glucose oxidase enzyme and results in an efficient glucose sensing device [30]. GOD

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is also immobilized into the GO sheets via peptide bonds between the amine groups of GOD and the residual carboxylic functionalities of GO [27].

Therefore, by combining these two mechanisms of immobilization (Schematic Figure 1 ab), we can have a big advantage in using the functionalized graphene for the right application (as enzymatic biosensors) and transduction mechanism.

Materials and Methods

Graphene oxide was prepared by the modified Hummer's method [20,21]. The graphite flakes (Sigma Aldrich PN 332461, 4 g) were first put in H_2SO_4 (98%, 12 mL) and kept at 80°C for 5 h. The resulting solution was cooled down to room temperature. Mild sonication was performed in a water bath for 2 h to further delaminate graphite into a few micron flakes. Sonication time and power is very critical as it defines the size of the resulting graphene oxide sheets. Excessive sonication leads to extremely small flakes. Then the solution was diluted with 0.5 L deionized (DI) water and left overnight. The solution was filtered by Nylon Millipore filters afterwards. The resulting powder was mixed with $KMnO_4$ and H_2SO_4 and put in a cooling bath under constant stirring for 1.5 h. The solution was diluted with DI water and 20 mL H_2O_2 (30%) was added to it.

The supernatant was collected after 12h and dispersed in dilute HCl in order to remove the metal ion residue and then recovering by centrifuge [20,21]. Recovered clean GO was again dispersed in water to make a homogeneous dispersion and was centrifuged at 8000 rpm for 40 mins in order to remove the multilayer fragments. A PIL was added into the aqueous dispersion of GO at a concentration of 1 mg mL^{-1}, followed by reduction by hydrazine [24]. This process resulted in aqueous dispersion of functionalized graphene sheets.

Graphene was coated on a hot platinum (Pt) wire by dip coating [31,32]. Glucose oxidase (GOD)enzymesolution, 10 mg/ml, was prepared in 10 mM Phosphate Buffered Saline, pH 7.4 using GOD (E.C. 1.1.3.4) from Aspergillus niger type GO3A, 360 U/mg (BBI Enzymes Ltd). GOD enzyme was electrostatically immobilized by dipping the graphene-coated metal wire into 10 mL of the enzyme solution for 15 minutes at room temperature and then it was dried in air for approximately 20 minutes. All the electrochemical measurements of GOD immobilized graphene were perform usinga Keithley 2400 source meter, connected via computer controlled programin glucose solutions ranging from 1μM-100mMversus an Ag/AgCl as reference electrode.

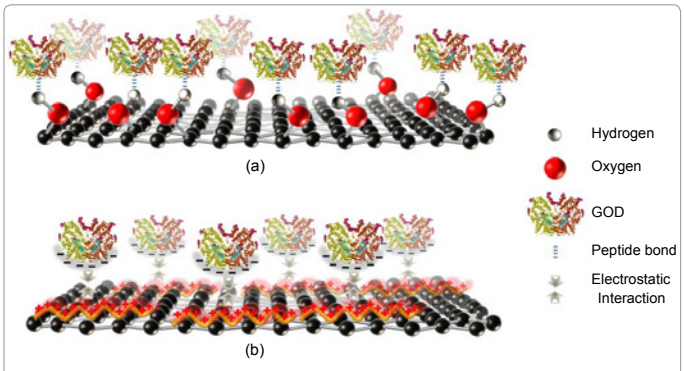

Figure 1: Schematic of the GOD immobilization on graphene(a) Peptide bond formation between remaining carboxylic groups of RGO and GOD (b)Electrostatic interaction between PIL functionalized graphene surface and GOD.

