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An ultrasensitive sandwich-type electrochemical immunosensor based on the signal amplification strategy of echinoidea-shaped Au@Ag-Cu_2O nanoparticles for prostate specific antigen detection

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ABSTRACT

Highly sensitive determination of tumor markers plays an important role in early diagnosis of cancer. Herein, a novel and ultrasensitive sandwich-type electrochemical immunosensor was fabricated for quantitative detection of prostate specific antigen (PSA). In this process, gold nanoparticles functionalized nitrogen-doped graphene quantum dots (Au@N-GQDs) was synthesized through a simple and green hydrothermal procedure to enhance conductivity, specific electrode surface area and quantity of immobilized primary antibodies (Ab_1). Subsequently, the prepared echinoidea-shaped nanocomposites (Au@Ag-Cu_2O) composed of Au@Ag core-shell nanoparticles and disordered cuprous oxide were prepared successfully to label the secondary antibodies (Ab_2), which convened the advantages of good biocompatibility and high specific surface area. Because of the synergetic effect present in Au, Ag and Cu_2O, the novel nanocomposites exhibited excellent electrocatalytic activity towards the reduction of hydrogen peroxide (H_2O_2) for the amplified detection of PSA. Therefore, the as-proposed immunosensor for the detection of PSA possessed wide dynamic range from 0.01 pg/mL to 100 ng/mL with a low detection limit of 0.003 pg/mL (S/N = 3). Furthermore, this sandwich-type immunosensor revealed high sensitivity, high selectivity and long-term stability, which had promising application in bioassay analysis.

1. Introduction

Recently, cancer outbreaks are occurring more frequently worldwide resulting in serious morbidity and mortality. In particular, prostate cancer has been recognized as the third most common cancer in men and the sixth most common cancer worldwide over the past decades (Grönberg, 2003; Li et al., 2017a). As the number of people suffered from prostate cancer has been increasing, early detection and prevention of this disease become significant. Prostate specific antigen (PSA, 34 kDa glycoprotein) synthesized merely by the prostate gland has been selected to be the important indicator for early detection of prostate cancer (Li et al., 2017b; Salimi et al., 2013). Therefore, the ability to develop a highly sensitive method and accurately detect the level of PSA in serum is highly desirable for early detection of prostate cancer (Kerman et al., 2007).

A series of methods have been used for the quantitative detection of PSA such as lectin-immunoassay (Kekki et al., 2017), “signal-on” electrochemiluminescence (ECL) (Wu et al., 2016), turn-on luminescence energy transfer (LET) (Zhang et al., 2015), site-specific enzymatic-cleavage-reaction-based biosensor (Choi et al., 2013), surface plasmon resonance (SPR) sensor (Ertürk et al., 2016), microfluidic enzyme linked immunosorbent assay (ELISA) (Adel Ahmed and Azzazy, 2013) and electrochemical immunosensor (Jang et al., 2015). Among them, electrochemical immunosensor has garnered considerable attention in the last decades due to their economic, sensitive, portable, easy-to-operate, and simple-to-construct properties (Yang et al., 2016c; Zhu et al., 2015). In particular, the sandwich-type electrochemical immunosensor based on highly specific interaction between antigen and antibody featuring better sensitivity and wider linear range is widely applied in clinical diagnosis and biochemical analysis (Jung et al., 2010; Liu et al., 2016). Therefore, we choose sandwich-type electrochemical immunosensor connected with the excellent catalytic properties of nanomaterials to achieve the optimization of this detection method.

Besides the aesthetic value of well-defined ordered structure, nanoarchitectures with multiple functions provide new tools to explore

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physical and chemical properties at the nanoscale (Li et al., 2015). In order to improve the sensitivity of immunosensor, various types of nanomaterials with perfect catalytic performance are used as labels or intermediates to fabricate the immunosensors, such as nanoparticles (NPs) (Yang et al., 2017), metal oxides (Lan et al., 2010), noble metal (Zhang et al., 2016). Herein, novel echinoidea-shaped Au@Ag-Cu_2O NPs were used as the label of secondary antibodies (Ab_2) towards H_2O_2 reduction to improve the sensitivity of the immunosensor. Gold nanoparticles (Au NPs) and silver nanoparticles (Ag NPs) have attracted widespread attention in the last decades due to their excellent biocompatibility and catalytic properties (Li et al., 2015; Zhang et al.). Primarily, in comparison with individual NPs, Au@Ag core-shell structure bimetallic NPs are synthesized to provide better catalytic performances because of their synergistic effect and electronic effect (Li et al., 2017c). However, with the diameter of 60 nm, Au@Ag core-shell NPs is incapable of loading plenty of Ab_2, which leads to the decrease of the catalytic activity towards H_2O_2 reduction. To overcome this disadvantage, it is essential to utilize new catalyst for H_2O_2 and new stabilizer for Ab_2. To date, Cu_2O, an environmental-friendly metal oxide, has gained a lot of attention due to its advantages, such as non-toxicity, low cost, easy to fabricate, sufficient mobility and excellent catalytic performance (Xu et al., 2015; Yang et al., 2016a). Constructing noble metal-metal oxide binary nanohybrids is one of the most effective methods to improve catalytic activity towards H_2O_2 reduction. Therefore, Cu_2O is selected to load onto the Au@Ag core-shell NPs. It is well-known that the electrocatalytic activity and stability of biomolecules are strongly dependent on the exposed facets. Hence, the resultant echinoidea-shaped structure provides a high specific surface area and more catalytically active sites, which hold great promise for improving sensitivity of this immunosensor (Luo et al., 2015). The synergistic effect among the structure of Au NPs, Ag NPs and Cu_2O can distinctly improve the performance of the immunosensor.

