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Facile fabrication of an ultrasensitive sandwich-type electrochemical immunosensor for the quantitative detection of alpha fetoprotein using multifunctional mesoporous silica as platform and label for signal amplification

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ABSTRACT

A novel and ultrasensitive sandwich-type electrochemical immunosensor was designed for the quantitative detection of alpha fetoprotein (AFP) using multifunctional mesoporous silica (MCM-41) as platform and label for signal amplification. MCM-41 has high specific surface area, high pore volume, large density of surface silanol groups (Si–OH) and good biocompatibility. MCM-41 functionalized with 3-aminopropyltriethoxysilane (APTES), gold nanoparticles (Au NPs) and toluidine blue (TB) could enhance electrochemical signals. Moreover, primary antibodies (Ab_1) and secondary antibodies (Ab_2) could be effectively immobilized onto the multifunctional MCM-41 by the interaction between Au NPs and amino groups ($-NH_2$) on antibodies. Using multifunctional MCM-41 as a platform and label could greatly simplify the fabrication process and result in a high sensitivity of the designed immunosensor. Under optimal conditions, the designed immunosensor exhibited a wide liner range from 10^{-4} ng/mL to 10^{3} ng/mL with a low detection limit of 0.05 pg/mL for AFP. The designed immunosensor showed acceptable selectivity, reproducibility and stability, which could provide potential applications in clinical monitoring of AFP.

1. Introduction

As a member of the albuminoid gene family, alpha fetoprotein (AFP) is a tumor-associated fetal protein produced by the fetal liver and yolk sac [1]. The expressional level of AFP is highly elevated in hepatocellular carcinoma [2], which is one of the most common malignancies in the world, generally followed by liver cirrhosis or chronic infection with hepatitis B or C virus [3]. Therefore, AFP has been considered as one of the most important tumor markers in diagnosing and targeting of hepatocellular carcinoma [4]. In addition, AFP may function as a direct or indirect factor associated with hepatoma growth [5].

In recent years, a number of methods were proposed for the quantitative detection of AFP in human serum, such as latex particle immunoassay [6], supplementation counterimmunoelectrophoresis [7], time-resolved fluorometry [8], electrochromatography [9], radioimmunoassay [10], chemiluminescent sandwich enzyme immunoassays [11] and enzyme-linked immunosorbent assay [12]. Compared with these methods, electrochemical immunoassays with fast analysis, low detection limit, high sensitivity and simple instrumentation have recently attracted extensive attention and have been extensively applied in the detection of tumor markers [13,14].

In order to improve the sensitivity of the electrochemical immunoassays, all kinds of mesoporous materials have been used for signal amplification in construction of electrochemical immunosensors [15,16]. Mesoporous silica (MCM-41) has many advantages including highly ordered hexagonal pores, high specific surface area, tunable pore diameter, high pore volume, large density of surface silanol groups (Si–OH) and good biocompatibility [17]. Moreover, MCM-41 has demonstrated functional applications as solid supports to anchor variety of guest compounds like metal nanoparticles and dyes [18]. All these advantages mentioned above could be beneficial to the effective immobilization of antibodies and redox probes in electrochemical immunoassays. Therefore, MCM-41 has potential applications in the fabrication of electrochemical immunosensors.

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In this work, an ultrasensitive sandwich-type electrochemical immunosensor was designed for the quantitative detection the AFP in human serum. MCM-41 functionalized with 3-aminopropyltriethoxysilane (APTES), gold nanoparticles (Au NPs) and toluidine blue (TB) was used as a platform and label for signal amplification in this strategy. TB was used to provide the electrochemical signal of the designed immunosensor as a kind of electron transfer mediators containing amino groups ($-NH_2$). Au NPs were decorated on the surface or in the pore of MCM-41 to increase the load of TB by the interaction between Au NPs and $-NH_2$ on TB. Moreover, primary antibodies (Ab_1) and secondary antibodies (Ab_2) could be effectively immobilized onto the multifunctional MCM-41 (Au@MCM-41/TB/Ab_1 and Au@MCM-41/TB/Ab_2) by the interaction between Au NPs and $-NH_2$ on antibodies. Therefore, using multifunctional MCM-41 as a platform and label could greatly simplify the fabrication process and enhance the sensitivity of the designed sandwich-type electrochemical immunosensor.

2. Materials and methods

2.1. Apparatus and reagents

All electrochemical measurements were performed on a CHI760D electrochemical workstation (Chenhua Instrument Shanghai Co., Ltd., China). Transmission electron microscope (TEM) images were obtained from a Hitachi H-600 microscope (Japan). Scanning electron microscope (SEM) images were obtained using Quanta FEG250 field emission environmental SEM (FEI, United States) operated at 4 KV. UV–vis measurements were carried out using a Lambda 35 UV/vis Spectrometer (Perkin-Elmer, United States). Surface area measurements were performed on Micromeritics ASAP 2020 surface area and porosity analyzer (Quantachrome, United States).

Human AFP antigen and antibody to human AFP were purchased from Shanghai Linc-Bio Science Co., Ltd., China. MCM-41 was purchased from Nanjing XF NANO Co., Ltd., China. APTES was purchased from Shanghai Aladdin Chemistry Co., Ltd., China. TB was purchased from Sinopharm Chemical Reagent Co., Ltd., China. Phosphate buffered saline (PBS) was used as an electrolyte for all electrochemistry measurements. All other reagents were of analytical grade and ultrapure water was used throughout the study.

