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A cascade reaction signal-amplified amperometric immunosensor platform for ultrasensitive detection of tumour marker

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ABSTRACT

A new strategy for signal amplification was introduced in a label-free amperometric immunoassay platform. Improvements in the signal occurred following the oxidation of glucose by glucose oxidase and catalysis by polythionine of the produced H_2O_2 from the previous oxidation reaction. In addition, a multifunctional conductive hydrogel, polypyrrole-polythionine-gold with glucose oxidase, was synthesised with a one-pot method using pyrrole and thionine as monomers, ammonium persulfate and $HAuCl_4$ as co-oxidant agents, and glucose oxidase as the doping agent. Combined with the hydrogel, a label-free amperometric immunoassay for the tumour marker neuron-specific enolase (NSE) was obtained. If operated at 0 V (vs. Ag/AgCl), the sensor exhibited a wide liner range from 100 ng mL^{-1} to 1 pg mL^{-1} and ultralow detection limit of 0.65 pg mL^{-1} (S/N = 3). The presented method was further used for the analysis of human serum samples and showed good consistency with detection results from an electrochemiluminescence immunoassay.

1. Introduction

Cancer diagnosis in humans has increased over the past decades with high fatality, metastatic, and recurrence rates. Early detection is a key issue in successful treatment of the disease, leading to enhanced patient survival rates [1–4]. Tumour markers are highly significant in cancer treatments, as they express mutated proteins that are associated with malignant growth and can be directly measured in diseased blood and tissues. Therefore, the determination of tumour markers plays an important role in early detection of cancers. Recently, great efforts have been made to detect tumour markers, such as enzyme-linked immunosorbent, fluorescence immunoassay, chemiluminescence, radio immunoassay, and electrochemiluminescence assays [5–10]. Although these assays have proven to be mostly successful, some inevitable drawbacks do exist concerning time-consuming processes and complicated manipulations. Thus, it is highly desirable to develop an immunoassay that is highly sensitive, efficient, low-cost, and user-friendly.

Electrochemical immunoassay, particularly for label-free amperometric immunoassay, is a rather sensitive, time-saving, and reliable method [11–16]. In general, the construction of label-free amperometric immunosensors is based on a sensing substrate with strong electrochemical signal, good conductivity, large specific surface area, and catalytic ability [17–20]. In this case, various nanocomposites, including noble metal nanocomposites, magnetic nanocomposites, carbon nanotubes, graphene, and conducting polymers, are commonly used to fabricate the immunosensing substrate. Numerous researches have made great efforts to obtain such composites and advancements in amperometric immunosensors; however, shortcomings still exist, such as the complex fabrication processes for producing nanotubes and graphene [21–24]. Given the above disadvantages, it is of great significance to fabricate a new type of composite that has simple preparation (specifically using the one-pot method), excellent conductivity, a strong electrochemical signal, excellent catalytic ability, and easy immobilisation of the antibody (preferably direct antibody immobilisation through noble metal nanoparticles). Recently, efforts have been pursued to prepare a label-free amperometric immunosensor with immunosensing substrates that possess the following functions: (1) strong electrochemical signal, (2) great conductivity, (3) signal amplification, and (4) antibody immobilisation without the assistance of chemical agents [25–29]. Because the fabrication of a multifunctional substrate is complicated and time-consuming, it is of great significance to prepare a label-free amperometric immunosensor for fabricating multifunctional substrates using a one-pot method that exhibits a strong electrochemical signal, good conductivity, signal amplifica-

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tion, and easy antibody immobilisation without the assistance of chemical agents [30–35].

Herein, we developed a label-free amperometric immunosensor platform based on a multifunctional conductive hydrogel, which was synthesised using pyrrole and thionine as monomers, $HAuCl_4$ and ammonium persulfate as co-oxidising agents, and glucose oxidase as the doping agent. The hydrogel exhibited a strong electrochemical signal at about 0.0 V (vs Ag/AgCl), good conductivity, large specific surface area, and signal enhancement via cascade reaction. The signal was enhanced by the catalysis of glucose by glucose oxidase to produce H_2O_2 and catalysis of H_2O_2 by polythionine. Based on these outstanding properties, the proposed platform presents excellent sensing performance for neuron-specific enolase (NSE) with a wide linear range, high sensitivity, and low detection limit.

2. Experimental

2.1. Materials and reagents

Neuron-specific enolase (NSE), alpha fetoprotein (AFP), and prostate specific antigen (PSA) were obtained from Shanghai LincBio Science Company. Ammonium persulfate, thionine, and pyrrole were obtained from Aladdin (Tianjin, China). Human immunoglobulin G (IgG) was obtained from Beijing Xinjingke Biotechnology Company. Glucose oxidase (50 KU), $HAuCl_4{\cdot}xH_2O$, glucose, uric acid (UA), and ascorbic acid (AA) were obtained from Alfa Aesar (Tianjin, China). H_2O_2 (30%), thionine, pyrrole, bovine serum albumin (BSA), polishing powder, $K_3Fe(CN)_6$, $K_4Fe(CN)_6$, NaH_2PO_4, Na_2HPO_4, KCl, and ethanol were achieved from Beijing Chemical Reagents Company (Beijing, China). Clinical human serum samples were kindly provided by the Capital Normal University Hospital (Beijing, China). All other reagents were of analytical grade and used without any further purification. All aqueous solutions were prepared with ultrapure water (resistivity > 18 MΩ cm).

