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Ultrasensitive electrochemical immunosensor based on orderly oriented conductive wires for the detection of human monocyte chemotactic protein-1 in serum

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ABSTRACT

For the first time, a simple, ultrasensitive and label-free electrochemical monocyte chemotactic protein-1 (MCP-1) immunosensor based on orderly oriented conductive wires has been developed. A conductive wire, which is similar to an electron-conducting tunnel, was designed with Au nanoparticles (AuNPs) joined to Au@Pt core–shell microspheres via a cysteamine (CA) crosslinker. To enhance the sensitivity of the immunosensor, Au nanoparticles were electrodeposited onto the gold electrode, and CA was self-assembled via strong Au–S covalent bonds, providing an appropriate surface and promoting electron transfer. Next, Au@Pt core–shell microspheres with large surface area were grafted onto the modified electrode to immobilize more MCP-1 antibodies. MCP-1 is an initiating factor and biomarker of atherosclerotic diseases. Under optimal experimental conditions, differential pulse voltammetry (DPV) current changes were used to detect MCP-1 with a broad linear range of 0.09–360 pg mL^{-1} and a low detection limit of 0.03 pgmL^{-1} ($S/N=3$). The proposed immunosensor exhibited good selectivity, reproducibility and reusability. When applied to spiked serum samples, the data for the developed immunosensor were in agreement with an enzyme linked immunosorbent assay, suggesting that the electrochemical immunosensor would be suitable for practical detection.

1. Introduction

Monocyte chemotactic protein-1(MCP-1), which is also known as cysteine–cysteine (CC) chemokine ligand 2 (CCL2), is an important member of the CC family of chemokines. MCP-1 plays a key role in the pathogenesis of cardiovascular disease (Gosling et al., 1999; Jacobson et al., 2000). In addition, MCP-1 binds to CC chemokine receptor-2 (CCR2) and promotes the migration and accumulation of monocytes in the vascular wall (Hartung et al., 2007). MCP-1 enhances the superoxide production of monocytes (Aukrust et al., 2001) by stimulating tissue factors (Schecter et al., 1997), and therefore converts the stable atherosclerotic plaque into an unstable one (Omer Satiroglu et al., 2011). Elevated serum MCP-1 levels are associated with atherosclerotic diseases, such as unstable angina (Koichi Nishiyama et al., 1998), myocardial infarction (McDermott et al., 2005) and in-stent restenosis (Shuichi Oshima et al., 2001). Probucol and its derivative (i.e., AGI-1067) inhibit gene expression of MCP-1 and are effective at preventing atherosclerosis in clinical studies and tested animal models (Tardif et al., 2003); MCP-$1^{-/-}$ mice have protected from atherosclerosis both in low-density lipoprotein receptor ($LDLR^{-/-}$) and apoB transgenic mice (Linton and Fazio, 2003). Therefore, MCP-1 may be employed as a biomarker (Omer Satiroglu et al., 2011) for the diagnosis and prediction of atherosclerotic diseases.

The conventional analytical methods used for the determination of MCP-1 primarily include enzyme-linked immunosorbent assays (ELISA) (Hayashi et al., 2006), immunohistochemistry (IHC) (Hartung et al., 2007), western blot (WB) (Pervin et al., 1998) and immunocytochemistry (ICC) (Paine et al., 1993). However, these methods are time-consuming and expensive labeling processes that require stringent laboratory conditions. Therefore, an alternative method is urgently needed for the sensitive detection of MCP-1.

Signal amplification is the primary concern for the detection of target molecules at quite low concentrations. AuNPs that exhibited unique properties such as excellent conductivity, good biocompatibility and large surface areas, have been widely used to enhance electron transfer. Herein, AuNPs were deposited onto the surface of a bare gold electrode to enlarge the electrochemically active sites, resulting in an increase in the functional density of

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molecules and to facilitate electron transfer. CA was widely used to construct sensors due to its excellent conductivity and feasible decoration (Lai et al., 2009; Pimenta-Martins et al., 2012). The cysteamine self-assembled monolayer was spontaneously chemisorbed on the gold substrates via the Au–S bond producing well-ordered amine array groups on the outside. The positive charge on the amine groups can attract negative redox probes and metal nanoparticles, enhancing the electron transfer and amplifying the current response. In addition, the amine group array exposed on the electrode surface was used to anchored Au@Pt core–shell microspheres via electrostatic interactions. Au@Pt core–shell microspheres with a large surface area increased the immobilized amounts of antibodies on the electrode to further enhance the sensitivity of the sensor. AuNPs joined to Au@Pt core–shell microspheres via cysteamine resulted in conductive wires, which are similar to the electron-transfer tunnel (Jiang et al., 2013) that was used in fabricating an ultrasensitive electrochemical sensor in this work.

