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Highly stable and sensitive glucose biosensor based on covalently assembled high density Au nanostructures

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ABSTRACT

We describe the development of a highly stable and sensitive glucose biosensor based on the nanohybrid materials derived from gold nanoparticles (AuNPs) and multi-walled carbon nanotubes (MWCNT). The biosensing platform was developed by using layer-by-layer (LBL) self-assembly of the nanohybrid materials and the enzyme glucose oxidase (GOx). A high density of AuNPs and MWCNT nanocomposite materials were constructed by alternate self assembly of thiol functionalized MWCNTs and AuNPs, followed by chemisoption of GOx. The surface morphology of multilayered AuNPs/MWCNT structure was characterized by field emission-scanning electron microscope (FE-SEM), and the surface coverage of AuNPs was investigated by cyclic voltammetry (CV), showing that 5 layers of assembly achieves the maximum particle density on electrode. The immobilization of GOx was monitored by electrochemical impedance spectroscopy (EIS). CV and amperometry methods were used to study the electrochemical oxidation of glucose at physiological pH 7.4. The Au electrode modified with five layers of AuNPs/MWCNT composites and GOx exhibited an excellent electrocatalytic activity towards oxidation of glucose, which presents a wide liner range from 20 μM to 10 mM, with a sensitivity of 19.27 μA mM^{-1} cm^{-2}. The detection limit of present modified electrode was found to be 2.3 μM (S/N = 3). In addition, the resulting biosensor showed a faster amperometric current response (within 3 s) and low apparent Michaelis–Menten constant (K_m^{app}). Our present study shows that the high density of AuNPs decorated MWCNT is a promising nanohybrid material for the construction of enzyme based electrochemical biosensors.

1. Introduction

Diabetes mellitus is a worldwide public disease resulting from insulin deficiency and hyperglycemia, reflected by blood glucose levels higher or lower than the normal range of 80–120 mg/dL (4.4–6.6 mM) (Wang, 2008). The complications caused by diabetes includes higher risks of heart disease, kidney failure, blindness, etc (Wang, 2001). Such complications can be greatly reduced through stringent personal control of blood glucose. The development of a highly stable and sensitive glucose biosensor is therefore of critical importance for the diagnosis and keen observation of blood glucose levels. In addition, the accurate detection of glucose is also of great interest in industrial applications such as food industry and bio-fermentation process (Wang et al., 2003). Much effort has been devoted so far on developing suitable techniques for precisely monitoring the glucose level with high sensitivity, good selectivity, high reliability and faster response.

Since the concept of enzyme electrode was first introduced by Clark and Lyons about 50 years ago (Clark and Lyons, 1962), glucose oxidase (GOx) based enzyme sensors have been widely employed to fabricate glucose biosensors. However, simple and efficient immobilization of high content of GOx on the electrode still remains a challenge for improving the performance of glucose biosensors. In the past years, a variety of nanomaterials have been widely used to structurally-modify the electrodes and immobilize GOx (Cheng et al., 2001; Deng et al., 2009; Guo and Li, 2005; Lee et al., 2008; Salimi et al., 2004; Sampath and Lev, 1996; Wang et al., 2009). Among them, gold nanoparticles (AuNPs) have drawn considerable attention because of their unique size, shape dependent physical, chemical and electronic properties when compared to the bulk gold (Daniel and Astruc, 2004; Xiao et al., 2003). It has been reported that, AuNPs can provide a suitable microenvironment, which is similar to the redox center of a native protein, to immobilize enzyme and retain their bioactivities (Zhao et al., 1992). Besides, owing to their special chemical properties, AuNPs allow a variety of functional groups, including –SH, $–NH_2$ and –CN, to bind to their surface covalently, which is favorable for the stable immobilization of biomolecules (Freeman et al., 1995; Grabar et al., 1995).

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In addition, due to their high conductivity, AuNPs can easily minimize the impedance on electrode interface and provide a necessary conduction pathway to facilitate the electron transfer between the immobilized enzyme and electrode surface (Pingarron et al., 2008). Taking account of these advantages, AuNPs were extensively used to construct glucose biosensors either alone or combined with other materials (Zhang et al., 2005a; Li et al., 2009; Ragupathy et al., 2009). However, it is difficult to attach a high density of AuNPs on the electrode surface due to the limited binding sites and low adsorption rate of AuNPs, which is not favorable for the immobilization of a large quantity of enzyme on the electrode. Consequently, the biosensor may result in low sensitivity and narrow linear range.

Layer-by-layer (LBL) is a simple and efficient method to immobilize high volume of enzyme on the electrode. Because of the simplicity of fabrication procedure, wide choice of materials and tailorable control of thickness, LBL based on electrostatic interaction has attracted tremendous interest in recent years (Chirea et al., 2007; Heath and Ratner, 2003). Wu et al. reported the amperometric glucose biosensor based on multilayered AuNPs, chitosan and GOx (Wu et al., 2007). Yan et al. fabricated a glucose biosensor via LBL self assembly of MWCNT, PDDA and GOx (Yan et al., 2007). Deng et al. constructed a glucose biosensor by assembling multilayered GOx and polyelectrolyte on MWCNTs (Deng et al., 2010). Komathi et al. proposed a glucose biosensor based on LBL assembly of MWCNT, conducting polymer, AuNPs and GOx (Komathi et al., 2009). However, the biosensors constructed via electrostatic adsorption have some major drawbacks such as instability in high ionic solution, film leaching over long time storage and long response time, which limited their application (Yan et al., 2007). While covalent binding of GOx to CNTs or AuNPs usually requires pretreatment for quite a long time with strong acid (e.g. H_2SO_4 and HNO_3) or HIO_4 (Zhang et al., 2004), which are harmful to the environment and human health. In addition, long time treatment is not favorable for industrial production. Thus, the optimal approach for the construction of glucose biosenosrs should be a mild condition which can offer both high stability and efficiency to immobilize large quantities of enzyme.

