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Ultrasensitive sandwich-type electrochemical immunosensor based on a novel signal amplification strategy using highly loaded palladium nanoparticles/carbon decorated magnetic microspheres as signal labels

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ABSTRACT

An ultrasensitive sandwich-type electrochemical immunosensor for quantitative detection of alpha fetoprotein (AFP) was proposed based on a novel signal amplification strategy in this work. Carbon decorated Fe_3O_4 magnetic microspheres (Fe_3O_4@C) with large specific surface area and good adsorption property were used as labels to anchor palladium nanoparticles (Pd NPs) and the secondary antibodies (Ab_2). Pd NPs were loaded on Fe_3O_4@C to obtain Fe_3O_4@C@Pd with core–shell structure by electrostatic attraction, which were further used to immobilize Ab_2 due to the bonding of Pd-NH_2. A signal amplification strategy was the noble metal nanoparticles, such as Pd NPs, exhibiting high electrocatalytic activities toward hydrogen peroxide (H_2O_2) reduction. This signal amplification was novel not only because of the great capacity, but also the ease of magnetic separation from the sample solution based on their magnetic property. Moreover, carboxyl-functionalized multi-walled carbon nanotubes (MWCNTs-COOH) were used for the immobilization of primary antibodies (Ab_1). Therefore, high sensitivity could be realized by the designed immunosensor based on this novel signal amplification strategy. Under optimal conditions, the immunosensor exhibited a wide linear range of 0.5 pg/mL to 10 ng/mL toward AFP with a detection limit of 0.16 pg/mL ($S/N=3$). Moreover, it revealed good selectivity, acceptable reproducibility and stability, indicating a potential application in clinical monitoring of tumor biomarkers.

1. Introduction

Tumor biomarkers could be found with formation of tumor in the body. The detection of tumor biomarkers is of great significance in early clinical diagnosis and disease prevention (Sakamoto et al., 2014). The sensitive and reliable detection of tumor marker is currently the subject of intensive studies (Fan et al., 2013; Li et al., 2013). Alpha fetoprotein (AFP) is a fetal serum protein which is primarily produced in the yolk sac and endodermal organ of the embryo (Matsunou et al., 1994). In adults, the content of AFP is physiologically diminished, but can be reproduced under some pathological conditions, including hepatic regeneration, carcinogenesis of germinal tumors, hepatocellular carcinoma, and a subset of extrahepatic adenocarcinoma referred to as AFP-producing adenocarcinoma (Ishikura et al., 1985). AFP-producing adenocarcinoma is a highly malignant subtype of adenocarcinoma, which accounts for 2.7–5.4% of primary gastric adenocarcinoma (Chang et al., 1992; Kono et al., 2008). It has been considered that the production of AFP in adenocarcinoma suggests enteroblastic differentiation or hepatoid differentiation of tumor cells (Furuya et al., 2011; Ishikura et al., 1985; Matsunou et al., 1994).

Enzyme-linked immunosorbent assay (Bi and Liu, 2013), high performance liquid chromatography (Huang et al., 2003), and single-strand conformation polymorphism assay (Bosari et al., 1995) have been developed to detect tumor markers, which are time-consuming, expensive and with low sensitivity. Recently, an electrochemical immunosensor based on the principle of highly biospecific recognition interactions between antigens and the corresponding antibodies (Jung et al., 2010) has been developed for detection of tumor markers in clinical diagnosis because of its high sensitivity, low cost, fast response and ease of handling and miniaturization (Wang et al., 2014). For a sandwich-type electrochemical immunosensor, what kind of labels was used to be conjugated to secondary antibodies (Ab_2) for signal amplification always attracts extensive attentions.

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In this study, an ultrasensitive sandwich-type electrochemical immunosensor based on a novel signal amplification strategy was proposed for the quantitative of alpha fetoprotein (AFP), where high loaded Fe_3O_4@C@Pd magnetic microspheres were used as signal labels.

Thus, magnetic microspheres have been widely used in sensor fabrication (Teymourian et al., 2013; Yang et al., 2014) and protein separation (Chen et al., 2009; Deng et al., 2009; Qi et al., 2009; Xu et al., 2006). Carbon decorated Fe_3O_4 magnetic microspheres (Fe_3O_4@C) with large specific surface area were used as a label matrix to capture palladium nanoparticles (Pd NPs) and the secondary antibodies (Ab_2). Meanwhile, carbon acted as electron mediator to accelerate the electron transfer. The noble metal nanoparticles like Pd NPs, exhibited high electrocatalytic activity toward hydrogen peroxide (H_2O_2) reduction (Kim et al., 2014). The high load Pd NPs would not only improve the catalytic current, but also capture a mass of Ab_2 by the bonding of Pd-NH_2.

The immobilization of primary antibodies (Ab_1) is another point for the signal amplification and ultrasensitive detection of a sandwich-type electrochemical immunosensor (Jeong et al., 2013). Multi-walled carbon nanotubes (MWCNTs) have attracted great interests due to their unique properties including fast electron transportation, high thermal conductivity (Shahrokhian et al., 2009). In this work, the obtained carboxyl-functionalized multi-walled carbon nanotubes (MWCNTs-COOH) exhibited several superiorities: (1) MWCNTs were shortened and produced carboxyl groups, which could introduce negative charges on the MWCNTs and improved their dispersion in water; (2) the functional groups on the outer walls of MWCNTs-COOH could capture more Ab_1, while retaining intact inner walls for highly conducting network (Liang et al., 2012). Therefore, signal amplification and ultrasensitive detection could be achieved by this designed sandwich-type electrochemical immunosensor for the quantitative of AFP in human serum.

