Available online at www.sciencedirect.com
ScienceDirect
Biomaterials 28 (2007) 2147–2154
Biomaterials
www.elsevier.com/locate/biomaterials

A piezoelectric immunosensor for the detection of α-fetoprotein using an interface of gold/hydroxyapatite hybrid nanomaterial

Yanjun Ding, Jia Liu, Hua Wang*, Guoli Shen*, Ruqin Yu

State Key Laboratory for Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, PR China

Received 24 September 2006; accepted 31 December 2006
Available online 24 January 2007

Abstract

The ideal immobilization methods that are suitable for binding immuno-active materials with high efficiency onto the sensing surface are the key target to pursue in the current biosensor design. In this paper, a new hybrid material formed by assembling gold nanoparticles (GNP) onto nano-sized hydroxyapatite (HA) has been employed for the interface design of piezoelectric immunosensor, on which the antibodies were bound. The detection performances of the resulting immunosensor were investigated by use of the antibody–antigen model system of α-Fetoprotein (AFP), an important indicator in the diagnosis of clinical cancers. The hybrid material was characterized by the UV–vis spectroscopy, the SEM and TEM measurements. The frequency and electrochemical impedance responses characteristics for the processes of immobilization and immunoreaction of anchored anti-AFP antibodies were studied in detail. The immunoresponse of the proposed immunosensor was compared with those antibodies immobilized by using HA or GNP alone. It was found that the developed sensing interface has some advantages such as the activation-free immobilization and the high antigen-binding activities of antibodies. The as-prepared immunosensor can allow for the determination of AFP in the concentration range of 15.3–600.0 ng ml^{-1}. Such an interface design with the nano-sized hybrid materials should be tailored as a new alternative used for biosensor design.

Keywords: Gold nanoparticles; Hydroxyapatite; Immunosensor; AFP

1. Introduction

The development of reliable bio-functional materials is of great importance in both biological and modern industrial fields. Many analysts in biological field focus on finding new materials with good biocompatibility for improving the behavior of biosensor. Metal nanoparticles, especially gold nanoparticles (GNP), can be used as efficient materials for protein immobilization and have already been widely used in biosensor fabrication [1,2]. Hydroxyapatite (HA, $Ca_5(PO_4)_3(OH)$), a bioceramic analogous to the mineral component of bone with great biocompatibility and particular multi-adsorbing sites, has attracted a lot of attention because of its extensive applications such as bone and tooth implants, adsorbents, protein separation and immunosensor [3–5]. Nanostructured HA particles with a higher surface area would be more desirable for their use in many fields. For example, HA supported palladium complexes have been reported as efficient heterogeneous catalyst for the oxidation of alcohol [6,7]. Conventionally, HA powders are synthesized by various methods including solid-state reaction, precipitation and hydrolysis of calcium phosphates and sol–gel [8]. In recent years, significant research effort has been made in developing non-silica-based inorganic hybrid materials for their potential applications in biology, electronics and information technology [9–12]. Cai et al. [12] have successfully prepared a porous calcium carbonate-gold nanoparticle hybrid material for enzymatic direct electrochemistry.

The quartz crystal microbalance (QCM) immunosensor has been a hot point in investigating biomolecular interactions and clinical bioassay because of its primary advantages including high sensitivity, label- or

*Corresponding authors. Tel.: +86 731 8821355; fax: +86 731 8822237.
E-mail addresses: huawang@hnu.cn (H. Wang), glshen@hnu.cn (G. Shen).

0142-9612/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2006.12.025

radiation-free entities and low cost. The QCM immunosensor as a mass-sensitive transducer device, according to the Sauberbrey's equation [13], exhibits extremely high detection sensitivity for biological macromolecules including protein and microbes [14]. The sensing interface designed for QCM immunosensor, however, remains a key challenge to be addressed before the device can be used on a wide scale. Conventional interfacial design methodologies have suffered from some problems such as low detection sensitivity and difficulty in regeneration due to partial loss of the bioactivity of irreversibly bound entities. Therefore, exploring an improved interfacial design strategy for piezoelectric immunosensors, which would guarantee desirably high loading and bioactivity of the biomolecules immobilized is of considerable interest.

In the present work, citrate-stabilized GNP were first bound to nanostructured HA particles yielding a new GNP/HA hybrid nanomaterial. The resulting hybrid material was expected to present particular structure with multi-adsorbing sites and multifunctional advantages such as high surface area, satisfactory biocompatibility, good solubility and dispersibility. Accordingly, we tried to assemble the hybrid materials on an amine-terminated film to immobilize antibodies in an attempt to develop a new interfacial design strategy for a piezoelectric immunosensor for the detection of α-fetoprotein (AFP), an important tumor marker relating to hepatocellular carcinoma. The antibody immobilization performances of the sensing interface with the hybrid material were investigated in comparison with those for using GNP or HA nanoparticles alone. The analytical characteristics of the as-prepared immunosensor are discussed in detail, including the linear range, selectivity, reproducibility and reusability.

