Materials Science and Engineering C 58 (2016) 666–674

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Fabrication of graphene/gold-modified screen-printed electrode for detection of carcinoembryonic antigen

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ARTICLE INFO

Article history:
Received 30 June 2015
Received in revised form 21 August 2015
Accepted 3 September 2015
Available online 7 September 2015

Keywords:
Biosensor
Electrochemistry
Gold nanoparticles
Graphene
Screen-printed electrode

ABSTRACT

Immunosensors based on gold nanoparticles and reduced graphene oxide (AuNPs/rGO)-modified screen-printed electrodes (SPEs) were successfully synthesized using an electrochemical deposition method. The modified SPEs were characterized using a field emission scanning electron microscope (FESEM) and Raman spectroscopy to analyze the morphology and composition of AuNPs and rGO. Both the FESEM and Raman spectroscopy revealed that the AuNPs were successfully anchored on the thin film of rGO deposited on the surface of the SPEs. Characterization with a ferri–ferrocyanide couple [$Fe(CN)_6^{3-/4-}$] showed that the electron transfer kinetic between the analyte and electrode was enhanced after the modification with the AuNPs/rGO composite on the electrode surface, in addition to increasing the effective surface area of the electrode. The modified SPE was immobilized with a sandwich type immunosensor to mimic the ELISA (enzyme-linked immunosorbent assay) immunoassay. The modified SPE that was fortified with the sandwich type immunosensor exhibited double electrochemical responses in the detection of carcinoembryonic antigen (CEA), with linear ranges of 0.5–50 ng/mL and 250–2000 ng/mL and limits of detection of 0.28 ng/mL and 181.5 ng/mL, respectively.

1. Introduction

Cancer diagnosis and treatment is gaining great attention globally because of the prevalence, high fatality rate, and possible recurrence of the disease after treatment [1]. Early cancer detection methods include screening methods such as a Papanicolau test for cervical cancer and mammography for breast cancer in women, prostate-specific antigen (PSA) level detection in a blood sample for men to detect prostate cancer, enzyme-linked immunosorbent assay (ELISA), radiation immunological assays (RIAs), occult blood detection for colon cancer, and instrumental approaches such as endoscopy, CT scans, X-ray, ultrasound imaging, MRI, time-resolved fluorescence, and chemiluminescence [2]. An increased level of a tumor marker in human serum is a reliable symptom associated with cancer patients. Thus, the determination of a tumor marker plays an important role in the early diagnosis of cancer [3]. In clinical assays, the detection methods for tumor markers, which include RIAs, time-resolved fluorescence, and chemiluminescence, have the disadvantages of being environmentally unfriendly, time-consuming, and difficult to automate, as well as having poor precision. The costs of specific instruments and reagents also limit their wide application in clinical laboratories. Hence, there is a need to develop immunosensors that are low-cost and effective, with real-time control [4].

Immunosensors have been known as a core development in the immunochemical field in clinical diagnosis because they combine the advantages of sensors, like high sensitivity, with high specific immune reactions [5]. The remarkable simultaneous monitoring of immunoreactions of immunosensors renders a dynamic analysis of immunoreactions possible [3]. There are five types of immunosensor detection devices: electrochemical (amperometric, potentiometric, capacitive, or impedimetric), optical (fluorescence, luminescence, or refractive index), microgravimetric, thermometric, and immunosensors coupled with other techniques such as flow injection analysis and capillary electrophoresis [6]. Electrochemical devices have traditionally received the major share of the attention in biosensor development [7]. Among the immunosensor devices, an electrochemical immunoassay has a low detection limit, requires a small analyte volume, simple instrumentation, and minimal manipulation, and the system can be easily miniaturized and integrated in biochips [8]. Apart from that, the advantages of high sensitivity, specificity, simplicity, and inherent miniaturization of electrochemical immunosensors make them a significant rival of the most advanced optical methods [9]. Although an electrochemical ELISA requires a suitable electrode to contact the analyte solution, many groups have been focusing on the potential miniaturization of an ELISA system in combination with various electrochemical methods [10]. Miniaturized systems offer many potential advantages over conventional assay platforms, including small sample volumes, low cost, short assay time, high throughput, and automation [11].

