Analytical Methods

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Journal:	*Analytical Methods*
Manuscript ID:	AY-ART-10-2014-002473.R2
Article Type:	Paper
Date Submitted by the Author:	11-Dec-2014

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Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

A Novel Photoelectrochemical Sensor for the Detection of α-Fetoprotein Based on Mesoporous TiO_2-CdS QDs Composite Film

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

In this article, a label-free photoelectrochemical immunosensor based on CdS-modified mesoporous TiO_2 films was developed for the first time. The CdS quantum dots could be deposited onto the mesoporous TiO_2 films by electrochemical deposition, which could enhance photocurrent intensity in visible region. Employing specific binding of antigen-antibody, α-fetoprotein (AFP) could be captured on the surface of the modified electrode. Based on the steric hindrances, the photocurrent intensity decreased with the increase in AFP concentration. The linear response up to 20 ng/mL was obtained for AFP detection and the detection limit was 0.02 ng/mL. Moreover, the practical determination of AFP in human serum was also investigated.

1 Introduction

Proteins are ubiquitous in all living organisms and play essential role in biochemical systems. In particular, many disease-marker proteins that are used to diagnose the early stage of disease or pathological condition, are often present at a very low concentration. Accordingly, the specific identification and sensitive detection of proteins are of tremendous importance in fundamental research and clinical diagnostic.

During recent years, the photoeletrochemical (PEC) analysis has become a new topic and technology for biological detection due to its remarkable sensitivity, simple instruments, easy integration and low-cost.[1-4] In the PEC technique, upon photoirradiation, the electron could transfer among analyte, semiconductor and electrode, and the change of photocurrent intensity could be recorded.[5] Owing to the completely separation of excitation source (light) and detection signal (photo-current), the photoeletrochemical method has low background and high sensitivity in many potential applications.[2,6] Some metal oxide semiconductors have been used widely as photoeletrochemical materials, such as ZnO, ZrO_2 and TiO_2.[7]

As a typical n-type semiconductor, TiO_2 owns the extensively applications because of the wide band gap, good biocompatibility and high photoeletrochemical activity under UV light irradiation.[8] A variety of the states of TiO_2 including TiO_2 nanorods and mesoporous TiO_2 have been tested in photoeletrochemical experiment and obtains the satisfactory results.[9-11] With the high pore volume, the mesoporous TiO_2 have large specific surface area and strong surface absorption ability. The antibody and other biomolecules could be easily combined on the TiO_2 surface and pore channel through electrostatic adsorption.[12] Compared with chemical binding between biomolecules and electrodes, electrostatic adsorption on mesoporous TiO_2 surface could make the operation simpler. However, the wide band gap (~3.2 eV) impedes the application of the TiO_2 semiconductor.[8] In particular, the UV light could damage the biological activity of many biomolecules such as DNA, antigen, antibody, etc.[13, 14] Therefore, it was difficult to achieve the photoeletrochemical immunoassay using TiO_2 which could only absorb the UV light. To address this drawback, different amplification strategies were developed,[15, 16] such as sensitized with dye, doped with other impurities and coupled with the narrow band gap semiconductor. As significant semiconductor material, quantum dots (QDs) have been widely used for photoeletrochemical detection due to their unique characteristics such as favourable size, broad excitation spectra and active surface property.[17-20] By combining TiO_2 and QDs together, the two semiconductor materials could provide the stability of photocatalyst activities.[21]

Herein, a label-free PEC immunosensor based on CdS-modified mesoporous TiO_2 films was presented for protein detection. AFP, an important protein for the diagnosis of hepatocellular carcinoma (HCC),[22] was selected as the model protein. In this work, the CdS QDs could be one-step achieved on the surface and channel of the mesoporous TiO_2 thin films by electrochemical deposition. The photocurrent intensity could be enhanced dramatically by the TiO_2 film modified with CdS QDs. After that, the illuminated light has been changed to the visible range.[23] The AFP antibody could be combined on CdS/TiO_2 electrode through immunoreaction. AFP could be quantified in the range from 0.05 ng/mL to 20 ng/mL with the detection limit of 0.02 ng/mL. Moreover, due to the high affinity and specificity of immunoassay, the label-free biosensing system exhibited not only high sensitivity and specificity but also excellent performance in real human serum assay.

2 Experimental

2.1 Reagents

Tetrabutyl titanate (TBOT, 5593-70-4), ascorbic acid (50-81-7), Chromic chloride ($CdCl_2$, 10108-64-2), Sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$, 10102-17-7) were purchased from Aladdin CHEMICAL (Shanghai, China); P123 (9003-11-6) were purchased from J&K CHEMICAL (Beijing, China); AFP antigen and AFP antibody were purchased from Biosynthesis Biotechnology co., Ltd (Beijing, China). Analytical reagent grade chemicals and deionized, doubly distilled water were used throughout.

