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Immobilization of antibodies on aldehyde-functionalized polymer/graphene films for the fabrication of a label-free electrochemical immunosensor

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ABSTRACT

Using poly-(epichlorohydrin) and 4-pyridinecarboxaldehyde, we synthesized a new aldehyde-functionalized polymer, which was applied to simplify antibody immobilization on the electrode surface, in which no additional chemical cross-linking is required. Combined with the advantages of graphene, a label-free electrochemical immunosensor based on graphene/aldehyde-containing polymer film for α-fetoprotein detection was fabricated. α-Fetoprotein was detected within the range from 0.05 to 35 ng mL^{-1} with a detection limit of 0.02 ng mL^{-1} obtained by 3 S/N. The proposed immunosensor is easy, label-free, specific and thus provides a good diagnostic tool for detection of α-fetoprotein and other cancer markers.

1. Introduction

Electrochemical immunosensors have received considerable attention due to their high sensitivity, inherent simplicity, rapid detection, miniaturization and low cost [1]. In the development of electrochemical immunosensors, a key step is immobilization of antibodies on the electrode surface for binding to its analyte. To achieve this, various of substances including self-assembled monolayers [2–4] and nanomaterials [5–7] were used widely. In addition, polymers were also extensively applied to immobilize antibodies on sensing interfaces for the fabrication of immunosensor [8–10]. This is due to its extraordinary stability, simplicity of synthesis from an inexpensive monomer, functional groups, and unique electrochemical properties. The use of polymers for antibody immobilization can be carried out via entrapment [11] and covalent attachment [12–14]. The entrapment method is easy to operate and can remain the activity of antibodies, but the antibodies would leak out from polymer film into bulk solution, resulting in a low sensitivity and bad accuracy of immunoassay. This problem can be overcome using covalent binding for obtaining a stable and sensitive biosensor. Traditionally, carboxyl or amino-functionalized polymers were applied to immobilize antibodies for the development of immunosensors [15–18]. Unfortunately, these functional groups need to be activated with the mixture of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and N-hydroxysuccinimide, or glutaraldehyde. Apparently, the activation step is trivial and time-consuming. Therefore, it is important to develop a new matrix for simplifying the procedure of immobilizing antibodies, in which no additional chemical cross-linker is required.

Poly(epichlorohydrin) (PECH) is biocompatibility, commercially available and very cheap. Moreover, chlorine atoms can be efficiently substituted by many nucleophilic reagents such as aliphatic carboxylates, sodium methoxide and series of substituted phenolates under mild conditions [19]. Thus, it has been used as a starting material for synthesizing novel functional polymers including well-defined DNA-mimicking brush polymers [20], self-assembled brush glycopolymers [21], and lipid-mimicking brush polymers [22]. To the best of our knowledge, PECH has not been used for synthesizing an aldehyde-functionalized polymer. Because aldehyde groups can bond to amino groups of antibodies directly, synthesizing a aldehyde-containing polymer by the use of poly(epichlorohydrin) is of great significant for the construction of immunosensors. The electrical conductivity of sensing interface makes a great effect on the sensitivity of immunosensors. Graphene (Gr) as a nanomaterial has attracted considerable attention owing to its high electrical conductivity, large specific surface area and good biocompatibility [23]. It has been used to develop label free immunosensors [24,25].

In this work, a new aldehyde-functionalized polymer (polymer-CHO) has been synthesized. Using polymer-CHO/graphene films, we developed a label free immunosensor for the detection of α-fetoprotein (AFP) as a model analyte. The aldehyde-functionalized polymer was

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applied to immobilize antibody directly, while the addition of graphene improved the conductivity of the sensing interface.

2. Experimental

2.1. Reagents and apparatus

α-Fetoprotein antigen (AFP), anti-α-fetoprotein antibody (Ab) were purchased from Biocell Company (Zhengzhou, China). Human immunoglobulin G (IgG), bovine serum albumin (BSA), and human serum albumin (HSA) were purchased from Beijing Dingguo Biotechnology Company (Beijing, China). Graphene (Gr) was purchased from Nanjing Xianfeng Nanotechology Co. (Nanjing, China). Poly(epichlorohydrin) (PECH, average Mw ~ 700,000), 4-pyridinecarboxaldehyde, and dimethylformamide used in this work were of analytical grade and purchased from Sigma-Aldrich. 0.1 M phosphate buffer solution (PBS, pH 7.2) was prepared using Na_2HPO_4 and NaH_2PO_4.

All electrochemical measurements, including electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV) were carried out with a CHI 660 electrochemistry workstation (Shanghai CH Instruments, China). A conventional three-electrode cell, consisting of a Pt electrode as counter electrode, a saturated calomel electrode (SCE) as reference electrode, and a glassy carbon electrode (GCE) modified with an aldehyde-functionalized polymer/graphene film as working electrode was used.

