

An electrochemical immunosensor for AFP measurement based on the magnetic Fe₃O₄@Au@CS nanomaterials

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Abstract: An ultrasensitive electrochemical immunosensor was fabricated based on the magnetic Fe₃O₄@Au@CS nanomaterials for alpha fetoprotein (AFP). First, the Fe₃O₄@Au@CS nanocomposites were prepared with Fe₃O₄@Au NPs as the core and chitosan (CS) as the shell. Then, The human AFP antibody (Ab1) was immobilized on Fe₃O₄@Au@CS as the capture probe, the mouse anti-human AFP antibody (Ab2) and horseradish peroxidase (HRP) were conjugated on the gold nanoparticles (Au NPs) as the detection probe. In the presence of AFP, the immunosensor Fe₃O₄@Au@CS-Ab1@AFP@Ab2-Au-HRP was formed via specific recognition of antigen and antibody. Due to the immobilized HRP catalyzed the decomposition of H₂O₂ resulting in a substantial current, the response signal of the sensor was recorded by the amperometric (i-t) method. The current response presented the linear relationship to the concentration of AFP, a dynamic range from 0.01 to 8 µg/mL, with the detection limit of 1.78 ng/mL.

1. Introduction

As we all know, hepatocellular carcinoma (HCC) has been the third cause of death in patients with cirrhosis. Alpha fetoprotein (AFP), the biomarker for HCC, is a plasma protein derived from embryonic liver cells [1]. In the serum of healthy human, the average level of AFP is barely detectable with a concentration less than 20 ng/mL, but it increases obviously to 400 ng/mL in nearly 75% HCC patients [2]. Moreover, the concentration of AFP in the human body is related to the stage of disease. Therefore, seeking a simple, sensitive and reliable analytical methods to identify AFP is absolutely vital for the early clinical diagnosis [3].

To date, various techniques have been developed for AFP detection, such as fluorescence immunoassay [4], enzyme-linked immunosorbent assay [5], electrochemiluminescence [6], electrochemical immunosensor [7] and so on. Among them, electrochemical immunosensors was widely applied in the detection of AFP owing to high sensitivity, facile operation, low cost and ease miniaturization.

Recent years, nanoparticles (NPs) provide a multifunctional platform for immunosensing interface on account of the high specific surface area, excellent electroconductibility and so on [8]. Significantly, composite nanomaterials have comprehensive biological and physical properties, which have widely used in the field of biosensing and catalysis, compared to single-component nanomaterials [9]. Fe₃O₄@Au NPs have been demonstrated to be the suitable candidates for not only acted as carrier for capture and recognition biomolecules, but also provided more active sites to accelerate the electron transfer because of great biocompatibility and upstanding electric conductivity [10]. Magnetic



nanocomposites utilizing chitosan (CS), a cationic alkaline polysaccharide polymer with unique physical and chemical properties, offer several potential benefits because of the biocompatibility and non-cytotoxicity of chitosan [11].

In this paper, the magnetic $\text{Fe}_3\text{O}_4@\text{Au}@\text{CS}$ composite nanomaterials were synthesized to fabricate the electrochemical immunosensor for AFP detection. The sensor used the Ab1 immobilized $\text{Fe}_3\text{O}_4@\text{Au}@\text{CS}$ as the capture probe and HRP and Ab2 functionalized Au NPs as the detection probe. In the presence of AFP, the sensor $\text{Fe}_3\text{O}_4@\text{Au}@\text{CS}-\text{Ab1}@\text{AFP}@\text{Ab2}-\text{Au}-\text{HRP}$ was formed via specific recognition reaction of antigen-antibody. Due to HRP could catalyze the decomposition of H_2O_2 , resulting in a substantial current, the amperometric (i-t) method was used to record the current signal of the immunosensor.

2. Materials and methods

2.1 Chemicals and reagents

Alpha fetoprotein (AFP), Human AFP antibody (Ab1), Horseradish peroxidase (HRP) and immunoglobulin G (Ig G) were supplied by Shanghai Linc-Bio Science Co., Ltd. (Shanghai, China). Chitosan (CS), Mouse anti human AFP antibody (Ab2), sodiumcitrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$), H_2O_2 , bovine serum albumin (BSA) and HAuCl_4 were purchased from Sangon Biotech (Shanghai, China). $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, hydroxylamine hydrochloride were obtained from XiLong Chemical Co., Ltd. (Guangzhou, China). All reagents were of analytical grade. Ultrapure water was obtained from the Milli-Q ultrapure water system (Millipore, USA).

