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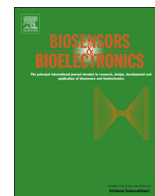
A nanoceria–platinum–graphene nanocomposite for electrochemical biosensing

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ABSTRACT

Most graphene–metal nanocomposites for biosensing are formed using noble metals. Recently, development of nanocomposites using rare earth metals has gained much attention. This paper reports on the development of a nanoceria–nanoplatinum–graphene hybrid nanocomposite as a base transducing layer for mediator-free enzymatic biosensors. The hybrid nanocomposite was shown to improve detection of superoxide or hydrogen peroxide when compared to other carbon–metal hybrid nanocomposites. Based on this finding, the nanocomposite was applied for biosensing by adding either a peroxide-producing oxidase (glucose oxidase), or a superoxide-producing oxidase (xanthine oxidase). Material analysis indicated that nanoceria and nanoplatinum were equally distributed along the surface of the hybrid material, ensuring detection of either superoxide or hydrogen peroxide produced by oxidase activity. Glucose biosensors demonstrated a sensitivity ($66.2 \pm 2.6\ \mu A\ mM^{-1}\ cm^{-2}$), response time ($6.3 \pm 3.4\ s$), and limit of detection ($1.3 \pm 0.6\ \mu M$) that were comparable to other graphene–mediated electrodes in the current literature. Remarkably, XOD biosensor sensitivity ($1164 \pm 332\ \mu A\ mM^{-1}$), response time ($5.0 \pm 1.5\ s$), and limit of detection ($0.2 \pm 0.1\ \mu M$) were higher than any reported biosensors using similar metal-decorated carbon nanomaterials. This material is the first demonstration of a highly efficient, diverse nanoceria/nanoplatinum/graphene hybrid nanocomposite for biosensing.

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1. Introduction

Rare earth metal oxides such as cerium oxide (CeO_2) have been used for decades as catalysts in devices such as solar cells, fuel cells, automobile catalytic converters, and oxygen storage systems (Adachi and Imanaka, 1997; F. Zhang et al., 2010a, 2010b; Lu et al., 2011). The catalytic activity of CeO_2 is due to fast transition between Ce(III) and Ce(IV), changing the oxygen vacancies and forming *n*-type hybrids (Joung et al., 2011). Various forms of nanoscale CeO_2 (known as nanoceria) have recently been shown to significantly improve electro and photocatalysis (Nyk et al., 2008; Neil et al., 2011). Zhou et al. (2005) developed CeO_2 nanorods for enhanced carbon monoxide (CO) conversion efficiency with applications in catalytic converters (Joung, et al., 2011; Jiang et al., 2012; Ji et al., 2013). Gaynor et al. (2013) developed a spectrophotometric device for enzyme-free detection of hydrogen peroxide (H_2O_2) using oxygen deficient nanoceria.

Graphene, composed of a monolayer of carbon atoms in a honeycomb allotrope, has received much attention in recent years due to its excellent mobility of charge carriers, high specific surface area, and superb electrical/thermal conduction (Bolotin et al., 2008; Geim and Novoselov, 2007; Geim, 2009; Li et al., 2008; Rao et al., 2009; Stankovich et al., 2006). Reduction of water soluble graphene oxide (GO) to reduced graphene oxide (RGO) produces a highly conductive material due to oxygen vacancies along the surface of allotropic sheets (Zhu et al., 2010). GO has been reduced using chemical agents such as hydrazine (Stankovich et al., 2007), hydroquinone (Wang et al., 2008), sodium borohydride (Sun et al., 2013), and ascorbic acid (Dua et al., 2010). Thermal treatment (Schniepp et al., 2006) and solar exfoliation (Aravind et al., 2011) have also been used to reduce GO.

