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Label-free electrochemical immunosensor based on gold–silicon carbide nanocomposites for sensitive detection of human chorionic gonadotrophin

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ABSTRACT

Uniform and highly dispersed gold–silicon carbide (Au@SiC) nanocomposites were prepared via simple way and used for fabrication of label-free electrochemical immunosensor for sensitive detection of human chorionic gonadotrophin (hCG). Using Au@SiC as electrode material and using ferricyanide as mediator, the proposed immunosensor provides a simple and economic method with higher sensitivity and a wider concentration range for detection of hCG. Under the optimal condition, the approach provided a good linear response range from 0.1 to 5 IU/L and 5 to 1000 IU/L with a low detection limit of 0.042 IU/L. The immunosensor showed good selectivity, acceptable stability and reproducibility. Satisfactory results were obtained for determination of hCG in human serum samples. The proposed method provides a promising platform of clinical immunoassay for other biomolecules. In addition, the bio-functionalization of SiC combined with other nanomaterials will provide promising approach for electrochemical sensing and biosensing platform.

1. Introduction

Human chorionic gonadotrophin (hCG) is a secretion of the placenta during pregnancy and gestational trophoblastic diseases (Marcillac et al., 1992). It is increased as a consequence of abnormal placental invasion and placental immaturity. Therefore, it is an important diagnostic marker of pregnancy and one of the most important carbohydrate tumor markers (Lu et al., 2012). Thus, exactly determining the concentration of hCG in urine or serum plays an important role in monitoring of trophoblastic diseases in all modern immunological pregnancy tests (Yang et al., 2011a).

Specific affinity between antibody and corresponding antigen, so-called immunoassay, provides a promising analytical method for clinical assay and biochemical analysis (Mani et al., 2009; Tang and Ren, 2008; Cui et al., 2007; Nourani et al., 2013). During recent years, conventional diagnostic methods, such as enzyme-linked immunosorbent assay, chemiluminescence, surface plasmon resonance, and quartz crystal microbalance, have been the main methods used for detection hCG (Zhou et al., 2010; Krishnan et al., 2011; Miller et al., 2011; Yang et al., 2010). Compared with conventional immunoassays, electrochemical immunoassay has exhibited several advantages, including simplicity of instrument, low cost, feasibility of miniaturization, and subsequent portability. Some strategies based on sandwich-type immunosensors (Yang et al., 2009; Wei et al., 2011; Viet et al., 2013) have been applied to the determination of hCG. In comparison to sandwich-type immunosensors, label-free immunosensors have obvious advantages that its fabrication is simple, easy-handle, and low-cost owing to its avoidance of tedious labeling operations (Wu et al., 2013). Up to now, various label-free immunosensors related to medical diagnosis (Zhuo et al., 2008; Song et al., 2010), environmental monitoring (Tran et al., 2012), and food safety monitoring (Li et al., 2011a) have been reported. Among them, electrochemical label-free immunosensors have been attracted increasing attention due to its high sensitivity, low cost, and ease of preparation.

In the design and fabrication of highly sensitive electrochemical immunosensors, antibody immobilization and signal amplification are the crucial steps (Sánchez et al., 2008). Many kinds of nanomaterial, including noble metal nanoparticles, carbon nanomaterials, semiconductor nanoparticles, metal oxide nanostructures, and hybrid nanostructures, have been developed to amplify

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electrochemical signal in order to improve the sensitivity of electrochemical immunosensor (Pei et al., 2013). Gold nanoparticles (Au NPs) are well-known bio-nanomaterials because of their large specific surface area, strong adsorption ability, well suitability, and good conductivity (Liu et al., 2005); it can strongly interact with biomaterials and has been utilized as an intermediator to immobilize antibody to efficiently retain its activity and to enhance current response in the construction of immunosensor (Huang et al., 2013). Chitosan (CS) is a polysaccharide derived by deacetylation of chitin. It possesses many advantages, such as excellent membrane-forming ability, high permeability towards water, good adhesion, and biocompatibility. Also, it has abundant reactive amino and hydroxyl functional groups. Therefore, it has been widely used as an immobilization matrix for biofabrication. Ferricyanide is an excellent redox mediator in the electrochemical immunosensor system. It can cause a lower background current and have a pair of redox peaks in the amperometric measurement. The electrochemical signal achieved by ferricyanide is very stable. Thus, using ferricyanide as mediator is beneficial for improving the stability of the immunosensor.

Silicon carbide (SiC) is a material that consists of the covalent bonding of Si and C atoms, in a tetrahedron form in which Si (or C) is the central atom. The high mechanical and chemical stability of the material are determined by the very short bond length, and hence, a very high bond strength present in the SiC structure (Deva Reddy et al., 2008). SiC has more than 200 polymorphic forms, called polytypes, but cubic (β)3C-SiC, hexagonal 4H-SiC, and (α)6H-SiC are the most common polytypes (Oliveros et al., 2013). As a kind of electronic matrices and a wide band gap semiconductor, SiC has been demonstrated attractive properties, such as high modulus, high strength, good corrosion/oxidation resistance, and good high-temperature strength (Belmonte et al., 2006; Van Dorp et al., 2009; Ferroni and Pezzotti 2002; Willander et al., 2006). Hence, SiC has great practical application in several scopes, such as catalysis oxidize, photocatalyst reaction, selective oxidation of hydrogen sulfide into elemental sulfur, and isomerization of linear saturated hydrocarbons (Salimi et al., 2009; Wu et al., 2011; Dai et al., 2012). However, the design and fabrication of electrochemical immunosensor based on SiC and its nanocomposite has not been developed.

