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Electrochemical immunosensor for detection of topoisomerase based on graphene–gold nanocomposites

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ABSTRACT

A facile electrochemical immunosensor based on graphene–three dimensional nanostructure gold nanocomposites (G–3D Au) using simple and rapid one-step electrochemical co-reduction technique was developed for sensitive detection of topoisomerase. The resultant G–3D Au nanocomposites were characterized by scanning electron microscopy, cyclic voltammetry and electrochemical impedance spectroscopy, and then were used as a substrate for construction of the "sandwich-type" immunosensor. Amperometric current–time curve was employed to monitor the immunoreaction on the protein modified electrode. The proposed method could respond to topoisomerase with a linear calibration range from 0.5 ng mL^{-1} to 50 ng mL^{-1} with a detection limit of 10 pg mL^{-1}. This new biosensor exhibited a fast amperometric response, high sensitivity and selectivity, and was successfully used in determining the topoisomerase which was added in human serum with a relative standard deviation ($n=5$) < 5%. The immunosensor served as a significant step toward the practical application of the immunosensor in clinical diagnosis and prognosis monitor.

1. Introduction

In recent years, electrochemical immunosensors have been of extensive interest in medical diagnosis and biological research due to their high sensitivity, small analyte volume, simple instrumentation, and minimal manipulation [1–6]. The introduction of nanomaterials and nanotechnologies has greatly improved the sensitivity and selectivity of electrochemical biosensors [7–13]. Recently, graphene–metal nanocomposites have attracted increasing attention due to their high electrocatalysis, biocompatibility and large active surface [14–16]. The graphene–metal nanocomposites are usually prepared by chemical or thermal reduction of mixtures of graphene (or graphite oxide) and metallic precursors [17–20]. However, in these methods, toxic reducing agents such as hydrazine hydrate or hydroquinone are introduced, thus extreme care should be made. Moreover, the chemical synthesis processes are time-consuming and require high temperature (95–100 °C) [21]. To overcome those drawbacks, the one-step electrochemical co-reduction of graphite oxide (GO) and metallic precursors has recently developed due to its simple, fast and green nature [22–24].

Topoisomerases are ubiquitous nuclear enzymes that regulate the overwinding or underwinding of DNA in eukaryotic cells. Several studies have demonstrated that topoisomerases could be used as prognostic markers for assessing the prognosis of different cancers such as osteosarcomas, breast ductal carcinomas and ovarian cancer, just to name a few examples [25–27]. Recently, many efforts have been made to develop various immunoassay methods including enzyme-linked immunosorbent assay (ELISA) [28], Western blot analysis [29] and immunohistochemistry [30] for detection of topoisomerases. However, these methods have some disadvantages such as tedious, time-consuming and expensive instruments. Therefore, it is necessary to develop a simple and sensitive method for quantitative detection of topoisomerases.

Herein, topoisomerase IIIβ was chosen as a detection topoisomerase model, and a highly sensitive electrochemical immunosensor based on graphene–three dimensional gold nanocomposites modified glassy carbon electrode (G–3D Au/GCE) was developed for detection of topoisomerase IIIβ (Scheme 1). The G–3D Au nanocomposites were

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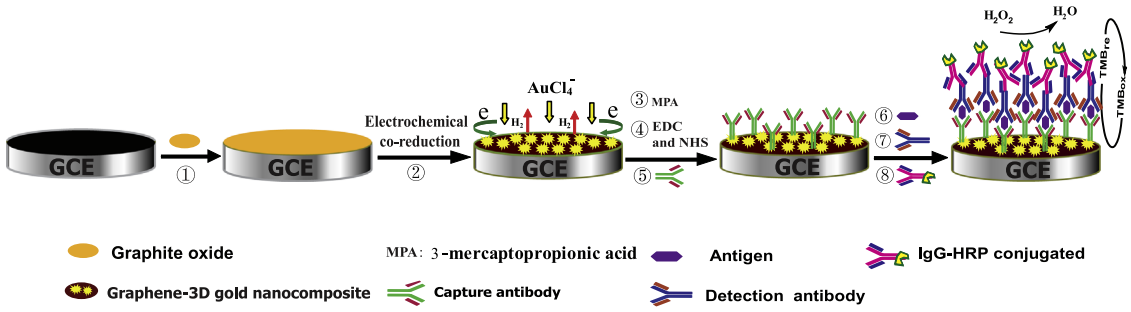

Scheme 1. Schematic representation of the fabrication procedure of the immunsensor based on graphene–3D nanostructure gold nanocomposite.

prepared by a one-step electrochemical co-reduction technique. The resultant G–3D Au/GCE was first covalently assembled with 3-mercaptopropionic acid (MPA) by Au–S bond, and then topoisomerase IIIβ primary antibodies (Ab1)/antigens/secondary antibodies (Ab2)/horseradish peroxidase (HRP) conjugated IgG were immobilized onto the electrode by immunoreaction to form an sandwich-type immunosensor. The electrochemical transduction was reached by the catalytic reduction of H_2O_2 by coupling with the redox reaction of 3,3′,5,5′-tetramethylbenzidine (TMB) at the electrode surface [31,32]. This method is cost-effective and versatile. The proposed immunosensing strategy allowed a rapid and sensitive means of topoisomerase IIIβ detection.