2.2 Apparatus

Photoelectrochemical measurement was taken with a MPI-EO analyzer (XiAn Remex Analysis Instrument Co. Ltd). Atomic Force Microscope (AFM) image was taken with a Being Nano-Instruments CSPM-4000 (Benyuan, China). X-ray measurements were taken on a D/max2500PC diffractometer (RIGAKU) using Cu-Kα radiation. Scanning electron microscopy (SEM) image was taken with a JSM-6700F instrument (HITACHI). The TiO_2/ITO films were taken with a spin coater (Siyouyen Electronics Technology Co. Ltd., Beijing, China). A three-electrode system was employed with Pt wire as an auxiliary electrode, Ag/AgCl as a reference electrode and indium tin oxide (ITO) glass as a working electrode. Prior to use, the ITO-coated glass was cleaned in an ultrasonic cleaner with each of the following solutions sequentially: deionized water (2 min, twice), ethanol (20 min), acetone (20 min) and deionized water (10 min, twice).

2.3 Synthesis of mesoporous TiO_2 thin films

The TiO_2 sol was prepared by the hydrolysis method described previously with a slight modification.[23] HCl solution (5 mL, 37%) was added to ethanol water solution (40 mL) under vigorous stirring. $Ti(OC_4H_9)_4$ (5 mL) was dissolved in ethanol (20 mL) at room temperature under vigorous stirring for 3 h. The $Ti(OC_4H_9)_4$ solution was added to HCl mixture solution at room temperature under vigorous stirring for 1.5h. P123 (1.8 g) used as the template was dissolved in ethanol (25 mL), then added to $Ti(OC_4H_9)_4$ solution. The solution was aged with stirring at room temperature for 30 min to obtain TiO_2 sol. ITO-coated glass was cleaned in an ultrasonic cleaner. TiO_2 sol was coated with spin coater operating at 300 rpm for 3 s, then the speed of spin coater was increased to 2000 rpm for 10 s. The film was aged at room temperature for 24 h, and then calcined at 480 °C for 2 h in order to move the template. The TiO_2 mesoporous film was then deposited onto 8 × 50 mm^2 sized slides of ITO.

2.4 Preparation of CdS-mesoporous TiO_2 composite films

The electrochemical deposition process was achieved with Pt wire as an auxiliary electrode, Ag/AgCl as a reference electrode and TiO_2/ITO as a working electrode in electrolyte containing $CdCl_2$ (0.1 M), $Na_2S_2O_3 \cdot 5H_2O$ (0.01 M) at pH=2 under 40 °C. The deposition potential was -0.7 V and deposition time was 25 min.

2.5 The construction of immunosensor

Dissolving chitosan (CS) powder in acetic acid (1%) and the CS solution (0.5 wt%) were obtained. CS solution (30 μL) was dropped on CdS/TiO_2/ITO electrode and dried at room temperature and the CdS/TiO_2/ITO electrod was washed with NaOH (0.1 M) and distilled water. Glutaraldehyde (GLD) (30 μL) was dropped on CS/CdS/TiO_2/ITO electrode and aged at room temperature for 30 min. The electrode was washed with distilled water. AFP antibody (30 μL, 50 μg/mL) was dropped on CS/CdS/TiO_2/ITO electrode and aged at 4 °C for 12 h. The electrode was washed with phosphate buffer solution (NaH_2PO_4-Na_2HPO_4, PBS, 0.01 M, pH=7.4). Finally, the electrode was incubated with Bovine Serum Albumin (BSA) for 30 min at 37 °C to block nonspecific binding sites. After washing with PBS, the electrode was incubated in different concentrations of AFP for 1 h at 37 °C, and then was used for PEC detection.

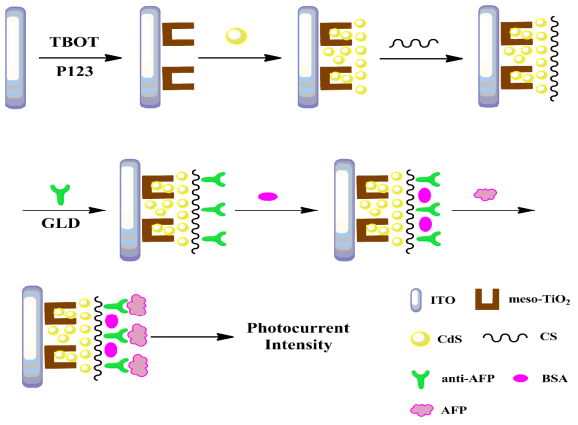
2.6 Analysis of AFP

The photoelectrochemical detection was achieved with Pt wire as an auxiliary electrode, Ag/AgCl as a reference electrode and anti-AFP/CdS/TiO_2/ITO as a working electrode in PBS buffer solution (0.01 M, pH=7.4) containing ascorbic acid (0.1 M) as electron donor which could stabilize the photocurrent signal with the illuminated light at 400 nm.