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http://dx.doi.org/10.1016/j.msec.2015.09.010
0928-4931/

Screen-printing technology, which has been adopted for microelectronics, is significantly used to fabricate electrodes for disposable electrochemical biosensors [12]. A screen-printed electrode (SPE) is simple, versatile, low cost, portable, easily operated, reliable, small sized, and capable of mass production. Therefore, it is applied widely in the electroanalytical chemistry field [13]. Furthermore, a screen-printed electrode avoids the cleaning process, unlike conventional electrodes such as a glassy carbon electrode (GCE) [14]. An SPE avoids the disadvantages of conventional three- and two-electrode systems, which need frequent re-calibration and are unstable and unsuitable for on-site analysis, because they can take several hours to complete. In addition, these must be performed by trained personnel and require numerous separations and washing steps. These drawbacks of conventional electrode systems make them less capable than screen-printed electrodes.

There have been numerous studies and developments in nanotechnology and the application of nanomaterials to the detection of cancer at an early stage. The unique physical, optical, and electrical properties of nanomaterials make them useful in immunosensing. Quantum dots, gold nanoparticles, magnetic nanoparticles, carbon nanotubes, gold nanowires, and many other materials are developed and modified for immunosensors to achieve a wider detection range and lower detection limit for biomarkers [2]. Gold nanoparticles are among the nanoparticles that have been widely used in analytical and biomedical areas because of their speed and ease of use in chemical synthesis, their narrow size distribution, and their convenient labeling of biomolecules [15]. Reduced graphene oxide (rGO) contains hydroxyl (–OH) and carboxylate (–COOH) groups in the structure, which enables interaction with metal nanoparticles to produce a metal nanoparticle–graphene based electrochemical sensor [16]. Insertion of metal nanoparticles prevents graphene layers from stacking to form graphite multi-layered structure [17]. Nanomaterials can be fabricated on an electrode surface through chemical reduction from an aqueous solution of chlorometallate anions, electrochemical deposition, and metal-vapor synthesis. Electrochemical-metal deposition is a convenient and fast method to prepare metal nanoparticles on large areas of conductive electrodes [18]. Green synthesis approach is commonly used to decorate rGO with Au, Pt and Ag nanoparticles to achieve enhanced mechanical, electrical and thermal properties [19].

In the present work, we developed an immunosensor using AuNPs/rGO-modified SPEs through the in-situ electrodeposition of graphene and the reduction of gold cation (Au^{3+}). After the modification of the SPE, a primary antibody, secondary antibody, and carcinoembryonic antigen (CEA) were immobilized on the electrode surface. The modified SPE was characterized using cyclic voltammetry (CV), a field emission scanning electron microscope (FESEM), and a Raman spectrometer. This work realized a convenient, low-cost, and one-step method for fabricating a disposable immunosensor.

2. Experimental

2.1. Chemicals and reagents

Graphite flakes were obtained from Ashbury Inc. (NJ, USA). Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$, 99%), and disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$, 99.5%) were purchased from Merck, Germany. Hydrogen tetrachloroaurate (III) trihydrate (ACS, 99.99%) was purchased from abcr GmbH & Co. KG, Germany. Hydrogen chloride (HCl, 37%) and bovine serum albumin (BSA, 96%) were obtained from Sigma-Aldrich. Hydrogen peroxide (H_2O_2, 35%) was purchased from Systerm, Malaysia. A primary antibody (mouse monoclonal, 2.000 mg/mL), carcinoembryonic antigen (CEA, 2.000 mg/mL), secondary antibody (rabbit polyclonal, 2.000 mg/mL), and secondary antibody labeled HRP (goat polyclonal — HRP, 0.500 mg/mL) were obtained from Abcam, USA. SPEs were purchased from DS Dropsens, Spain.