2.2. Preparation of aldehyde-functionalized polymer (polymer-CHO)

Aldehyde-functionalized polymer can be synthesized according to reported literature [26]. The synthetic route was shown in Fig. 1. Typically, under the protection of argon, 1.24 g poly(epichlorohydrin) was dissolved in 20 mL andydrous dimethylformamide at 60 °C, and then 1.3 g 4-pyridinecarbox-aldehyde was added. The mixture was stirred at 120 °C for 24 h. Most of the solvent was removed by constant rotation under vacuum. The crude polymer was dried at 50 °C in a vacuum overnight. The dried polymer was redissolved in dichloromethane and precipitated three times with ethanol. Finally a brown solid (0.5 g) was obtained after drying under vacuum at 50 °C for 24 h.

2.3. Preparation of the electrochemical immunosensor

A GCE (3 mm in diameter) was polished repeatedly with 0.3 and 0.05 μm alumina slurries sequentially, followed by successive sonication in distilled water and ethanol, respectively, then dried in air at room temperature prior to modifying. Synthesized aldehyde-containing polymer was dissolved in dimethylformamide to form a 0.5 wt.% solution. 0.5 mg graphene were added into 0.5 mL above polymer-CHO solution. The solution mixture was ultrasonicated for 1 h to obtain a homogeneous of graphene/polymer-CHO composite. Then, 10 μL of polymer-CHO/graphene composite was dropped on the electrode and dried in air at room temperature. Then, 10 μL of antibody solution (80 μg mL^{-1}) was dropped on the modified electrode and incubated for 60 min, followed by washing with ultrapure water to remove unspecific physically adsorption. In order to eliminate non-specific binding effect and block the remaining active sites, the electrode modified with antibody was incubated with 2.0 wt.% BSA for 40 min at room temperature. The as-prepared immunosensor (Ab/polymer-CHO/graphene/GCE) was stored at 4 °C for further detection of analyte. The whole process of electrochemical immunosensor fabrication was shown in Fig. 2.

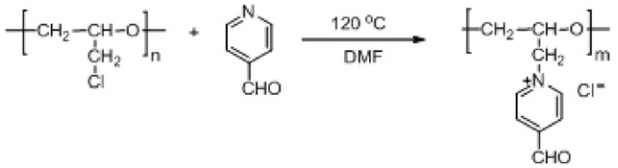

Fig. 1. Synthetic route for aldehyde-functionalized polymer.

2.4. Electrochemical measurements

All electrochemical experiments were carried out in a conventional electrochemical cell containing a three-electrode system. Electrochemical impedance spectra (EIS) and differential pulse voltammetry (DPV) measurements were performed in 10 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ solution. EIS measurements were carried out in the frequency range from 10^{-1} to 10^5 Hz under an open potential. The amplitude of the alternative voltage was 5.0 mV. DPV measurements were carried out under the following: the potential range was from -0.4 to 0.1 V, pulse amplitude was 0.05 V, pulse width was 0.05 s, and sample width was 0.02 s. The peak current of DPV was used for quantitative detection of AFP.

3. Results and discussion

3.1. Characterization of polymer-CHO, graphene and polymer-CHO/graphene

IR-spectra of poly(epichlorohydrin) (PECH) and aldehyde-functionalized polymer were measured respectively. Fig. 3A showed aldehyde-functionalized polymer has an obvious aldehyde peak at 1730 cm^{-1} (curve a) compared with the spectrum of PECH (curve b). This result demonstrated that the aldehyde-functionalized polymer had been synthesized successfully. Additionally, the morphologies of graphene (Fig. 3B) and polymer-CHO/graphene (Fig. 3C) composite were investigated by TEM.

3.2. Electrochemical properties of the modified electrodes

Electrochemical impedance is a useful method to investigate the electrochemical properties of the modified electrodes. Fig. 4 showed the Nyquist plots of impedance spectra of different electrodes in 10 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ solution. The semicircle diameter in the impedance spectra equals to the electron-transfer resistance (Ret) [27]. As can be seen from Fig. 4, the impedance spectra of bare GCE showed an almost straight line (curve a). When the electrode was modified by aldehyde-functionalized polymer (polymer-CHO/GCE), Ret increased because aldehyde-containing polymer blocked electron transfer of $[Fe(CN)_6]^{3-/4-}$ to the electrode (curve b). However, when polymer-CHO/graphene composite was modified onto the electrode (polymer-CHO/graphene/GCE), the Ret decreased (curve c). It was probably because graphene improved the electron transfer between the electrode and electroactive indicator [28]. After antibodies were assembled on the polymer-CHO/graphene film (Ab/polymer-CHO/graphene/GCE), the Ret increased (curve d), which illustrated that the antibodies had been successfully immobilized on the surface of electrode. The introduction of the antigen would result in the further increase of the Ret (curve e). It should be ascribe to the inert blocking layer of antigen or antibody hinders the diffusion of ferricyanide toward the electrode surface.

3.3. Optimization of experimental conditions

Using the DPV peak current of the immunosensor toward 10 ng mL^{-1} antigen as an analyte, we investigated the influence factors including antibody concentration, antibody immobilization time, and immunoreaction time.

The amounts of antibody immobilized on the electrode surface will influence the binding sites for analyte and thus influence the sensitivity