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Ultrasensitive photoelectrochemical immunoassay through tag induced exciton trapping

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ABSTRACT

The development of photoelectrochemical (PEC) sensors with novel principles is of significance in realizing sensitive and low-cost detection. This work uses CuO NPs labeled antibody to construct a simple and sensitive sandwich-type immunobiosensor for the detection of protein. The detection signal is produced by dissolving the CuO NPs to release copper ions, which are then added on a quantum dots (QDs) modified F-doped tin oxide to quench the photocurrent of QDs via copper ion-induced formation of exciton trapping. The formed exciton trapping blocks the escape of photoelectron and thus leads to a "signal off" PEC method for sensitive immunoassay. The proposed method shows a detectable range from 0.05 to 500 ng/mL for α-fetoprotein (AFP) with a detection limit (LOD) of 0.038 ng/mL. This work further extends the application of exciton trapping-based PEC biosensing strategy in bioanalysis. The sensitive analytical performance of the designed route implies a promising potential of the PEC sensing in clinical diagnosis.

1. Introduction

As the levels of tumor markers in serum are associated with the stages of tumors, sensitive and accurate determination of tumor-related biomarkers is critical to clinical diagnosis [1]. In particular, the clinical measurement of tumor biomarkers shows great promise for early diagnosis, cancer monitoring, and highly reliable prediction [2]. It also offers opportunities for understanding the fundamental biological process involved in monitoring patient response to the therapy method [3]. As a tumor marker, α-fetoprotein (AFP) is an oncofetal glycoprotein with a molecular mass of approximately 70 kDa [4], and is mainly produced by the liver, yolk sac, and gastrointestinal tract of a human fetus. It may be found at high levels in the sera of adults having certain malignancies. The increased AFP concentration in adult plasma is usually considered as an early indication of hepatocellular carcinoma [5] or endodermal sinus tumor [6]. Thus, developing a rapid and sensitive detection method for AFP is of great importance in clinical research. Many conventional methods, including enzyme-linked immunosorbent assay (ELISA) [7], electrochemistry [8], electrochemiluminescence (ECL) [9], mass spectrometry [10], quartz crystal microbalance (QCM) [11], and surface plasmon resonance (SPR) immunoassays [12], have been reported for the detection of AFP. Except the high accuracy, some of these techniques involve the disadvantages such as relatively sophisticated instruments, significant sample volume, limited sensitivity, and clinically unrealistic expense and long detection time. Therefore, there is a real need to develop operationally simple, highly sensitive, and inexpensive methods to detect the levels of biomarkers for low-cost and convenient clinical diagnosis.

As a highly sensitive detection methodology, PEC sensing is a newly developed technology which has drawn growing interest in many fields, such as environmental monitoring and bioanalysis [13–21]. Owing to the separation of excitation signal and detection signal, PEC sensing strategy has plenty of advantages such as low background, low potential different from electrochemiluminescence analysis, which leads to a good analytical performance. Moreover, this strategy can be very easily combined with general immunosensing methods for highly sensitive immunoassay of biomarkers [22–24].

Based on the quantum photoelectric effect of quantum dots (QDs), a "signal on" visual method has been proposed for immunoassay of protein via the formation of insoluble reduction product of nitro blue tetrazolium by the photoelectron escaped from the QDs labeled to the secondary antibody under light excitation [24], and a "signal off" PEC method has also been reported for selective sensing of trace Cu^{2+} by the analyte-induced formation of exciton trapping [25]. This work further combined the "signal off" PEC method with sandwich-type immunosensing strategy to develop a simple and sensitive PEC

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immunoassay method by using CuO NPs to label the secondary antibody. As shown in Fig. 1, the CdTe QDs was coated on the surface of F-doped tin oxide (FTO) electrode to form CdTe QDs/FTO electrode, which could produce the photocurrent by the photo-induced exciton process [25]. Meanwhile, the CuO NPs were dissolved with acid after immunocomplex was formed, and the obtaining Cu^{2+} solution was dropped on the CdTe QDs/FTO electrode to induce the exciton trapping sites, which blocked the escape of photoelectron and thus quenched the photocurrent of QDs. The designed "signal off" immunoassay method exhibited good performance. It extended the application of PEC sensing strategy, and possessed promising potential in clinical diagnosis and detection of low-abundant protein.

2. Experimental

2.1. Materials and reagents

Meso-2,3-Dimercaptosuccinic acid (DMSA), cadmium chloride ($CdCl_2 \cdot 2.5H_2O$) and CuO NPs (40 nm) were purchased from Alfa Aesar China Ltd. Tellurium rod (4 mm in diameter) was purchased from Leshan Kayada Photoelectricity Co., Ltd. α-Fetoprotein (AFP), anti-AFP antibody and bovine serum albumin (BSA) were purchased from Nanjing Olive Twigs Biotech. Co., Ltd. (China). FTO electrode was purchased from Beijing Midwest Group Technology Co., Ltd. (China). All other chemicals were of analytical grade without further purification. Phosphate buffer saline (PBS, 0.01 M, pH 7.0) was used throughout the photoelectrochemical (PEC) detection. All aqueous solutions were prepared using ultra-pure water obtained from a Millipore system (≥ 18 MΩ, Milli-Q, Millipore). Clinical serum samples were obtained from Jiangsu Cancer Hospital.