2. Experimental section

2.1. Materials and reagents

Human AFP, antibody to human AFP (anti-AFP) and bovine serum albumin (BSA, 96–99%) were obtained from Shanghai Linc-Bio Science Co., Ltd., China. Multi-walled carbon nanotubes were purchased from TIMESNARO Co. Ltd (Chengdu, China). 1-(3-(Dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride (EDC, 98.5%), N-hydroxysuccinimide (NHS, 98%) and sodium tetrachloropalladate (II) (Na_2PdCl_4) were purchased from Shanghai Aladdin Chemistry Co., Ltd., China. Ethylene glycol, hydrogen peroxide, $FeCl_3 \cdot 6H_2O$ and $NaBH_4$ were acquired from Sinopharm Chemical Regent Co. Ltd. Phosphate buffered solutions (PBS) were prepared by compounding the solution of KH_2PO_4 (1/15 mol/L) and Na_2HPO_4 (1/15 mol/L) to appropriate pH values. PBS was used as electrolyte for all electrochemistry measurements. Ultrapure water was used in all runs. All other chemicals were of analytical grade and used without further purification.

2.2. Apparatus

All electrochemical measurements were performed on a CHI760D electrochemical workstation (Chenhua Instrument Shanghai Co., Ltd., China). A conventional three-electrode configuration was used: a glassy carbon electrode (GCE, 4 mm diameter) as working electrode, a saturated calomel electrode (SCE) as the reference electrode and a Pt wire as the counter electrode. Scanning electron microscope (SEM) and Energy Dispersive X-Ray Spectroscopy (EDS) were recorded by JEOL JSM-6700 F microscope (Japan). Transmission electron microscope (TEM) images were obtained from a JEOL-2100 microscope (Japan). Fourier transform infrared spectroscopy (FTIR) spectrum was obtained from VERTEX 70 spectrometer (Bruker, Germany).

2.3. Synthesis of palladium nanoparticles

Firstly, 20 mL of aqueous solution containing Na_2PdCl_4 (73.6 mg) and trisodium citrate (73.5 mg) was prepared where trisodium citrate acted as a capping agent to restrict the particle growth. Then, 0.6 mL of $NaBH_4$ solution (0.01 M) was added at once into the palladium solution under constant stirring and lasted for another 30 s. Finally the solution would turn brown black color, which indicated the successful formation of Pd nanoparticles (Pd NPs).

2.4. Synthesis of Fe_3O_4@C@Pd magnetic microspheres

The Fe_3O_4@C magnetic nanoparticles were synthesized through hydrothermal reaction according to the published reference (Qi et al., 2010). Fe_3O_4@C was dispersed into an aqueous solution of poly(diallyldimethylammonium chloride) (PDDA) (0.20%) that contained 2.0×10^{-2} M Tris and 2.0×10^{-2} M NaCl and the resulting dispersion was stirred for 20 min. Residual PDDA was removed by using a magnet and the PDDA electrostatic adsorbed microspheres were rinsed with water for three times. The obtained magnetic microspheres (20 mg) were redispersed in 30 mL of the as-synthesized solution of Pd NPs and the mixture was stirred for another 20 min. The presence of a layer of adsorbed positively charged PDDA on the Fe_3O_4@C magnetic microspheres ensured the efficient adsorption of negatively charged Pd NPs. After the magnetic separation, the obtained Fe_3O_4@C@Pd was washed with ultrapure water three times and dried under vacuum at 50 °C for 12 h.

2.5. Synthesis of carboxyl-functionalized Multi-walled carbon nanotubes

MWCNTs-COOH were synthesized according to the previous literature (Dong et al., 2012). Briefly, MWCNTs were dispersed in HNO_3 (30%) and then refluxed for 24 h at 140 °C to shorten the nanotubes and to produce carboxylic groups focusing on the open ends as well as the sidewalls, which introduced negative charges on the MWCNTs and improved water dispersibility.

2.6. Preparation of the Fe_3O_4@C@Pd-Ab_2 labels

The preparation process of Fe_3O_4@C@Pd-Ab_2 is presented in Scheme 1A. 2 mg of Fe_3O_4@C@Pd magnetic microspheres were dispersed into 20 mL of ultrapure water, and then ultrasonic treatment lasted for 30 min. The supernatant was removed with the help of a magnet. Fe_3O_4@C@Pd (2 mg) was added to Ab_2 dispersion (10 μg/mL, 1 mL) and incubated for 12 h at 4 °C. After the oscillation, it was centrifuged for 15 min at 4 °C with 6500 rpm in refrigerated centrifuge and the supernatant was removed afterwards. Then the obtained immunocomplex was stored in 1 mL of PBS solution (pH=7.0) at 4 °C before use.

2.7. Fabrication of the immunosensor

The preparation process of the immunosensor is displayed in Scheme 1B. In the current experiment, the glassy carbon electrode (GCE) was polished with alumina powders (0.05 μm). Afterwards, 6 μL of MWCNTs-COOH solutions were dropped onto the surface of GCE. The carboxyl group of MWCNTs-COOH was activated with EDC/NHS (Liu et al., 2013) for 20 min. Subsequently, 6 μL of Ab_1