Biosensors and Bioelectronics 35 (2012) 63–68

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Layer-by-layer assembly of chemical reduced graphene and carbon nanotubes for sensitive electrochemical immunoassay

Yan Liu[a,b], Yang Liu[a,**], Hongbin Feng[a], Yongmin Wu[a], Lokesh Joshi[c], Xiangqun Zeng[d], Jinghong Li[a,*]

[a] Department of Chemistry, Beijing Key Laboratory for Microanalytical Methods and Instrumentation, Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology, Tsinghua University, Beijing 100084, China
[b] Department of Chemistry and Chemical Engineering, Yangtze Normal University, Fuling, Chongqing 408100, China
[c] Alimentary Glycoscience Research Cluster, National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Ireland
[d] Chemistry Department, Oakland University, Rochester, MI 48309, USA

ARTICLE INFO

Article history:
Received 28 November 2011
Received in revised form 2 February 2012
Accepted 6 February 2012
Available online 7 March 2012

Keywords:
Layer-by-layer
Graphene
Carbon nanotubes
Electrochemical immunoassay
IgG

ABSTRACT

In this work, uniform and stable multi-walled carbon nanotubes (MWCT) and chemically reduced graphene (GR) composite electrode interface was fabricated by using layer-by-layer assembly method. The performances of these GR–MWCT assembled electrode interfaces were studied by electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). It was demonstrated that the assembled composite film significantly improved the interfacial electron transfer rate compared with that of GR or MWCT modified electrode. Based on the GR–MWCT assembled interface, a sandwich-type electrochemical immunosensor was constructed using human IgG as a model target. In this assay, human IgG was fixed as the target antigen, the HRP-conjugated IgG as the probing antibody and hydroquinone as the electron mediator. The detection limit of the immunosensor was 0.2 ng mL^{-1} (signal-to-noise ratio of 3). A good linear relationship between the current signals and the concentrations of Human IgG was achieved from 1 ng mL^{-1} to 500 ng mL^{-1}. Moreover, this electrochemical immunosensor exhibited excellent selectivity, stability and reproducibility, and can be used to accurately detect IgG concentration in human serum samples. The results suggest that the electrochemical immunosensor based on GR–MWCT assembled composite will be promising in the point-of-care diagnostics application of clinical screening of multiple diseases.

1. Introduction

Sensitive and accurate detection of disease-related proteins is a fundamental requirement in the field of modern biomedicine, clinical diagnostics and therapeutic analysis, which will offer opportunities for the understanding of disease related biological processes (Kitano, 2002; Weston and Hood, 2004; Xiao et al., 2005). Immunoassay, which is one of the most commonly used methods based on the highly specific interaction between antigen and antibody, is conducted on the clinical serum sample to measure disease-associated biomarkers. Until now, several immunoassay methods including chemiluminescence immunoassay (Fu et al., 2006; Voller et al., 1978), enzyme-linked immunosorbent assay (ELISA) (Yates et al., 1999), radioimmunoassay (Goldsmith, 1975), fluorescence immunoassay (Cesaro-Tadic et al., 2004; Matsuya et al., 2003), mass spectrometric immunoassay (Aebersold and Mann, 2003; Hu et al., 2007), electrophoretic immunoassay (Schmalzing and Nashabeh, 1997) and immune polymerase chain reaction (PCR) assay (Saito et al., 1999) have been developed. Recently, electrochemical immunosensor has attracted much attention especially in the point-of-care applications due to the high sensitivity, simplicity, ease of miniaturization, and low-cost of both the sensors and the instrumentation, and several electrochemical immunosensors have been reported (Ala-Kleme et al., 2006; Bourigua et al., 2010; Chen et al., 2010b; Miao and Bard, 2004; Pumera et al., 2007; Wan et al., 2010). Although great progresses have been made, it is still a challenge to develop novel detection technologies and enhance the detection sensitivity due to the increasing demand for inexpensive, operationally simple, early and ultrasensitive profiling of early clinical disease diagnostic including cancer biomarkers.

