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Single-layer CVD-grown graphene decorated with metal nanoparticles as a promising biosensing platform

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ABSTRACT

A new approach to the development of a single-layer graphene sensor decorated with metal nanoparticles is presented. Chemical vapor deposition is used to grow single layer graphene on copper. Decoration of the single-layer graphene is achieved by electroless deposition of Au nanoparticles using the copper substrate as a source of electrons. Transfer of the decorated single-layer graphene on glassy carbon electrodes offers a sensitive platform for biosensor development. As a proof of concept, 10 units of glucose oxidase were deposited on the surface in a Nafion matrix to stabilize the enzyme as well as to prevent interference from ascorbic acid and uric acid. Amperometric linear response calibration in the $\mu mol\,l^{-1}$ is obtained. The presented methodology enables highly sensitive platforms for biosensor development, providing a scalable roll-to-roll production with a much more reproducible scheme when compared to the graphene biosensors reported previously based on drop-cast of multi-layer graphene suspensions.

1. Introduction

Graphene has attracted great interest in different scientific areas in the last years. One of the areas where it has experienced major interest is in its use as sensing platform (Pumera et al., 2010). Graphene is considered as an ideal material for electrochemical sensors because of its intrinsic properties in terms of large 2D electrical conductivity and surface area, two factors that would allow better performance in terms of signal to noise ratio and sensitivity. Another advantage of graphene when compared to other multidimensional carbon allotropes, such as carbon nanotubes, is the fact that graphene does not contain metal impurities that dominate and foul the electrochemical behavior of the transducer (Pumera and Miyahara, 2009). However in the majority of works presented as graphene sensors the employed material consists of multilayered carbon sheets, and thus the term graphene is misleading. The synthetic route in most cases is the reduction of graphite oxide following the Hummers method (Hummers and Offeman, 1958) with a final drop cast of the obtained flakes on the transducer electrode. Further decoration of these flakes with metal nanoparticles (NP) has also been extensively presented using reducing agents in solution or external electron sources (Baby et al., 2010; Lightcap et al., 2010; Hong et al., 2010; Wu et al., 2009). Those widely spread methods do not allow good reproducibility in the fabrication process in terms of number of layers, stacking on the transducer when drying or homogeneous transducer coverage, and thus do not lend themselves easily to mass production.

Here we present a synthetic route based on chemical vapor deposition (CVD) of single-layer graphene using copper as catalyst and its further decoration with Au nanoparticles by a simple one-step electroless deposition and transfer onto the transducer. CVD offers the advantage of producing high quality graphene on copper with the possibility of scaling into roll-to-roll processes (Bae et al., 2010), and in our case it also allows the use of the catalytic Cu foil as electron source for the subsequent metal nanoparticle decoration of graphene by electroless deposition. Improved transfer of CVD grown graphene from copper has been reported recently, (Li et al., 2009a) permitting an effective way to insure complete coverage of any desired electrical transducer by single layer graphene. Sensitivity of the developed graphene-based platform has been explored using the widely used enzyme glucose oxidase (GOD) as a study case. For this purpose, 10 enzymatic units were deposited on top of Au NP decorated graphene using Nafion as immobilizing matrix. To the best of our knowledge, the use of single layer graphene, decorated with noble metal nanoparticles by an electroless deposition, as a biosensing platform has not been reported previously. The detection limit achieved with the here presented biosensor is 4 μM.

2. Experimental

Fig. 1 illustrates the whole synthesis process from graphene growth to final transfer onto the GCE electrode and enzyme immobilization.

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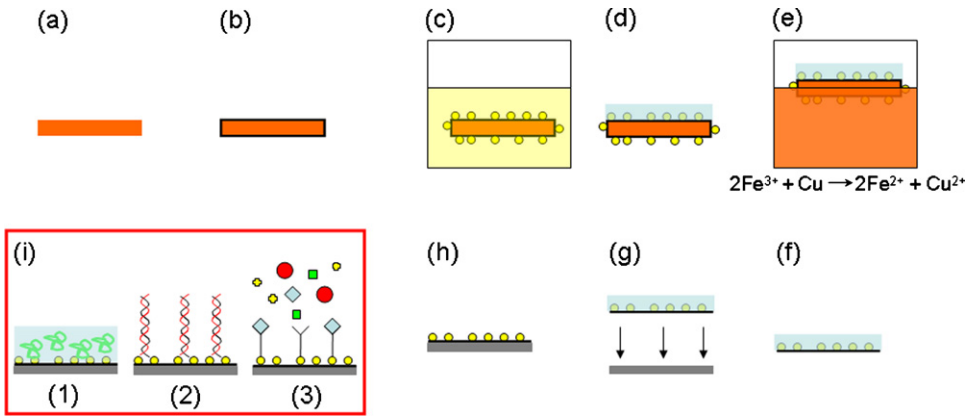

