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Ultrasensitive electrochemical immunoassay for squamous cell carcinoma antigen using dumbbell-like Pt–Fe_3O_4 nanoparticles as signal amplification

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ABSTRACT

Dumbbell-like Pt–Fe_3O_4 nanoparticles (NPs) were synthesized and used as a novel kind of label for the preparation of electrochemical immunosensor, which is applied to the detection of cancer biomarker squamous cell carcinoma antigen (SCC-Ag). The signal amplification strategy, using the synergetic effect present in Pt–Fe_3O_4 to increase the reduction ability of the NPs toward H_2O_2, improved the sensitivity of the immunosensor. Nitrogen-doped graphene sheets (N-GS) were synthesized from graphite oxides through thermal annealing of graphite oxides in ammonia, which was used to immobilize primary anti-SCC antibody (Ab_1). Secondary anti-SCC antibody (Ab_2) was adsorbed onto the Pt–Fe_3O_4 NPs. The immunosensor was prepared through a sandwich structure and displayed a wide linear range (0.05–18 ng/mL), low detection limit (15.3 pg/mL), good reproducibility and stability. The method has been applied to the analysis of clinical serum samples with satisfactory results. These labels for immunosensors can provide many potential applications for the detection of different biomolecules.

1. Introduction

Squamous cell carcinoma antigen (SCC-Ag), a glycoprotein with isoforms ranging from 45 to 55 kDa, is a subfraction of tumor antigen TA-4 isolated from a cervical squamous cell carcinoma (Takeuchi et al., 2003; Yasumatsu et al., 2001). Initially, SCC-Ag was discovered as a serological marker for advanced squamous cell tumors in the cervix and was later found to be associated with other types of cancer with epithelial or endodermal origins, including lung cancer, head and neck cancer, melanomas, and hepatocellular carcinoma. The serum level of SCC-Ag is increased in parallel to the growth of the tumor size or the recurrence of the disease (Schedel et al., 2011). Therefore, measurement of the serum level of SCC-Ag has been used clinically for the diagnosis and the management of SCC other uterine cervix as well as other various organs. Enzyme-linked immunosorbent assay (ELISA) (Erickson et al., 2010; Chang et al., 2004), radioimmunoassay (RIA) (Neunteufel et al., 1990) and chemiluminescence enzyme immunoassay (CLEIA) are often used to detect the total SCC-Ag in serum. However, the above methods have limitations such as environmental pollution, poor reproducibility, low sensitivity, high cost and narrow linear range. Electrochemical immunosensor, especially sandwich-type immunosensor, due to its high sensitivity and selectivity, has recently gained growing interest and found wide applications in different fields (Gan et al., 2011; Ahirwal and Mitra, 2010; Zhang et al., 2011b; Ricci et al., 2012). Thus, we developed a convenient sandwich-type immunosensor for the sensitive detection of SCC-Ag. To increase the sensitivity of the immunosensors, there are two basic issues. The first is to increase the capture of primary antibody onto electrode surface and the second is to select the signal amplification strategy.

Doping of graphene shows the possibility of opening the bandgap and modulating conducting types by substituting carbon atoms with foreign atoms. The dopant atoms can tailor electronic band structure and the physicochemical property of graphene and lead to widespread potential applications (Zhang et al., 2011a; Guo et al., 2010; Sheng et al., 2012). In order to enhance the sensitivity of the sensor, nitrogen-doped graphene sheets (N-GS) were introduced during the modification of electrochemical sensor owing to their unique properties involving large surface area, strong excellent conductivity, subtle electronic property and catalytic ability.

Dumbbell-like NPs are referred to as those with two different functional NPs in intimate contact. The two best known categories are dumbbell-like NPs of noble metal and quantum dots (such as Au–CdS) or dumbbell-like NPs of noble metal and magnetic NPs (such as Pt–Fe_3O_4) (Lin et al., 2011). The interfacial interactions that

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originate from electron transfer across the nanometer contact at the interface of these two NPs can induce new properties that are not present in the individual component NPs (Wang et al., 2009a). For example, Au NPs are normally chemically inert, but Au NPs deposited on a metal-oxide support have shown high catalytic activity for CO oxidation. Wang et al. (2009b) reported Pt NPs in the Pt–Fe_3O_4 structure show a 20-fold increase in mass activity toward oxygen reduction reaction compared with the single component Pt NPs. As a result of this interaction, the dumbbell-like NPs have shown interesting catalytic, optical, and magnetic properties and offer an ideal nanosystem for catalytic and biomedical applications.

To the best of our knowledge, there is no report focusing on electrochemical detection of SCC-Ag based on dumbbell-like Pt–Fe_3O_4 nanoparticles as label. In this work, the dumbbell-like Pt–Fe_3O_4 NPs were prepared and indeed showed synergetic effect in catalyzing H_2O_2 reduction, which is more active than Pt or Fe_3O_4 alone. With the secondary antibody (Ab_2) adsorbed onto Pt, the resulting Pt–Fe_3O_4–Ab_2 was used as label for the preparation of immunosensor to detect SCC-Ag. The sandwich-type structure is formed by immobilizing the primary SCC antibody (Ab_1) onto N-GS modified glassy carbon electrode through cross-link of glutaraldehyde. The large surface area of N-GS can increase Ab_1 loading, the good conductivity of N-GS can promote electron transfer, and the synergetic effect of dumbbell-like Pt–Fe_3O_4 NPs in catalyzing H_2O_2 reduction, which resulted in the high sensitivity of the immunosensor. Therefore, this simple, economic and sensitive immunosensing approach could find wide potential application in clinical analysis.

