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Sandwich-type amperometric immunosensor using functionalized magnetic graphene loaded gold and silver core-shell nanocomposites for the detection of Carcinoembryonic antigen

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ABSTRACT

Herein, a novel and sensitive sandwich-type electrochemical immunosensor was fabricated for quantitative detection of carcinoembryonic antigen (CEA). In order to construct the base of the immunosensor, the gold nanoparticles (Au NPs) were immobilized on the surface of bare glassy carbon electrode (GCE) through electrochemical reduction of $HAuCl_4$ solution. The electrodeposited gold nanoparticles (D-Au NPs) not only effective improve immobilization of primary anti-CEA antibody (Ab_1) but also accelerate the electron transfer on the electrode interface due to the high specific surface area, good biocompatibility and superior electrical conductivity. Moreover, the amino functionalized magnetic graphene loaded gold and silver core-shell nanoparticles to adsorb nickel ion (GS-Fe_3O_4/Au@Ag/Ni^{2+}), which were used as a novel label to load the secondary anti-CEA antibody (Ab_2). The resultant nanocomposites possess high specific surface area, excellent electrochemical property, good biocompatibility and superior auxiliary catalytic activity due to the synergetic effect. The signal amplification strategy, using the synergetic effect present in Au@Ag/Fe_3O_4-GS/Ni^{2+} to improve immobilization of Ab_2 and increase the reduction ability of the nanocomposites towards H_2O_2, improved the sensitivity of the immunosensor. Under the optimal conditions, a linear relationship between current signals and the concentrations of CEA was obtained in the range from 0.1 pg/mL to 100 ng/mL and the detection limit of CEA was 0.0697 pg/mL (signal-to-noise ratio of 3). Furthermore, the as-proposed immunosensor showed excellent performance in detection of human serum samples. The results suggest that the proposed immunosensor will be promising in the diagnostics application for accurately quantitative detection of CEA.

1. Introduction

The reliable and sensitive determination of tumor markers is currently the subject of intensive studies due to it play a crucial role for early clinical [1], since the precise and early diagnosis of tumor markers could greatly improve the treatment efficiency of many cancers [2]. Carcinoembryonic antigen (CEA), an acidic glycoprotein having a molecular mass of ca. 200 kDa, is one of the most widely used tumor markers. Generally, the average concentration of CEA is obviously higher in the blood of the patients who suffer from some cancers such as breast cancer [3], colon cancer [4], ovarian carcinoma [5] than that in healthy individuals. Therefore, the accurate and sensitive determination of CEA in human body is very important for prophylaxis and treatment the above-mentioned cancers [6].

Nowadays, various detection assays have been used to detect tumor markers, such as enzyme-linked immunosorbent assay [7], fluorescence [8,9], high performance liquid chromatography [10] and colorimetric sensors [11]. Compared with these methods, electrochemical immunosensors have become one of the most popular analytical methods for the detection of tumor markers due to the advantages of rapid, high sensitivity, simple pretreatment procedure and simple operation [12–14]. Among all of the electrochemical immunosensors, sandwich-type immunosensors especially have attracted great research interests and found wide applications in many fields due to its high sensitivity and selectivity. For sandwich-type immunosensors, the signal amplification strategy is mainly based on coupling different kinds of labels to secondary antibody (Ab_2). Therefore, developing new labels and strategies to fabricate an electrochemical immunosensor for CEA

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detection is crucial.

