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MIPs-graphene nanoplatelets-MWCNTs modified glassy carbon electrode for the determination of cardiac troponin I

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ABSTRACT

An electrochemical sensor with high selectivity in addition to sensitivity was developed for the determination of cardiac troponin I (cTnI), based on the modification of cTnI imprinted polymer film on a glassy carbon electrode (GCE). The sensor was fabricated by layer-by-layer assembled graphene nanoplatelets (GS), multiwalled carbon nanotubes (MWCNTs), chitosan (CS), glutaraldehyde (GA) composites, which can increase the electronic transfer rate and the active surface area to capture a larger number of antigenic proteins. MWCNTs/GS based imprinted polymers (MIPs/MWCNTs/GS) were synthesized by means of methacrylic acid (MAA) as the monomer, ethylene glycol dimethacrylate (EGDMA) as the cross linker α,α′-azobisisobutyronitrile (AIBN) as the initiator and cTnI as the template. In comparison with conventional methods, the proposed electrochemical sensor is highly sensitive for cTnI, providing a better linear response range from 0.005 to 60 ng cm^{-3} and a lower limit of detection (LOD) of 0.0008 ng cm^{-3} under optimal experimental conditions. In addition, the electrochemical sensor exhibited good specificity, acceptable reproducibility and stability. Moreover, satisfactory results were obtained in real human serum samples, indicating that the developed method has the potential to find application in clinical detection of cTnI as an alternative approach.

1. Introduction

It is known to all that myocardial infarction (MI) is a leading cause of death. Recently, electrocardiographic investigation has been the main method for the diagnosis, however, only half of patients reveal electrocardiogram changes [1,2]. As a result, there has been considerable interest in developing new methods for MI diagnosis [3–6]. Human cardiac troponin I (cTnI), a cardiac muscle protein, compared with other myocardial injury biochemical markers, such as creatine kinase-MB isoenzyme and myoglobin, has been recognized as a principle diagnostic marker for myocardial damage because of its higher specificity [7]. Thus, rapid, sensitive detection of cTnI is extremely important for early detection of MI. Existing methods of diagnosis for cTnI rely heavily on expensive and time-consuming laboratory tests, including immunoenzymometric assays (ELISA) [8], chemiluminescentimmunoassays [9], fluoro-immunoassays [10], electrical detections [11], surface plasmon resonance detection (SPR) [12], colorimetric protein array [13] and so on. However, they are far from sensitivity and specificity [14]. In order to meet the increasing demand of quick diagnosis and clinical therapeutics, we are in dire need of a certain device that has fast response time, high sensitivity, stable characteristics and more importantly ease of operation and fabrication. Electrochemical detection offers several advantages, such as ease of use, low cost, direct detection, miniaturization, and fast response times [15–21].

As addressed previously, the detection of biomarkers plays an important role in basic medical research as well as in clinical diagnostics [22,23]. Molecularly imprinting, which happened as followings: a polymer synthesized with templates through a polymerization process; some or all of the templates removed for selective recognition sites; recognition in the spaces vacated by the templating species [22–24], has received widespread attention and development over recent years in design, preparation, characterization and application fields. Molecularly imprinted polymers (MIPs) in the quantitative detection of molecules, complexes, and macromolecular assemblies, including biomarkers, has reflected the gradual maturation of molecular imprinting technology (MIT) [25]. Hitherto, a great deal of MIPs based electrochemical sensors have been reported [26,27]. Mainly, there are two approaches to fabricate MIPs based sensors. One is to directly prepare MIPs on an

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electrode surface through electrochemical [28], optical [29] and thermal technologies [30], etc. The other is to coat the as-prepared MIPs on an electrode surface with the help of binder analogs [26,27]. MIPs synthesized on the electrode surface directly has attracted considerable interests for its advantages such as sensitive, rapid, straight-forward preparation and low cost [31]. Therefore, different electrochemical MIPs sensors [32–34] have been developed and extensively applied to the determination of biomarkers [35–37].

Nanomaterials, such as graphene nanoplatelets (GS) and multiwalled carbon nanotubes (MWCNTs), processing many advantages, like large surface area, excellent conductivity, low cost, and electrocatalytic activity [38–41], have offered alternative approaches for sensitive and low-cost detection of proteins in MIT [36,42–47]. Recently, many researchers have demonstrated that GS could serve as a reinforcing element with substrate materials to offer better catalytic properties due to the synergistic effect, such as MWCNTs, in fabricating nanocomposites than sole GS [48,49]. Chitosan (CS) is an abundant natural cationic biopolymer which possesses a lot amino groups, excellent film-forming ability, adhesion and biocompatibility, has become one of the most interesting substrate materials in the chemical modification of electrode surface [50,51]. Composite materials based on CS and nanomaterials in MIT applications can thus increase the selectivity, sensitivity and the binding kinetic properties [52,53], prevent the leakage of nanomaterials as well as improve the electronic transmission rate and enlarge the surface area to capture more target protein antigen through the glutaraldehyde (GA) cross-linker [54,55].

Up to now, only one reference about the MIPs-based electrochemical sensor to detect cTnI, the concentration range was from 0.05 to 5.00 nM and the limit of detection (LOD) was found to be 0.027 nM [56]. To broad the linear as well as improve the sensitivity, we developed an ultrasensitive electrochemical sensor for cTnI determination with advantages of MIPs and the nanocomposite of GS, MWCNTs and CS. The CS dotted MWCNTs-GS composite possess large surface area and excellent conductivity, which can greatly amplified the sensor's sensitivity, improved the electronic transmission rate, shorten the responding time, and displayed superb selectivity too. The sensor displayed broader linear and lower detection limit compared with the existed methods. Furthermore, this test method was successfully employed to recognize and detect cTnI in the real human serum, showing potential applications.