2. Materials and methods

2.1. Chemicals and materials

Mouse monoclonal anti-topoisomerase IIIβ (primary antibodies, Ab1, 100 ug) and rabbit purified polyclonal anti-topoisomerase IIIβ (secondary antibodies, Ab2, 50 ug) were purchased from Abnova Corporation (Taipei, Taiwan). The topoisomerase IIIβ recombinant protein (antigen, 10 ug) and the standard antigens solution (topoisomerase IIIβ 293T overexpression lysate) were obtained from Abnova Corporation (Taipei, Taiwan). The IgG-HRP conjugate was purchased from Abnova Corporation (Taipei, Taiwan). The antibody dilution was 10 mM phosphate-buffered saline (PBS buffer) containing 0.08 M NaCl (pH 7.4). The antibodies were aliquoted and stored at −20 °C. The purified antibodies were diluted upon usage in PBS suitable for the immobilizing and capture reactions. A commercial human topoisomerase IIIβ ELISA kit was purchased from CUSABIO (Wuhan, China), stored at 4 °C and used following the instructions given by the producers.

Natural powder graphite (sized ≤ 30 μm) was purchased from Shanghai Chemical Reagent Company. Tetrachloroaurate(III) tetrahydrate ($HAuCl_4 \cdot 4H_2O$, 47.8% Au), tris-(hydroxymethyl)aminomethane, 3-mercaptopropionic acid (MPA), nitric acid, sulfuric acid, potassium permanganate, ethanol, N-Hydroxysuccinimide (NHS), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were provided by Sinopharm Chemical Reagent Co., Ltd. (China). Bovine serum albumin (BSA) was from Sigma-Aldrich (St. Louis, MO, USA). TMB substrate (Neogen K-blue low activity substrate) was purchased from Neogen (U.S.). All the chemicals were of analytical reagent grade and used without further purification. All solutions were prepared with Milli Q water (18 MΩ cm resistivity) from a Millipore system.

2.2. Apparatus

All electrochemical measurements were performed on a CHI 760D Electrochemical Workstation (CH Instrument, USA). Electrochemical experiments were carried out with a conventional three-electrode system comprising a platinum wire auxiliary electrode, an Ag/AgCl (with saturated KCl) reference electrode, and the modified glassy carbon electrode (GCE, diameter: 3 mm) as working electrode. Scanning electron microscopic (SEM) images were obtained from a Hitachi S-4800 scanning electron microscopy (Tokyo, Japan).

2.3. Preparation of G–3D Au/GCE

GO was synthesized from natural powder graphite by a modified Hummers' method [33,34]. Briefly, graphite powders were first oxidized by potassium permanganate in the presence of concentrated nitric acid and sulfuric acid for 30 min. After oxidation of graphite, the mixture was added to excess water, washed with a 5% HCl aqueous solution, and then repeatedly washed with water until the pH of filtrate reached neutral. The as-synthesized GO was suspended in water to give a brown dispersion, which was dialyzed for 1 week to completely remove residual salts and acids. Exfoliated graphite oxide (exfoliated GO) was obtained by ultrasound of the 1.0 mg mL^{-1} GO dispersion using a Sonifier. The obtained brown dispersion was then centrifuged at 3000 rpm for 5 min to remove any unexfoliated graphite oxide (an extremely small amount). The method to prepare G–3D Au/GCE was the same as that described in our previous report [35]. Briefly, GCE was polished successively with 1.0, 0.3 and 0.05 μm alumina powder to form a smooth, shiny surface. Then it was cleaned ultrasonically in 1:1 HNO_3, ethanol and Milli-Q water for 1 min, respectively, and dried with blowing N_2. An 8 μL exfoliated GO suspension was spread on a pretreated bare GCE using a micropipette tip. The film was dried in a vacuum desiccator. The GO-coated electrode was immersed in 2.8 mM $HAuCl_4$ and 0.1 M H_2SO_4 solution and a one-step electrochemical co-reduction was performed by cyclic voltammetry (CV) in a potential range from 0.0 to −1.5 V for a time of 900 s. The resultant G–3D Au/GCE was sonicated in deionized water and electrochemically cleaned by cycling the electrode potential between −0.35 and 1.5 V at 0.1 V s^{-1} in 0.5 M H_2SO_4 solution. The real surface of the clean electrode was calculated from the CV in 0.5 M H_2SO_4 solution.

2.4. Fabrication of the immunosensor

The cleaned G–3D Au/GCE was first dipped in 5 mM MPA aqueous solution for 24 h at room temperature. After thoroughly rinsed with deionized water to remove physically adsorbed MPA, it was immersed in a solution with 20 mg mL^{-1} of EDC and 10 mg mL^{-1} of NHS for 40 min. The activated MPA/G–3D Au/GCE was thoroughly rinsed with PBS, and then incubated overnight with 8 μl of Ab1 (0.1 mg mL^{-1}) to yield sensing interfaces. A blocking treatment aimed at preventing unspecific responses during the sample incubation was carried out by casting 15 μl of 5% (v/w) BSA at room temperature for 1 h, followed by washing carefully with PBS. Subsequently, 8 μl of the topoisomerase IIIβ antigen solution with different concentrations was added onto the