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Triple tumor markers assay based on carbon–gold nanocomposite

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ABSTRACT

A sandwich-format electrochemical immunosensor for simultaneous determination of three cancer biomarkers using the carbon–gold nanocomposite (CGN) as immunoprobes was introduced. The CGN were fabricated through a simple microwave-assisted carbonization of glucose and deposition of gold nanoparticles (AuNPs). This nanocomposite showed great adsorption ability to the redox probes such as some organic dyes and metal ions, due to the abundant reactive oxygen functional groups on its surface. The AuNPs decorated on the nanocomposite provided extra binding sites for the three antibodies carcinoembryonic antigen (CEA), prostate specific antigen (PSA), α-fetoprotein (AFP), respectively. The ionic liquid reduced graphene oxide was combined with poly(sodium-p-styrenesulfonate) as substrate to attach the three different antibodies through electrostatic adsorption. Three separate signals can be detected directly in a single run through square wave voltammetry. Under optimized conditions, the electrochemical immunosensor exhibited good sensitivity and selectivity for the simultaneous determination of CEA, PSA and AFP with linear ranges of 0.01–100 ng mL^{-1}. The detection limit for CEA, PSA and AFP is 2.7, 4.8 and 3.1 pg mL^{-1}, respectively. This method was applied for the analysis of CEA, PSA and AFP levels in clinical serum samples, and the results were in good agreement with those of enzyme linked immunosorbent assay (ELISA). This approach gives a promising simple and sensitive immunoassay strategy for the identification and validation of specific early cancer.

1. Introduction

For the cancer patients, the early detection of cancer can contribute to the early diagnosis and treatment of malignancies and lead to a higher chance of survival. In recent years, protein biomarkers have begun to act an increasingly important role in the management of patients with malignancy. The quantified detection of protein biomarkers such as tumor markers in serum thus holds enormous promise, both in detecting the disease early and in subsequently tracking disease progression in response to therapy. This increased the demand of a more sensitive and fast detection of tumor markers. In addition, because of the inherent complexity of cancers, the different type cancers could commonly altered in different series of tumor markers. It was agreed that the simultaneous multiplexed immunoassay of a panel of targets can provide a more accurate and reliable diagnosis.

Electrochemical immunoassay with the advantages of innate high sensitivity and simplicity has become a powerful analytical tool for the specific and sensitive detection of clinical samples (Liu and Ju, 2008). The traditional electrochemical immunoassay for one or two analytes has become common and lots of works have been done (Cao et al., 2013; Chen and Ma, 2014; Gao et al., 2015; Jia et al., 2014; Lian et al., 2013; Liu and Ma, 2013; Liu et al., 2014; Wang et al., 2014). However, many cancers were associated with multiple tumor markers. Such as the liver cancer and ovarian cancer, they were all specific to three tumor markers (Li et al., 2012; Petru et al., 1990). It is often a time-consuming and complicated work for diagnostic cancer screening. Therefore, the simultaneous multiplexed analyzes that could cover more tumor markers in a single run is urgently needed, for the advantages of shortened analysis time, decreased sample and simplified analytical procedure volume (Wu et al., 2007). But the simultaneous multiplexed analyses always face the difficulties of fabricating immunoprobes with different redox activity, particularly for the analyses covered more than two biomarkers in a single run. The voltammetric peaks of the redox probes need to be distinguishable and separate from each other. And in order to prevent the interference from blood serum substance, such as dopamine and ascorbic acid, the potential should be under 0.1 V (Chen et al., 2010; Wang et al., 2002). To solve this issue, it will be of great significance to develop new materials which can generally adsorb three or more redox probes and meanwhile possess abundant activity sites to bind antibody.

Recently, lots of attentions have been paid to the carbon nanomaterials such as carbonaceous materials due to their large surface area, excellent biocompatibility, and fascinating

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electrocatalytic activity (Gao et al., 2013; Hu et al., 2008; Liu et al., 2008; Makowski et al., 2008; Rezan et al., 2009; Xu et al., 2011). Carbon nanosphere obtained through hydrothermal carbonization usually got abundant reactive oxygen functional groups, showed good adsorption capacity to redox probes such as some organic dyes and metal ions. In addition, its intrinsic properties can be finely tuned by changing parameters such as bulk structure, diameter and chemical composition. These make the carbon nanosphere ideal material in the application of immunoprobes and biosensor platform for the simultaneous multiplexed assay. However, the carbon materials commonly face the problems of complicated synthesis process, hydrophobicity and poor dispersion (Tang et al., 2009). The traditional hydrothermal carbonization methods of carbohydrates could hardly control the size of the colloidal spheres, and will cost more than 12 h (Hu et al., 2010).

In this work, a carbon–gold nanocomposite (CGN) was introduced. The carbon sphere was obtained by the microwave-assisted carbonization of glucose in several minutes. The growth mechanism of the carbon spheres was confirmed to the LaMer model (LaMer, 1952; Sun and Li, 2004). The hydrothermal conditions will lead to aromatization and carbonization of glucose. Firstly, the glucose was dehydrated into furan-like molecules, such as furfural aldehyde or 5-(hydroxymethyl)-2-furaldehyde. Subsequently a cross-linking was induced by the intermolecular dehydration. The resulting nuclei grew uniformly and isotropically by diffusion of solutes toward the particle surfaces. The immobilization of AuNPs was achieved through the in-situ formation, by dispersion the carbon nanosphere in the presence of $HAuCl_4$. With the aid of the second step microwave irritation, the AuNPs could be deposited on the obtained carbon sphere at last. The as-prepared CGN has abundant reactive oxygen functional groups, which are easy to adsorb different electrochemical redox probes. In addition, antibody can be easily fixed on the AuNPs of CGN. Herein, the as-prepared CGNs were used to fabricate three electrochemical immunoprobes through adsorbing thionin (Thi), 2,3-diaminophenazine (DAP) and Cd^{2+}, and fixing three kind of antibodies, respectively. The obtained three new electrochemical immunoprobes exhibited electrochemical windows can effectively avoid the interference from other substances in serum and were applied for simultaneous detection of three tumor markers carcinoembryonic antigen (CEA), prostate specific antigen (PSA), and α-fetoprotein (AFP). The results were well consistent with those of ELISA.