2. Experimental section

2.1. Materials and reagents

SCC-Ag and SCC antibody were purchased from Sangon Biotech Co., Ltd.. (Shanghai, China). Platinum acetylacetonate (98%) was purchased from J&K Scientific LTD. Oleylamine and octadecane were purchased from Aladdin. Oleic acid, graphite powder, $K_3[Fe(CN)_6]$ and hexane were purchased from Sinopharm Chemical Reagent Co., Ltd.. Glutaraldehyde and BSA (96–99%) were purchased from Sigma-Aldrich. Phosphate buffered saline (PBS, 0.1 mol/L containing 0.1 mol/L NaCl, pH 7.4) was used as an electrolyte for all electrochemistry measurement. Doubly distilled water was used throughout the experiments.

2.2. Apparatus

All electrochemical measurements were performed on a CHI 760D electrochemical workstation (Shanghai CH Instruments Co., China). Electrochemical impedance spectroscopy (EIS) was obtained from the impedance measurement unit (IM6e, ZAHNER elektrik, Germany). Transmission electron microscope (TEM) images were obtained from a Hitachi H-800 microscope (Japan).

2.3. Preparation of nitrogen-doped graphene sheets

Graphite oxides were prepared from graphite powder by a modified Hummers' method (Marcano et al., 2010). Nitrogen-doped graphene sheets were synthesized from graphite oxides through thermal annealing of graphite oxides in ammonia (Long et al., 2010). The detailed synthetic steps and characterization were shown in our previous study (Fan et al., 2012).

2.4. Preparation of dumbbell-like Pt–Fe_3O_4 nanoparticles

Dumbbell-like Pt–Fe_3O_4 nanoparticles were synthesized by a modified one-step method (Sahoo et al., 2011). In brief, 2 mmol of platinum acetylacetonate, 6 mmol of oleic acid, 6 mmol of oleylamine and 20 mL of octadecane were mixed under a constant flow of argon. The solution was heated to 120 °C with a constant rate of 3 °C/min. $Fe(CO)_5$ (8 mmol) was added after reaching to this temperature, and then the temperature was raised to 280 °C and kept for 20 min. The product was precipitated by addition of excess of ethanol and collected by centrifugation (9000 rpm, 10 min). Finally, the nanoparticles were repeatedly washed by dissolving them in hexane, precipitating them with ethanol and centrifugation (9000 rpm, 10 min, RT). Finally, the resulting dumbbell-like Pt–Fe_3O_4 nanoparticle was obtained by dissolving in toluene and then drying in vacuum.

2.5. Preparation of Pt–Fe_3O_4–Ab_2 label

The as synthesized Pt–Fe_3O_4 NPs (1 mg) were dispersed in 1.0 mL of CTAB (0.018 g) solution, and stirred for 0.5 h. Then, secondary antibody (Ab_2) and pH 7.4 PBS were added into the solution and the mixture was allowed to react at room temperature under stirring for 24 h, followed by centrifugation. Ab_2 could be immobilized on Pt–Fe_3O_4 nanoparticles through adsorption and it has been proved that amino groups in SCC Ab_2 can be bound strongly to Pt (Mandal et al., 2004). The resulting Pt–Fe_3O_4–Ab_2 was washed with pH 7.4 PBS and then redispersed in buffer and stored at 4 °C before use.

2.6. Modification of electrodes

Fig. 1 shows the fabrication procedure of the immunosensors. A glassy carbon electrode with 3-mm diameter was polished to a mirror-like finish with 1.0, 0.3 and 0.05 μm alumina powder and then thoroughly cleaned before use. First, 6.0 μL of N-GS solution (1.0 mg/mL) which was dispersed in chitosan (0.5 wt%) was added onto the electrode and then dried. The electrode was then thoroughly rinsed with PBS to remove unbounded particles. To immobilize the antibody onto electrode surface, 6.0 μL of glutaraldehyde (2.5%, v/v) was dropped onto the electrode surface and incubated for 1 h. Then, 6.0 μL of 10 μg/mL Ab_1 was added onto electrode surface and incubated for 1 h. Excess antibodies were washed with PBS and the electrode was incubated in 1 wt% bovine serum albumin (BSA) solution for another 30 min to eliminate nonspecific binding sites. Subsequently, SCC-Ag solution with varying concentrations was added to the electrode surface and incubated for 1 h, and then the electrode was washed extensively to remove unbound SCC-Ag molecules. Finally, the prepared Pt–Fe_3O_4–Ab_2 solution was dropped onto the electrode surface and bound via the specific antibody–antigen interaction. The amount of label captured was in accordance with the SCC-Ag concentration. The immunosensor was incubated for another 1 h, and then rinsed three times to remove unbound Ab_2 on the outer surface. After washing, the prepared electrode was stored at 4 °C prior to use.

2.7. Characterization of the immunosensor

A conventional three-electrode system was used for all electrochemical measurements: a glassy carbon electrode as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum wire electrode as the counter electrode. pH 7.4 PBS was used for all the electrochemical measurements. Cyclic voltametry (CV) was recorded in PBS at 100 mV/s. For amperometric measurement of the immunosensor,