2. Experimental

2.1. Apparatus

The QCM (AT-cut, 9 MHz, gold electrode) was supplied by Chenxing Radio Equipments (Beijing, China). One side of the crystals was sealed with an O-ring of silicon rubber covered by plastic plate forming an air compartment isolated from aqueous solution in order to stabilize the oscillation frequency in the solution. The one-side-sealed QCM was mounted in a laboratory-made reaction cell, where the test solution was gently agitated by a magnetic stirrer (Model JB-2, Shanghai, China). The resonance frequency was monitored with quartz crystal analyzer (QCA 922, Princeton Applied Research, USA). The particle surface areas of particles were performed with Surface Area Analyzer (SA3100, Beckman Coulter Company). Electrochemical faradic impedance spectroscopy (EIS) measurements were performed with a VMP multichannel potentiostat supplied by Princeton Applied Research (Oak Ridge, TN, USA); electrochemical experiments were conducted using a conventional three-electrode format, with the prepared gold electrode (1.0 mm in diameter), a platinum wire and a saturated calomel electrode as the working electrode, the counter and the reference electrode, respectively. The experimental temperature was controlled with a thermostat (model CSS501, Chongqing Experimental Equipments, Chongqing, China). Transmission electron microscopic (TEM) images were taken by Hitachi H-800 (Japan) and copper grids were purchased from Scientific Instruments of the Chinese Academy of Sciences (Beijing, China). UV–vis spectra were recorded by Shimadzu Multispec-1501 (Japan).

2.2. Reagents and materials

Cysteamine was obtained from Sigma (UK). Calcium nitrate [$Ca(NO_3)_2 \cdot 4H_2O$], diammonium hydrogen phosphate [$(NH_4)_2HPO_4$], ammonia [$NH_3 \cdot H_2O$] and triethanolamine [$(HOCH_2CH_2)_3N$] were supplied by Henan Jiaozuo Chemicals (Henan, China). $HAuCl_4 \cdot 4H_2O$ was purchased from Shanghai Chemical Reagents (Shanghai, China). Rabbit anti-α-fetoprotein (anti-AFP) antibody was obtained from Zhongshan Biotechnology Company (Beijing, China). Purified AFP antigen of human serum and serums of cancer patients were provided by Hunan Provincial Tumor Hospital (Hunan, China). Bovine serum albumin (BSA) was purchased from Shensuo Biological Products (Shanghai, China). Phosphate-buffered saline (PBS) solution with various pHs was prepared using 0.01 M Na_2HPO_4 and 0.01 M KH_2PO_4. All other reagents were of analytical grade. All the solutions were prepared with doubly distilled water.

2.3. Preparation of GNP and HA nanocrystal

GNP were prepared following the method of Turkevich and Xu et al. [15,16] by adding 5 ml of 1% sodium citrate solution to 95 ml of a boiling chlorauric solution that contained 5 mg of Au. The solution eventually changed to salmon pink. The GNPs prepared were of a diameter of 20 ± 3 nm, as estamated from TEM images.

Nanosized HA particles were synthesized by precipitation process using $Ca(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2HPO_4$ at normal temperature (25 °C) and atmosphere pressure in a water-ethanol reactive system according to the literature [17]. Briefly, 0.5 M of Ca $(NO_3)_2$ (150 ml) and 0.5 M of $(NH_4)_2HPO_4$ (20 ml) prepared by ethanol and distilled water, respectively, were added to a round-bottom flask and stirred at 1200 rpm at the reaction temperature. This mixture was then added to 70 ml of the $(NH_4)_2HPO_4$ at a rate of 2–4 ml/min, as a result, the total molar ratio of Ca $(NO_3)_2$ and KH_2PO_4 was adjusted to 1.67. The pH of the above solution was controlled at about 10 using ammonia during the course of the $(NH_4)_2HPO_4$ addition. The temperatures used were 25 °C. After the addition of $(NH_4)_2HPO_4$ ended, the reactants were stirred for another 2 h, and the suspension was left to settle for 24 h. The resulting liquid crystalline mixture was centrifuged, washed with distilled water and then with ethanol, and finally dried at 90 °C in an oven [18]. The nanosized HA particles were mainly plate-like with the average width below 20–25 nm and length in the range of 50–100 nm, as estimated from TEM images.

2.4. Preparation of GNP/HA hybrid material

HA (200 mg) was dispersed in 100 ml of an Au colloid solution (pH 7.0) and sonicated for 30 min. After centrifugation, the GNP/HA hybrid materials were obtained. Finally, the composites were further washed with distilled water three times and dried at 90 °C in an oven.

2.5. Preparation of AFP immunosensor

Prior to the modification and measurements, each of the piezoelectric quartz crystal was cleaned in fresh piranha solution (70% H_2SO_4, 30% H_2O_2) (warning: piranha solution reacts violently with organic solution) followed by rinsing with water. Then, self-assembled monolayers were formed on the surface of the quartz crystal by immersing the crystal into a solution of 0.02 M cysteamine for 2 h. Thereafter, the crystal was immersed in a suspension of GNP/HAp particles overnight at 4 °C or for 1 h at 37 °C, rinsed with water and dried in air. The crystal was then immersed in AFP antibody solution (1:1.5 dilution ratio) to be incubated for 1 h at 37 °C, washed with PBS (pH 7.0) and water and again dried in air. Subsequently, 30 μl of 10 mg ml^{-1} BSA was introduced accordingly to block the nonspecific binding sites on the crystal surface.