Composites of graphene with various metal oxides have been developed for use in devices such as fuel cells, sensors, batteries, and solar cells (Akhavan, 2011; Bai and Shen, 2012; Du et al., 2011; Liang et al., 2011; Luo et al., 2012; Wang et al., 2009; Wu et al., 2010a; Yang et al., 2011; Zhang et al., 2010). Graphene–metal nanocomposites have unique properties that are significantly improved over the bulk metal and metal oxide alone (Liang et al., 2011; Wu et al., 2010a, 2010b; Kim et al., 2009; Stankovich et al., 2006; Li et al., 2012; Huang et al., 2012). This effect is due to

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the ability of metal oxides to maintain the interplanar spacing of the graphene sheets, preventing aggregation into graphitic structures and improving electron transport (Huang et al., 2011).

In the last 3 years, a number of researchers have created nanoceria–graphene nanocomposites that could be useful in biosensing. Jiang et al. (2013) demonstrated tunable synthesis of ceria nanocrystal/graphene hybrid nanostructures (including nanorods, nanoparticles, and nanocubes). The material demonstrated excellent photocatalytic and electrocatalytic effects. Wang et al. (2011) demonstrated use of nanoceria–graphene composites for high efficiency batteries; the material showed extremely high specific capacitance, power density, and behaved as a super capacitor. Jiang et al. (2012) synthesized crystalline CeO2 nanocubes hybridized with graphene sheets using a hydrothermal method. The nanocomposites were immobilized on a glassy carbon electrode and used to detect uric acid and ascorbic acid. Wang et al. (2011) synthesized CeO2–graphene nanocomposites using stimuli-responsive polymers. The only report of nanoceria hybrid composites used together with immobilized proteins was Asati et al. (2009); nanoceria-based immunoassays were developed for catalysis of organic dyes such as tetramethyl benzidin. While each of these materials is promising for biosensing applications, validation studies are needed to confirm the catalytic behavior of nanoceria–graphene composites are conserved once proteins are immobilized on the surface.

This paper demonstrates use of nanoceria–graphene–nanoplatinum hybrid nanocomposite as a platform for developing electrochemical biosensors based on oxidase enzymes (Fig. 1). The simple recipe can be reproduced at room temperature using common/inexpensive materials, and produces a transducer platform for fabrication of high quality biosensors with excellent electroactive surface area, sensitivity, response time, and limit of detection. The platform is demonstrated for development of glucose and xanthine biosensors.

2. Methods

2.1. Materials and reagents

Graphene oxide was obtained from ACS Material; L-ascorbic acid and methanol were purchased from Fisher Scientific; cerium (IV) oxide (nanoparticles dispersion, < 25 nm particle size, 10 wt%

in H2O), chloroplatinic acid 8 wt%, glucose oxidase (GOx), and xanthine oxidase (XOD) were procured from Sigma-Aldrich; D(+); glucose, xanthine (98%), hydrogen peroxide (35%), tetraethyl orthosilicate (98% TEOS), hydrochloric acid (37% HCl), and potassium nitrate (KNO3) were acquired from Acros organics; potassium ferrocyanide trihydrate (K4Fe(CN)6) was purchased from EMD chemicals; laponite RD was obtained from Southern Clay Products; PBS, and TRIZMA buffers were procured from Mediatech, Inc.

2.2. Nanomaterial deposition

Prior to analysis, Pt/Ir working electrodes (BASI MF-2013, 1.6 mm diameter, 7.5 cm length, 6 mm shaft diameter, CTFE plastic body) were polished with 3 μm, and 1 μm polycrystalline diamond suspensions (Buehler®, USA). Electrodes were then rinsed with methanol, and polished with 0.05 μm alumina slurry (Buehler®, USA). After polishing, all electrodes were ultrasonicated in deionized water for 15 min.

