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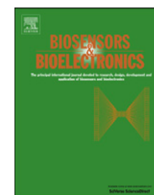

Sensitive immunosensor for the label-free determination of tumor marker based on carbon nanotubes/mesoporous silica and graphene modified electrode

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ABSTRACT

A novel label-free immunoassay strategy for sensitive detection of α-fetoprotein (AFP) was proposed based on controlled fabrication of single-wall carbon nanotubes (CNTs) inside the channels of mesoporous silica (MPS). The silanol groups on the internal pore walls of MPS were grafted with amino groups, while the silanol groups on the external surface were blocked by trimethylchlorosilane (TMCS). Thus, CNTs and the monoclonal antibodies of AFP (anti-AFP) could be confined inside the mesopores of MPS by the covalent linking of the carboxyl and amino groups. For the preparation of immunosensing electrode, graphene sheets (GS) and anti-AFP/CNTs/TMCS–MPS were coated on the electrode surface based on layer by layer assembly. After dipping the anti-AFP/CNTs/TMCS–MPS/GS/GCE into the sample solution, the immunoconjugates formed after the immunological reaction, which resulted in the increment of spatial blocking and impedance of the immunosensing interface. Thus, the peak current decreased with the increasing concentration of AFP. CNTs inside the mesopores could promote the electron transportation through the pore channel. Meanwhile, modified GS with distinctive conduction capacity could also improve the electrochemical response. Under the optimal experimental conditions, the label-free immunosensor could detect AFP in a linear range from 0.1 to 100 ng mL^{-1} with a detection limit of 0.06 ng mL^{-1} (3σ).

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1. Introduction

The determination of tumor markers was of immense significance in the early diagnosis of cancer, evaluating the extent of disease and monitoring the response of tumors to therapy. Alpha fetoprotein (AFP), as one of the most important tumor markers, has been widely studied recently for the assistant diagnosis of hepatocellular cancer, yolk sac cancer and liver metastasis (Fu et al., 2006; Liu et al., 2007). Numerous immunoassay methods, especially immunosensors combined with various transducers, have been developed to detect the levels of tumor markers (Wu et al., 2007a).

In the traditional immunoassay, antigen- or antibody–enzyme conjugates were usually prepared and used as the labels (Jia et al., 2009; Chena et al., 2006). However, the labeling process was often complicated and time-consuming (Swift and Cramb, 2008; Qiu et al., 2009). Based on monitoring the changes in interfacial or electronic performance before and after the immunological reaction, label-free strategies have been gained increasing attention due to the simple, rapid and low cost detection process (Qiu et al., 2009; Zhang et al., 2008; Vollmer and Arnold 2008). Usually, the label-free electrochemical immunosensor depended on the real-time detection of specific antigen–antibody interaction by the electrochemical impedance spectroscopy (Daniels and Pourmand 2007; Wang et al., 2004; Thavarungkul et al., 2007; Ionescu et al., 2007; Tang et al., 2007), the capacitance (Loyprasert et al., 2008) or amperometric (Liang et al. 2009) measurements. Great efforts have been made to enhance detection sensitivity and/or obtain low detection limits. In our previous work, a sensitive label-free strategy for the detection of AFP has been proposed by confining gold nanoparticles inside the channels of MPS to improve the electric performance (Lin et al., 2012). In the current work, we focused on the signal amplification to improve the detection sensitivity by using graphene sheets (GS) and single-wall carbon nanotubes (CNTs).

GS has been interestingly utilized as the carrier of signal amplifying probe (Zhong et al., 2010; Li et al., 2011a; Liu et al., 2011; Kuila et al., 2011) and modification material in the fabrication of electrochemical immunosensor (Yang et al., 2010; Tang et al., 2011; Li et al., 2011b) because of its marvelous properties

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such as high conductivity, large surface area and low cost (Wang and Musameh 2003). CNTs, with unique electronic properties, high chemical stability and mechanical strength, has also shown the prospect on the fabrication of biosensors (Lin et al., 2009; Lu et al., 2010; Chen et al., 2000; Bhandari et al., 2009). It has been reported that CNTs sonicated in the mixture of HNO_3 and H_2SO_4 could cause severe etching of the graphitic surfaces (both outer and inner) of the CNTs, resulting in shorter tubes with opened ends (Rosca et al., 2005; Datsyuk et al., 2008). Luo et al. reported the usage of the acid-pretreated CNTs with carboxyl groups as nanoreserviors for drug delivery (Luo et al., 2011). In the current work, acidic pretreated single-wall CNTs with shorter length were filled into the channels of mesoporous silica (MPS) to enhance electric performance. By blocking the Si–OH groups on the external surface of MPS with trimethylchlorosilane (TMCS), the amino functional groups were grafted onto the internal pore walls by aminopropyltriethoxyl silane (APTS). Thus, CNTs and monoclonal antibodies of AFP (anti-AFP) could be captured into the pore channels by the covalent linking between the amino and carboxyl groups. Anti-AFP/CNTs/TMCS–MPS and GS were coated on the electrode surface to prepare the label-free immunosensor based on a layer by layer assembly. The label-free determination of AFP could be realized by monitoring the changes of the electrochemical signals before and after the immunological reaction. After the immunosensor was incubated with sample AFP solution, the nonconductive immunoconjugates formed, which resulted in increasing impedance. Thus, the electron transfer was blocked and the electrochemical signals decreased. The electronic performance could be enhanced by the synthetic effects of CNTs and GS. The proposed strategy displayed an excellent selectivity for the detection of AFP in human serum.