2.2. Apparatus

Scanning electron microscope (SEM) and energy dispersive X-ray spectroscopy (EDS) were determined with a Hitachi SU8010 SEM. Thermogravimetry was analysed on TGA/SDTA851 from METTLER TOLEDO. X-ray photoelectron spectroscopy (XPS) was conducted using an Escalab 250 X-ray Photoelectron Spectroscope (Thermofisher, American) employing a monochromatic Al Kα radiation. Electrochemical measurements were conducted on a CHI-660E electrochemical workstation (Chenhua Instruments Co., Shanghai, China). A three-electrode system was used in the experiment with a glassy carbon electrode (GCE) (4 mm in diameter) as the working electrode, a Ag/AgCl electrode (saturated KCl) as reference electrode and a Pt wire as counter-electrode, respectively.

2.3. Synthesis of the multifunctional conductive hydrogel

The multifunctional conductive hydrogels were synthesised by directly mixing solutions A and B. Solution A was prepared by adding thionine (1% (m:m)), pyrrole (2% (m:m)), glucose oxidase (0.5% (m:m)) in 2 mL of deionized water. The solution was then agitated with ultrasound to form a clear solution. Solution B was prepared by dissolving 0.286 g ammonium persulfate and $HAuCl_4$ (4% (m:m)) in 1 mL of deionized water. Next, 10 μL of the mixed solution was coated onto the glassy carbon electrode (GCE) with a diameter of 4 mm. The colour of the clear solution changed to atrovirens, and a hydrogel formed within 5 min. The coated electrode was allowed to react in the refrigerator for 10 min and was then was immersed in deionized water for approximately 30 min to purify the hydrogel film and dried at 37 °C for 4 h.

2.4. Fabrication of the label-free amperometric immunosensor platform

Prior to the functionalisation procedure, the GCE was polished with 0.05-μm alumina polishing powder and ultrasonically treated with ultrapure water three times and dried at 37 °C. After that, 10 μL of the mixed solution was coated onto the GCE, one solution containing pyrrole monomer and thionine and the other containing the polymerization initiator. The coated solution was gelated to form a hydrogel film within 5 min. Subsequently, the electrode was immersed in deionized water for approximately 30 min to purify the hydrogel film and dried at 37 °C for 4 h. The electrode modified with hydrogel was socked in 0.5 mM $HAuCl_4$ solution containing 0.1 M KCl, then a depositional potential of −0.2 V for 30 s was used to form Au film. After that, the modified GCE was rinsed with ultrapure water, then was dipped in anti-NSE (200 μg mL^{-1}) aqueous solution and incubated in a moisture-saturated environment overnight at 4 °C. Finally, the modified electrode was further incubated with a solution of 1% BSA (m:m) for 1 h at 37 °C to block the remaining active sites against non-specific absorption. After that, the modified electrode was thoroughly rinsed with ultrapure water to obtain the immunosensor.

3. Results and discussion

3.1. Principle of the label-free amperometric immunosensor

The sensing mechanism of the proposed label-free amperometric immunosensor platform based on AuNPs-modified multifunctional conductive hydrogel is illustrated in Scheme 1. Multifunctional conductive hydrogels were found to be hierarchically porous while providing good conductivity, large specific surface area, and strong redox signal. Moreover, a cascade reaction signal-amplified strategy was introduced to achieve good analytical performance. The glucose oxidase catalysed glucose to produce H_2O_2, resulting in a signal increase. Furthermore, the produced hydrogen peroxide was catalysed by polythionine nanoparticles to further enhance the signal. AuNPs were homogeneously electrodeposited in the hydrogel to improve conductivity and immobilise the antibody. Thus, hydrogel-based electrodes can be used as a highly sensitive platform to detect different tumour biomarkers by immobilising different antibodies.

The electrochemical immunosensor was fabricated using the hydrogel as a sensing substrate, which provides a strong electrochemical signal, enhances the electron transfer ability, signal amplification, and direct antibody immobilisation. After immobilising anti-NSE and blocking with BSA, an amperometric immunosensing interface was obtained (Scheme 1).

3.2. Characterization of the multifunctional conductive hydrogel

The hydrogel exhibited an atroviren colour (Fig. S1). The gold nanoparticles were uniformly distributed on the hydrogels, which were found to be hierarchically porous before and after AuNPs electrochemical deposition. The chemical compositions of the hydrogels before and after being coated with AuNPs were analysed by EDS and XPS. C, N, O, and S elements were found in the hydrogel, while Au was detected after electrochemical deposition of AuNPs (Fig. 1). Moreover, the spectrum in Fig. S2 reveals the presence of C, O, S, N, and Au atoms in the composite. The Au4f doublet (84.1 eV and 87.8 eV) in Fig. S2 B is consistent with Au^0 state, indicating that AuNPs were formed in the hydrogel. After polymerisation and purification through extensive rinsing with ultrapure water, the