In the present study, we report a three dimensional (3-D) assembly of high density AuNPs architecture, which is constructed by LBL covalent attachment of (3-mercaptopropyl) triethoxysilane (MPTS) functionalized MWCNTs and AuNPs. This nanohybrid material can act as a suitable matrix to immobilize GOx for the fabrication of glucose biosensor. The proposed biosensor successfully overcame the problems mentioned above, which provides a stable biosensing interface, mild enzyme immobilizing environment and efficient fabrication process. MWCNTs were used in the present work to construct the 3-D structure due to their high mechanical strength, chemical stability, large surface area, and biocompatibility (Wang, 2005). In addition, MWCNTs can also facilitate the shuttling of electrons between the enzyme and the electrode surface (Agui et al., 2008). However, the poor solubility of MWCNT in most solvents is a major barrier for constructing MWCNT-based structure on electrode. In this study, MPTS was employed to covalently functionalize MWCNTs, the high density of –SH groups presented on the side walls of MWCNT not only can stabilize the MWCNT dispersion, but also provide a large amount of AuNP binding sites. The surface coverage of AuNP on electrode was found to be 90%. To the best of our knowledge, such high density of AuNPs covalently assembled on electrode surface was achieved for the first time. The resulting biosensor has achieved a remarkably enhanced sensitivity, which is much superior to multilayed GOx/MWCNT or GOx/AuNPs-based electrodes.

2. Experimental

2.1. Chemicals

Glucose, glucose oxidase from *Aspergillus niger* (GOx, 100,000–250,000 units/g), (3-Mercaptopropyl)triethoxysilane (MPTS), hydrogen tetrachloroaurate trihydrate ($HAuCl_4{\cdot}3H_2O$) and human blood serum were purchased from Sigma–Aldrich. MWCNT-COOH (OD ~50 nm, length ~20 μm, –COOH 1.23 wt%, purity 95 wt%) were obtained from Chengdu Organic Chemicals Co. Ltd., China. The human blood serum was diluted ten-fold before use. All other chemicals used in this investigation were of analytical grade and all the experimental solutions were prepared by using double distilled water.

2.2. Preparation of AuNPs and multilayered AuNPs/MWCNT electrode

AuNPs with an average diameter of ~13 nm were prepared according to the reported literature (Dubas and Schlenoff, 2001). The purification of MWCNT, preparation of multilayered AuNPs/MWCNT electrode (Wang et al., 2008; Zhang et al., 2005a) and instrumentations are discussed in supporting information.

3. Results and discussion

3.1. Characterizations of the AuNPs/MWCNT assembly

In this study, MWCNTs were first functionalized by MPTS through hydroxyl-carboxyl dehydrate reaction (Guo et al., 2010). The attached MPTS can expose large arrays of –SH groups on the end and side walls of the MWCNTs through hydrolysis, followed by self polymerization (Supplementary Scheme S1A). Then, MWCNT–Si–SH can easily adsorb on the surface of Au electrode and AuNPs through the S–H bond cleavage (Nuzzo et al., 1987). Fig. 1(A) shows one layer of MWCNTs self-assembled on the Au electrode surface through gold-thiol interaction. As can be seen the picture, the MWCNTs were uniformly distributed on the electrode surface. Afterwards, significant amount of AuNPs could easily immobilize on the end and side walls of MWCNTs via the same mechanism (Supplementary Scheme S1B). As a result, one layer of the AuNPs/MWCNT nanocomposite film was formed on the electrode surface (Fig. 1(B)). The AuNPs tethered on MWCNTs further provided the large binding sites of MWCNT–Si–SH, which allowed for the self assembly of subsequent layers. The high density of multilayered AuNPs/MWCNT nanohybrid structure was obtained on the Au electrode surface by repeating the modification process for several times. Fig. 1(C) shows the electrode modified with five layers of AuNPs/MWCNT nanocomposite architectures and depicts that the coverage of AuNPs/MWCNT on the electrode surface increased significantly compared with Fig. 1(B). In addition, the multilayered nanohybrid film shows a large surface area and high density of nanoporous structure, which allows for further immobilization of GOx and provides high accessibility toward the target molecules.

3.2. Surface coverage of AuNPs

The AuNPs coverage on the electrode surface was studied by scanning cyclic voltammograms (CV) potential range between −0.6 and 0.7 V in 0.1 M NaOH (Kumar and Zou, 2005). Fig. 2(A) shows the CVs of 1–5 layers of AuNPs/MWCNTs nanocomposite modified electrodes. The particle coverage (θ_p) is defined as the ratio between the electrochemically accessible AuNPs area and the geometric area of the Au electrode in contact with the electrolyte