2. Experimental

2.1. Reagents

Cardiac troponin (cTnI), Carcinoembryonic antigen (CEA), Bovine serum albumin (BSA), Neuron specific enolase (NSE), Human immunodeficiency virus p24 (HIV-p24), Human chorionic gonadotropin (HCG) and cTnI ELISA kit were purchased from LincBio Science Co. Ltd (Shanghai, China). Methacrylic acid (MAA), 2,2′-azobisisobutyronitrile (AIBN), ethylene glycol dimethacrylate (EGDMA), Chitosan (CS, 99% deacetylation) and Glutaraldehyde (GA, 25% aqueous solution) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, US). Acetic acid and methanol were from J&K SCIENTIFIC Ltd (Beijing, China). Multi-walled carbon nanotubes (MWCNTs) were purchased from Nanoport Co. Ltd. (Shenzhen, China), graphite (GS) was obtained from Nanjing XFNANO Materials Tech. Co. Ltd. (Nanjing, China).

Tris-HCl buffered solution (20 mM, pH7.5) was prepared by dissolving the Trisbase into hyperpure water and then adjusted pH by adding suitable volumes of concentrated hydrochloric acid freshly prepared. 0.1 M PBS (pH7) was prepared by mixing the stock solutions of KH_2PO_4 and K_2HPO_4. Hyperpure water (resistivity = 18.2 M Ω·cm) was used through-out the experiment.

2.2. Apparatus

Electrochemical measurements of cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed on a CHI 660B electrochemical workstation (Chenhua, Shanghai, China). The electrochemical cell consisted of a conventional three electrode system with platinum wire as auxiliary electrode, saturated calomel electrode (SCE) as reference electrode and the modified or unmodified glass carbon electrode (3 mm diameter, GCE) as working electrode. CV characterization was performed in PBS containing 5 mmol dm^{-3} $Fe(CN)_6^{3-/4-}$ (1:1) and 0.1 mol L^{-1} KCl at a scanning rate of 50 mV s^{-1}. DPV were conducted in the same solution to the CV characterization from −0.2 V to 0.6 V at a scan rate of 50 mV s^{-1}. The surface of the modified electrode was characterized by SEM (FE-SEM; Zeiss Ultra55, Germany), and the samples were sputtered with a thin layer of gold before imaging. Infrared microspectrography were collected by a Fourier transformation infra-red spectrometer coupled with infra-red microscope (EQUINOX 55, Bruker, Germany) under room temperature/humidity control after background correction.

2.3. Preparation of MIPs and NIPs

Prior to the fabrication, a bare GCE was successively polished using 0.05 μm alumina powder and then ultrasonically cleaned in absolute alcohol and ultrapure water for 5 min to get a mirror like surface, followed by electrochemical cycling in 0.5 M H_2SO_4 for 100 cycles in the range of −1.5–1.5 V, 100 mV s^{-1}. 1.0 mg GS was dispersed into 1 mL DMF to form 1.0 mg cm^{-3} GS dispersion. MWCNTs were treated with nitric acid and sulfuric acid, the acid-treated MWCNTs were then dispersed into ultrapure water (0.5 mg cm^{-3}). The MIPs/MWCNTs/GS/GCE was prepared by the following steps: First of all, 5 μl GS, 5 μl MWCNTs and 1.0 μl of 0.25 mg cm^{-3} amino-riched CS were dropped on the electrode surface in file and then dried under infrared lamp between each step. 5 μl 2.5% GA (in 50 mM, pH 7.4 phosphate buffer) were grafted onto the electrode through the imine bond with CS for 2 h and washed with ultrapure water, then the aldehyde-riched electrode was activated with 5 μl of 50 μg cm^{-3} cTnI, reacted at room temperature for 1 h and then kept at 4 °C overnight in a one hundred percent humidity environment to form an imide group between the aldehyde-groups on GA and the amino-groups on cTnI. They were washed with pH 7.5 Tris–HCl to remove unspecific physically adsorption. Then, immersed the electrodes into a solution containing 2 ml 1 mol dm^{-3} MAA as the functional monomer and 0.07 mol dm^{-3} EGDMA as the cross-linker, in Tris-HCl buffer, pH 7.5. After that, 1 ml of 0.06 mol dm^{-3} AIBN solution was added to initiate the polymerization mainly through hydrogen bonds between them to form a highly lattice-like structure. The reaction was kept at room temperature for 5 h with N_2, then the sensor was thoroughly washed with hyperpure water several times. Finally, the product was eluted by methanol/acetic acid (9/1, V/V) for 4 h to break hydrogen bonds, releasing the protein from the imprinted layer. The MIP was finally conditioned in Tris-HCl buffer, pH 7.5 for a while to wash away the cTnI released by methanol/acetic acid (9/1, V/V) treatment. The preparation of stepwise procedure of the MIP sensor is showed in Fig. 1. The optimization of concerntration of MAA, the crosslinker, polymerization, elution and readsorption time were shown in supporting information read as Fig. S1.

For comparison, a non-imprinted film modified electrode (NIPs/MWCNTs/GS/GCE) was prepared similarly to the procedure only without the cTnI in the polymerization process.