In this work, a sandwich-type electrochemical immunosensor was fabricated for quantitative detection CEA using functionalized magnetic graphene loaded gold and silver core-shell nanoparticles to adsorb nickel ion (GS-Fe_3O_4/Au@Ag/Ni^{2+}) as signal labels. Graphene sheets (GS), which has large surface area, excellent conductivity, chemical stability and biocompatibility [15], have attracted a far-ranging interest in the fabrication of immunosensor [16,17]. Simultaneously, graphene oxide (GO) possess many oxygen-containing functional groups which make the water-solubility better and make it easy to form a stable chemical bond with various materials, such as, magnetic nanostructures, metallic or catalytic [18,19]. Furthermore, ferriferous oxide nanoparticles (Fe_3O_4 NPs) have attracted a considerable interest due to its good biocompatibility [20,21] and great auxiliary electrocatalytic properties towards the reduction of hydrogen peroxide (H_2O_2) [22]. Among the noble metal nanoparticles, gold nanoparticles (Au NPs) and silver nanoparticles (Ag NPs) were used to promote electron transfer between proteins and electrodes. They also exhibit good biocompatibility [23] and superior auxiliary catalytic activity towards the reduction of H_2O_2 [24,25]. Compared with single metal NPs, bimetallic NPs with a core-shell structure show distinctly unique characteristics than their monometallic counterparts, due to the synergetic effect of the second metal [26]. In addition, Au@Ag NPs can increase the load of antibody by forming stable Au—N and Ag—N bond between Au@Ag NPs and $-NH_2$ on antibodies [27]. Simultaneously, the amino functionalized magnetic graphenes composite material (NH_2-Fe_3O_4-GS) showed good adsorption properties for Ni^{2+} [28,29]. The adsorbed Ni^{2+}, can further promote the redox behaviour of H_2O_2, which can be used to amplify the detection signal. The synergetic effect present in GS-Fe_3O_4/Au@Ag/Ni^{2+} enhances the reduction ability of NPs towards H_2O_2 and it was used for the efficient labels.

For the sandwich-type electrochemical immunosensor, the immobilization of primary antibody (Ab_1) is another key policy to achieve signal amplification and increase the sensitivity [30]. Au NPs have been widely applied as the immobilization matrix for fabrication of biosensor due to its unique properties, including fast electron transportation, large specific surface area and good biocompatibility [31–33]. In this research, the electrodeposited gold nanoparticles (D-Au NPs) were used as substrate material for the immobilization of Ab_1. With these advantages, the sensitivity and stability of the immunosensor were effectively improved.

In this work, a novel sandwich-type immunosensor was fabricated successfully basing on D-Au NPs as the substrate material and GS-Fe_3O_4/Au@Ag/Ni^{2+} as the label of Ab_2. The proposed immunosensor show high sensitivity, good selectivity and stability for the quantitative detection of CEA, holding a great potential in clinical and diagnostic applications.

2. Experimental section

2.1. Apparatus and reagents

All electrochemical measurements were performed with a CHI760E electrochemical workstation (Shanghai Chenhua Instrument Co., Ltd., China), which including a conventional three-electrode system: a modified glassy carbon electrode (GCE, Φ = 4 mm) as a working electrode, a saturated calomel electrode (SCE) as the reference electrode, and the platinum wire electrode as the counter electrode. Energy-dispersive X-ray spectroscopy (EDX) analysis and scanning electron microscopy (SEM) images and were recorded using Quanta FEG250 field emission environmental SEM (FEI, United States). Fourier transform infrared spectroscopy (FTIR) spectrum was collected using VERTEX 70 spectrometer (Bruker, Germany).

CEA antibody and antigen, immunoglobulin G (IgG), α-1-fetoprotein (AFP) and thyroid stimulating hormone (TSH) were purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd., China. Bovine serum albumin (BSA, 96%–99%) and Hydrogen tetra chloroaurate (III) hydrate ($HAuCl_4{\cdot}4H_2O$) was obtained from Beijing Sigma-Aldrich co., Ltd., China. Graphite flakes, silver nitrate ($AgNO_3$, 99.9%) and Nickel (II) nitrate hexahydrate ($Ni(NO_3)_2{\cdot}6H_2O$, 98%) were purchased from sinopharm chemical reagent Co., Ltd., China. Potassium ferrocyanide ($K_4Fe(CN)_6$, 99%), potassium ferricyanide ($K_3Fe(CN)_6$, 99%), sodium citrate (99%) and hydrochloric acid (99%) were purchased from Aladdin Co., Ltd., China. 3-aminopropyltriethoxysilane (APTES, 98%) were purchased Shanghai Aladdin Chemistry Co., Ltd., China. $FeCl_3{\cdot}6H_2O$ (99%) was purchased from Damao Chemical Reagent Tianjin Co., Ltd., China. Phosphate buffered saline (PBS, 1/15 $mol{\cdot}L^{-1}$ Na_2HPO_4 (99%) and 1/15 $mol{\cdot}L^{-1}$ KH_2PO_4 (99.5%) was used as electrolyte for electrochemical measurements which was purged with nitrogen gas for 20 min to remove the dissolved oxygen. All other reagents were at analytical grade and ultrapure water (18.25 MΩ) was used throughout the study.