2.2 Apparatus

All electrochemical measurements were conducted on CHI660D electrochemical workstation (Shanghai Chenhua Instrument, China). The three-electrode system, consisting of a magnetic glassy carbon electrode (MGCE) as working electrode, a platinum wire as auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode, was used in experiments. The amperometric (i-t) was employed in (10 mmol/L, pH 7.4) PBS solution containing 2.0 mmol/L hydroquinone under the potential of -0.2V with 50 mV/s scanning rate.

2.3 Preparation of $\text{Fe}_3\text{O}_4@\text{Au}@\text{CS}$ nanomaterials

The Fe_3O_4 NPs were prepared by hydrothermal method using H_2O_2 as oxidizer [12]. The $\text{Fe}_3\text{O}_4@\text{Au}$ NPs were prepared by one-step reduction process with hydroxylamine hydrochloride as deoxidizer and Fe_3O_4 NPs as magnetic core according to a previous paper [2]. Subsequently, the $\text{Fe}_3\text{O}_4@\text{Au}@\text{CS}$ nanocomposites were prepared with $\text{Fe}_3\text{O}_4@\text{Au}$ NPs as the core and chitosan (CS) as the shell. 0.2 g chitosan was dissolved in 10 mL 1% acetic acid solution and stirred well. Then 5 mL 1 mg/mL $\text{Fe}_3\text{O}_4@\text{Au}$ NPs solution was added to the mixture, sonicated for 4 h. Finally, the prepared solution was separated by magnetic separation and stored at 4 °C before use.

2.4 Preparation of $\text{Fe}_3\text{O}_4@\text{Au}@\text{CS}-\text{Ab1}$

Firstly, 150 μg Ab1 was added dropwise into the 1 mg/mL $\text{Fe}_3\text{O}_4@\text{Au}@\text{CS}$ suspension with mildly stirred and incubated at 25 °C for 2 h. Secondly, 1% BSA was added to block excess nonspecific sites of the $\text{Fe}_3\text{O}_4@\text{Au}@\text{CS}$ NPs and adjust the concentration to 1%, with shaking for 1 h. Finally, the $\text{Fe}_3\text{O}_4@\text{Au}@\text{CS}-\text{Ab1}$ complex was separated by PBS containing 1% BSA and resuspended in pH 7.4 PBS containing 1% BSA to form a 1 mg/mL solution and stored at 4 °C before use.

2.5 Preparation of $\text{Ab2}-\text{Au}-\text{HRP}$

The pH of the Au NPs solution was adjusted to 9.0 with 0.1 mol/L Na_2CO_3 solution. Then 30 μg Ab2 and 100 μg HRP were added to the Au NPs solution, stirred for 30 min. Afterwards, 1% BSA was added and incubated for 1 h to block out excess active sites. Then, the $\text{Ab2}-\text{Au}-\text{HRP}$ complex was centrifuged with 4800 rpm for 30 min. Finally, the resulting complex was aspirated supernatant and

dispersed in pH 7.4 PBS solution containing 1% BSA to form a 30 $\mu\text{g/mL}$ solution and stored at 4 $^{\circ}\text{C}$ until use.

2.6 Fabrication of the immunocomplex

10 μL $\text{Fe}_3\text{O}_4@\text{Au}@CS\text{-Ab1}$ (1.0 mg/mL) solution was dropped into the round hole of a polystyrene micro plate. 10 μL of certain concentration AFP antigen solution and 10 μL Ab2-Au-HRP (30 $\mu\text{g/mL}$) solution was dropped into the microporous, reacted for 20 min at 37 $^{\circ}\text{C}$. With the specific interaction of antibody-antigen, the immunocomplex of $\text{Fe}_3\text{O}_4@\text{Au}@CS\text{-Ab1}@AFP@Ab2\text{-Au-HRP}$ was formed.

