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Label-free electrochemical immunosensor based on conductive Ag contained EMT-style nano-zeolites and the application for α-fetoprotein detection

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ABSTRACT

Early detection of tumor markers is of great significance, while the common used immunosensors always meet the contradiction between the conductivity and limited surface area for immobilizing more antibodies. Herein, to address this problem, the conductive silver containing EMT zeolite nanoparticles (NPs) were prepared by ion exchange and further applied in Alpha-fetoprotein (AFP) detection. Since no template was involved in the nanosized EMT zeolite synthesis at low temperature, excessive silver ions can be introduced inside the channels of zeolite and bring greatly enhanced conductivity. Besides the native porous feature of zeolite, the silver containing EMT based immunosensors shows very good performance in AFP detection, which well compensates the conductivity and surface area. In human serum, the proposed immunosensor also shows linear and even higher response to AFP relative to that in PBS. This kind of zeolite based immunosensor protocol is potentially attractive for clinical immunoassay.

1. Introduction

Alpha-fetoprotein (AFP, an oncogenic glycoprotein) is the only standard serum marker for the detection of hepatocellular carcinoma (HCC) [1]. Therefore, it is essential for early diagnosis, curative effect evaluation, therapeutic prognosis monitoring and long-term survival evaluation from HCC cancers [2–4]. Generally, the AFP concentration in healthy human serum is below 25 ng/mL, while if the rise of serum AFP level is routinely taken as an abnormality in adults and over 500 ng/mL indicates the malignant HCC [5]. There are the various novel methods for detecting elevated AFP concentration, such as enzyme-linked immunosorbent assay (ELISA) [6], electrochemiluminescent (ECL) immunoassay [7], radioimmunoassay (RIA) [8], and fluoroimmunoassay [9], while the electrochemical immunosensor has attracted more attention due to the virtue of high sensitivity and specificity, excellent detection limits, fast response, and easy handling [6,10,11]. The key factor of electrochemical immunosensor, especially for a label-free type, is the design of the electrode surface, which should facilitate the fast and stable immobilization of tested biomolecules. As the in-depth cancer marker detecting studies, both conductivity and surface area of electrode surface should be considered, since conductivity of electrode materials determines the sensitivity and morphology control is important for a large surface area to immobilize more antibodies. With the utilization of different types of nanomaterials, important advances in this aspect have been made such as metal nanoparticles [12], magnetic nanomaterials [13], carbon materials [14], etc. in order to improve electrochemical signal of biocatalytic events occurring on the electrode surface. In addition, hierarchical nanocomposites also have attracted great attentions in the electrode design strategy for the nonenzymatic biosensing applications, such as the Ag modified NiO nanowires [15], graphene oxide based CuO nanoparticles [16] and Au/graphene oxide composites [17]. To improve specific surface area, electrodes based on one-

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dimensional semiconductor and inverse opals were also developed and applied in label-free AFP immunosensors [5].

However, it is still a challenge to compensate the two factors among various immunosensors for better detection behaviors, because electrical conductivity is always restricted to the small surface area of semiconductor and metal. The motivation of the present work rests on developing new conductive and highly porous materials which are suitable for biosensing with enhanced performances.

Zeolite is an important class of inorganic crystalline materials for catalysis, sorption and ion exchanges processes [18] The wide use of these materials is due to their special structures and related properties, such as accessible intracrystalline volume and thus a much larger specific surface area with active sites. In addition, nanosized zeolites offer a fast response and facile processing that make them particularly appropriate for sensing devices. Consequently there are different types of sensing and detection techniques based on zeolites, including humidity sensors [19,20] and gas sensor [21–23], and most of them focus on the porous feature for molecule separation. Zeolite also found the applications in biosensing, such as lysophosphatidic acid, glucose, H_2O_2 detection [24–26]. However, because of the nonconductive nature of zeolite, electrical biosensing was never mentioned before.

Inspired by the successful synthesis of ultrasmall EMT nanocrystals by S. Mintova et al. at the low temperature [27], we further prepared silver loaded EMT-type nanozeolites by ion exchange and the followed reduction [28]. The ultra-small EMT-type zeolite ($Na_{36}Al_{36}Si_{36}H_{48}O_{168}$) is a hexagonal polytype of cubic faujasite (FAU) synthesized from an organic template-free Na-rich initial system. Usually, zeolites in big sizes and with stable structures are more popular due to the applications of petroleum refining and petrochemical industry [29,30], for these reasons, high temperature and pressure are always required in the preparation for high stable structure. However, since EMT-type zeolite nanocrystals can be prepared at room temperature, the metastable structure is resulted, which facilities the high ion exchange efficiency. In addition, because of the same valence state as Na^+, the nanosize is more favoured to a fast ion exchange and highly loaded silver amount in EMT zeolite is desired, which makes the conductivity of zeolite possible.