2.2. Synthesis of AuNPs–rGO-modified SPE

Graphene oxide (GO) was synthesized with Hummer's method [20]. First, 1.22 mL of 8.2 g/L synthesized GO was added into a 10 mL volumetric flask. Then, 1.5 mL of 1.0 mM $HAuCl_4$ solution was added into the volumetric flask. The volumetric flask was diluted with 0.1 M (pH 9.2) phosphate buffer solution (PBS). The final solution had a GO concentration of 1.0 mg/mL. After that, the solution was sonicated at a high rate for around 30 s. A bare SPE was washed with ethanol and deionized water. Then, 5 μL of the as-prepared solution was drop casted on the SPE and it was allowed to dry overnight at 25 °C. The SPE was then electrodeposited using a CV potentiostat at potentials from 0 V to −1.5 V in a 0.1 M KCl solution in order to simultaneously reduce the GO to rGO and the Au^{3+} ions to AuNPs nanocomposite. The modified SPE was denoted as AuNPs/rGO-3. A 0.5 mg/mL concentration of GO/AuNPs solution was prepared by repeating the steps, except 0.61 mL of GO was added. This modified SPE was labeled as AuNPs/rGO-2. Another 0.1 mg/mL concentration of GO/AuNPs solution was prepared by diluting 1 mL of the 1.0 mg/mL GO/AuNPs solution in a 10 mL volumetric flask with 0.1 M PBS. This modified SPE was named as AuNPs/rGO-1.

2.3. Immobilization of antibody

The immobilization process of immunoassay was based on the previous work [21]. The modified SPE was dipped in a solution containing 1 mL of N-hydroxysulfosuccinimide (NHS) and 2 mL of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), which acted as a cross-linker between the primary antibody and AuNPs/rGO nanocomposite. The modified SPE was incubated with the primary antibody (captured antibody) for 2 h at 25 °C. After that, the modified SPE was washed twice with 0.1 M PBS. The modified SPE was blocked with 1% BSA for 2 h at 25 °C. A diluted CEA solution was added to the modified SPE and incubated for 2 h at room temperature. Again, the modified SPE was washed twice with PBS 0.1 M. The modified SPE was incubated with secondary antibody (detection antibody) for 2 h at room temperature. The modified SPE was washed twice with PBS 0.1 M. The modified SPE was incubated with the HRP for 2 h at room temperature. The modified SPE was washed twice with 0.1 M PBS. H_2O_2 was added to the modified SPE in 0.1 M PBS solution, and the signal produced by the enzyme–substrate reaction was measured.

2.4. Characterizations

The morphology and microstructure of the modified SPEs were characterized using the field emission scanning electron microscope (FESEM: FEI Quanta 400F), and a Raman spectrometer (Renishaw in-Via Raman microscope using laser excitation at $\lambda = 514$ nm).

3. Results and discussions

3.1. Morphology and microstructure of AuNPs/rGO modified SPE

Fig. 1 shows the FESEM images of the rGO-modified SPE, AuNPs-modified SPE, and AuNPs/rGO-modified SPE. The morphology of the graphene deposited on the carbon electrode revealed a layer with a typical crumpled and wrinkled structure coating the surface of the carbon electrode [22]. The initially deposited AuNPs acted as nucleation centers for the further reduction of Au^{3+} ions, and hence the size of the AuNPs increased. On the other hand, the AuNPs on the rGO film were not aggregated because of the presence of oxygen functional groups [23]. AuNPs with an average size of 143.77 nm were formed by the electrochemical reduction. The AuNPs were anchored on the graphene film in a well distributed manner. The presence of AuNPs on the graphene film was also supported by a small peak for the Au element shown in the EDX spectrum.