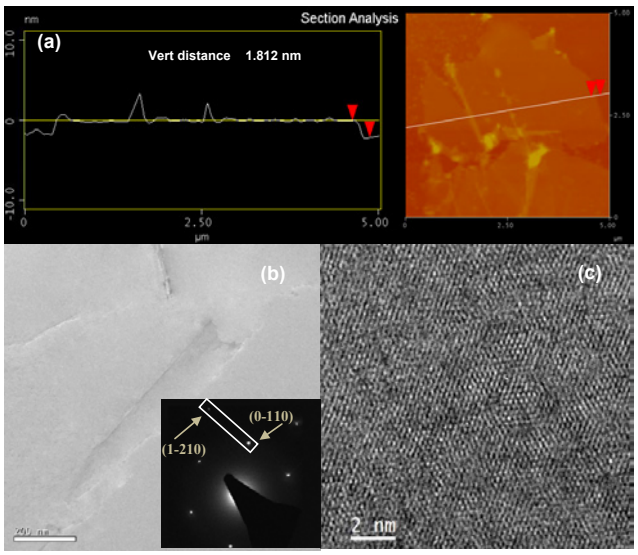

Figure 2: (a) AFM image of graphene nanosheets showing height profile(b) Low resolution TEM image of a typical wrinkled graphene sheet. Inset shows hexagonal skeleton carbon ring arrangement of carbon atom. (c) High resolution TEM illustrates good crystal quality of nanosheet.

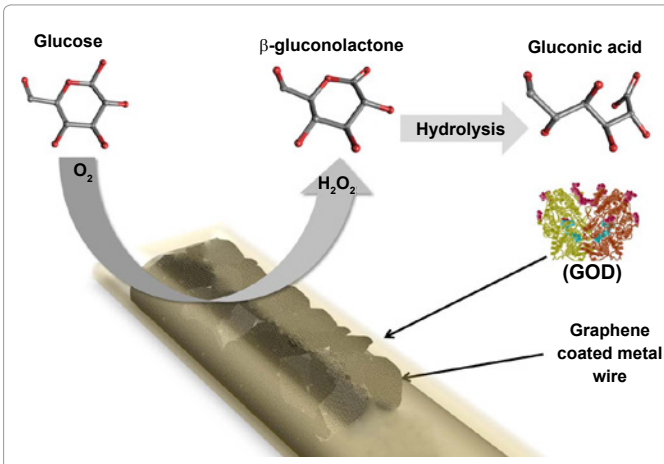

Figure 3: Schematic illustration of the glucose sensing setup using working electrode comprised of graphene sheets coated with GOD, along with possible electrochemical reaction near the electrode.

Results and Discussion

AFM image of our chemically reduced GO is shown in Figure 2. The graphene sheets were found to be fractionally thicker than the theoretical thickness of a single graphene layer owing to the adsorption of the PIL on the surface of the graphene sheets by non-covalent π–π interactions between the imidazolium rings of the PIL and graphene sheets [24,33,34]. The physical adsorption the ionic liquids along the surface of the graphene sheets has been verified by X-ray photoelectron spectroscopy (XPS) analysis, previously [34].

The sample for the transmission electron microscopy (TEM) was prepared by simple drop casting of the graphene dispersion onto the holey carbon grid. A fan heater was utilized with an objective to dry the holey carbon grid containing graphene nanosheets. The bright-field low resolution TEM results are shown in figure 2(b); selected area electron diffraction (SAED) pattern displays good crystalline quality of graphene

nanosheets with regular arrangement of hexagonal atomic packing of carbon, inset of Figure 2(b). The method of diffraction intensity ratios in the SAED pattern agrees with the previously reported results related to mono-layer graphene nanosheets [35]. High resolution TEM image demonstrates the presence of carbon lattice in Figure 2(c).

Graphene deposition onto the Pt wire was carried out by dip-coating. The metal wire can be dipped into the graphene solution for some time so as to allow the absorption of graphene planes on the surface of the metal [31,32]. The binding force between graphene and Pt, Pd and Ni surface is found to be higher than that for other metal surfaces [36]. Recent experimental observations also suggest a relatively strong adhesion between the metal (Pt and Pd) and RGO [37].

