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Ultrasensitive electrochemical immunosensor for SCCA detection based on ternary Pt/PdCu nanocube anchored on three-dimensional graphene framework for signal amplification

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ABSTRACT

In this study, a novel and ultrasensitive sandwich-type electrochemical immunosensor was designed for the quantitative detection of squamous cell carcinoma antigen (SCCA) based on the β-cyclodextrin functionalized graphene nanosheet (CD-GN) and the ternary hollow Pt/PdCu nanocube anchored on three-dimensional graphene framework (Pt/PdCu-3DGF). CD-GN exhibited high specific surface area and good dispersibility and stability in water, which were beneficial to fix captured antibodies (Ab_1) through the supramolecular host-guest interaction between CD and Ab_1. The abundant oxygen-containing functional groups on 3DGF provided binding sites for anchoring noble metal nanoparticles. Pt/PdCu-3DGF could capture detected antibodies via the interaction of Pd-NH_2 and Pt-NH_2. Furthermore, the ternary metal nanoparticles exhibited high electrocatalytic activity toward the reduction of hydrogen peroxide. Under optimal conditions, the fabricated immunosensor showed a sensitive response to SCCA with two linear ranges. The linear ranges are 0.0001–1 ng/mL and 1–30 ng/mL with a detection limit of 25 fg/mL. Additionally, the proposed immunosensor showed good reproducibility and stability.

1. Introduction

Squamous cell carcinoma antigen (SCCA) is a kind of TA-4 subtypes (Williams et al., 2013) and exists in normal epithelial cells with low level. As for the epithelia of cancerous tissue, the amount of SCCA is found to be over-expressed (Pontisso et al., 2004). And a large number of reports have indicated that the level of SCCA increased along with the incidence of cerivical squamous cell carcinoma (CSCC) (Crombach et al., 1989; Kato, 1992). SCCA is well recognized as the tumor marker for CSCC (Duk et al., 1989). In the past years, many methods and strategies have been developed for the detection of SCCA, such as chemiluminescence immunoassay (Brabant et al., 2003; Tanaka and Matsunaga, 2000), fluoroimmunoassay (Wu et al., 2007), radioimmunoassay (Barnes et al., 2000), enzyme-linked immunosorbent assay (Butler, 2000) and surface plasmon resonance (Pattnaik, 2005). In consideration of the highly biospecific interaction between antigens and the corresponding antibodies (Pei et al., 2001), electrochemical immunosensors have attracted widespread interest due to the advantages of high sensitivity, rapid detection, excellent selectivity and low manufacturing cost.

Recently, noble metal nanoparticles have been applied in electrochemical sensors on account of their catalytic performance toward hydrogen peroxide (H_2O_2) reduction (Wu et al., 2013). Pt-based nanoparticle catalysts have been widely used in fuel cells and show high electrocatalytic activity and stability (Mu et al., 2005; Mukerjee and Srinivasan, 1993; Steigerwalt et al., 2002). Considering the high cost, most of the recent efforts have been focused on the improvement of the catalytic efficiency of Pt-based catalysts with relatively lower Pt content (Demarconnay et al., 2007; Zhang et al., 2012). Pd and Pd-based catalysts, with relatively large reserves and low price, are also excellent candidates for development of immunosensor (Gao et al., 2014). However, the chemically inert Pd was unstable when exposed to hostile electrochemical environments where the surface Pd atoms dissolved and migrated, resulting in aggregation of nanoparticles and reduction of surface area, activity, and power density (Yang et al., 2010). In order to reduce the costs and retain the catalytic efficiency of noble metal nanoparticles, the hollow ternary Pt/PdCu nanocubes was introduced to 3D graphene by solvothermal approach (Yang et al., 2011). Graphene is a charming supporting material for biosensor applications, largely due to its excellent electrocatalytic activity and the fast electron transfer process

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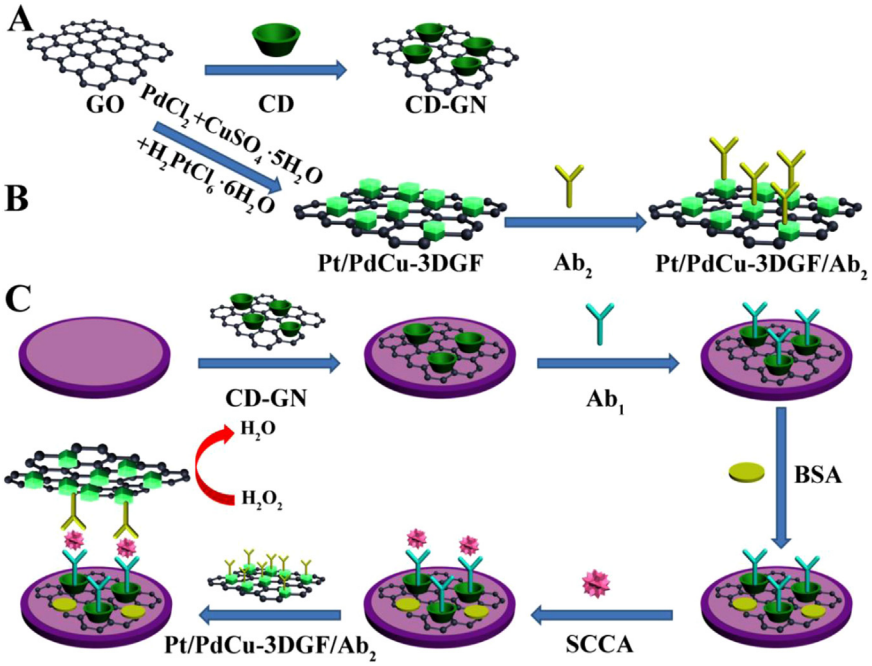