2.7 Electrochemical measurements

The immunocomplex was carefully extracted from the micro porous and dropped on the pretreated MGCE. Then, the electrodes were placed in pH 7.4 PBS solution containing 2 mmol/L hydroquinone. The amperometric (i-t) was employed to record the current response at the potential of -0.2V with 50 mV/s scanning rate at 25 $^{\circ}\text{C}$. Finally, 100 μL 0.1 mol/L H_2O_2 was added to the buffer solution and the AFP was quantified according to the response of two currents before and after the addition of H_2O_2 .

3. Result and discussion

3.1 Analytical principle of electrochemical immunosensor for AFP detection

Figure 1 illustrated the fabrication process of the prepared immunosensor for AFP detection. The Ab1 was used as the capture probe that was immobilized on the surface of $\text{Fe}_3\text{O}_4@\text{Au}@CS$. The Ab2 and HRP functionalized Au were used as the detection probe. The successful construction of immunosensor was attributed not only to the specific interaction of antibody-antigen, but also to the excellent electroconductivity and high specific surface area of $\text{Fe}_3\text{O}_4@\text{Au}@CS$ NPs, which effectively accelerate electron transfer and enhance signal amplification. When the H_2O_2 was added, due to the immobilized HRP could catalyse the decomposition of H_2O_2 generating a detection signal, the amperometric (i-t) method was used to monitor the response signal of the sensor for AFP detection.

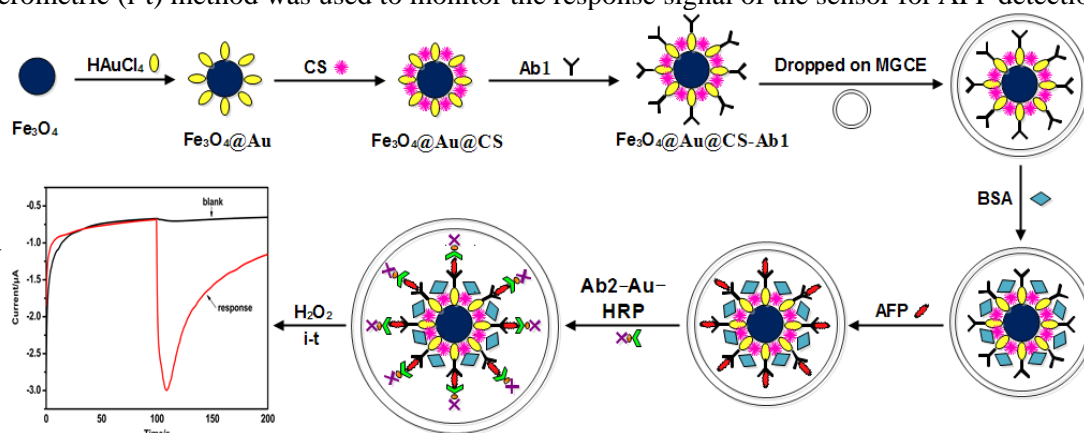


Figure 1 Principle of the immunosensor detection for AFP

Figure 2 illustrated the current signal of the developed immunosensor for AFP detection. As shown in Figure 2, compared to the absence of AFP (Figure 2, curve a), there was a significant current signal (2.31 μA) with the presence of AFP (Figure 2, curve b), which implied that the immunosensor has been successfully fabricated.

