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Ultrasensitive electrochemiluminescence immunosensor based on nanoporous gold electrode and Ru-AuNPs/graphene as signal labels

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ABSTRACT

In the paper, a simple and sensitive sandwich-type electrochemiluminescence (ECL) immunosensor was proposed for the detection of cancer antigen 125 (CA 125) on a nanoporous gold (NPG) modified glassy carbon electrode (GCE). The NPG was easily prepared by a selective dissolution of silver from silver/gold alloy in nitric acid. The fabrication of $Ru(bpy)_3^{2+}$-gold nanoparticles (Ru-AuNPs) aggregates was owing to the electrostatic interactions between citrate-capped AuNPs and Ru in aqueous medium. The Ru-AuNPs composite assembled with poly(diallyldimethylammonium chloride) functionalized graphene nanosheets (GR) (Ru-AuNPs/GR) was used as ECL labels, which has large surface area, good biocompatibility and electronic conductivity. The ECL intensity observed by the application of as-prepared Ru-AuNPs/GR composites was enhanced 6-fold compared to those of Ru-AuNPs. The primary antibody of CA 125 (Ab_1) was first immobilized on the NPG modified electrode, then the antigen and the secondary antibody (Ab_2) were conjugated to form a sandwich-type immunocomplex through the specific interaction. The proposed ECL immunosensor provided a wide linear response range over 0.01–100 U mL^{-1} with a detection limit of 0.005 U mL^{-1}. The ECL immunosensor showed high sensitivity, good reproducibility, satisfied regeneration and selectivity, and may exhibit an attractive approach for other analyte determination.

1. Introduction

Ovarian cancer has a high morbidity rate, since the disease is often neglected until the disease deteriorates to an untreatable stage. Epithelium ovarian cancer (EOC) [1] is the most common form of ovarian cancer leading to the highest death rate within gynecological cancers. Cancer antigen 125 (CA 125) is the primarily used diagnostic biomarker for EOC [2], but the early diagnosis rate (diagnosed stage I) is low [3]. So far, many methods, including chemiluminescence enzyme-linked immuno sorbent assay (CL-ELISA) [4], electrochemical immunoassay [5], fluorescence immunoassay [6], and chemiluminescent immunoassay [7–9] have been used for CA 125 measurements. Recently, electrochemiluminescence (ECL) has become an important and powerful analytical tool in many fields, such as environmental pollutant determination, pharmaceutical analysis, and immunoassay [10–14]. ECL method has many advantages over photoluminescence techniques, such as low cost, rapid determination, wide range of analytes and high sensitivity [15]. Based on their specific antigen–antibody recognition, ECL immunoassay has become one of the most effective analytical methods in use for detection and quantification of bio-molecules [16–18]. Consequently, to enhance the sensitivity, ECL was employed to measure CA 125 in this work.

Recently, nanoporous metals have aroused great attention due to their high surface area, low density, and three-dimensional (3D) bicontinuous pore-ligament structure [19,20]. Nanoporous gold (NPG) has been one of the most popular nanoporous metals for their good stability, high conductivity, and good biocompatibility [21]. NPG can be prepared by using a simple dealloying method, by which silver was dissoluted from silver/gold alloys in nitric acid, giving free-standing noble metal membranes with controllable three-dimensional porosity [22]. In this work, we used the NPG modified glassy carbon electrode (GCE) as a solid support for the immobilization of the primary antibody.

Graphene (GR), a single layer of carbon atoms in a closely packed honeycomb two-dimensional (2D) lattice [23], has recently attracted enormous attention owing to its unique charge carrier mobility [24,25], thermal conductivity [26], mechanical properties [27], and specific surface area [28]. However, the poor biocompatibility of GR limits their further application in designing biosensors because of its hydrophobicity and aggregative trend in water [29]. Liu et al. [30] reported that the positive poly(diallyldimethylammonium chloride) (PDDA) functionalized GR exhibited good conductivity, solubility and biocompatibility. Owing to its superior properties including high sensitivity and stability under moderate conditions in aqueous solution [31,32],

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tris(bipyridine)-ruthenium(II) ($Ru(bpy)_3^{2+}$) has been widely used in ECL analysis among all the ECL systems. Sun and colleagues proposed the formation of $Ru(bpy)_3^{2+}$-gold nanoparticles (Ru-AuNPs) aggregates [33]. Herein, for higher sensitivity, a new signal amplification strategy based on Ru-AuNPs and GR (Ru-AuNPs/GR) was proposed, and the Ru-AuNPs/GR composites exhibit good physical and chemical properties, combining the advantages of favorable biocompatibility and superior conductivity.

In this work, we have successfully proposed a sensitive ECL immunosensor for CA 125 detection via self-assembly and NPG GCE. When the electrode was modified by the NPG, the first antibody (Ab_1) assembled on them, then bovine serum albumin (BSA) was used to block nonspecific binding sites. After dropping the CA 125, the Ru-AuNPs/GR labeled second antibody (Ab_2) was successively conjugated to shape a sandwich-type immunocomplex via the specific reaction. The as-prepared Ru-AuNPs/GR could display better biocompatibility, more electronic conductivity and enhance the ECL signal. The experimental results indicated that the ECL immunosensor exhibited simple instrumentation, wide linear range, low detection limit and excellent analytical performance.

2. Materials and methods

2.1. Reagents

Cancer antigen 125 (CA 125), the primary mouse anti-CA 125 (Ab_1) and the secondary mouse anti-CA 125 (Ab_2) were gotten from Shanghai Linc-Bio Science Co. Ltd. (Shanghai, China). Bovine serum albumin (BSA, 96–99%), and poly(diallyldimethylammonium chloride) (PDDA) were obtained from Shanghai Reagent Company (Shanghai, China). Tris(2,2′-bipyridyl)dichlororuthenium(II) hexahydrate (Rubpy), tripropylamine (TPA), and gold chloride ($HAuCl_4$) were all bought from Alfa Aesar China Ltd. 100 nm-thick white gold foils (Au/Ag alloy, 50:50 wt%) were purchased from Monarch.

