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Effective immobilization of Au nanoparticles on TiO_2 loaded graphene for a novel sandwich-type immunosensor

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ABSTRACT

A novel and sensitive sandwich immunosensor for amperometric determination of carcinoembryonic antigen (CEA) was designed using Au nanoparticles-titanium dioxide-graphene (AuNPs-TiO_2-graphene) nanocomposites. Dopamine-functionalized graphene was firstly prepared by π-stacking interaction, and TiO_2 was then attached to the surface of dopamine-graphene by the specificity and high affinity of enediol ligands to Ti (IV). Afterwards, AuNPs-TiO_2-graphene nanocomposites were synthesized with photo-reduction approach under ultraviolet irradiation. The morphology and conductivity of the as-prepared nanocomposites were characterized by transmission electron microscopy, Fourier transform infrared spectra, X-ray powder diffraction, cyclic voltammetry and electrochemical impedance spectroscopy. Taking the advantage of large specific surface area and excellent biocompatibility, AuNPs could covalently link horseradish peroxidase labeled secondary antibody (HRP-Ab_2) through the interaction between AuNPs and mercapto or primary amine groups of HRP-Ab_2 for sandwich-type immunosensor construction. Under optimum conditions, the modified electrode exhibited a linear current response to CEA concentration in a wide range of 0.005–200 ng mL^{-1} ($R^2 = 0.994$) with low detection limit of 3.33 pg mL^{-1} (S/N = 3).

1. Introduction

Carcinoembryonic antigen (CEA) is a glycoprotein that can be used as a eurytopic tumor marker in lung cancer, intestinal cancer, pancreatic cancer and other gynecological cancers. In general, CEA exists at a low concentration in serum of normal human, while its level can be elevated in cancer patients' serum (Ahn et al., 2017; Benchimol et al., 1989; Zamcheck and Matin, 1981). Therefore, it is of importance to measure CEA level in human serum.

Electrochemical immunosensor is a kind of biosensor combining electrochemical analysis method with immunological technology (Chen et al., 2016; Wang et al., 2017; Yang et al., 2017). To construct an electrochemical immunosensor, effective immobilization of biological recognition molecules on detection interface is one of the most important steps (Shen et al., 2015). Graphene, a two-dimension carbon material, possesses unique physicochemical properties such as high specific surface area, good conductivity and biocompatibility. Recently, graphene-based nanocomposites are rapidly becoming ideal functional materials in many fields (Ambrosi et al., 2014; Balandin et al., 2008; Karimi et al., 2015; Li et al., 2015). It's worth noting that the combination of graphene with metals can provide an electrochemical sensing platform with enhanced electronic and catalytic properties. The coupling and synergistic effects of graphene and metal can offer graphene a wider application field (Wang et al., 2015b; Wei et al., 2016; Yin et al., 2015; Zhang et al., 2016). Firstly, the presence of metal nanoparticles on graphene sheets can increase the spacing of graphene sheets to reduce the Van der Waals' force among graphene sheets and also preserve the physiochemical properties of graphene. Secondly, graphene-metal nanocomposites can be introduced to the sensor interface for sensitive detection of biological molecules through the biological recognition process of sandwich-type (Sun et al., 2015; Wang et al., 2015a). Especially, as an efficient nanomaterial for biomolecule immobilization, Au nanoparticles (AuNPs) bear good electrical conductivity, large specific surface area and excellent biocompatibility (Zhou et al., 2015). Meanwhile, the introduction of AuNPs onto the electrode interface can effectively accelerate electron transfer, leading to the improvement of biosensor performance (Jia et al., 2014; Wang et al., 2013; Yang et al., 2015).