Fig. 1. Schematic presentation of the complete biosensor construction. From a copper foil (a) single layer graphene is grown by CVD (b). Au nanoparticle decoration is obtained by electroless deposition (c). PMMA spin-coating is performed (d) before copper etching (e–f). Transfer to the transducer (g) and PMMA removal with acetone (h) is performed prior to final biosensor fabrication (i). Examples of possible biosensors developed by this method are (1) enzymatic biosensors, (2) DNA-based biosensors, (3) immunological biosensors.

2.1. Reagents and materials

Copper foils 0.025 mm thick 2 cm × 8 cm in area 99.8% metal basis (Alfa Aesar) were used as the substrate. H_2 and CH_4 (Praxair) used in graphene growth were directly connected into the low pressure CVD reactor. $KAuCl_4$ (98% pure, Sigma) was dissolved in deionized (DI) water (Barnstead Nanopure) to the final desired concentration. Poly(methylmethacrylate) (PMMA, from Sigma), used in the transfer of the decorated graphene layer, was dissolved in toluene (Fisher Chemical) to a final concentration of 8 wt%. Phosphate buffer solution (PBS) at pH 7.0 was prepared by dissolution of the corresponding disodium salt (Fisher Chemical) with HCl 5 M final pH adjustment. Nafion 5% perfluorinated ion-exchange resin (Aldrich) was diluted in DI water to a final concentration of 1%. Glucose, ascorbic acid and uric acid (highest purity available from Sigma) were dissolved in PBS at the desired concentration for the calibration and interference studies of the biosensor. Glucose oxidase (from Aspergillus Niger 168800 U/g) was purchased from Sigma and prepared as described below.

2.2. Graphene growth by chemical vapor deposition

Graphene was grown on the copper foil following the procedure reported previously (Li et al., 2009b) A 2 cm × 8 cm sheet was introduced into a 1 in. diameter quartz tube and heated to 1000 °C under vacuum in a 30 sccm H_2 flow and annealed for 30 min. After annealing a 60 sccm CH_4 flow was added to the H_2 flow. Graphene growth was carried out for 30 min before cooling down with both H_2 and CH_4 flowing until temperature was below 600 °C. Graphene on copper was taken out from the CVD reactor when the temperature reached 100 °C.

2.3. Graphene decoration and transfer

Electroless deposition was used for the decoration of graphene. This technique is based on the spontaneous oxidation–reduction reaction between a surface and a metal ion in solution. For the reaction to be spontaneous, the reduction potential of the surface needs to be lower than that from the ion in solution. Then, the surface oxidizes while the metallic ion gets reduced. In our case the copper substrate used as catalyst during graphene growth presents a standard reduction potential of +0.34 V, meaning that any metal with standard reduction potential higher than copper (E^0 = +0.34 V) can be deposited by electroless deposition, opening a wide range of possible metal catalyst, such as Au, Pt, Pd and Ag.

For the decoration with Au nanoclusters, the 2 cm × 8 cm sheet was immersed in a 2 mM $KAuCl_4$ dissolution for 5 min. Rinsing in DI water was performed by triplicate and dried by N_2. After the decoration, the Cu/graphene/Au sheet was cut into 2 cm × 2 cm pieces prior to PMMA spin coating at 2000 rpm for one minute. For the transfer onto the glassy carbon electrodes (GCE) (CHI 104, 3 mm diameter), 3 mm × 3 mm pieces were cut and floated on a concentrated $FeCl_3$ dissolution as the copper-etching agent. Rinsing of the PMMA/Au/graphene in DI water was performed before the final placement on the GCE electrode surface.

2.4. Biosensor construction

Three milligram GOD (Sigma) 168,800 U/g were dissolved in 100 μL consisting of 50 μL DI water and 50 μL Nafion 1% in a similar way as in previous works (Baby et al., 2010). The final GOD concentration was 5000 U/ml while the final Nafion concentration was 0.5% due to the 1:2 dilution in DI water. 2 μL of which were drop-casted on top of the 3 mm × 3 mm decorated graphene on GCE and let dry at room temperature. Because of the known impermeability of Nafion towards AA and UA 2 extra μL of 1% Nafion were drop-casted on top to ensure enzyme entrapment as well as to provide AA and UA blockage that might provide interference signal by direct oxidation on the electrode surface. Electrodes were rinsed in phosphate buffer 0.l M at pH 7.0 prior to use. Biosensors were kept at 4 °C when not in use.