Herein, we pursued a simple electrochemical immunosensor for the direct assay of MCP-1. First, AuNPs were electrodeposited on the surface of the bare gold electrode. Next, Au@Pt core–shell microspheres were attached via CA. After the antibodies were immobilized on the Au@Pt modified electrode, BSA was used to block the nonspecific binding sites. To the best of our knowledge, this is the first time that an electrochemical sensor was used for the detection of MCP-1. The experimental results indicated that the sensor exhibited a high sensitivity, wide linear range and excellent analytical performance, which will be promising for the diagnosis and prognosis of cardiovascular disease.

2. Experimental

2.1. Materials and reagents

A human monocyte chemotactic protein-1 (MCP-1) ELISA Kit was purchased from Zhenjiang Hope Biotechnology Corporation (Zhenjiang, China). Cysteamine hydrochloride was purchased from Tianjin Heowns Biochem LLC. $HAuCl_4 \cdot 3H_2O$, $H_2PtCl_4 \cdot 6H_2O$ and ascorbic acid ($C_6H_6O_8$) were obtained from Sigma-Aldrich (Beijing, China). Bovine serum albumin (BSA, 96–99%) was purchased from Sigma (St. Louis, USA, www.sigmaaldrich.com). Doubly-distilled water was obtained from a Millipore Mill-Q purification system. The other chemicals were analytical grade and used without further purification.

2.2. Apparatus and measurements

The morphologies of Au nanoparticles and Au@Pt core–shell microspheres were characterized by transmission electron microscopy (TEM, Hitachi-7500158) and UV–vis absorption spectroscopy (UV-2450). The electrochemical experiments, including differential pulse voltammetry (DPV), electrochemical impedance spectroscopy (EIS), square wave voltammetry (SWV) and cyclic voltammetry (CV) were performed with a CHI660E electrochemical workstation at room temperature using a conventional three-electrode system (Shanghai Chenhua Apparatus Corporation, China) composed of a modified gold electrode (GE, 4 mm in diameter) as a working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum wire as an auxiliary electrode. All of the electrochemical measurements were carried out in 10 mM PBS (pH 7.4) containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_5]$ (1:1) and 0.1 M KCl.

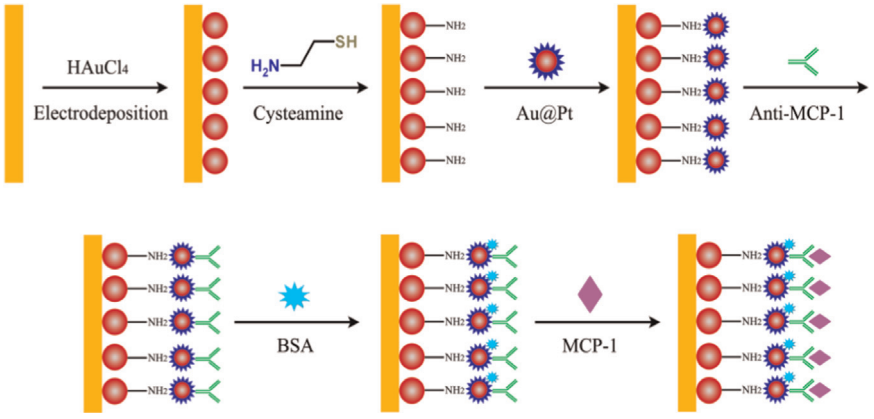
2.3. Preparation of the Au@Pt core–shell microspheres

The detail of preparation of the Au@Pt core–shell microspheres can be found in Supplementary information (S11).

2.4. Fabrication of the biosensor

Prior to the fabrication of the immunosensor, the bare gold electrode (GE) was immersed in a fresh mixture of a piranha solution (1:3, v/v, H_2O_2 and H_2SO_4) for 5 min followed by rinsing with double-distilled water. Then, the gold electrode was successively polished with 1, 0.3 and 0.05 μm alumina powders and cleaned ultrasonically in anhydrous ethanol. Finally, the gold electrode was subjected to an electrochemical cleaning fluid (0.5 M sulfuric acid) at the scanning potential between 0.0 V and 1.6 V at a scan rate of 100 mV s^{-1} until the stable cyclic voltammogram for the clean gold electrode was obtained (Elshafey et al., 2013a).

The fabrication of the electrochemical immunosensor is outlined in Scheme 1. Initially, the pretreated gold electrode was modified with AuNPs using electrodeposition in a 1.0 wt% $HAuCl_4$ solution at −0.25 V for 60 s (Jiang et al., 2013). Next, the self-assembled monolayer of cysteamine was formed by incubating 8 μL of 50 mM cysteamine with the AuNPs modified gold electrode (AuNPs/GE) for three hours at room temperature followed by washing the modified electrode to remove unbounded cysteamine residues. Then, the Au@Pt core–shell microspheres (8 μL) were dropped onto the cysteamine layer modified electrode (CA/AuNPs/GE) at 4 °C for 12 h. The modified electrode was incubated with 8 μL of the MCP-1 antibodies at 4 °C for 12 h followed by rinsing



Scheme 1. Schematic representation of the electrochemical immunosensor.