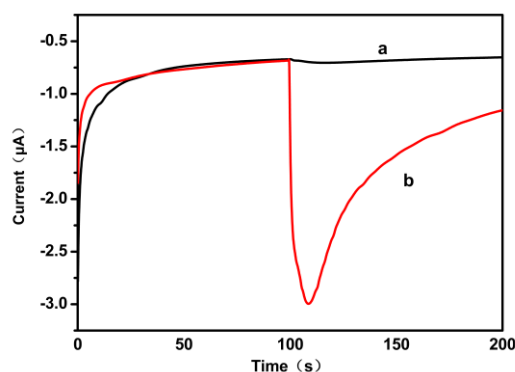


Figure 2 Current response of the immunosensor with AFP

3.2 Linear dynamic range of electrochemical immunosensor

The developed immunosensor was exposed to AFP solutions with different concentrations and the current response was recorded using the amperometric method in pH 7.4 PBS solution containing 2 mmol/L hydroquinone at -0.2 V. Figure 3A showed the electrochemical signal of the immunosensor for AFP detection covering the concentration ranged from 0.01 to 8 $\mu\text{g/mL}$. The current response of the sensor increased along with increasing concentration of AFP, which implied the formation between AFP and AFP antibody. In Figure 3B, the current signal presented linear relationship with the concentration of AFP from 0.01 to 8 $\mu\text{g/mL}$. The linear regression equation was $Y = 0.16679X + 0.17813$ (X indicated the concentration of AFP and Y was the peak current) with a correlation coefficient of 0.99715. More specifically, lower detection limits (LOD) was calculated according to the formula $\text{LOD} = 3(\text{SD}/B)$ (SD indicated the standard deviation of the response and B was the slope of the standard curve). The limits of detection reached 1.78 ng/mL. It means that the proposed biosensor possessed high sensitivity, good reproducibility and low detection, attributing to the excellent electron transfer capability and high specific surface area of $\text{Fe}_3\text{O}_4@\text{Au}@\text{CS}$ NPs.

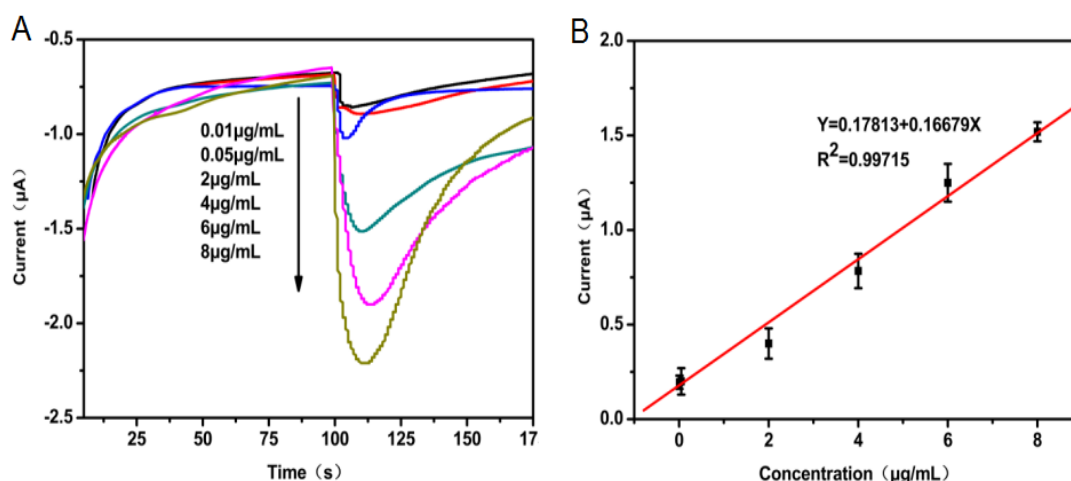


Figure 3 Amperometric current responses for different concentrations of AFP (A) and calibration curve of the immunosensor for different concentrations of AFP (B)

4. Conclusion

In summary, an ultrasensitive electrochemical immunosensor based on the magnetic $\text{Fe}_3\text{O}_4@\text{Au}@\text{CS}$ composite nanomaterials was prepared, using $\text{Fe}_3\text{O}_4@\text{Au}@\text{CS}-\text{Ab1}$ as the capture probe and $\text{Ab2}-\text{Au}-\text{HRP}$ as detection probe. Combining $\text{Fe}_3\text{O}_4@\text{Au}@\text{CS}$ NPs (high electron transfer capability

and large specific surface area), HRP (good catalytic performance) and AFP antibody (high affinity and specificity), the proposed immunosensor showed excellent performance with highly sensitive and selective, simple operation and long-term stability. The amperometric current response presented linear relationship with the concentration of AFP, ranged from 0.01 to 8 $\mu\text{g/mL}$. The detection limit was 1.78 ng/mL. In addition, this electrochemical immunosensor provided a simple and specific method for AFP detection and offered great inspiration for the application of nanomaterials in biosensors.

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