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Platinum porous nanoparticles hybrid with metal ions as probes for simultaneous detection of multiplex cancer biomarkers

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ABSTRACT

In this work, platinum porous nanoparticles (PtPNPs) absorbed metal ions as electrochemical signals were fabricated. Clean-surface PtPNPs were prepared by a surfactant-free method and decorated with amino groups via 2-aminoethanethiol. Amino capped PtPNPs complexation with Cd^{2+} and Cu^{2+} to form PtPNPs-Cd^{2+} and PtPNPs-Cu^{2+} hybrids, respectively. Anti-CEA and Anti-AFP separately labeled with PtPNPs-Cd^{2+} and PtPNPs-Cu^{2+} were used as distinguishable signal tags for capturing antigens. The metal ions were detected in a single run through differential pulse voltammetry (DPV) without acid dissolution, electric potentials and peak heights of which reflected the identity and concentrations of the corresponding antigen. Ionic liquid reduced graphene oxide (IL-rGO) modified glassy carbon electrode (GCE) was used as a substrate, which was rich in amino groups to immobilize antibodies by glutaraldehyde through cross-link between aldehyde groups and amino groups. Using the proposed probes and platform, a novel sandwich-type electrochemical immunosensor for simultaneous detecting carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) was successfully developed. This immunoassay possessed good linearity from 0.05 ng mL^{-1} to 200 ng mL^{-1} for both CEA and AFP. The detection limit of CEA was 0.002 ng mL^{-1} and that of AFP was 0.05 ng mL^{-1} ($S/N=3$). Furthermore, analysis of clinical serum samples using this immunosensor was well consistent with the data determined by the enzyme-linked immunosorbent assay (ELISA). It suggested that the proposed electrochemical immunoassay provided a potential application of clinical screening for early-stage cancers.

1. Introduction

A biomarker is regarded as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention (Atkinson et al., 2001), the concentrations of which increase or decrease in serum, tissue, or saliva, can be indicative of disease states (Chikkaveeraiah et al., 2012). The detection of biomarkers provides potential for early cancer detection and monitoring therapeutic effects. The broad range of clinically relevant concentrations for different biomarkers presents challenges for multiplexed detection. Meanwhile, simultaneous measurements of a panel of protein biomarkers will offer more credible diagnostic results rather than a single test, reduce medical cost per assay and improve assay efficiency (Wulfkuhle et al., 2003; Kulasingam and Diamandis, 2008; Rusling et al., 2010).

Electrochemical methods for detection of protein biomarkers are of high sensitivity, simple equipment, and easy operation, which include potentiometric immunosensors and amperometric immunosensors such as stripping voltammetry (Liu et al., 2004), square-wave voltammetry (Feng et al., 2012a; Feng et al., 2012b), differential pulse voltammetry (Gao et al., 2013; Chen et al., 2013), amperometry and cydic voltammetry amperometry (Wei et al., 2009; Fragoso et al., 2011; Chikkaveeraiah et al., 2011). Stripping voltammetry is a sensitive electrochemical technique for measuring trace metals, which includes a preconcentration step combined with a stripping step (Achterberg and Braungardt., 1999). Suitable potentials are applied to strip metal ions, which can be used to discriminate among multiple metal species and allow simultaneous measurement of four to six metals in multiple component solution. For biomarkers, this type of multiplexed measurement can be achieved by attaching different metal nanoparticles or quantum dots to the respective secondary antibodies (Chikkaveeraiah et al., 2012). However, the preparations of metal nanoparticles are often complicated and hydrophobic surfaces of metal nanoparticles need hydrophilic treatment for labeling antibodies. Furthermore, the metal nanoparticles need to be dissolved by strong acid before electrochemical detection.