For an electrochemical immunosensor, its performance is critically dependent on the properties of electrode interface. The structure, conductivity, surface area and biocompatibility of the electrode interface play a central role on the electron transfer, mass transport, biomolecule activities on the interface electrochemical signal transduction (Chen et al., 2006; Jacobs et al., 2010; Lai et al., 2011; Lu et al., 2006; Warsinke, 2009). On the other hand, stable

* Corresponding author. Tel.: +86 10 62776949; fax: +86 10 62782485.
** Corresponding author.
E-mail addresses: liu-yang@mail.tsinghua.edu.cn (Y. Liu), jhli@mail.tsinghua.edu.cn (J. Li).

0956-5663/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2012.02.007

immobilization and retaining the activity of biomolecules on the electrode interface are of fundamental importance for engineering reliable biosensors (Chen and Li, 2006). Recently, nanomaterials, the building blocks of nanotechnology, have opened new horizons for electrochemical interface as well as high performance devices due to their good biocompatibility, enhanced electrocatalytic activity, large surface area, excellent conductivity, and so on. The organization of nanomaterials would open a new pathway for engineering intelligent multifunctional interfaces for the construction of high performance biosensors (Jie et al., 2008, 2010; Pinijsuwan et al., 2008; Wu et al., 2010).

Carbon nanomaterials have attracted considerable attention in electrochemical biosensors because of their extraordinary physical properties and remarkable conductivities. Carbon nanotubes are formed by rolling graphite sheets, and they have quasi-one dimensional (1D) structures. Due to their great chemical stability, large aspect ratio, excellent electrical conductivity, and extremely high mechanical strength and stiffness, carbon nanotubes are widely used in the development of high-performance electrochemical sensors (Ahammad et al., 2009; Bareket et al., 2010; Jacobs et al., 2010; Nie et al., 2009). Recently, graphene, another new carbon materials, attracted much attention since its fascinating two-dimensional (2D) structure, unusual electrochemical properties, large accessible surface area as well as good biocompatibility (Chen et al., 2010a; Du et al., 2011; Geim and Novoselov, 2007; Jacobs et al., 2010; Liu et al., 2008; Lu et al., 2009; Wang et al., 2010; Yang et al., 2010). Graphene presents excellent electron transfer ability for enzymes and excellent catalytic behavior towards small molecules such as dopamine, H_2O_2, O_2, NADH and TNT (Kong et al., 2011; Lu et al., 2011; Tang et al., 2009; Wang et al., 2009; Zhou et al., 2009). Chemically reduced graphene (GR), which is fabricated through chemical reduction of colloidal dispersion of graphene oxide, possesses the outstanding electronic features. In addition, a small quantity of functional groups such as hydroxyl, carboxyl and epoxy also remain on its edges and surface, which make GR negatively charged and is ideal to be functionalized to build multifunctional nanostructure hybrid materials in the applications of high efficiency photoelectronic energy conversion module and sensing devices (Bai et al., 2011; Du et al., 2011; Wan et al., 2011).

In this work, we fabricated a composite electrode interface based on the quasi 1D structure of multi-walled carbon nanotubes (MWCT) and 2D plane structure of graphene by a layer-by-layer assembly method through the electrostatic adsorption between positively charged poly(diallyldimethylammonium chloride) (PDDA) and negatively charged MWCT and GR. The performance of the composite electrode interface was studied by electrochemical impedance spectroscopy and cyclic voltammetry, and improved electron transfer ability was observed compared with those only MWCTs or GR used. Moreover, a sandwich-type electrochemical immunosensor was constructed based on the assembled composite interface, using human IgG as a model target antigen, human IgG antibody as the capture probe fixed on the composite interface, the HRP-conjugated human IgG antibody as the probes and hydroquinone as the electron mediator. The results were also correlated with standard ELISA method. The as-prepared electrochemical immunosensor platform provides a strategy to fabricate high performances electrode interface and shows great promise in the point-of-care diagnostics application of clinical screening of disease-associated cancer biomarkers.