Most biomolecule recognition-based systems require immobilization of specific molecules with controlled structural order and composition (Oliveros et al., 2013). Surface functionalization provides many advantages in the development of semiconductor based biosensors. In addition, surface functionalization is one of the main tools used for covalent biomolecule immobilization. SiC is a very promising and interesting material for surface functionalization because the formation of a very thin native oxide on its surface facilitates the successful surface termination that is the prerequisite in the realization of devices. However, the surface functionalization of SiC with carboxyl group has not been explored. It has been reported that if SiC is appropriately doped, the conductivity of this material dramatically increases and exhibits electrical characteristics similar to carbon materials (Chu et al., 1995). In the work performed by Wu et al., they were able to resolve the overlapping voltammetric responses of ascorbic acid (AA), dopamine (DA) and uric acid (UA) on a SiC-coated glassy carbon electrode (GCE), and the selective determination of DA in the presence of AA and UA with a high sensitivity (Wu et al., 2011). Salimi et al. used SiC nanoparticles to modify a GCE to detect insulin via electrocatalytic oxidation with high sensitivity, excellent catalytic activity, short response time, and long term stability (Salimi et al., 2009). These findings led to the construction of different SiC electrode applications.

In the present paper, gold–silicon carbide (Au@SiC) nanocomposites were prepared via simple way and used for fabrication of label-free electrochemical immunosensor. Using Au@SiC as electrode material and using ferricyanide as mediator, the proposed label-free immunosensor provides a simple and economic method with higher sensitivity and a wider concentration range for detection of hCG and could find potential application in clinical analysis.

2. Materials and methods

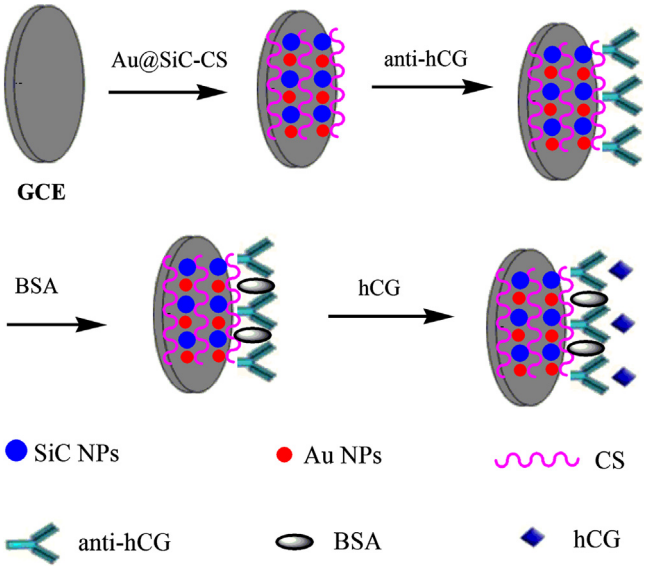
2.1. Reagents and apparatus

SiC was purchased from Nanjing Aipurei Nano-Material Company (Nanjing, China). Montmorillonite and kaolin (KL) were purchased from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China). SiO_2 was prepared from montmorillonite according to a previous report (Yang et al., 2013). Anti-hCG, hCG, luteinizing hormone (LH), thyroid stimulating hormone (TSH), and follicle-stimulating hormone (FSH) were obtained from National Institutes for Food and Drug Control (Beijing, China). Bovine serum albumin (BSA), gold chloride ($HAuCl_4$), and chitosan (CS), were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade. Phosphate buffer (PBS, 0.1 M, pH 7.4) containing 2 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl was used as working solution. All aqueous solutions were prepared with deionized water (18 MΩ/cm).

Cyclic voltammetry (CV) experiments were performed with a CHI 660E Electrochemical Workstation from Shanghai Chenhua Instrument (Shanghai, China) and conducted using a three-electrode system, with the proposed immunosensor as working electrode, a platinum wire as the counter electrode, a saturated calomel electrode (SCE) as the reference electrode. SiC and Au@SiC were characterized by a QUNT200 scanning electron microscopy (SEM, USA), a JEM 2100 transmission electron microscopy (TEM, Japan), a Rigaku TTR III X-ray diffractometer (XRD, Japan), and a Thermo Fisher SCIENTIFIC Nicolet IS10 Fourier transform infrared spectrometry (FTIR, USA). The size of Au@SiC NPs was determined by dynamic light scattering (DLS) using a Zetasizer Nano-ZS90 (Malvern Instruments, UK).

2.2. Preparation of Au@SiC

SiC was carboxyl functionalized as previously reported (An et al., 2007) with the modification of replacing SiO_2 with SiC before using.



Scheme 1. Schematic illustration of the stepwise immunosensor fabrication process.