2.2. Apparatus

A CHI 660D electrochemical workstation (CHI, USA) was used to synthesize DMSA capped CdTe QDs. PEC detection was performed on a Zahner intensity modulated photo-spectrometer (Zahner, German) with a LW405 LED light as the accessory light source. The X-ray photoelectron spectra (XPS) were gained by a PHI5000 VersaProbe X-ray photoelectron spectrometer (ULVAC-PHI Co. Japan). The X-ray source was Al target with an applied power of 25 W 15 kV.

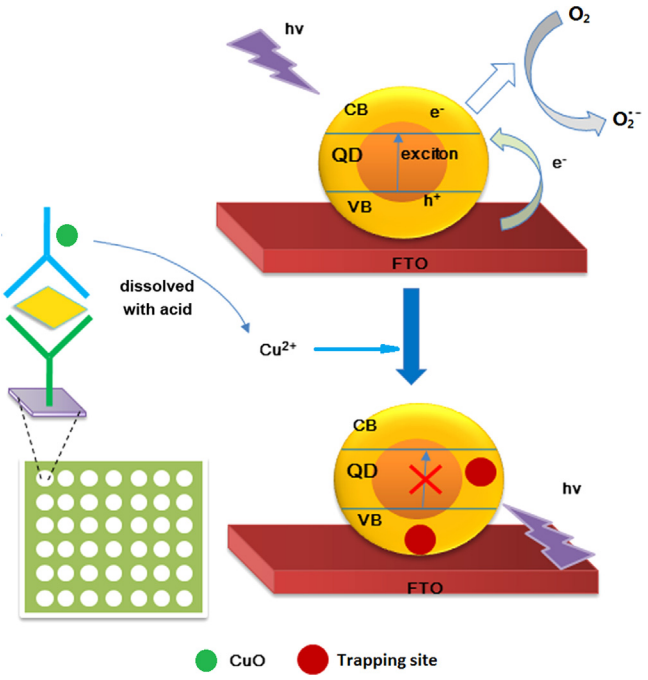

Fig. 1. Schematic illustration of photoelectrochemical immunoassay using CuO NPs-labeled antibody and copper ion-induced formation of exciton trapping on a CdTe/FTO electrode.

2.3. Synthesis of DMSA-capped CdTe QDs

The DMSA capped CdTe QDs were synthesized with an electrolysis method according the previous report [26] using a CHI 660D electrochemical workstation (CH Instruments Inc.). Firstly, 6.5 mg of DMSA, 200 μL of 1 M NaOH, and 120 μL of 0.1 M $CdCl_2$ were added into 20 mL of water in sequence. After being bubbled with highly pure N_2 for 20 min, this solution was used as electrolyte by applying a constant potential of −1.0 V (vs. saturated calomel electrode) on a Te electrode until an electric charge of 0.5 C was reached. During the electrolysis process, the solution was continuously bubbled with highly pure N_2. After the resulting solution was refluxed at 50 °C for 24 h, an equal volume of isopropyl alcohol was added, and the mixture was centrifuged at 8000 rpm for 5 min. The obtained precipitate was washed with a 1:1 mixture of isopropyl alcohol and water and then redissolved in 20 mL of water, which was kept at 4 °C prior to use. After storage for 2 months, the solution remained clear and stable.

2.4. Preparation of CuO NPs-labeled antibody and immunoreaction wells

1 mg of CuO NPs was dispersed into 1 mL 0.01 M of PBS by ultrasonication for 10 min. 500 μL 0.2 mg/mL AFP antibody was then added into the dispersion over a course of 3 min and vortexed for 3 h at 500 rpm. The mixture was centrifuged for 10 min at 10,000 rpm to obtain the CuO NPs-labeled antibody, which was re-dispersed in 1.5 mL PBS, vortexed for 3 min, and centrifuged for 10 min at 200 rpm to rid the excess CuO NPs as a precipitate. 200 μL 10% BSA in PBS was finally added and vortexed for 30 min to stabilize the solution. The CuO NPs-labeled antibody was stored at 4 °C prior to use.

100 μL 50 μg/ml primary antibody was added to each well of 96-well plate and incubated at 4 °C overnight. The wells were washed with PBST (PBS including 0.1% Tween 20, 3 × 200 μL) and blocked with 5% BSA (200 μL) for 1 h at 37 °C, which were then washed with PBST (3 × 200 μL) to obtain the immunoreaction wells.

2.5. Detection protocol

After 10 μL AFP solutions or samples were added into the wells and incubated for 1 h at 37 °C, the wells were washed with PBST (3 × 200 μL) and 10 μL CuO NPs-labeled antibody was added in each well, which were incubated for 1 h at 37 °C and thrice washed with water. 20 μL HCl (1 mmol/L) was then added in every well to react at 500 rpm for 10 min. 10 μL of the obtained solution was finally used to perform the PEC detection in 10 mL pH 7.0 PBS at an applied voltage of −0.2 V vs. SCE and an illuminating wavelength of 405 nm and intensity of 50 W m^{-2} with an CdTe QDs/FTO electrode as working, a platinum wire electrode as the auxiliary and a saturated calomel electrode as the reference electrodes. The CdTe QDs/FTO electrode was prepared by dropping 10 μL of DMSA-capped CdTe QDs solution and drying at 37 °C for 20 min.

3. Results and discussion

3.1. PEC biosensing mechanism

The PEC response of the CdTe QDs/FTO electrode comes from the light-induced formation of excitons under light excitation, which release negatively charged electrons into the vacant conduction and then are adopted by dissolved oxygen [25] (Fig. 1). The unpassivated surface of QDs possesses low surface energy level