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In situ deposition of Prussian blue on mesoporous carbon nanosphere for sensitive electrochemical immunoassay

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ABSTRACT

A Prussian blue (PB) functionalized mesoporous carbon nanosphere (MCN) composite was prepared for loading signal antibody and high-content glucose oxidase (GOD) to obtain a new nanoprobe for sensitive electrochemical immunoassay. The MCN nanocarrier with an average diameter of 180 nm was synthesized by using mesoporous silica nanosphere as a hard template in combination with a hydrothermal carbonization method. This hydrophilic carbon nanomaterial provided an ideal platform for in situ deposition of high-content PB to form the MCN-PB nanocomposite. Based on the step-wise assembly of polyelectrolyte and gold nanoparticles (Au NPs) on the negative-charged nanocomposite, signal antibody and high-content GOD were loaded on this nanocarrier to obtain the nanoprobe. After a sandwich immunoreaction at an Au NPs-modified screen-printed carbon electrode based immunosensor, the nanoprobes were quantitatively captured on the electrode surface to produce sensitive electrochemical response with a PB-mediated GOD catalytic reaction for immunoassay. The high loading of PB and GOD on the nanoprobe greatly amplified the electrochemical signal, leading to the development of a new immunoassay method with high sensitivity. Using human immunoglobulin G as a model analyte, excellent analytical performance including a wide linear range from 0.01 to 100 ng/mL and a low detection limit down to 7.8 pg/mL was obtained. Additionally, the immunosensor showed high specificity, satisfactory stability and repeatability as well as acceptable reliability. The PB-mediated GOD electrochemical system well excluded the conventional interference from the dissolved oxygen. Thus this immunoassay method provides great potentials for practical applications.

1. Introduction

The development of electrochemical chip based immunosensors for sensitive measurement of protein biomarkers has shown great application potentials for point-of-care diagnosis (Wan et al., 2013; Rusling, 2013; Lai et al., 2014a). In order to achieve the accurate measurement of low-abundant biomarkers for early disease diagnosis, various strategies, especially various nano signal amplification strategies have been developed recently. Commonly, the excellent electroconductivity and biocompatibility of nanomaterials enable their wide application in the construction of versatile nanosensing surfaces with good bioaffinity and electron transfer ability (Malhotra et al., 2010; Haque et al., 2012). More importantly, the high specific surface area of nanomaterials makes them to be served as ideal nanocarriers for loading high-content signal tags and designing various useful nanoprobes for immunoassays (Song et al., 2010; Chikkaveeraiah et al., 2012; Ji et al., 2015). Up to now a great variety of nanomaterials, especially various carbon nanomaterials such as carbon nanotubes (CNTs) (Yu et al., 2006; Lai et al., 2011) and graphene (Du et al., 2011; Wen et al., 2014), have been widely used for the nanoprobe preparation owing to their exceptional electrical, thermal, chemical and mechanical properties, which dramatically enhanced the signal responses of the sandwich immunorecognition events and thus greatly improved the analytical sensitivity of these immunoassay methods.

As a kind of useful carbon nanomaterial, carbon nanosphere has also shown successful application in this field. For example, Cui (Cui et al., 2008) and Xu (Xu et al., 2012) designed two types of nanoprobes for electrochemical immunoassay using carbon nanospheres to load high-content horseradish peroxidase (HRP) and gold nanoparticle (Au NP) tags. Significantly, in order to further

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improve the analytical performance of immunoassay methods for achieving higher sensitivity, an increasing number of researches are focused on the development of new nanocarriers, especially mesoporous nanomaterials which possess larger specific surface area and are able to load higher content of signal tags in the latest years (Yang et al., 2010; Li et al., 2011a). Although mesoporous carbon nanosphere (MCN) might be the best candidate as the mesoporous nanocarrier, its irregular particle shape with large average size over 1 μm and the inherent hydrophobicity accompanied with the conventional preparation under rather harsh conditions greatly limit its applications in the bioassay field (Wan et al., 2008; Hu et al., 2010; Gu et al., 2011). As a cheap, mild and absolutely "green" approach newly rising in recent years, hydrothermal carbonization provides a new route for synthesis of various carbon nanomaterials with tunable structure and size character. Additionally, it can also produce abundant oxygen-containing groups such as COOH, OH, C=O on the surface of the materials (Titirici and Antonietti, 2010; Fang et al., 2010; Li et al., 2011b). These groups greatly improve the water dispersibility of carbon nanomaterials and also provide an ideal interface for further functionalization with various signal tags.