2. Experimental

2.1. Reagents

Ferrocenecarboxylic acid (FCA) and the silane reagents of tetraethyl orthosilicate (TEOS), TMCS and APTS were purchased from Sigma-Aldrich Chemical Co. $EO_{20}PO_{70}EO_{20}$ (P123), 1-ethyl-3-(3-dimethyl aminopropyl)-carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were obtained from ACROS. Trimethylbenzene (TMB) was purchased from Shanghai Chemical Reagent Co. (China). Graphite powder was purchased from BASF Chemical Co. (Tianjin, China). Single-walled carbon nanotubes (CNTs, length: 5–30 μm) was obtained from Deke Daojin Tech Co. (Beijing, China). Bovine serum albumin (BSA) was purchased from Shanghai Aibi Chemical Reagent Co. (China). The standard solutions of AFP (0–200 ng mL^{-1}) and carcionembryonic antigen (CEA, 0–80 ng mL^{-1}) were purchased from Zhengzhou Bosai Bio-tech Co. (China). Other chemicals were of analytical grade and used without further purification. All solutions were prepared with doubly-distilled water. EDC/NHS solution was prepared by mixing 5 mM EDC and 8 mM NHS at a volume ratio of 1:1 in prior to use. 0.1 M phosphate buffered solutions (PBS) at various pH values were prepared by mixing the stock solutions of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 at different volume ratio to the appropriate pH value.

2.2. Apparatus

Electrochemical measurements were performed on a CHI 832B electrochemical analyzer (Shanghai Chenhua Ins.c, China) with a three-electrode system comprised of platinum wire as an auxiliary electrode, saturated calomel electrode (SCE) as a reference and modified glassy carbon electrode (GCE) as a working electrode, respectively. Differential pulse voltametry (DPV) and cyclic voltammetric (CV) measurements were done in a conventional electrochemical cell at room temperature. Electrochemical impedance spectroscopy (EIS) measurements were carried out on a CHI 660 electrochemical workstation (Shanghai Chenhua Ins.c, China). Transmission electron micrograph (TEM) was recorded on JEM-1200EX TEM (JEOL, Japan).

2.3. Preparation of graphene sheets

Graphene oxide was prepared by a modified Hummers method according to the literature (Zhang et al., 2011). After ultrasonic dispersing 0.2 g graphene oxide in 80 mL H_2O for 1 h, 1.15 mL of 50% hydrazine hydrate was dropped into the suspension for the reducing reaction. The mixture was kept stirring at 50 °C for 25 h. After filtering and washing with water, graphene sheets (GS) were obtained and re-dispersed in water at a concentration of 1.0 mg mL^{-1}.

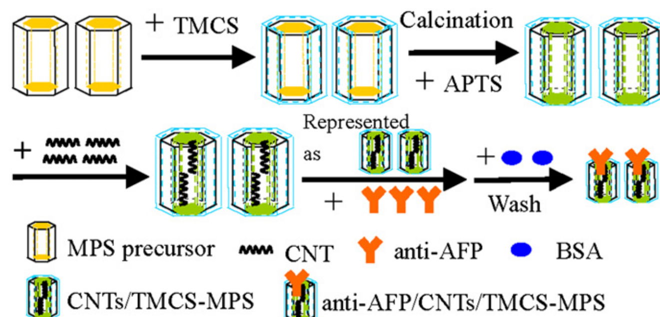
2.4. Loading CNTs into TMCS–MPSlo

TMCS–MPS was prepared according to our previous method (Lin et al., 2011a; Lin et al., 2012). In order to break CNTs into shorter tubes and open their ends with carboxyl functional groups, 200 mg of CNTs was dispersed in 100 mL concentrated HNO_3 and H_2SO_4 (volume ratio 1:3) solution and sonicated for 2 h (Luo et al., 2011). Afterwards, the suspension was kept at room temperature overnight, then washed with water until the pH was neutral. Finally, the hydrophilic CNTs with carboxyl groups were collected and dried at 60 °C.

0.1 mL of EDC/NHS solution was added into 0.5 mL of TMCS–MPS (2.5 mg mL^{-1}), and then mixed with 0.5 mL of the shortened CNTs (0.25 mg mL^{-1}). The above mixture was kept stirring for 12 h. By the covalent bonding of the carboxyl groups of CNTs and amino groups on the MPS inner walls, CNTs could be captured into the channels of the MPS. After rinsed with water, CNTs/TMCS–MPS was obtained.

2.5. Fabrication of AFP immunosensor

As shown in Scheme 1, 0.5 mL of monoclonal antibody of AFP (anti-AFP, 100 ng mL^{-1}) was mixed with 0.5 mL EDC/NHS firstly. And secondly, 1.0 mL of CNTs/TMCS–MPS (2.0 mg mL^{-1}) was added and kept stirring for 30 min. The molecules of anti-AFP were immobilized into the channels of MPS owing to the reaction between the carboxyl groups of anti-AFP and the amino groups of TMCS–MPS (Wu et al., 2007b; Suni 2008). After rinsed and centrifuged with distilled water, bovine serum albumin (BSA, wt. 1%) was employed to block the non-specific activity sites of CNTs/TMCS–MPS. Then, the immunological probe for the label-free



Scheme 1. Preparation of the label-free immunological probe.

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