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Ultrasensitive sandwich-type electrochemical immunosensor based on dual signal amplification strategy using multifunctional graphene nanocomposites as labels for quantitative detection of tissue polypeptide antigen

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ABSTRACT

Detection of tissue polypeptide antigen (TPA) in human serum plays an important role in monitoring the occurrence of cancers. In this work, a sandwich-type electrochemical immunosensor based on a novel dual signal amplification strategy was employed for quantitative detection of TPA. Multifunctional graphene nanocomposites (Au@MGN) were designed as labels to achieve a high sensitivity and low limit of detection (LOD), which dominated the dual signal amplification strategy. On one hand, introduction of graphene could accelerate the electron transfer capability of magnetic graphene nanocomposites (MGN) than single ferriferrous oxide nanoparticles (Fe_3O_4 NPs). On the other hand, introduction of gold nanoparticles (Au NPs) could build a synergetic effect between Fe_3O_4 NPs and Au NPs, which resulted in the increasing of electrocatalytic properties of Au@MGN. In short, due to the excellent electrochemical property of Au@MGN, high electrocatalytic current responses toward the reduction of hydrogen peroxide (H_2O_2) were achieved. Under optimum conditions, the proposed sandwich-type electrochemical immunosensor exhibited a wide linear range from 10^{-5} ng/mL to 10^2 ng/mL with a low LOD of 7.5 fg/mL for TPA. The designed immunosensor displayed an excellent analytical performance with good reproducibility, high selectivity and stability, indicating potential application promising in clinical monitoring of tumor markers.

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1. Introduction

Tissue polypeptide antigen (TPA) is a protein produced and released by proliferating cells that possesses several characteristics for an ideal tumor marker [1]. Evaluating the content of TPA in human serum is helpful to the monitoring the occurrence of cancer, such as breast cancer [2], ovarian carcinoma [3], squamous cell lung cancer [4] and oral squamous cell carcinoma [5]. Nowadays, electrochemical immunosensor based on specific interaction between antigens and antibodies has attracted extensive attentions and become a popular technique for the detection of tumor markers [6,7]. Because it has good portability, low cost, low power requirements, high sensitivity, and high compatibility with advance micromachining technologies [8].

As the major analytical model of electrochemical immunosensor, sandwich-type electrochemical immunosensor was the most widely applied technology [9–12]. It is employed a sandwich format in which analytes are captured and detected by excess immobilized primary antibodies (Ab_1) and secondary antibodies (Ab_2) respectively, which builds a direct relationship between concentrations of antigens and electrochemical signal intensity [13]. Different signal amplification strategies have been created to improve the sensitivity and decrease the limit of detection (LOD) of the sandwich-type electrochemical immunosensor [8,14–17]. As a result, a wide variety of multifunctional nanomaterials have been designed as labels for different signal amplification strategies. In this regard, Yuan's group developed lots of work, such as using single-walled carbon nanohorns/hollow Pt chains complex [18] and hemin/G-quadruplex DNAzyme concatamers functionalized Pt@Pd nanowires [19] as labels, which both achieved a high sensitivity and LOD of tumor markers. In a word, signal amplification strategy based on novel multifunctional nanomaterials is crucial for ultrasensitive sandwich-type electrochemical immunosensors.

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Ferriferrous oxide nanoparticles (Fe_3O_4 NPs) have attracted a considerable interest because of its good biocompatibility [20] and good electrocatalytic properties toward the reduction of hydrogen peroxide (H_2O_2) [21]. A series of nanomaterials based on Fe_3O_4 NPs have been designed for signal amplification strategy in our group's previous work, such as dumbbell-like Au–Fe_3O_4 nanoparticles [22], Fe_3O_4 nanoparticles-loaded PEG–PLA polymeric vesicles [23], ferrocene functionalized iron oxide nanoparticles [13] and dumbbell-like Pt–Fe_3O_4 nanoparticles [24]. In addition, Ai's group also has done relative works in using Fe_3O_4 NPs nanocomposites as labels for signal amplification strategy [17,25].

Inspired by these works, novel multifunctional graphene nanocomposites (Au@MGN) based on Fe_3O_4 NPs were used as labels for quantitative detection of TPA. The Au@MGN could achieve dual signal amplification strategy for the fabrication of sandwich-type electrochemical immunosensors. First, graphene exhibiting fast electron transportation, high thermal conductivity, excellent mechanical stiffness and good biocompatibility [26] was introduced to combine with Fe_3O_4 NPs by chemical reaction. The obtained magnetic graphene nanocomposites (MGN) have a better electron transfer capability. Second, gold nanoparticles (Au NPs) were employed to functionalize the MGN to produce synergetic effect, which could result in the increasing of electrocatalytic properties toward the reduction of H_2O_2. The introduction of graphene and Au NPs toward Fe_3O_4 NPs was the key of dual signal amplification strategy. Moreover, the introduction of Au NPs was beneficial to promote the biocompatibility of nanomaterials and increase the conjunction with antibodies. Consequently, the high sensitivity and low LOD could be achieved in this novel sandwich-type electrochemical immunosensor.