Nanomaterials such as noble metal nanoparticles, magnetic particles, quantum dots, and carbon nanotubes have a perfect perspective for their volume effects and surface effects, which could enhance biological activity and adhesiveness, and are commonly used to increase the sensitivity of electrochemical

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detection (Choi et al., 2010). Platinum porous nanoparticles (PtPNPs) have shown exceptional performance in catalysis, sensors, optics and biomedicine owing to their large surface areas, large number of active site and excellent biocompatibility (Wang et al., 2013). Based on the high catalysis activity, platinum nanoparticles can be used in multiple-label assays with horseradish peroxiadase to reduce H_2O_2 for signal amplification (Song et al., 2010). Metal nanocrystals can be used as tracers in square-wave anodic stripping voltammetry for simultaneous detection of multiple proteins, which required a mercury film electrode and an acid dissolution step (Liu et al., 2004). However, to the best of our knowledge, PtPNPs adsorbed metal ions as probes for electrochemical immunoassays via amino groups complexation with metal ions have not been reported in literatures. The metal ions can be detected directly in a single run through differential pulse voltammetry (DPV) without acid dissolution, whose position and height of the peaks reflected the identity and concentrations of the corresponding antigens.

Graphene is an ideal material for electrochemistry (Liang and Zhi, 2009; Yang et al., 2010; Shao et al., 2010) because of its very large 2-D electrical conductivity, a tunable band gap, room-temperature Hall effect, high mechanical strength, and high elasticity and thermal conductivity. It is also a biocompatible nanomaterial (Chen et al., 2008) which can provide uniform and great electroactive site and large surface area for immobilization of biomolecules. Reduced graphene exhibits lower over-potentials and larger electrochemical response than graphite, which is associated with the oxygen-containing functional groups on the surface of the reduced graphene (Pumera et al., 2010). Recently, our group reported a one-step approach to synthesize graphene oxide–thionine–Au nanocomposites in a mild condition for fabrication of a simple label-free electrochemical immunosensor to detect CEA with an ultra low detection and wide concentration range (Han et al., 2013). Another sandwich-type electrochemical immumosensor was developed for simultaneous detection of CEA and AFP using an Au-ionic liquid functionalized reduced graphene oxide nanocomposite as immunosensing matrix and chitosan coated prussian blue nanoparticles or cadmium hexacyanoferrate nanoparticles loaded AuNPs as distinguishable signal tags, which exhibited high selectivity and sensitivity (Liu and Ma., 2013). Based on the previous work, liquid reduced graphene oxide (IL-rGO) was chosen as a substrate material. Surface of IL-rGO is rich in amino groups, which both allowed the IL-rGO well disperses in water to yield a colloidal stable suspension and also helped antibodies immobilization by glutaraldehyde through cross-link between aldehyde groups and amino groups.

Herein novel electrochemical labels were developed by combining PtPNPs as braced structure with metal ion as electrochemical signals. Using 2-aminoethanethiol, PtPNPs absorbed Cd^{2+} and Cu^{2+} to form PtPNPs-Cd^{2+} and PtPNPs-Cu^{2+} hybrids for directly labeling antibodies. The metal ions can be detected in a single run by differential pulse voltammetry (DPV), which can avoid acid dissolution in stripping voltammetry and simplified the experimental process. Glassy carbon electrode (GCE) was modified with IL-rGO to immobilize capture antibodies using glutaraldehyde for signal amplification. Base on the probes and platform, an accurate and sensitive electrochemical immunoassay for simultaneous detecting CEA and AFP was fabricated.