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TiO_2 is an ideal photocatalyst with excellent properties of photoelectric conversion (Ishiguro et al., 2011; Kalathil et al., 2013; Khan et al., 2013). Under ultraviolet lamp irradiation, the electron transition from valence band to conduction band of TiO_2 forms free electrons, which can act as good reducing agents. In this way, AuNPs can be prepared using photocatalytic method, avoiding cumbersome experimental steps and harsh experimental conditions in conventional AuNPs preparation process (Cui et al., 2016; Williams et al., 2008). In addition, short and unequal Ti-O bond lengths in nano-TiO_2 make Ti-O bond strong polarity, so water molecules can easily be absorbed and polarized on TiO_2 surface. The polyhydroxy and hydrophilic surface of Ti atom would be easily replaced by hydroxyl in the enediol ligands and form a five-membered ring structure with Ti atom (Jin et al., 2015; Rajh et al., 2002). Thus, an aromatic molecule with enediol ligands can functionalize graphene for the immobilization of TiO_2.

Herein, a highly sensitive sandwich-type amperometric immunosensor has been fabricated for the determination of CEA using horseradish peroxidase-labeled secondary antibody (HRP-Ab_2) immobilized AuNPs-TiO_2-graphene nanocomposites. The AuNPs-TiO_2-graphene nanocomposites can act as carriers of signal amplification with good biological microenvironment, which can immobilize a large amount of enzyme-labeled antibody for a wide detection range and high sensitivity.

2. Experimental

2.1. Chemicals and materials

Mouse anti-human monoclonal antibody to CEA (anti-CEA), horseradish peroxidase labeled secondary antibody (HRP-Ab_2) and CEA were obtained from Linc-Bio Science Co., Ltd. (Shanghai, China). Commercial TiO_2 (P25) was purchased from Degussa Co., Ltd. (Germany). Graphene, lyophilized bovine serum albumin (BSA, ~68 kDa, ≥98%), dopamine and $HAuCl_4·4H_2O$ were provided by Sigma-Aldrich (USA). Hydroquinone (HQ), H_2O_2 (30%), $K_3Fe(CN)_6$ and $K_4Fe(CN)_6·3H_2O$ were bought from Sinopharm Chemical Reagent Co., Ltd. (China). Milli-Q water (18.25 MΩ cm) was used throughout the experiments.

2.2. Instruments and characterizations

Transmission electron microscopy (TEM) images were carried out at an acceleration voltage of 100 kV on Hitachi H-600 (Japan). Fourier transform infrared (FTIR) spectra were run on AVATAR 370 Fourier spectrometer (USA). X-ray powder diffraction (XRD) patterns were obtained with Rigaku D/max-2500 using Cu Kα radiation. Electrochemical measurements were performed on CHI 660D electrochemical workstation (Shanghai CH Instrument Co., China). A conventional three-electrode system was used with a saturated calomel electrode as the reference electrode, a platinum sheet as the counter electrode, and a modified glassy carbon electrode (GCE, 3 mm in diameter) as the working electrode. Differential pulse voltammograms (DPV) were recorded by applying a negative-going potential scan with pulse amplitude of 0.05 V, pulse period of 0.2 V and voltage increment of 0.004 V. Electrochemical impedance spectroscopy (EIS) measurements were tested on a Solartron 1255B Frequency Response Analyzer/SI 1287 Electrochemical Interface (Scribner Associates, Inc., USA) using 5 mM $[Fe(CN)_6]^{3-/4-}$ as the electrochemical probe. EIS was recorded when the electrochemical system reach steady state. 5 mV amplitude of sine voltage signal was applied to the three-electrode system under open circuit potential and frequency varying from 0.1 Hz to 100 kHz.