2. Experimental section

2.1. Reagents and Materials

CEA, PSA, AFP was purchased from Biosynthesis Biotechnology Company (Beijing, China). Monoclonal anti-CEA, anti-PSA and anti-AFP capture antibodies, monoclonal anti-CEA, anti-PSA and anti-AFP labeled antibodies were purchased from Linc-Bio Company (Shanghai, China). The hydrogen tetrachloroaurate hydrate ($HAuCl_4 \cdot xH_2O$, 99.9%), acetic acid (AA), uric acid (UA), sodium citrate, glucose, thionine acetate, DAP and poly(sodium-p-styrenesulfonate) (PSS) were purchased from Alfa Aesar China (Tianjin, China). Ionic liquid (e.g., 1-aminopropyl-3-methylimidazolium chloride) was purchased from Shanghai Chengjie Chemical Co. Ltd. (Shanghai, China). Graphene oxide was purchased from JCNANO (Nanjing, China). NaH_2PO_4, Na_2HPO_4, $Cd(NO_3)_2$ potassium ferricyanide ($K_3[Fe(CN)_6]$), potassium ferrocyanide ($K_4[Fe(CN)_6]$), K_2CO_3 and bovine serum albumin (BSA) were purchased from Beijing Chemical Reagents Company (Beijing, China). Clinical serum samples were obtained from Hospital of Capital Normal University, China. The ELISA date of serum samples were obtained from Deyi clinical testing center, Beijing. Ultrapure water (resistivity > 18 MΩ cm) was used throughout the experiment and all the reagents were of analytical grade and used as received.

2.2. Apparatus

During all experiment procedures, the water used was purified through Olst ultrapure K8 apparatus (Olst, Ltd., resistivity > 18 MΩ cm). The synthesis of CGN was conducted by microwave reaction instrument (CEM, American). Zeta potential analysis was performed on Nano ZS Zetasizer (Malvern Instruments Co. British). All the electrochemical experiments were carried out on CHI1140 electrochemical workstation (Chenhua Instruments Co., Shanghai, China). Three-electrode system consisting of an Ag/AgCl electrode (saturated KCl) as the reference electrode, a platinum wire as the auxiliary electrode and a glassy carbon electrode (GCE) (4 mm diameter) as the working electrode was used in experiment.

2.3. Preparation of CGN

In previous works, the carbon–gold structure materials were synthesized using different method (Cui et al., 2008; Jerzy et al., 2012). Here we introduced a more simple strategy. Briefly, 4 mL (wt. 1%) glucose aqueous solution was mixed with 200 μL (wt. 10%) sodium citrate aqueous solution. The mixture was reacted in microwave reaction instrument (250 W) at 160 °C for 10 min and then cooled down to the room temperature. As programmed, the microwave reactor automatically controlled the reaction temperatures. The color of mixture changed from pale to dark brown, indicating the carbonization of carbohydrates was happened. Then resulting mixture was centrifuged and washed with water several times, then dispersed in 4 mL ultrapure water. A sonication of 45 min was carried on until the turbid mixture was transformed into a homogeneous solution. The mixture was mixed with 100 μL (wt. 1%) $HAuCl_4$ aqueous solution and reacted in microwave reaction instrument (250 W) at 100 °C for 15 min, then cooled down to the room temperature. Subsequently, centrifuged and washed with water several times, redispersed in 4 mL ultrapure water. The CGN was obtained.

2.4. Preparation of the Ionic liquid reduced graphene oxide

Graphene possessed excellent electron-transfer ability and exceptional thermal stability (Kang et al., 2009; Shan et al., 2009; Tang et al., 2011). Ionic liquid reduced graphene oxide (IL-rGO) was synthesized according to our previous work. Briefly, 50 mg ionic liquid was added to 50 mL dispersion of graphene oxide in water (0.5 mg mL^{-1}). 50 mg KOH was added. A sonication was carried on until the mixture was transformed from turbid to a homogeneous solution. Then, the solution was vigorously stirred under 80 °C for 24 h. The obtained mixture was centrifuged and washed with ethanol and water repeatedly for six times, then dispersed in ultrapure water (0.5 mg mL^{-1}) for further use.

2.5. Preparation of immunoprobes

Three equal parts of 4 mL CGN were mixed with 100 μL 20 mM Thi, DAP and $Cd(NO_3)_2$, respectively. Then were stirred 5 h under room temperature. After centrifuged and washed with water for several times, three obtained bioconjugates CGN-Thi, CGN-DAP and CGN-Cd^{2+} were re-dispersed in 4 mL ultrapure water. Under gently stirring, three labeled antibodies of CEA, PSA and AFP (200 μL, 1 mg mL^{-1}) were added to the CGN dispersion, respectively, and stirring at room temperature for 2 h, bioconjugates of CGN-Thi-anti-CEA, CGN-DAP-anti-PSA and CGN-Cd^{2+}-anti-AFP were obtained. After centrifugation and re-dispersed in 2 mL