2.5. Characterization

Scanning electron microscopy (SEM) images were taken using a field-effect mySEM microscope (Novel X) operated at 1 kV. Raman spectroscopy (JYHoriba LabRAM) was performed in backscattering configuration with an excitation line provided by a HeNe laser (632.8 nm wavelength, 10 mW at the sample) through an Olympus BX41 confocal microscope (100× objective, numerical aperture = 0.8). Electrochemical characterization by cyclic voltammetry and chronoamperometry were performed using a CHI 660D (CH Instruments) potentiostat. The biosensor was mounted on a GCE (CH Instruments CHI104) and used as working electrode with a Ag/AgCl (CH Instruments CHI111) and Pt wire (CH Instruments CHI115) as the reference and counter electrodes respectively. Cyclic voltammetry was performed in the range of −0.5 V to +0.9 V (vs. Ag/AgCl) at 50 mV/s scan rate. Final calibrations were obtained at a fixed potential of +0.8 V (vs. Ag/AgCl) by adding the right amount of stock dissolutions into a 25 ml PBS dissolution.

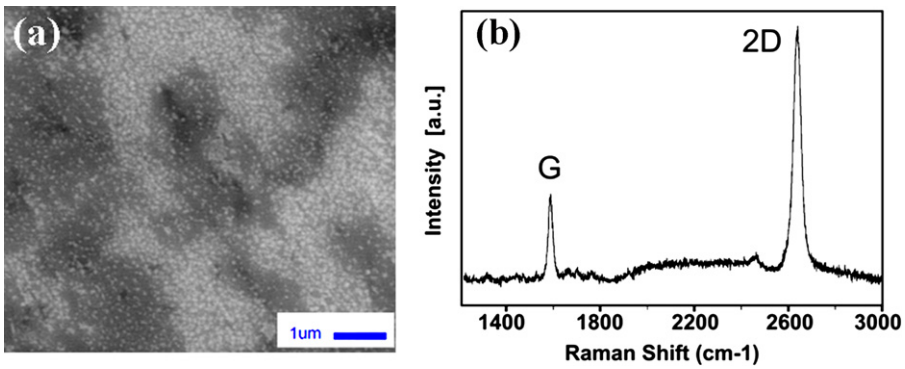

Fig. 2. (a) Scanning electron microscopy image of a Au nanoparticle decorated graphene after transfer on SiO_2-coated Si substrate. (b) Raman spectrum of the CVD grown graphene showing the typical G to 2D ratio of single layer graphene.

3. Results and discussion

3.1. Graphene/Au nanoparticle characterization

Fig. 2a shows a typical scanning electron microscope image from a gold decorated graphene sheet prepared and transferred by the above mentioned methods. Transfer was conducted onto a 100 nm SiO_2 on Si for SEM imaging purposes only. Fig. 2b shows the Raman spectrum of the CVD-grown graphene on copper. Peak position and G to 2D peak intensity ratio indicate single layer graphene as shown elsewhere (Ferrari et al., 2006).

3.2. Biosensor characterization

The biosensor fabricated for the determination of glucose is based on the use of the enzyme glucose oxidase [Wilson and Turner, 1992]. These enzyme catalyses the oxidation of glucose to gluconic acid, producing hydrogen peroxide as subproduct that can be monitored by electrochemical techniques and directly correlated to the actual glucose concentration in solution. Previous works show the catalytic effect of noble metals in decreasing the necessary potential for hydrogen peroxide oxidation [Cespedes et al., 1993]. It is also well known that higher surface area provided by nanoparticles enhance the sensitivity of this process [Luo et al., 2006]. In the here presented graphene–Au nanoparticle material the enhancement of the signal is also increased by the high signal to noise ratio that graphene presents, due to its intrinsic properties in electrical conductivity [Stankovich et al., 2006]. The combination of metal nanoparticles with graphene provides a promising approach for high sensitivity biosensing platform development.