2.3. Preparation of HRP-Ab_2-AuNPs-TiO_2-graphene nanocomposites

HRP-Ab_2-AuNPs-TiO_2-graphene nanocomposites were prepared as follows. First, 1 mg graphene and 1 mg dopamine were added into 1 mL water, and the mixture was continuously sonicated for 1 h to promote the exfoliation of graphene sheets and the anchorage of dopamine. Then, 1 mg TiO_2 (in 1 mL water) dispersion was added into the mixture, followed by stirring vigorously for 20 min to obtain TiO_2-dopamine-graphene nanocomposites. Afterwards, the resulting dispersion was purified by repeated centrifugation (10,000 rpm) and washed with 10 mL water (3 times) to remove excessive dopamine. Subsequently, 50 μL of 0.2 g L^{-1} $HAuCl_4$ was dropwise added into the TiO_2-dopamine-graphene suspension solution under magnetic stirring, and the homogenous dispersion was vigorously stirred for 1 h under ultraviolet irradiation to obtain AuNPs-TiO_2-graphene nanocomposites. Finally, 10 μL HRP-Ab_2 solution was added to AuNPs-TiO_2-graphene dispersion under stirring, followed by incubation at 4 °C for 24 h. The final product (termed as HRP-Ab_2-AuNPs-TiO_2-graphene) was collected and stored at 4 °C for further use.

2.4. Fabrication of sandwich-type immunosensor

Prior to surface modification, GCE was polished to a mirror face, and then cleaned by successively ultrasonicating in 1:1 HNO_3, ethanol and water for 3 min, respectively. Then, the electrode was immersed in 0.1 M KNO_3 + 0.2 g L^{-1} $HAuCl_4$ solution to electrodeposit AuNPs by cyclic sweeping in the potential range of −0.5 to 0 V (vs. SCE) at 50 mV s^{-1} for 50 segments. The obtained AuNPs/GCE was rinsed with 10 mM phosphate buffer saline (PBS, pH 7.4) and dried at room temperature. Subsequently, 20 μL of 20 μg mL^{-1} anti-CEA was dropped on the surface of AuNPs/GCE, and incubated for 12 h at 35 °C under 100% humidity to obtain anti-CEA/AuNPs/GCE. After washing with buffer to remove physically adsorbed anti-CEA, the modified electrode was incubated with 20 μL of 0.1% BSA at 35 °C for 30 min to block the nonspecific binding sites (BSA/anti-CEA/AuNPs/GCE) and stored at 4 °C for further use.

The as-prepared electrode was incubated for 30 min with different concentrations of CEA for antigen-antibody specific recognition. Afterwards, the sandwich immunosensor was fabricated by incubating the electrode with 8 μL HRP-Ab_2-AuNPs-TiO_2-graphene bioconjugate at 35 °C for 1 h, followed by washing with 10 mM PBS (pH 7.4) to remove nonspecifically bound conjugates. Immunosensor was placed in electrochemical cell containing 10 mM PBS (pH 7.4) + 0.9% NaCl + 2 mM H_2O_2 + 2.5 mM HQ, and CEA detection was performed by measuring the increase of electrocatalytic peak current of HRP/H_2O_2 toward HQ reduction.

Scheme 1 displays the schematic diagram of sandwich immunosensor based on HRP-Ab_2-AuNPs-TiO_2-graphene nanocomposites for CEA determination.

2.5. Sample preparation

Three serum samples from healthy individuals were provided by Shanghai University Hospital. Before electrochemical detection, the serum was treated with perchloric acid (2.5% v/v) for 1 min and centrifuged at 4000 rpm for 5 min to remove redundant protein. Then, the supernatant was diluted 10 times with 10 mM PBS (pH 7.4) + 0.9% NaCl and stored at 4 °C for CEA detection.

3. Results and discussion

3.1. Characterization of TiO_2-dopamine-graphene and AuNPs-TiO_2-graphene nanocomposites

Fig. 1 is the TEM micrographs of TiO_2-dopamine-graphene (A, B) and AuNPs-TiO_2-graphene (C, D). As shown in Fig. 1A & B, TiO_2 with ~30 nm in diameter was dispersed on graphene sheets, and no TiO_2 was observed outside the graphene sheets, proving that the enediol ligands on dopamine were indeed effective in modifying TiO_2 on graphene sheets. Under ultraviolet irradiation, AuNPs-TiO_2-graphene was