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A disposable paper-based microfluidic immunosensor based on reduced graphene oxide-tetraethylene pentamine/Au nanocomposite decorated carbon screen-printed electrodes

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ABSTRACT

A novel, disposable and sensitive microfluidic paper-based electrochemical immunosensor (μ-PEI) was developed by using gold nanoparticles (Au NPs) decorated reduced graphene oxide-tetraethylene pentamine (rGO-TEPA/Au nanocomposite) as electrode materials. The rGO-TEPA greatly amplified the current response due to its excellent conductivity and large surface area. Au NPs was decorated on the surface of rGO-TEPA to increase the biocompatibility and retained good stability for rGO-TEPA/Au nanocomposite, which was used as an effective sensor platform for anchoring the capturing antibodies (Ab_1) and accelerating the electron transfer to the screen-printed electrodes (SPEs). The simple and disposable paper-based microfluidic channel patterned on the rGO-TEPA/Au nanocomposite modified SPEs (SPEs/rGO-TEPA/Au) was designed for combination of immunochromatography and immunofiltration simultaneously. With the combination of portable SPEs/rGO-TEPA/Au and simple paper-based microfluidic devices, horseradish peroxidase (HRP) and labeled signal antibodies (Ab_2) co-immobilized gold nanorods (HRP-GNRs-Ab_2) was explored as the tracers for square wave voltammetry (SWV) detection. Using Alpha-Fetoprotein (AFP) as a model analyte, the proposed μ-PEI exhibited a satisfactory performance like simple fabrication, high stability, selectivity, wide linear range (0.01 ng mL^{-1}–100.0 ng mL^{-1}) with a low detection limit (0.005 ng mL^{-1}). Furthermore, human serum were applied to the obtained μ-PEI and determined with satisfactory results.

1. Introduction

Cancer is one of the most fatal diseases for human beings worldwide [1]. The detection of tumor markers is crucial for early diagnosis and treatment of cancers [2]. AFP is a major plasma protein, which is widely used as a tumor marker for the diagnosis of hepatocellular carcinoma. The elevated AFP in adult plasma could indicate an increased risk of tumor, principally hepatoma and teratoma [3]. What's more, the AFP level is often related to the stage of disease [4]. Rapidly and sensitively monitor the level of AFP is highly important for reliable predictions and early tumor treatment. Therefore, the development of simple, cheap, sensitive, high reliable and fully disposable point-of-care testing (POCT) devices for AFP determination is highly demanded in early clinical diagnostic application.

Immunoassay based on the antibody-antigen interaction is one of the widely used analytical methods in the quantitative detection of biomarkers. Generally, detection of biomarkers is often performed on microplate in a well-equipped laboratory by the enzyme-linked immunosorbent assay (ELISA) [5]. However, for the complex operations, long analysis time and inaccessible to untrained personnel have motivated research relates to a wide variety of different analytical methods such as fluorescence, chemiluminescence, electrochemiluminescence and electrochemical immunosensing for the application in POCT [2,6,7]. Among the many analytical methods, electrochemical immunosensing technique stands out due to easy signal quantification, fast analysis, simple instrumentation, low cost, multiplexing possibilities, and convenient miniaturization [8,9]. Screen-printing technique

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is especially reliable for electrochemical biosensors and suitable for POCT due to its low cost, mass-production possibility and high inter-batch reproducibility [8,10,11]. Paper-based microfluidic immunoassay devices have recently emerged as an ideal platform for POCT due to the advantages such as inexpensive, thin, lightweight, disposable, low sample consuming, easy fabrication and functionalization [12–15]. The combination of portable SPEs with simple paper-based microfluidics is attractive, which could not only be attributed to the low-cost and mass production property, but also because it can make direct control over little sample volumes and potential integration of detection steps possibility [5,7,16–19].

Signal amplification technology based on nanomaterials such as carbon nanotubes (CNTs), graphene and nanometallic materials are being developed to increase the sensitivity of immunosensor [20,21]. Reduced graphene oxide (rGO), a monolayer of carbon atoms packed into a closed, honeycombed and two-dimensional lattice, has attracted great interest in the development of advanced electrochemical biosensors due to its specific properties such as high conductivity, high surface area-to-volume ratio and good biocompatibility [22]. Reduced graphene oxide-tetraethylene pentamine (rGO-TEPA), a combination of rGO and TEPA through covalent bonding, not only keeps the excellent properties of rGO, but also enhances the rGO's stability [1,22]. Besides, in-suit decoration of Au NPs on the surface of rGO-TEPA could further increase the biocompatibility of rGO-TEPA, which can ensure the direct and stable immobilization of rGO-TEPA/Au nanocomposite on the SPEs as an effective sensor platform in the immunosensor fabrication process.