In this work, based on the existing huge surface area, the conductivity of the Ag loaded EMT nanocrystals could be further applied in label-free, electrochemical immunosensor for AFP detection. The prepared Ag-EMT based immunosensor can not only provide a larger effective surface area compared to the conventional sensors, but also act as a rapid electron transfer channel, which ensures high sensitivity and selectivity, wide detection range, low detection limit, long-term stability, and, high accuracy to detect AFP in real serum.

2. Experimental section

2.1. Materials and apparatus

NaOH, Na_2SiO_3, KH_2PO_4, KCl, Na_2HPO_4, sodium aluminate, $K_3[Fe(CN)_6]$ and NaCl were purchased from Beijing Chemical Works. H_2O in this study was deionized water. Also, the AFP, monoclonal antibody (Ab), carcinoembryonic antigen (CEA), and prostate specific acid phosphatase (PSAP) were purchased from Beijing Boisynthese Biotechnology Co. (Beijing, China). Chitosan (CS) and ascorbic acid (AA) were purchased from Sigam-Aldrich. $NaAlO_2$ (56.7%Al_2O_3, 39.5%Na_2O) were purchased from Strem-chemicals. Bovine serum albumin (BSA 96–99%) was obtained from Beijing DingGuo Biotechnology Co. In addition, human serum used in this study is based calibration lyophilized serum which produced by Fuxing Changzheng Medical Science co., LTD, Shanghai, China.

2.1.1. Characterization

Transmission electron microscope (TEM) and high-resolution TEM (HR-TEM) images were recorded on a JEM-2010 transmission electron microscope under a working voltage of 200 kV. UV3600-Vis absorption spectra were measured by Shimadzu UV-3101PC UV–vis scanning spectrophotometer ranging of 200–1200 nm. All electrochemistry experiments were conducted by using a three-electrode electrochemical cell (Chen Hua Instruments Co. Ltd., Shanghai, China) with a 4 mm diameter of GCE, a platinum wire electrode, and calomel electrode which were based as working, counter and reference electrode. EIS and CV were performed on a model CHI660D electrochemical workstation (ChenHua Instruments Co. Ltd., Shanghai, China) with PBS solution (pH 7.4, including Na_2HPO_4, KH_2PO_4 and H_2O) containing 5 mM $K_3[Fe(CN)_6]$.

2.2. Synthesis and characterization of silver-EMT zeolites

The ultra-small hexagonal EMT nanocrystal (diameter of 10–20 nm, Horvath-Kawazoe in 1 nm) was prepared as following protocol: solution A was prepared by dissolving 3.024 g sodium aluminate and 3.286 g sodium hydroxide in 49.99 g of distilled water followed by mixing with 11.45 g of sodium hydroxide; solution B was prepared by dissolving 19.213 g sodium silicate and 6.466 g sodium hydroxide in 40 g of distilled water. After the two kind of solution became completely transparent, solution A was poured slowly into solution B under vigorously stirring at 0 °C for 2 h. Then, the colloidal obtained by hydro-thermal method at 37 °C in a conventional oven for 36 h from template-free colloidal precursors. The final material is suspension with centrifuging, washing, and filtering for four times. The crystalline EMT zeolite sample was finally purified to pH = 8 after 4 h' centrifuging, washing, and filtering, the final material is in the state of suspension.

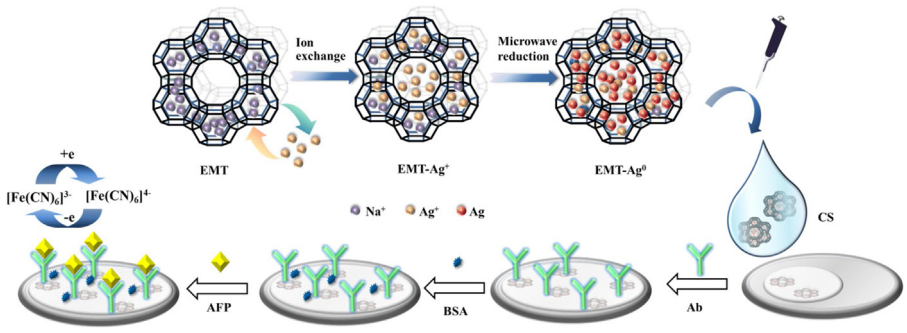

Fig. 1. Preparation process of Ag ion exchanged EMT zeolite and schematic presentation of the immunosensor fabrication.

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