Amorphous nanoplatinum clusters (nPt) were formed on the surface of the electrode via sonoelectrodeposition at 10 V for 30 s in a solution of 1.44% chloroplatinic acid and 0.002% lead acetate according to our previously published methods (McLamore et al., 2011; Shi et al., 2011; Vanegas et al., 2014). A graphene/nanoceria suspension was prepared by mixing 1 mL of cerium (IV) oxide nanoparticle suspension (mean nanoparticle size=12 nm) with 2 mg of single-layer graphene oxide (SLGO) powder and 8 mg of L-ascorbic acid. The suspension was ultrasonicated for 30 min. Immediately after sonication, a 2 μL aliquot of the nanoparticle suspension was spin-coated at 2600 rpm on nPt-modified electrodes, air-dried, and then rinsed. Finally, another layer of nPt was electrodeposited on the nCe–RGO composite by sonoelectrodeposition (42 kHz) at 10 V for 30 s.

2.3. Enzyme immobilization

Laponite hydrogel (1%) was prepared in TRIZMA buffer and ultrasonicated for 2 h. Sol–gel was prepared by mixing 5 g of TEOS with 4.33 g of deionized water and 2.3 g of HCl (0.04 M); sol–gel was vortex-agitated for 1 h. Approximately 10 mg of enzyme (GOx or XOD) was mixed with 20 μL of laponite hydrogel. An aliquot (2 μL) of the resulting hydrogel was spin coated on the surface of nanomaterial modified electrodes at 2600 rpm for 10 min. Finally, the tip of the electrode was immersed in sol–gel for 10 min and then air dried for 5 min. The final sol–gel layer was included to reduce leaching of enzymes from the hydrogel layer.

2.4. Electrochemical analysis

Electrochemical characterization was performed using a 3 electrode cell stand (C-3, BASi, West Lafayette, IN). Cyclic voltammetry was carried out in 4 mM Fe(CN)6/1 M KNO3 solutions at a switching potential of 600 mV, versus a Ag/AgCl reference electrode with 5 s quiet time, and scan rates of 20, 50, 100, 125, 150, and 200 mV/s. The electroactive surface area of the nanomaterial mediated electrodes was determined using the Randles–Sevcik equation (scheme 1):

$$i_p = (2.69 \times 10^5)n^{3/2}D^{1/2}CAv^{1/2} \qquad \text{(scheme1)}$$

where i_p is the redox peak obtained from the cyclic voltammogram, n is the number of transferred electrons in the redox reaction, D is the diffusion coefficient, C is the molar concentration of the working solution, A is the electroactive surface area of the electrode and v is the potential scan rate.

DC potential amperometry (DCPA) was conducted in PBS at a working potential of +300 mV versus a Ag/AgCl reference electrode with a sampling rate of 1 kHz. The working solution was polarized for 1 h; the current output was measured at constant
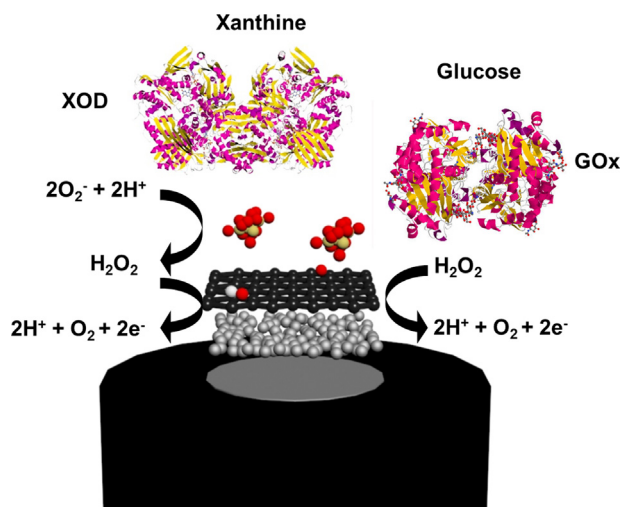
Fig. 1. The hybrid nanocomposite catalyzes detection of superoxide (nanoceria) or hydrogen peroxide (graphene–platinum). In this paper, oxidase enzymes that produce either superoxide (xanthine oxidase) or hydrogen peroxide (glucose oxidase) as a byproduct are demonstrated.