Owing to the lack of electrochemical activity between antigens and antibodies, some mediators or enzymes such as ferrocene derivatives, HRP, glucose oxidase (GOx) and alkaline phosphatase (ALP) are utilized as tracers to achieve electron-transfer in most immunoassay systems [20,23,24]. HRP is often used to conjugate with antibodies as a signal probe in immunoassays because of the advantages such as smaller, more stable and less expensive [25]. Besides, different kind of substrates could be catalytically oxidized by HRP to produce colored, luminescent, fluorimetric and electroactive products, which could allow the detection of analytes using optical, fluorescence, chemiluminescence or electrochemical techniques. What's more, various nanomaterials such as CNTs, carbon nanospheres, carboxylated magnetic beads, graphene oxide and Au NPs demonstrate excellent function of signal amplification and were developed for ultrasensitive detection of nucleic acids and proteins [7,26]. Therefore, most of them were used as carriers for loading a large amount of signal tags (dyes, enzymes, or quantum dots) toward an individual sandwich immunoreaction for promoting the sensitivity of detection [27,28].

Herein, a novel, integrated and disposable sandwich immunosensor for sensitive electrochemical detection of AFP was prepared through the combination of SPEs and paper-based microfluidic channels. To enhance the stability, sensitivity and conductivity of the disposable μ-PEI, rGO-TEPA/Au nanocomposite was synthesized as electrode materials and HRP-GNRs-Ab_2 was prepared as signal probe for electrochemical detection. The simple paper-based microfluidic channels integrated sample zone, detection zone and adsorption zone together was for sampling, infiltration and chromatography control. AFP, which shows great significance in early diagnosis of hepatocellular carcinoma, was used as a model analyte. The stability, selectivity, linear range and limit of detection were characterized with a satisfactory performance. This sensitive immunoassay approach could provide great potential in low-cost and POCT applications for the expandable detection of other cancer biomarkers.

2. Experimental section

2.1. Chemicals and materials

AFP (2.0 mg mL^{-1}), capturing and labeling mouse monoclonal anti-AFP antibodies (2.0 mg mL^{-1}) were bought from Abcam Inc (Cambridge, MA). rGO-TEPA was obtained from Nanjing XFNANO Materials TECH Co., Ltd. (China). $HAuCl_4{\cdot}4H_2O$, Tween-20, hexadecyl trimethyl ammonium bromide (CTAB), *o*-phenylenediamine (OPD), HRP, H_2O_2 (30 wt.%), phosphate buffer saline (PBS, 0.02 M, pH 7.4), *N*, *N*-dimethylformamide (DMF), sodium borohydride ($NaBH_4$) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich. Human serum was provided by the People's Hospital of Guilin. Whatman No. 1 filter paper was bought from GE Healthcare Worldwide. SU-8 2007 negative photoresist was purchased from MicroChem Corp. (Newton, MA). All solutions used in this study were prepared with deionized water purified by a MilliQ Water Purification System (Milli-Pore, Bedford, MA, USA). All chemical reagents used in this study were of analytical grade. Electrochemical substrate for SWV detection consisted of 10.0 mM OPD and 2.0 mM H_2O_2. The original AFP solution was diluted with PBS for the following experiments.

2.2. Apparatus

UV–vis spectra were obtained on a UH-5300 spectrophotometer (Hitachi, Japan). The surface morphologies and structures of rGO-TEPA and rGO-TEPA/Au nanocomposite were characterized using field emission scanning electron microscope (FESEM, JSM-7610F, Japan) and transmission electron microscope (TEM, JEM-2100, Japan). All electrochemical measurements were performed on a CHI660D workstation (CH Instruments Co., Shanghai, China). Disposable SPEs were used with modified carbon electrode as the working electrode, Ag/AgCl electrode as the reference electrode, and another carbon electrode as the counter electrode. The connection between SPEs and the CHI660 electrochemical analyzer was a simple sensor connector. All SWV measurements were carried out from −0.8 to −0.4 V with a step potential of 4 mV, amplitude of 25 mV, and a frequency of 15 Hz.

2.3. Preparation of HRP-GNRs-Ab_2

The preparation procedure of HRP-GNRs-Ab_2 bioconjugates is shown in Scheme 1. The GNRs were synthesized firstly with a seed-mediated growth procedure [29,30]. Briefly, 5.0 mL of $HAuCl_4$ (0.5 mM) was mixed with 5.0 mL of CTAB solution (0.2 M). 0.6 mL of 0.01 M fresh $NaBH_4$ was diluted to 1.0 mL. The color of mixed solution changed immediately from yellow to brownish yellow after the injection of the above diluted $NaBH_4$ solution with vigorous stirring (1200 rpm). 2 min later, the stirring was stopped and the prepared seed solution was aged 30 min for further use.

The growth solution was prepared as follows: 1.4 g of CTAB and 0.2468 g of sodium oleate (NaOL) were completely dissolved in 50 mL of hot water (50 °C). 3.6 mL of $AgNO_3$ solution (4 mM) was added after the solution cooled down to 30 °C. The mixture was kept undisturbed at 30 °C for 15 min followed by adding 50 mL of $HAuCl_4$ solution (1 mM). The mixed solution changed from yellow to colorless gradually after 90 min of stirring (700 rpm). Afterwards, 0.3 mL of HCl (37 wt.%) was introduced with another 15 min of stirring (400 rpm) to adjust the pH. 0.25 mL of ascorbic acid was injected to the solution and stirred vigorously for 30 s. Finally, 80 μL of prepared seed solution was added into the above growth solution with gently stirring for another 30 s. The obtained mixture was left undisturbed at 30 °C for 12 h. The final suspension was centrifuged at 7000 rpm for 30 min to obtain GNRs, which were redispersed in 25 mL of nanopure water.