Selective glucose measurements were performed by atwo electrode electrochemical method. A Graphene-decorated electrode coated with enzyme served as the working electrode, and an Ag/AgCl electrode was used as reference electrode. The electrochemical measurements were started 30 minutes after immobilizing the graphene working electrode with GOD. The response of the glucose probe was measured with a Keithley 2400 source meter, connected via computer controlled lab view program. The construction of a two-electrode electrochemical cell was as follows:

reference electrode | reference electrolyte || test electrolyte | indicator electrode

Amperometry is relatively sensitive electrochemical technique; the output signal is the current that is linearly dependent on the analyte concentration by applying a constant biased. Graphene decorated electrode was tested amperometrically to measure the current at an accumulation voltage of 1V. Good sensitivity and selectivity has been realized by proper binding of GOD enzyme with functionalized graphene sheets. The analyte solution modification corresponds to abrupt current change and these changes can be related to the concentration of ions in the test electrolyte via a calibration procedure. Figure 4a depicts the calibration curve of the current versus concentration of the glucose ranging from 1 μM to 100 mM. We see that the sensor deviates from the linearity for the concentrations above 1000 μM but shows very good linear behavior below that limit. A good linearity up to 1000 μM very easily covers the clinical range of glucose in human blood [38]. Inset of the Figure 4s displays the results of three experiments for the same glucose-sensing electrode for the range between 15 μM to 1000 μM. It shows good repeatability and linearity for various glucose concentrations in this range. The sensitivity of the enzyme immobilized graphene electrode is determined by the slope of the calibration curve and was found to be 5.59 μA/ decade. A constant potential is applied to the GOD immobilized graphene electrode which assists hydrogen peroxide production from the oxidation of glucose. To test the sensitivity and stability of our constructed graphene based glucose sensor, we performed measurements while changing the glucose concentration in the buffer solution around the working electrode. Current response at the successive addition of glucose is recorded as shown in Figure 4b. The current at the graphene modified electrode increased with the gradual injection of glucose, pointing out that the current response arises from the oxidation of hydrogen peroxide produced during the enzyme reaction rather than from direct oxidation of glucose at the electrode [27]. The output response with stability is shown in Figure 5, indicating that the proposed sensor is fast and sufficiently stable.

After the amperometric measurements, the GOD immobilized GO electrode was tested potentiometrically. The response of the electrochemical potential difference of the GOD-graphene/metal to the changes in buffer electrolyte glucose was measured for the range of 1 μM to 100 mM and shows that this glucose dependence is linear and has sensitivity equal to 1.18 V/decade at 23°C (Figure 6). This linear dependence implies that the sensor configuration can provide a large dynamic range.

The sensing mechanism of the electrochemical glucose sensors is based on an enzymatic reaction catalysed by glucose oxidase (GOD) with β d -glucose, according to the following reaction:

$$H_2O + O_2 + d-Glucose \xrightarrow{GOD} d-gluconolactone + H_2O_2 \quad (1)$$

As a result of this reaction, d-gluconolactone and hydrogen peroxide are produced. These two products with the oxygen consumption can be used for glucose determination. With H_2O availability in the reaction, gluconolactone is spontaneously converted to gluconic acid (by hydrolysis reaction), resulting in a decrease of pH was used for the indirect determination of the glucose concentration with charged