Among the electrochemical tags, Prussian blue (PB) is a well-known "artificial peroxidase" that has gained distinctive attentions in the biosensing field owing to its low cost and selective electrocatalytic reduction of H_2O_2 in the coexistence of oxygen (Karyakin et al., 2000; Li et al., 2007). Previously, we designed a glucose oxidase (GOD) functionalized CNT nanoprobe for the signal tracing of a screen-printed carbon electrode (SPCE) based immunosensor by coupling with PB-mediated enzymatic cycle, which successfully overcame the interference problem of dissolved oxygen in the conventional HRP-based electrochemical immunoassays (Lai et al., 2009). However, the large background current from the PB immobilized on the electrode surface restricted the sensitivity in some degree. Additionally, PB was chemically synthesized by mixing ferric and hexacyanoferrate ions with the different oxidation state of iron: either $Fe^{3+}+[Fe^{II}(CN)_6]^{4-}$ or $Fe^{2+}+[Fe^{III}(CN)_6]^{3-}$ (Karyakin, 2001; Ricci and Palleschi, 2005). However, the reaction between the two agents was too fast to be able to control the product, which resulted in its poor repeatability for the sensing applications. Recent research has shown that the inherent weak reduction property of carbon nanomaterials including CNTs and graphene can slowly reduce ferric ions resulting in the formation of PB nanocrystals in the presence of $[Fe^{III}(CN)_6]^{3-}$ (Zhai et al., 2009; Jin et al., 2010). This phenomenon provides a promising way for the controllable preparation of PB-carbon nanocomposites.

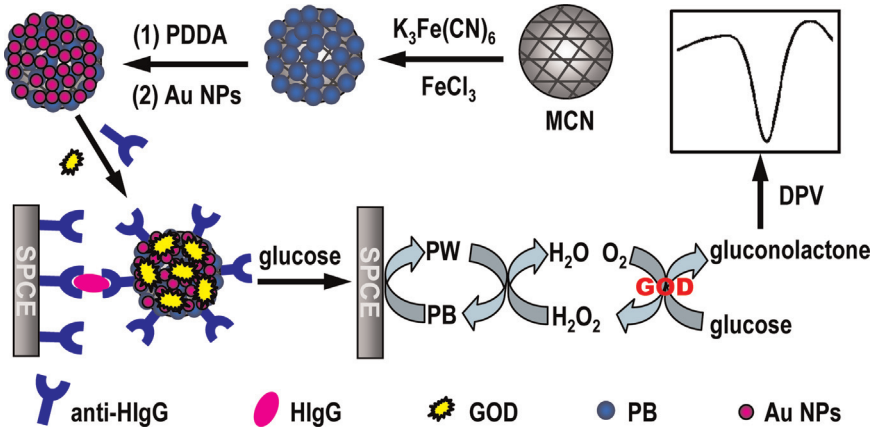
In view of this, in this work a kind of MCN with an average diameter of 180 nm was firstly synthesized using mesoporous silica nanosphere as template in combination with the hydrothermal carbonization method. PB was then in situ deposited on this nanocarrier to obtain a MCN-PB nanocomposite. After the step-wise assembly of polyelectrolyte and Au NPs for further loading signal antibody and high-content GOD on this nanocomposite, a new nanoprobe was successfully prepared for the electrochemical immunoassay (Scheme 1). Upon performing the sandwich immunoreaction at an Au NPs-modified SPCE-based immunosensor, the nanoprobes were quantitatively captured on the electrode surface to form the immunocomplex and then produced sensitive electrochemical signal for immunoassay through the PB-mediated GOD catalytic reaction, which excluded the interference from dissolved oxygen completely. Due to the high loading of PB and GOD on the nanoprobe for signal amplification as well as the enzymatically catalytic reaction, a sensitive immunoassay method was successfully developed for the accurate measurement of the protein analyte of human immunoglobulin G (HIgG).

2. Materials and methods

2.1. Materials and reagents

Human immunoglobulin G (HIgG), mouse immunoglobulin G (MIgG) and polyclonal rabbit anti-human immunoglobulin G (anti-HIgG) were purchased from Wuhan Boster Biological Technology Ltd. Carcinoembryonic antigen (CEA) was purchased from Xiamen Boson Biotechnology Ltd. Glucose oxidase (GOD, from Aspergillus niger), tetraethoxysilane (TEOS), Pluronic® F-127, hexadecyltrimethylammonium bromide (CTAB), furfural, poly(diallyldimethylammonium chloride) (PDDA, 20%, w/w in water), bovine serum albumin (BSA) and human serum albumin (HSA) were obtained from Sigma-Aldrich Chemical Co. Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) and trisodium citrate were obtained from Shanghai Reagent Company. The bovine serum was obtained from Beijing Solarbio Science & Technology Ltd. All other reagents were of analytical reagent grade and used without further purification. Doubly distilled water was used throughout the experiments.

Phosphate-buffered saline (PBS) solutions at various pH values were prepared by mixing the stock solutions of 50 mM NaH_2PO_4 and Na_2HPO_4. A 50 mM pH 7.0 PBS containing 0.05% (*w*/*v*) Tween-20 (PBST) was used as washing buffer, and a 50 mM pH 7.0 PBS containing 2% (*w*/*v*) BSA was used as blocking solution. In addition, a 50 mM pH 6.9 PBS containing 10 mM glucose was used as the substrate solution.



Scheme 1. Schematic representation of the preparation of MCN-PB based nanoprobe and the electrochemical detection strategy of the immunoassay method.