3 Results and discussion

3.1 Characterization of the development of the immunosensor

The preparation process of the immunosensor and the detection strategy are shown in Scheme 1. The mesoporous TiO_2 thin film was first obtained on the surface of the ITO electrode by calcined procedure. CdS QDs were deposited on TiO_2/ITO electrode through electrochemical deposition process. As a result, the illuminated light of TiO_2/ITO electrode was changed to the visible range, and the photocurrent intensity was enhanced dramatically. The anti-AFP was anchored on the surface of the CS/CdS/TiO_2/ITO by the covalent conjugation. After blocking with BSA, the assembled ITO electrode was introduced to the immunoreaction with the exposed part of AFP by an incubation period. With the increasing of the concentration of AFP, the photocurrent intensity would be decreased.



Scheme 1 Schematic diagram of the PEC immunosensor fabrication process.

Figure 1 showed the surface topography of ITO electrode modified with different materials through the AFM images. Figure 1A presented the image of mesoporous TiO_2/ITO. Figure 1B showed the image of TiO_2/ITO electrode modified with CdS QDs. The coverage of CdS QDs generated the different topography compared with TiO_2/ITO electrode, and the films became thicker clearly. The SEM results (Figure S1) also indicated that the CdS QDs was successfully deposited onto the surface of TiO_2/ITO electrode. Figure 1C showed the surface topography after the covalent immobilization of anti-AFP on CdS/TiO_2 composite films. After combined with anti-AFP, the thickness and surface topography in AFM images changed. It indicated that the anti-AFP was immobilized on the CdS/TiO_2/ITO electrode successfully. When the AFP antigen was captured on the electrode, the surface intensity of the electrode increased. The AFM image of the electrode surface combined with AFP antigen was shown in Figure 1D. After comparing between part C and part D, it was shown that the surface topography of part D was more compact and different.

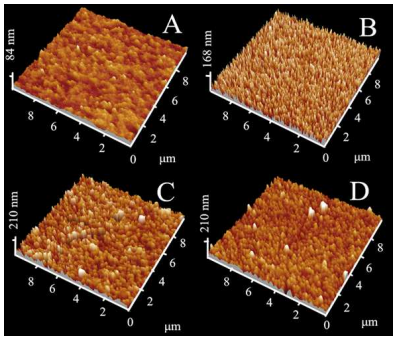


Fig. 1 AFM topography images of (A) mesoporous TiO_2/ITO; (B) CdS/TiO_2/ITO; (C) anti-AFP/CdS/TiO_2/ITO; (D) AFP/anti-AFP/CdS/TiO_2/ITO.

As shown in Figure 2A, the low-angle diffraction peak at 2θ=1.0 confirmed the presence of mesoporous structure in the TiO_2 film on the ITO glass.[24] After modified with CdS QDs on the TiO_2 film, the low-angle diffraction peak at 2θ=1.0 was lost (as shown in Figure 2C), which might be caused by pore filling with CdS QDs. The characteristic peaks of (101), (004), (200), (105) were attributed to diffraction peaks of anatase TiO_2 (as shown in Figure 2B and Figure 2D).[25, 26]

The TiO_2 semiconductor could only absorb the UV light, which limited its application. CdS was a low band gap semiconductor material. However, coupling of TiO_2 with CdS QDs could make TiO_2 absorb visible light and greatly improve the photocurrent efficiency. The mesoporous TiO_2 thin films on ITO electrode were modified with the CdS QDs by electrochemical deposition. The photocurrent intensity comparison between the TiO_2/ITO electrode (b) and CdS/TiO_2/ITO electrode (a) in 0.01 M PBS buffer solution (pH=7.4) containing 0.1 M ascorbic acid solution as electron donor with a light excitation at 400 nm was shown in Figure S2 (Supporting Information). The photocurrent intensity was enhanced clearly and indicated that the CdS QDs had been successfully deposited on the TiO_2/ITO electrode. The wavelengths scanning were shown in Figure S3 and Figure S4 (Supporting Information). It could be seen that CdS QDs expanded the absorption of TiO_2 film to from UV region to visible region.