2. Experimental

2.1. Reagents and materials

Carcinoembryonic antigen (CEA) was purchased from Biosynthsis Biotechnology Company (Beijing, China). Alpha-fetoprotein (AFP), mouse monoclonal anti-CEA and anti-AFP capture antibodies were purchased from Linc-Bio Company (Shanghai, China). Chloroplatinic acid hexahydrate ($HPtCl_4 \cdot 6H_2O$, 99.9%) was purchased from Acros Organics China. Ascorbic acid (AA), D-(+)-glucose (Glu), and cadmium chloride ($CdCl_2$) were obtained from Alfa Aesar China (Tianjin). 2-Aminoethanethiol were purchased from Tianjin Heowns Biochem LLC. Human immunoglobulin G (IgG) was purchased from Chenwen Biological Company (Beijing, China). Glutaraldehyde were purchased from Tianjin Fuchen Chemical Reagents Factory. IL-NH_2 (e.g. 1-a minopropyl-3-methylimidazolium chloride) was purchased from Shanghai Chengjie Chemical Co. L td. (Shanghai, China). Graphene oxide was obtained from Nanjing Jcnano technology Co. Ltd. (Nanjing, China). Cupric chloride dehydrate ($CuCl_2 \cdot 2H_2O$), albumin from bovine serum (BSA), urea acid (UA), KOH, NaH_2PO_4, Na_2HPO_4, acetic acid (HAc), potassium ferricyanide ($K_3Fe(CN)_6$) and potassium ferrocyanide ($K_4Fe(CN)_6$) were obtained from Beijing Chemical Reagents Company (Beijing, China). Sodium acetate (NaAc) was obtained from Tianjin Guangfu Fine Chemical Research Institute. Clinical human serum samples were obtained from Capital Normal University Hospital (Beijing, China). All other reagents were of analytical grade and used without further purification. Deionized-distilled water used in all precedures was purified through an Olst ultrapure K8 apparatus (Olst, Ltd., resistivity $> 18\ M\Omega$).

2.2. Apparatus

Transmission electron microscopy (TEM) images were obtained from a JEOL-100CX electron microscope under 80 kV accelerating voltage (H7650, Hitachi, Japan). Scanning electron microscopy (SEM) images were obtained from a Hitachi S-480 0 SEM equipped with an energy dispersive X-ray spectrometer (EDS). X-ray photoelectron spectroscopy (XPS) analysis was obtained from an ESCALAB 250 X-ray photoelectron spectroscope (Thermofisher, American). All electrochemical immunoassay measurements were performed on CHI-830 electrochemical work station (Chen-hua Instruments Co., Shanghai, China). A three-electrode system was used in the experiment with a 4 mm diameter glassy carbon electrode (GCE) modified as the working electrode, an silver chloride reference electrode and a Platinum electrode as counter-electrode. Supersonic cleaner was purchased from Kunshan Ultrasonic Instruments Co., Ltd. High speed centrifuge was purchased from Shanghai Anting Scientific Instrument Factory.

2.3. Preparation of antibodies–PtPNPs–metal ion probes

First, PtPNPs were synthesized according to the literature (Wang et al., 2013). Briefly, 1 M KOH solution (0.05 mL), 10 mM fresh ascorbic acid (3 mL) aqueous solution, and 2 mL H_2O were sequentially injected into a conical flask. Then 2 mM chloroplatinic acid solution (4 mL) was added. The mixture was blended for about 1 min and then located in 60 °C thermostated container for 1 h. The resulting PtPNPs were centrifuged and washed with absolute ethanol and deionized-distilled water for at least three times and then dispersed in 2 mL deionized-distilled water.

For ion adsorption, 1 mL 0.1 mM mercapto-ethylamine aqueous solution was added to 2 mL colloid of PtPNPs, and then 1 ml 0.025 mM $CdCl_2$ aqueous solution was added. The mixture was dispersed by ultrasonic for several minutes and stirred vigorously over night at room temperature. The fleshly formed PtPNPs hybrid with Cd^{2+} was rinsed with deionized-distilled water for at least three times and then dispersed in 1 mL phosphate buffer (0.01 M, pH = 7.0). The PtPNPs hybrid with Cu^{2+} was synthesized using the similar method. Then 100 μL of anti-CEA (1 mg/mL) solution was added into the PtPNPs-Cd^{2+} hybrids and 100 μL anti-AFP (1 mg/mL) solution was added into the PtPNPs-Cu^{2+} hybrids,