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Amperometric carbohydrate antigen 19-9 immunosensor based on three dimensional ordered macroporous magnetic Au film coupling direct electrochemistry of horseradish peroxidase

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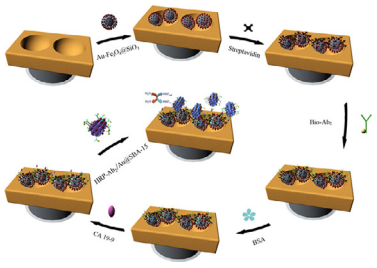
HIGHLIGHTS

- Three dimensional ordered macroporous magnetic electrode was newly used in electrochemical immunosensor.
- The large surface area of macroporous magnetic electrode could improve the immobilized amount of antibody.
- Au nanoparticles functionalized SBA-15 was used to immobilize enzyme labeled Ab_2 and enzyme.
- Macroporous magnetic electrode and Au nanoparticles composite facilitated the direct electron transfer of enzyme.
- The immunoassay avoided adding electron transfer mediator, simplifying the procedure.

GRAPHICAL ABSTRACT



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ABSTRACT

A sandwich-type electrochemical immunosensor for the detection of carbohydrate antigen 19-9 (CA 19-9) antigen based on the immobilization of primary antibody (Ab_1) on three dimensional ordered macroporous magnetic (3DOMM) electrode, and the direct electrochemistry of horseradish peroxidase (HRP) that was used as both the label of secondary antibody (Ab_2) and the blocking reagent. The 3DOMM electrode was fabricated by introducing core–shell Au–SiO_2@Fe_3O_4 nanospheres onto the surface of three dimensional ordered macroporous (3DOM) Au electrode via the application of an external magnet. Au nanoparticles functionalized SBA-15 (Au@SBA-15) was conjugated to the HRP labeled secondary antibody (HRP-Ab_2) through the Au–SH or Au–NH_3^+ interaction, and HRP was also used as the block reagent. The formation of antigen–antibody complex made the combination of Au@SBA-15 and 3DOMM exhibit remarkable synergistic effects for accelerating direct electron transfer (DET) between HRP and the electrode. Under the optimal conditions, the DET current signal increased proportionally to CA 19-9 concentration in the range of 0.05 to 15.65 U mL^{-1} with a detection limit of 0.01 U mL^{-1}. Moreover, the immunosensor showed high selectivity, good stability, satisfactory reproducibility and regeneration. Importantly, the developed method was used to assay clinical serum specimens, achieving a good relation with those obtained from the commercialized electrochemiluminescent method.

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1. Introduction

The early cancer diagnosis plays a crucial role in screening, evaluating and therapeutic treatment efficacy, which requires highly sensitive methods for the accurate determination of specific protein biomarkers [1]. Conventional detection methods for cancer biomarkers include enzyme-linked immunosorbent assay (ELISA) [2], radio-immunoassay (RIA) [3] and chemiluminescence enzyme immunoassay (CLEIA) [4]. However, the above methods have limitations such as environmental pollution, poor reproducibility, high cost and require time-consuming separations, which cannot meet the increasing clinical demands for the rapid detections. In addition, these techniques do not work well for the detection of cancer markers with ultra-low biogenic concentrations. Hence, developing a simple, rapid and sensitive method with low cost is a challenge for cancer diagnosis.

Recently, electrochemical immunosensor, especially sandwich-type immunosensor, has exhibited several advantages of simple pre-treatment, rapid detection, low cost and high sensitivity in the detection of cancer markers [5–7]. Despite many advances in this field, the exploration of new strategies for the improvement of the simplification and sensitivity of the immunoassays is still being pursued. In the design and fabrication of electrochemical immunosensors, antibody immobilization and signal amplification are the crucial steps. Furthermore, the sensitivity of an electrochemical immunosensor for antigen detection at very low concentrations can be enhanced by increasing the loading amount of antibodies as well as controlling the orientation of immobilized antibodies on electrode surfaces [8]. Various types of nanostructured materials have been used in the fabrication of immunosensors, due to their unique optical, electrical, catalytic and magnetic properties [9–12].