Substrate material plays an important role in signal amplification of the sandwich-type immunosensor. In this study, the nitrogen-doped graphene quantum dots (N-GQDs) supported Au NPs (Au@N-GQDs) are selected as the substrate material in the designed immunosensor. Recently, graphene quantum dots (GQDs) have been applied as a substrate due to the advantages of large specific surface area, biocompatibility, low toxicity and excellent electrical conductivity (Ju and Chen, 2015). The N atom, having a comparable atomic size and five valence electrons bonding with carbon atoms, has been widely used for chemical doping of carbon nanomaterials. Doping of GQDs with substituent N heteroatoms could effectively modulate the band gap of graphene to introduce new properties. Carbon materials can serve as reducing agents for the reduction of metal ions to metal nanoparticles (Dey et al., 2013), and the N atoms can increase the number of anchoring sites for the adsorption of metal ions. Compared with GQDs, the chemically bonded N atoms in N-GQDs could produce some new phenomena and properties including altering the electronic characteristics and increasing the number of anchoring sites for the adsorption of metal ions (Chizari et al., 2010; Li et al., 2012). Besides, lots of oxygen-containing functional groups in N-GQDs make it better to be with water-solubility and easier to form a stable chemical bond with various materials. As a result, with good biocompatibility and superior electrical conductivity, Au NPs supported by N-GQDs is selected as the substrate material in the designed immunosensor.

Herein, a novel sandwich-type immunosensor is fabricated successfully based on Au@N-GQDs as the substrate material and Au@Ag-Cu_2O as the label of Ab_2. The sandwich-type electrochemical immunosensor was based on the highly specific interaction between antigen and antibody and the fact that Au@Ag-Cu_2O was used as the label of secondary antibodies (Ab_2) for the reduction of H_2O_2, which effectually amplified the current signal in detection of PSA. In terms of novel nanomaterial, the designed immunosensor has shown its high sensitivity and excellent stability for quantitative determination of PSA.

2. Materials and methods

2.1. Reagents

The mixed Na_2HPO_4 and KH_2PO_4 solution were prepared to form phosphate buffered saline (PBS) that used as the electrolyte in the process of electrochemical measurements. Bovine serum albumin (BSA, 96–99%) was purchased from Sigma reagent Co., Ltd. (St. Louis, MO, USA). PSA and PSA antibody (Ab_1, Ab_2) were obtained from Linc-Bio Science Co., Ltd (Shanghai, China). $K_3[Fe(CN)_6]$ and silver nitrate ($AgNO_3$) were bought from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Chloroauric acid ($HAuCl_4$, 99%) and anhydrous copper (II) chloride ($CuCl_2$, 97%) were obtained from Sigma-Aldrich Co., Ltd. (Beijing, China). Citric acid (CA) and dicyandiamide (DCD) were purchased from Bodi Chemical Reagent Co., Ltd. (Beijing, China). Cetyltrimethylammonium bromide (CTAB, 98%), cetyltrimethylammonium chloride (CTAC, 95%), ascorbic acid (AA, 99.7%), sodium dodecyl sulfate (SDS, 99.6%) and hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$, 99%) were purchased from Shanghai Macklin Biochemical Co., Ltd. sodium hydroxide (NaOH) and trisodium citrate dehydrate ($Na_3C_6H_5O_7{\cdot}2H_2O$) were bought from Shuangshuang Chemical Co., Ltd (Yantai, China). All the experimental processes used ultrapure water (18.25 MΩ cm, 24 °C). All chemical reagents were of analytical grade. The prepared solutions were stored at 4 °C prior to use.

2.2. Apparatus

CHI760E electrochemical workstation (a conventional three-electrode system) was used to record all the electrochemical measurements, which consisted of a glassy carbon electrode (GCE,4 mm in diameter) as working electrode, a saturated calomel electrode as the reference electrode, and the platinum wire electrode as the counter electrode (Chenhua Instrument Shanghai Co., Ltd, China). Transmission electron microscope (TEM) images and energy-dispersive X-ray spectroscopy (EDX) analysis were collected using a Tecnai G^2 F20 (America).

2.3. Preparation of Au@N-GQDs

N-GQDs were prepared by a literature procedure (Ju and Chen, 2015). Briefly, 2.0 g of CA, 1.0 g of DCD and 5 mL ultrapure water were transferred into a 25 mL Teflon-lined autoclave and heated with 12 h at 180 °C. Eventually, the product was dispersed in 100 mL of ultrapure water, and the N-GQDs were collected by removing the large dots through centrifugation at 10,000 rpm for 10 min.

Au@N-GQDs was synthesized through a simple hydrothermal method (Peng et al., 2012; Song and Chen, 2014). Typically, $HAuCl_4$ (1 wt%, 0.158 mL) was added into a 15 mL ultrapure water with 20 mg of N-GQDs. The pH was adjusted to 10 with NaOH (0.1 mg/mL), and the mixture was transferred to Teflon-lined autoclave and hydrothermally treated at 160 °C for 6 h. Black precipitates appeared in the flask as a result of Au on N-GQDs to form Au@N-GQDs NPs. The precipitates were collected, washed extensively with ultrapure water and ethanol, and dried in a vacuum oven at room temperature for 12 h.

2.4. Preparation of Au@Ag-Cu_2O

Firstly, Au NPs were prepared according to the classical method (Frens, 1973). In brief, $HAuCl_4$ (1 wt%, 1.0 mL) and ultrapure water (99 mL) was refluxed and heated to boiling. Then sodium citrate (10 mg/mL, 2.5 mL) was rapidly added into the solution and stirred for another 15 min. The resultant wine-red solution was cooled down to room temperature and stored at 4 °C for further usage.

Secondly, Au@Ag core-shell NPs were synthesized following a literature procedure (Chiang and Huang, 2015). Briefly, CTAC