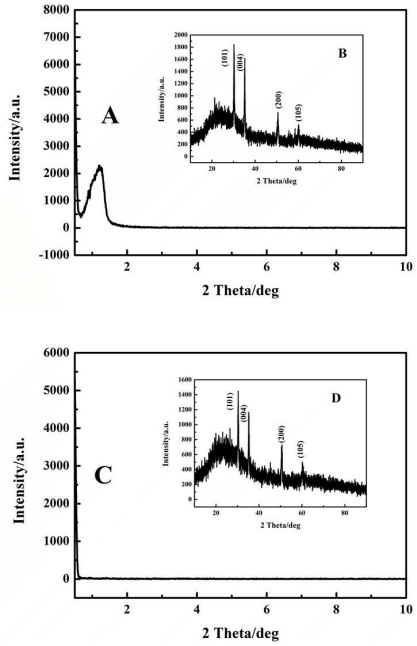


Fig. 2 (A) Low-angle X-ray Diffraction pattern and (B) wide-angle X-ray Diffraction pattern of TiO_2 film on the ITO glass. (C) Low-angle X-ray Diffraction pattern and (D) wide-angle X-ray Diffraction pattern of CdS/TiO_2/ITO film.

3.2 Feasibility of the assay

As an effective method to characterize surface modifications and assembling process on electrode, the electrochemical impedance spectroscopy (EIS) of the development of the immunosensor has been shown in Figure 3A. The CdS/TiO_2/ITO electrode (a) showed a small semicircle. After immobilizing chitosan (CS) on CdS/TiO_2/ITO electrode, the impedance spectrum (b) was increased. When the anti-AFP (c) was covalently combined with the CdS/TiO_2/ITO electrode, the resistance Ret increased. After blocked with BSA (d) and the specific immunoreaction between anti-AFP and AFP (e), the Ret still increased gradually. The reason of the increasing of the resistance Ret was that the layer on the electrode increased in the experiment process. The electron transfer between the electrode and the electrolyte solution was obstructed.

As shown in Fig. 3B, the change of photocurrent intensity was recorded for charactering the fabrication process of the photoelectrochemical immunoassay sensor. The current intensity of CdS/TiO_2/ITO (a) electrode was about 3000 nA. The current intensity was reduced after immobilized with anti-AFP (c) through covalently combined with the -NH_2 of chitosan (CS) (b) and blocking with BSA (d). After that, the photocurrent intensity was reduced clearly because of the specific recognition to AFP (e). The photocurrent intensity has been reduced after immobilized with biomolecule, because the biomolecule could increase the steric hindrances on the surface of electrode. Thus, it indicated that the biomolecule had been successfully immobilized on the composite electrode. At the same time, the change of

photocurrent intensity could be used to quantitative analysis the biomolecule in clinical practice.

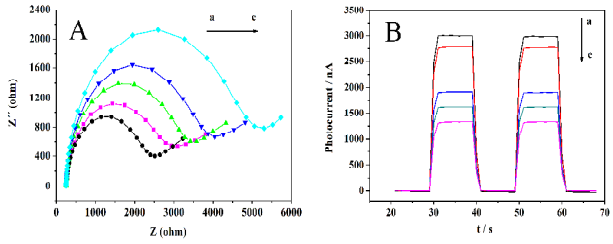


Fig. 3 EIS of the immunosensor and photocurrent responses of the immunosensor.

3.3 The condition optimization of the immunosensor

The optimization of the deposition time was shown in Figure S5 (Supporting Information). The effect of the deposition time on the photocurrent responses was examined at the ranges from 5 to 35 min. It was found that the photocurrent intensity was enhanced along with the increasing of deposition time. However, as the time increasing, the photocurrent intensity became lower, because the films on ITO electrode became thicker than before. It indicated that the optimizing deposition time is 25 min.

The optimization of the concentration of anti-AFP was shown in Figure S6 (Supporting Information). In order to study the influence of the amount of the anti-AFP immobilized on the on the surface of ITO electrode, different concentrations of anti-AFP were added. I_0 and I were the photocurrent intensity of CS/CdS/TiO_2/ITO and anti-AFP/CS/CdS/TiO_2/ITO. With the increasing of the concentration of anti-AFP, the $(I_0\text{-}I)/I_0$ was reduced. When the concentration of anti-AFP exceeded 50 μg/mL, the $(I_0\text{-}I)/I_0$ kept unchanged. Therefore, the optimizing concentration of anti-AFP was 50 μg/mL.