2. Materials and methods

2.1. Apparatus and reagents

All electrochemical measurements were performed on a CHI760E electrochemical workstation (Huakeputian Technology Beijing Co., Ltd., China). A conventional three-electrode system was used for all electrochemical measurements: a glassy carbon electrode (GCE, 4 mm in diameter) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum wire electrode as the counter electrode. Scanning electron microscope (SEM) images and energy dispersive X-ray spectral data (EDX) were obtained using Quanta FEG250 field emission environmental SEM (FEI, United States) operated at 4 kV. Fourier transform infrared spectroscopy (FTIR) spectrum was obtained from VERTEX 70 spectrometer (Bruker, Germany). X-ray powder diffraction (XRD) was performed with a D8 advance X-ray diffractometer (Bruker AXS, Germany).

Human TPA and antibody to human TPA (anti-TPA) were purchased from Shanghai Linc-Bio Science Co., Ltd., China. Phosphate buffered saline (PBS, 1/15 M Na_2HPO_4 and KH_2PO_4) was used as an electrolyte for all electrochemistry measurement, which was purged with nitrogen gas for 20 min to remove the dissolved oxygen. $FeCl_3{\cdot}6H_2O$ was purchased from Damao Chemical Reagent Tianjin Co., Ltd., China. $AuCl_3{\cdot}HCl{\cdot}4H_2O$ was purchased from Sinopharm Chemical Reagent Shanghai Co., Ltd., China. All other reagents were of analytical grade and ultrapure water was used throughout the study.

2.2. Preparation of the multifunctional graphene nanocomposites

Graphene oxide (GO) was synthesized by an improved Hummers method [27]. In brief, a mixture of concentrated H_2SO_4 (36 mL) and H_3PO_4 (4 mL) was added to a mixture of graphite flakes (0.3 g) and $KMnO_4$ (1.8 g), producing a slight exotherm to 35–40 °C. The reaction was then heated to 50 °C and stirred for 12 h. The reaction was cooled to room temperature and poured onto ice (40 mL) with 30% H_2O_2 (0.3 mL). The mixture was centrifuged and the supernatant was decanted away. For workup, the remaining solid material was washed in succession with water, 30% HCl, ethanol and ether. The obtained solid was dried in vacuum overnight.

In a typical synthesis of MGN [28], $FeCl_3{\cdot}6H_2O$ (0.5 g) was dissolved in ethylene glycol (10 mL) to form a clear solution, followed by the addition of NaAc (1.5 g), ethanediamine (5 mL) and GO (0.5 g). The mixture was stirred vigorously for 30 min and then sealed in a teflon-lined stainless steel autoclave. The autoclave was heated to and maintained at 200 °C for 8 h, and allowed to cool to room temperature. The resulting black powder was obtained after being washed several times and dried at 35 °C under high vacuum overnight.

Au NPs were synthesized by the classical Frens method [29]. In brief, a solution of $HAuCl_4$ (0.01 wt%, 100 mL) was heated to boiling, and then a solution of trisodium citrate (1 wt%, 1.5 mL) was added. The boiling solution turned a brilliant ruby-red in around 15 min, indicating the formation of Au NPs, and then it was cooled to room temperature.

A mixture of MGN (20 mg) and the prepared Au NPs solution (40 mL) was shaked for 12 h. The final product was obtained by being washed several times and dried at 35 °C under high vacuum overnight.

2.3. Fabrication of the immunosensor

Fig. 1A shows the preparation procedure of the Ab_2 combined Au@MGN (Au@MGN-Ab_2) labels. A solution of Au@MGN (2 mg/mL, 1 mL) was added to Ab_2 dispersion (10 μg/mL, 1 mL) and shaked for 12 h at 4 °C. Following by the process of magnetic separation, the resulting Au@MGN-Ab_2 labels were dispersed in 1 mL of PBS at pH = 7.4 and stored at 4 °C.

Fig. 1B shows the schematic diagram of the proposed sandwich-type electrochemical immunosensors. A GCE was polished to a mirror-like finish with 1.0, 0.3 and 0.05 μm alumina powder and then thoroughly cleaned before using. First, Au NPs were electrodeposited on the surface of GCE, which process was performed in $HAuCl_4$ aqueous solution (1 wt%, 2 mL) at a constant potential of −0.2 V for 30 s [30]. Following that, Ab_1 (10 μg/mL, 6 μL) was added onto the electrode. After incubating at 4 °C and being washed, 3 μL of 1 wt% bovine serum albumin (BSA) solution was added to eliminate nonspecific binding sites. Next, the electrode was washed and incubated with varying concentrations of TPA (6 μL) for 1 h at room temperature, and then the electrode was washed extensively to remove unbounded TPA molecules. Finally, 6 μL of the prepared Au@MGN-Ab_2 labels buffer solution was incubated onto the electrode surface for 1 h at room temperature, and the electrode was washed thoroughly for measurement. For amperometric *I–T* curve to record the amperometric response, a detection potential of −0.4 V was selected. 5 mM H_2O_2 was added into the PBS after the back ground current was stabilized.

3. Results and discussion

3.1. Characterization of different nanomaterials

To verify that the Au@MGN have better electrocatalytic properties toward the reduction of H_2O_2 than other nanomaterials without being multifunctionalized, Fe_3O_4 NPs [20] and Au NPs functionalized graphene nanosheets (Au@GN) [15] were synthesized for the comparison of different signal amplification strategies.

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