The combination of nanostructured materials with electrochemical immunoassay opens new horizons for highly sensitive detection of biomarkers [13]. Recently, nanoporous metals have aroused great attention due to their high surface area, low density and 3D bicontinuous pore–ligament structure [14]. Nanoporous Au has been one of the most popular nanoporous metals due to its good stability, high conductivity and good biocompatibility [15]. In our previous works, we have constructed several electrochemical immunosensors based on a 3D ordered macroporous (3DOM) Au film electrode [16,17]. An ideal method of developing 3DOM Au with controlled size and spatial arrangement of pores is to use colloidal crystal films as templates for subsequent electrodeposition [18]. Magnetic beads (MBs) are recognized as a powerful and versatile tool for the development of immunosensing platforms. Their large active surface area enhances the immobilization of high biomolecules loadings onto the solid phase of the transducer through the application of a magnetic field, exhibiting a great convenience for separation [19]. The use of Fe_3O_4 nanospheres has motivated great interest in bio-applications due to their advantages such as narrow size distributions, controllable size and the possibility to be functionalized easily [20]. However, the pure Fe_3O_4 nanospheres are very likely to aggregate. As a result, multilayered nanosphere with a magnetic core and biocompatible shell is a more attractive composite system in biosensing [21]. To the best of our knowledge, there is no report focusing on electrochemical immunosensing of cancer biomarker by using 3DOM magnetic (3DOMM) electrode as the substrate.

Silica nanomaterials with ordered structures have not only all the virtues of inorganic material but also a large specific surface area, well-defined tunable pore sizes and good biocompatibility, which provides great opportunities for the construction of immunosensors [22,23]. One of the best-known discovered mesoporous silica materials is the Santa Barbara Amorphous, SBA-15, which has been used for immobilization of enzymes, nanoparticles and antibodies [24–26]. Here, Au nanoparticles functionalized SBA-15 (Au@SBA-15) composite with good biocompatibility and excellent conductivity was employed to construct a labeling system for the fabrication of electrochemical detection platform.

In this study, carbohydrate antigen 19-9 (CA 19-9) was chosen as a cancer biomarker model to evaluate the electrochemical immunoassay. CA 19-9 is a preferred label for pancreatic cancer, which is a highly lethiferous sarcomata and difficult to be diagnosed early in current clinical medicine. Therefore, the highly sensitive determination of serum CA 19-9 levels is of great significance. In present clinic, CA 19-9 immunoassay has become a gold standard for pancreatic cancer diagnosis [27]. For the first time, 3DOMM Au electrode was fabricated as the immobilization substrate of primary antibody (Ab_1) by the application of an external magnet. The Au–SiO_2@Fe_3O_4 nanospheres with small size of about 70 nm could be immobilized into the nanopores of 3DOM Au electrode with the pore size of about 400 nm. Both the large active surface area of 3DOMM and the streptavidin (SA)–biotin (bio) complex could enhance the coupled amount of Ab_1 on the electrode surface. Au@SBA-15 microparticles have high conductivity, large surface area and highly open mesopores and, as a result, show high performance in immobilizing enzyme and accelerating direct electron transfer (DET) between enzyme and the electrode. The combination of Au@SBA-15 microparticles and 3DOMM exhibited remarkable synergistic effects for accelerating DET between horseradish peroxidase (HRP) and the electrode. With the Au@SBA-15 microparticles as immobilization matrix of HRP and HRP labeled secondary antibody (HRP-Ab_2), a CA 19-9 electrochemical immunosensor was proposed based on the DET of HRP. Compared with the traditional immunoassays, the omission of mediator greatly simplified the assay system. Meanwhile, HRP served as both labeling and blocking reagent, providing the improved signal to detect CA 19-9.

2. Experimental

2.1. Reagents

Bio-Ab_1, CA 19-9, HRP-Ab_2 and pure Ab_2 were supplied by Roche Co., Ltd. (Shanghai, China). Bovine serum albumin (BSA, 96–99%) and SA were purchased from Baoman Bio-tech Co., Ltd. (Shanghai, China). Monodispersed SiO_2 spheres with a diameter of 500 nm were purchased from Alfa Aesar. Phosphate buffer saline (PBS, 0.1 M) with various pH values was prepared by mixing a stock standard solution of NaH_2PO_4 and Na_2HPO_4, which was used as electrolyte for all electrochemistry measurements. The washing buffer (PBST) was PBS (0.1 M, pH 6.0) containing 0.05% (w/v) Tween 20. $EO_{20}PO_{70}EO_{20}$ (P123), poly(diallyldimethylammonium chloride) (PDDA, 20 wt%) and glutaraldehyde (Glu, 25 wt%) were purchased from Sigma-Aldrich. Stock solution of 3 wt% PDDA was prepared in double distilled water with Tris and NaCl. The supporting electrolyte solution of cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) measurements was 2 mM $[Fe(CN)_6]^{4-/3-}$ (1:1) solution containing 0.1 M KCl. All other chemicals were of analytical reagent grade and used without further purification. Double distilled water was used throughout the experiments. Human serum samples were obtained from the Zhangjiagang Hospital of Traditional Chinese Medicine and used as received.

2.2. Apparatus

Cyclic voltammetry (CV) and chronoamperometry were performed with a CHI 660D electrochemical workstation (Shanghai CH Instruments Co.). A conventional three-electrode system comprised a platinum wire auxiliary electrode, a saturated calomel