All chemicals and solvents used were analytical grade available and were used as received. Ultrapure water was prepared by a Milli-Q system (Millipore) and used throughout. Phosphate buffered solutions (PBS, pH 7.4) were prepared using 0.01 mol L^{-1} KH_2PO_4 and 0.01 mol L^{-1} Na_2HPO_4. The CA 125 was stored at 4 °C, and its standard solution was prepared daily with PBS solution.

2.2. Apparatus

The ECL measurements were carried out on a flow injection luminescence analyzer (IFFM-E, Xi'an Remex Electronic Instrument High-Tech Ltd., Xi'an, China) with the voltage of the photomultiplier tube (PMT) set at 800 V. Cyclic voltammetric measurements (CVs) were performed with a CHI 760D electrochemical workstation (Shanghai CH Instruments, China). Transmission electron microscopy (TEM) images of NPG, Ru-AuNPs, GR, and Ru-AuNPs/GR were obtained from a Hitachi H-800 microscope (Japan). Electrochemical impedance spectroscopy (EIS) was carried out on an IM6x electrochemical station (Zahner, Germany). All experiments were carried out with a conventional three-electrode system with the modified glassy carbon electrode (GCE, 3 mm in diameter) as the working electrode, a platinum counter electrode and an Ag/AgCl (sat. KCl) reference electrode.

2.3. Fabrication of NPG electrode

NPG was prepared according to the reported method [22] by selective dissolution (dealloying) of silver from Ag/Au alloy. Briefly, a piece of commercially available white gold leaf (Ag/Au alloy, 50:50 wt%, 100 nm thick) was dispersed in 1:1 concentrated nitric acid for 10–15 min at a constant temperature (25 °C). Upon silver dissolution, gold atoms left behind would self-organize into an interconnected network of pores and ligaments. Then the NPG foil was carefully coated onto the pretreated GCE using a variation of electroless deposition. The leaf adhered on the GCE surface via physical adsorption after being washed repeatedly with Milli-Q water to remove the NO_3^- and Ag^+, which would interfere with signal detection during the ECL analysis. The electrode was then intentionally parched in infrared light for 1 h. Thus, NPG was modified onto the GCE to get the NPG electrode.

2.4. Preparation of Ru-AuNPs composite

AuNPs with a diameter of about 12 nm were prepared by citrate reduction of $HAuCl_4$ in aqueous solution according to a well-known method [34]. The Ru-AuNPs composite was prepared on the basis of the reported method [33]. In a typical experiment, 2 mL of 0.5 mg mL^{-1} $Ru(bpy)_3^{2+}$ aqueous solution was added into 5 mL of AuNPs solution under stirring in room temperature. Several minutes later, a mass of black precipitate was formed. The formed precipitate was collected by centrifugation, washed three times with water, and finally suspended in 2 mL of water.

2.5. Preparation of Ru-AuNPs/GR

Graphite oxide (GO) was prepared from natural graphite powder according to a modified Hummer's method [35]. GR was prepared according to the reported method [36]. First, 0.5 mL of 20% PDDA solution was added to 100 mL of 0.5% GO solution and stirred for 30 min. Then 0.5 mL of 80% hydrazine hydrate was added and maintained stirring for 24 h at 90 °C. Finally, the black GR could be obtained by filtration and washing with Milli-Q water, and then redispersed readily in water upon mild sonication, forming a black suspension with a final concentration of 1.0 mg mL^{-1}. Then, 2 mL of the as-prepared GR dispersion was added into 10 mL of Ru-AuNPs suspension and sonicated for 30 min. After centrifugation, the obtained Ru-AuNPs/GR composites were further washed with Milli-Q water for several times and redispersed in water for use.

2.6. Preparation of Ru-AuNPs/GR composites labeled Ab_2

Zhu et al. [37] have reported that the AuNPs can link with amino-groups directly without any cross linkers, so the Ab_2 can be attached to the Ru-AuNPs/GR steadily through electrostatic interactions and interaction between AuNPs and $-NH_2$ groups of Ab_2. To generate Ru-AuNPs/GR composites labels, 1 mL of the Ru-AuNPs/GR composites were mixed with 1 mL of Ab_2 solution (anti-CA 125, 50 μg mL^{-1}). After incubation at 4 °C for 24 h, the residual antibody was removed by centrifugation and washing with 0.01 mol L^{-1} PBS for three times. After that, the Ru-AuNPs/GR composite labeled Ab_2 was redispersed in 2 mL of 1% BSA solution to block nonspecific binding sites and kept stirring for 2 h. Then the suspension was centrifuged and washed with PBS for several times, and was dispersed in 0.01 mol L^{-1} pH 7.4 PBS to a final volume of 2 mL and stored at 4 °C for use.

2.7. Fabrication of the sandwich-type ECL immunosensor

The steps for constructing the ECL immunosensor were shown in Scheme 1. A GCE electrode of 3 mm diameter was polished carefully with 1.0, 0.3 and 0.05 μm alumina powder on the fine abrasive paper. After removal of the trace alumina from the electrode surface, the electrode was rinsed thoroughly with water and cleaned by ethanol ultrasonically and then allowed to dry at room temperature. After that, the cleaned electrode was fabricated with the NPG foil. Then 5 μL of 20 μg mL^{-1} Ab_1 (50 mmol L^{-1} PBS, pH 7.4)