2.2. Preparation of the amino-functionalized MCM-41

Amino-functionalized MCM-41 (NH_2-MCM-41) was synthesized following the procedure reported previously with some modification [19]. Briefly, MCM-41 (0.5 g) was dispersed in 20 mL of anhydrous toluene with 0.5 mL of APTES and heated to 70 °C under stirring for 1.5 h, then centrifuged and dried at 110 °C. After the reaction of APTES and Si–OH on MCM-41, a free-flowing powdery material was obtained. The NH_2-MCM-41 was shown to contain $-NH_2$ by the ninhydrin test [20].

2.3. Preparation of the gold nanoparticles decorated MCM-41

Gold nanoparticles decorated MCM-41 (Au@MCM-41) was prepared by a simple method. Briefly, 0.8 mL of 1 wt% $HAuCl_4$ was added dropwise into 100 mL of aqueous solution containing 5 mg of $NaBH_4$ under stirring in ice bath. A ruby-red uniformly dispersed solution containing Au NPs in an approximate concentration of 0.2 mM was obtained. 20 mL of ultrapure water containing 20 mg of NH_2-MCM-41 was under continuous ultrasound for 1 h to disperse uniformly, which was added into 100 mL of prepared Au NPs solution under stirring. The Au@MCM-41 was obtained by the interaction between Au NPs and $-NH_2$ on MCM-41 after mild centrifugation and dried in vacuum at 35 °C.

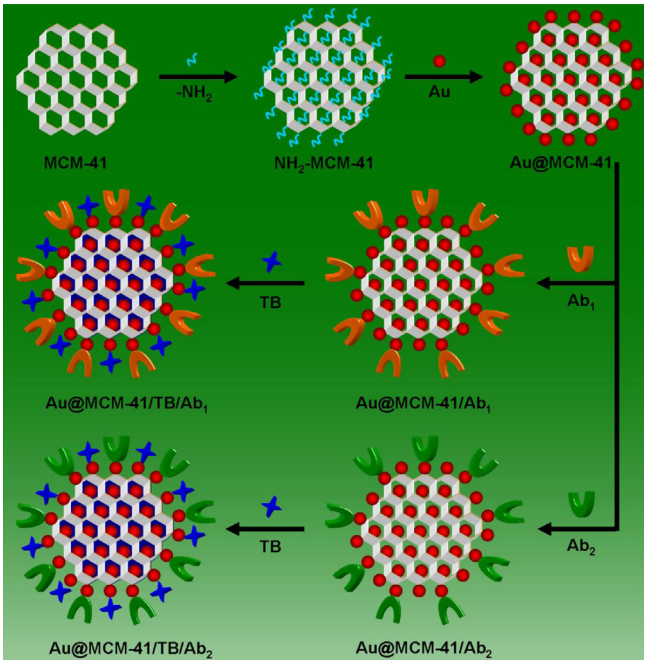

Fig. 1. The preparation procedures of Au@MCM-41/TB/Ab_1 and Au@MCM-41/TB/Ab_2.

2.4. Preparation of the Au@MCM-41/TB/Ab_1 and Au@MCM-41/TB/Ab_2

Fig. 1 shows the preparation procedures of Au@MCM-41/TB/Ab_1 and Au@MCM-41/TB/Ab_2. In brief, Au@MCM-41 (1 mg/mL, 1 mL) was added into the solution of Ab_1 (10 μg/mL, 1 mL) and Ab_2 (10 μg/mL, 1 mL) and stirred for 12 h at 4 °C. After centrifugation, a solution of TB (2 mg/mL, 1 mL) was added into the obtained precipitate (Au@MCM-41/Ab_1 and Au@MCM-41/Ab_2), and stirred for another 12 h at 4 °C. Following centrifugation, the resulting Au@MCM-41/TB/Ab_1 and Au@MCM-41/TB/Ab_2 were dispersed in 0.5 mL of PBS at pH 7.4 and stored at 4 °C.

2.5. Fabrication of the immunosensor

Fig. 2 shows the schematic diagram of the designed sandwich-type electrochemical immunosensor. A glassy carbon electrode (GCE) with 4 mm diameter was polished to a mirror-like finish with 1.0, 0.3 and 0.05 mm alumina powder successively and then thoroughly washed with ultrapure water before use. First, 6 μL of Au@MCM-41/TB/Ab_1 solution was added onto the electrode and dried at 4 °C. After washing, 3 μL of 1 wt% bovine serum albumin (BSA) solution was added and incubated for 1 h to eliminate nonspecific binding sites. Following that, the electrode was washed and incubated with a varying concentration of AFP for 1 h at room temperature, and then the electrode was washed extensively to remove unbounded AFP molecules. Finally, 6 μL of Au@MCM-41/TB/Ab_2 solution was added onto the electrode surface for another 1 h at room temperature, the electrode was washed thoroughly and ready for measurement.

2.6. Detection of AFP

A conventional three-electrode system was used for all electrochemical measurements: a GCE as the working electrode, a