2.2. Preparation of the NH_2-GS-Fe_3O_4

Graphene oxide (GO) was synthesized according to an improved Hummer's method [34]. In brief, graphite flakes (0.6 g) and $KMnO_4$ (3.6 g) were dispersed in a mixture of concentrated H_2SO_4 (72 mL) and H_3PO_4 (8 mL). Subsequently, the reaction was heated to 50 °C and maintained at this temperature for 12 h with stirred. The mixture was cooled to room temperature after reaction and poured onto ice (80 mL) with 30% H_2O_2 (0.6 mL). Then, the mixture was centrifuged and removed the supernatant. After that, the remaining solid material was thoroughly washed with water, 30% HCl, ethanol and ether. Finally, the obtained solid material was dried in vacuum overnight.

GS-Fe_3O_4 was synthesized according to a protocol described previously [29,35]. $FeCl_3{\cdot}6H_2O$ (0.5 g) was dissolved in ethylene glycol (10 mL) to form a clear solution, then, NaAc (1.5 g), ethanediamine (5 mL) and GO (0.5 g) was added into the mixture orderly and dissolved under stirred vigorously for 30 min. Subsequently, the mixture was transferred into the teflon-lined stainless steel autoclave. The autoclave was heated to and maintained at 200 °C for 8 h and cooled down to room temperature after reaction. The prepared compound sample was thoroughly washed to remove the impurities and separated via a strong magnet. The resulting GS-Fe_3O_4 was dried under high vacuum overnight. It should be noted that the GO was translated into the graphene sheet (GS) in the process of reaction.

The amino-functionalized GS-Fe_3O_4 (NH_2-GS-Fe_3O_4) was synthesized by an improved method [36,37]. Briefly, GS-Fe_3O_4 (0.1 g) was dispersed in a solution of ethanol (10 mL) containing APTES (0.1 mL). Subsequently, the solution was heated to 70 °C and kept for 1.5 h. Finally, the NH_2-GS-Fe_3O_4 was obtained by magnetic separation and dried under high vacuum overnight.

2.3. Preparation of GS-Fe_3O_4/Au@Ag

The preparation of Au NPs was referred to the classical Frens method [38]. In brief, Sodium citrate (1.5 mL, 10 mg/mL) was added to the aqueous solution (100 mL) containing $HAuCl_4$ (1 wt%, 1 mL). Then, the mixture was heated to reflux and kept for 15 min. A wine red solution of Au NPs was obtained after being cooled to room temperature and stored at 4 °C. Au@Ag NPs was synthesized according an to the literature previously [39]. 1.0 mL of ascorbic acid (100 mmol/L) and 0.5 mL of $AgNO_3$ (10 mmol/L) were added into 20 mL of hexadecyl trimethyl ammonium bromide (CTAB) solution (50 mmol/L). After that, 2 mL of the Au NPs solution was added into the mixed solution. Then, 0.1 mL of NaOH aqueous solution (1 mol/L) was added drop-wise to the above solution under stirred vigorously. In this way, the color of the solution changed from red to bright golden yellow, indicating the success preparation of Au@Ag NPs.

The prepared NH_2-GS-Fe_3O_4 (30 mg) was dispersed in the Au@Ag NPs (120 mL) solution. The suspension was stirred for 24 h and