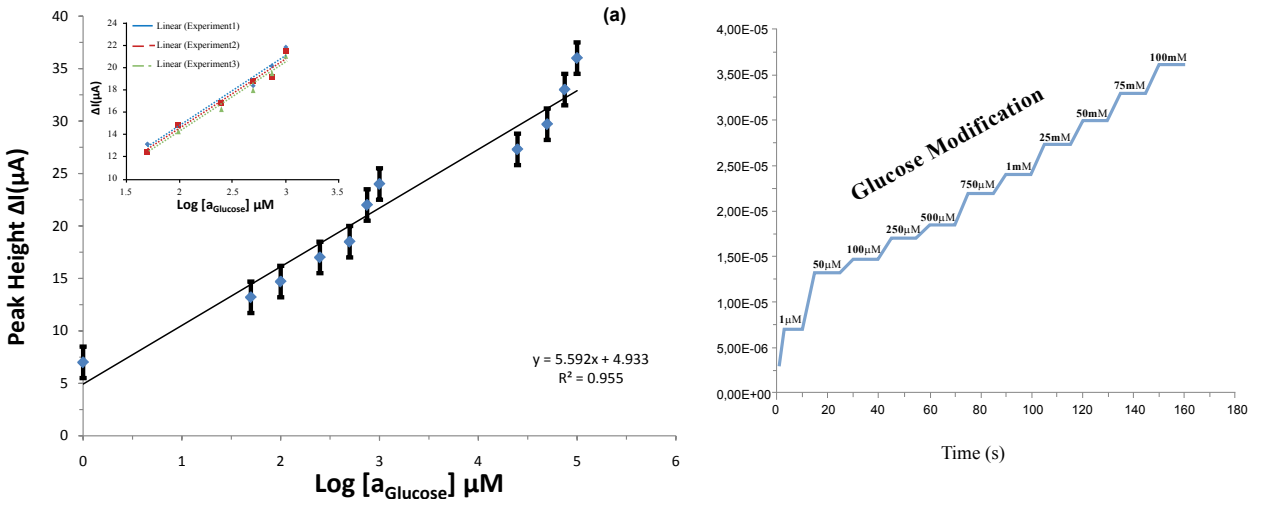

Figure 4: (a) Typical amperometric response at the GOD immobilized graphene decorated electrode versus glucose concentration, (b)The amperometric response versus time as the glucose concentration is modified surrounding the graphene decorated electrode.

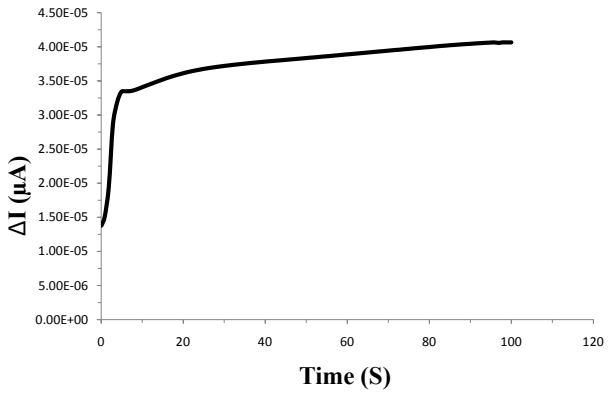

Figure 5: The output response versus time showing the stability of the sensor.

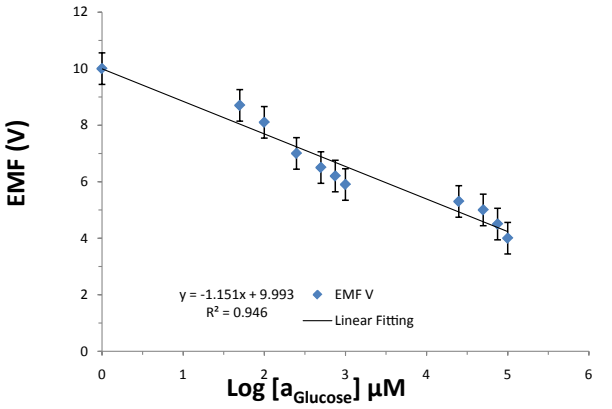

Figure 6: Calibration curve showing the electrochemical potential difference, for the enzyme coated graphene electrode with Ag/AgCl reference electrode versus Logarithmic concentration range for glucose solution variation over the range of 1μM to 100 mM.

products of gluconate$^-$ and a proton (H^+) according to the following equation:

$$\text{d}-\text{Gluconolactone}\ \text{Gluconate}^- + \text{H}^+ \xrightarrow{\text{Spontaneous}} \quad (2)$$

Typical biosensors usually measure the amount of hydrogen peroxide produced by the reaction between glucose and GO. Alternatively, some sensors (including our biosensor) measure the amount of oxygen consumed, or a change in pH which is accompanied by the production of gluconic acid that is produced from gluconolactone show in equation (2) [38-40].

Conclusion

In summary, we demonstrate a novel and efficient glucose sensor based on functionalized graphene. Interaction between carboxyl acid groups of RGO and amines of glucose oxidase plus the presence of positively charged PIL resulted in an effective GOD immobilization. The resulting electrochemical sensor exhibits a broad linear range up to 100 mM glucose concentration and with a sensitivity of 5.59 μA/ decade and a stable output response. Thus, sensor based on functionalized graphene can be seen as an effective candidate for the detection of sugar concentration, paving the way to its potential applications in the clinical diagnosis.

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