Fig. 3A shows the cyclic voltammograms obtained with the GCE/graphene/AuNP/GOD/Nafion biosensor at different glucose concentrations. It is clearly observed that as glucose concentration increases the reduction signal corresponding to O_2 reduction decreases dramatically because of the depletion of oxygen during enzymatic reaction of glucose oxidase. Also an oxidation wave at positive potentials ($E \geq +0.75$ V) corresponding to H_2O_2 oxidation appears as glucose concentration increases. Because of the small amount of enzymatic units per biosensor (10 U) saturation is easily achieved in the millimolar range. Calibrations at +0.8 V in a lower concentration range to test the sensitivity of the biosensor were performed by adding small volumes of concentrated glucose into 25 ml PBS pH 7.0. Fig. 3B shows the typical response curve obtained when 20 additions of 25 μL plus 9 additions of 50 μL of glucose 10 mM where performed to a 25 ml PBS pH 7.0 while stirring at 750 rpm. An inset in Fig. 3B plots the averaged curve of 3 calibrations performed in three consecutive days. Base currents were subtracted prior to averaging. Error bars ($\alpha = 0.05$ 95% confidence level) are given for each concentration. The obtained calibration curve is $i = (3.08 \pm 0.16)$ [glucose] + (-23.174 ± 25.10), $r = 0.9995 \pm 0.002$. As can be observed, an excellent linear range is obtained in the range of 10–366 μM. After this value the signal becomes saturated because of the small amount of enzyme present in the biosensor surface. The limit of detection achieved with the here presented biosensor is 4 μM.

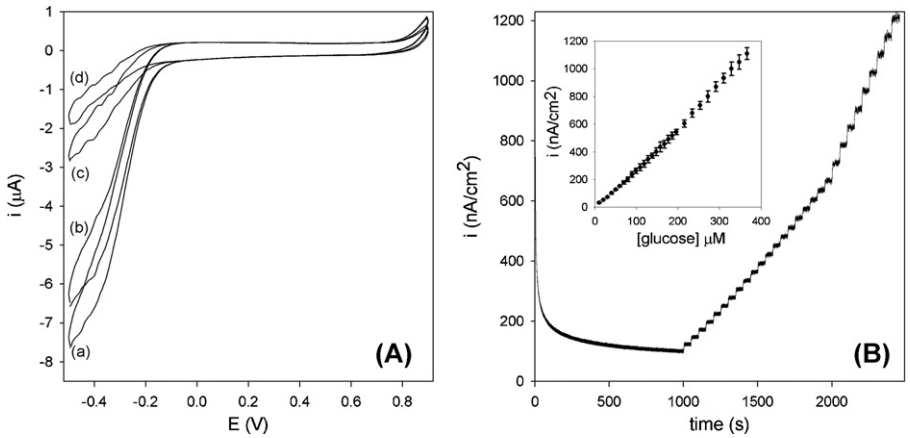

Fig. 3. (A) Cyclic voltammetries obtained from the developed GCE/graphene/AuNP/GOD/Nafion biosensor at different glucose concentrations. (a) Baseline, (b) 0.4 mM, (c) 2 mM, and d) 4 mM glucose in 0.1 M PBS buffer at pH 7.0. Scan rate was 50 mV/s. (B) Response curve of the developed GCE/graphene/AuNP/GOD/Nafion biosensor. Inset in (B) Calibration plot of the biosensor obtained from averaging three calibrations from three consecutive days.

3.3. Interference study

An interference study of the presented biosensor was performed because of the known interference of biomolecules such as ascorbic acid (AA) and uric acid (UA) in glucose biosensors. Additions of concentrated AA and UA to a final concentration of 0.2 and 0.1 mM respectively were intercalated between additions of glucose in the 0 to 20 mM range during regular amperometric detection as presented previously. Fig. S1 in the Supplementary information shows the obtained current at +0.8 V during the glucose and AA and UA additions. It can be observed that no signal interference was obtained when adding AA or UA in a concentration range three orders of magnitude higher than glucose. Considering that AA and UA are electroactive species and thus they could be directly oxidized on the electrode surface, the absence of interference means that the Nafion layer prevents the two interferents from reaching the surface ing excellent stability for the proposed biosensor.

4. Conclusion

A new method for the synthesis of single layer graphene decorated with metal nanoparticles is presented. The versatility of the fabrication methodology implies numerous advantages including scalability for mass production, possible deposition of a wide range of metals by changing the plating dissolution composition, wide range of possible biosensor development by using different biomarkers/biomolecules with the capacity to specifically bind to metals (such as modified DNA strings, peptides, and enzymes) and biocompatibility of the used nanomaterials. Enzymatic glucose determination in the μM range is presented as proof of concept. Future work will explore the use of the developed graphene AuNP platform in combination with engineered biomolecules that specifically bind to AuNP as demonstrated previously [Jaworski et al., 2011].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.12.018.

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