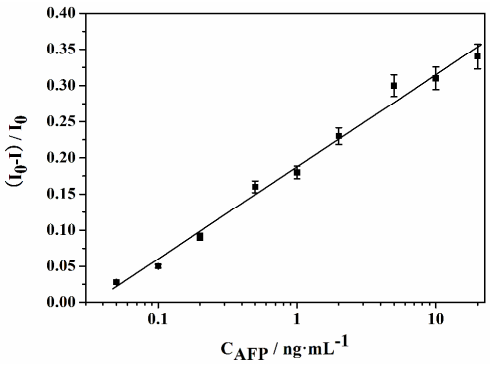


Fig. 4 Calibration curve corresponding to the analysis of different concentrations of AFP 0.05 ng/mL to 20 ng/mL. I_0 and I were the photocurrent intensity of BSA/anti-AFP/CS/ CdS/TiO_2/ITO and AFP/BSA/anti-AFP/CS/CdS/TiO_2/ITO.

3.4 The analytical performance of the immunosensor

After incubating with different concentration of AFP, the process of quantifying behavior has been achieved. Figure 4 showed the linear relationship between the photocurrent intensity and the concentration of AFP. The regression equation was $(I_0\text{-}I)/I_0$=0.1876+0.1273lgC (ng/mL), where I_0 and I were the photocurrent intensity of BSA/anti-AFP/CS/CdS/TiO_2/ITO and AFP/BSA/anti-AFP/CS/CdS/TiO_2/ITO, with a regression coefficient of 0.9947. The immunoassay showed the linear range for AFP from 0.05 ng/mL to 20 ng/mL with the detection limit of 0.02 ng/mL.

3.5 Selectivity of the AFP assay

Control experiments were performed by recording the photocurrent intensity of carcino embryonie antigen (CEA) (10 ng/mL), Tn antigen (10 ng/mL), AFP (1.0 ng/mL) and the mixed sample included CEA (10 ng/mL), Tn antigen (10 ng/mL) and AFP (1.0 ng/mL) were shown in Figure 5. The photocurrent intensity has been changed by adding AFP because of the recognition effect between anti-AFP and AFP. The $(I_0\text{-}I)/I_0$ value of CEA and Tn antigen were much lower than that of AFP. Moreover, the photocurrent intensity of the AFP and the mixed sample were almost the same because of the specific immunobinding. It indicated a good selectivity for this immunoassay sensor.

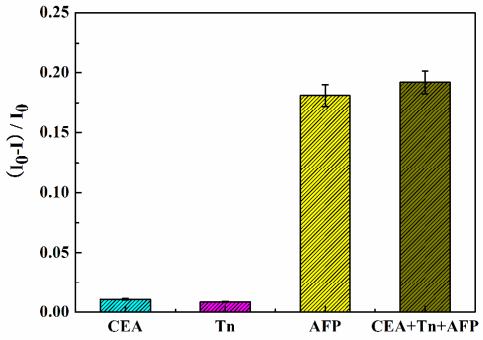


Fig. 5 Selectivity toward different analytes (CEA, Tn, AFP).

3.6 Practical determination of AFP in human serum

To demonstrate the feasibility of the immunoassay system for the practical clinical analysis, the standard addition was performed by adding the standard concentration of AFP into human serum samples. As shown in Table S1 (Supporting Information), the recoveries ranged from 94.6% to 109%, and the average recoveries were 103%. The relative deviation was 3.5%. All the results indicated the reliability of this assay. Therefore, this method could be used to monitor the content of ATP in human serum without the interference of other substance.

4 Conclusions

In this work, a label-free photoelectrochemical immunosensor has been established. Because of the large specific surface area of mesoporous TiO_2, the adsorption capacity was enhanced. It is more easily to modify CdS QDs on the mesoporous TiO_2 films. The photocurrent intensity has been enhanced and the illuminated light could be changed to the visible range by electrochemical deposition of the CdS QDs on mesoporous TiO_2 films. In this analysis process, the directly quantitative analysis of AFP has been achieved without other label molecule, and the good sensitivity and selectivity was obtained. Therefore, the proposed immunosensor exhibited ease of operation, low-cost, high

efficiency and excellent sensitivity. More importantly, the results in real human serum assay implied that the strategy could be an useful analytical tool in biological research and clinical diagnostics.

Acknowledgements

This research was supported by the National Nature Science Foundation of China (Nos. 21005044), Excellent Young Scientists Encouragement Foundation of Shandong Province (BS2011CL017), and the Program for Changjiang Scholars and Innovative Research Team in University (PCSIRT).

Notes and references

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† Electronic Supplementary Information (ESI) available. See DOI: 10.1039/ c0xx00000x /

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