2. Experimental

2.1. Regents and materials

Graphite powder (99.99995%, 325 mesh), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxylsuccinimide (NHS), bovine serum albumin (BSA) and H_2O_2 (30%) were purchased from Alfa Aesar. Poly(diallyldimethylammonium chloride) (PDDA, MW ~100,000 was purchased from Sigma–Aldrich and used as received without further purification. The goat anti-human IgG (Ab1), goat anti-human IgM (anti-IgM), human IgG (hIgG) (Ag), HRP conjugated goat anti-human IgG (Ab2-HRP), Adenosine 5′-triphosphate disodium salt (ATP) and hIgG enzyme-linked immunosorbent assay (ELISA) kits were obtained from Dingguo Biological Products Company (Beijing, China). Clinical serum samples of human IgG with different concentrations were provided by the Affiliated Hospital of Tsinghua University. Phosphate buffer solutions (PBS, 0.067 mol L^{-1}) were prepared with Na_2HPO_4 and NaH_2PO_4 and used as electrolyte. Other regents of analytical grade were obtained from Beijing Chemical Company (Beijing, China). The chemical-reduced graphene was synthesized and characterized (Fig. S1 in Supporting information) according to our previous works (Tang et al., 2009; Wang et al., 2009). The MWCTs (with 95% purity) were obtained from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China) and were refluxed in concentrated nitric acid for about 5 h, filtered, washed with double-distilled water before using.

2.2. Assembly of the composite electrode interface based on GR and carbon nanotubes.

Prior to fabricating immunosensors, a glassy carbon electrode (GC) (diameter of 3 mm) was successively polished with 1.0, 0.3, and 0.05 μm α-Al_2O_3 powder and ultrasonic cleaned with ethanol and water. The polished GC electrode was then immersed in a PDDA aqueous solution (10 mg mL^{-1}) with 20 mM NaCl for 30 min and followed by washing with distilled water and drying with nitrogen flow. PDDA modified electrode was immersed in a GR dispersed solution (1 mg mL^{-1}) for 30 min to adsorb a GR layer on the electrode. The modified electrode was dipped again in the PDDA solution and kept for 30 min. After washing with distilled water and drying with nitrogen flow, the modified electrode was then immersed into a MWCTs dispersed aqueous solution (1 mg mL^{-1}) for 30 min. The above procedures were repeated three times to get a unique and stable composite assembled electrode.

2.3. Preparation of the electrochemical immunosensors

To fabricate the electrochemical immunosensors, the above assembled electrode was immersed in a solution containing 25 mg mL^{-1} of EDC and 20 mg mL^{-1} of NHS for 40 min. Then, the above electrode was dipped into a PBS solution with 0.5 g L^{-1} Ab1 for 1 h, followed by washing with PBS solution (pH 7.4). The Ab1 immobilized electrode was finally immersed in 3% BSA for 30 min at 37 °C to block the excess active groups on the surface followed by washing, and used for the Ag detection. The electrode was stored at 4 °C until used.

2.4. Procedures for the detection of human IgG

A sandwich immunoassay was conducted for the detection of hIgG (Ag). The Ab1 immobilized electrode was first incubated with hIgG standard antigen solutions at different concentrations at 37 °C, followed by washing thoroughly with PBS buffer. Secondly, the above electrode was further incubated with 25 μL of Ab2-HRP solution for 1 h at 37 °C, followed by similar washing steps as above. The electrochemical immunoassay was then conducted in a PBS solution with 3 mmol L^{-1} hydroquinone and 3 mmol L^{-1} H_2O_2. An Ag/AgCl electrode with saturated KCl solution and platinum wire were used as the reference electrode and counter electrode, respectively, in all electrochemical measurements. The measurement of