Scheme 1. The preparation procedures of CD-GN (A), Ab_2-Pt/PdCu-3DGF (B) and immunosensor (C).

among its basal plane. (Lin et al., 2005). And compared with the 2D graphene nanosheet, 3D porous graphene is of worth noting due to its strong mechanical stability, high electrically conductivity and thermal stability (Chen et al., 2011; Shao et al., 2013). Furthermore, benefited from the higher specific areas, the 3D graphene with abundant oxygen-containing functional groups also provide much more binding sites for anchoring of metal nanoparticles (Xin et al., 2011; Xu et al., 2008). Besides, 3D graphene can be synthesized by hydrothermal treatment (Xu et al., 2010) which is compatible with the solvothermal approach to Pt/PdCu ternary alloy nanocubes. The Pt/PdCu hollow nanocubes with specific geometric structure own low densities and high surface areas which enable them to bind more detection antibodies (Ab_2) via the interaction of Pt-NH_2 and Pd-NH_2. And the ternary alloy has superb electrocatalytic activity toward H_2O_2 reduction.

For sandwich-type electrochemical immunosensor, the immobilization of captured antibodies (Ab_1) is one key point for the signal amplification and ultrasensitive detection (Jeong et al., 2013). Graphene-based platform is designed to improve sensitivity. Graphene nanosheet (GN) has drawn much attention owing to its high surface area, low cost and high conductivity (Guo et al., 2011; Shao et al., 2010). In the reduction process, graphene nanosheet tends to form irreversible agglomerates via van der Waals interactions due to the loss of oxygen containing functional groups (Xu et al., 2008). In order to solve this problem, β-cyclodextrin (β-CD) is introduced into graphene oxide (GO) before it is fully reduced. β-CD is a kind of toroidal biological macromolecules with a hydrophobic inner cavity and a hydrophilic hydroxyl exterior (Bardi et al., 2000). The high supramolecular recognition characteristic enable them to bind selectively with various guest molecules to form a stable host-guest complex (Boger et al., 1978; Pitha et al., 1986). Therefore, β-CD can not only prevent the stacking of GN but also improve the molecular recognition ability of GN. Meanwhile, β-CD functionalized graphene nanosheet (CD-GN) remains soluble in water, thus avoiding the aggregation(Guo et al., 2010). By the host-guest interaction, Ab_1 containing amino functional group can enter into the cavities of CD to form stable host-guest inclusion complexes (Jiang et al., 2015).

In this work, a novel sandwich-type electrochemical immunoassay was developed using CD-GN as a sensor platform and ternary hollow Pt/PdCu nanocube anchored on 3DGF as labels. The electro-catalysis of Pt/PdCu-3DGF toward H_2O_2 reduction was applied to generate an electrochemical signal for immunoassay. The highlights are as follows: (i) The CD-GN can promote the electron transfer on theelectrode surface and it is easy to capture a great deal of Ab_1 owing to the host-guest interaction and great specific surface area. (ii) Pt/PdCu-3DGF is easily labeled with Ab_2 due to the noble metal character. This strategy with a high current response, two linear ranges (from 0.0001 to 30 ng/mL and from 1 to 30 ng/mL) and a low detection limit for monitoring SCCA (25 pg/mL) shows potential applications in the clinical detection of cancer biomarkers.

2. Experimental

2.1. Regents and apparatus

SCCA Ab_1, and SCCA Ab_2 were purchased from Beijing DINGGUO CHANGSHENG biotechnology CO. LTD. (China). $PdCl_2$ and $H_2PtCl_6 \cdot 6H_2O$ were purchased from Shanghai Aladdin Chemistry Co., Ltd., China. Bovine serum albumin (BSA) was obtained from Sigma-Aldrich (Beijing, China). Phosphate buffered saline (PBS, 1/15 mol/L KH_2PO_4 and 1/15 mol/L Na_2HPO_4) was used as electrolyte for all electrochemical measurements. All other chemical reagents were analytical reagents grade and directly used without further purification.

All electrochemical measurements were achieved on CHI 760D electrochemical workstation (Shanghai Chenhua Instrument Co. Ltd., China). Transmission electron microscope (TEM) images were recorded by a JEOL-1400 microscope (JEOL, Japan). Scanning electron microscope (SEM) images were obtained from JSM-6700F microscope (JEOL, Japan). FT-IR spectra were collected using a FT-IR-410 infrared spectrometer (JASCO, Japan). Energy dispersive X-ray spectroscopy (EDS) was recorded by JEOL JSM-6700 F microscope (Japan).