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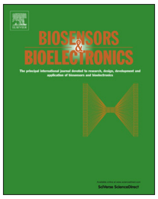

A network signal amplification strategy of ultrasensitive photoelectrochemical immunosensing carcinoembryonic antigen based on CdSe/melamine network as label

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ABSTRACT

Taking advantage of CdSe/melamine network as label and Au-TiO_2 as substrate, this work developed a novel kind of signal amplification strategy for fabricating photoelectrochemical (PEC) immunoassay. The melamine, a star-shaped triamino molecule, was firstly used for readily capturing CdSe QDs and forming a CdSe/melamine network, which was formed through strong interactions between the carboxyl groups of TGA-stabilized CdSe QDs and the three amino groups of each melamine molecule. In this strategy, the primary antibody (Ab_1) was immobilized onto Au-TiO_2 substrate, which made the photoelectric conversion efficiency increase significantly. After the formed Ab_2-CdSe/melamine network labels were captured onto the electrode surface via the specific antibody-antigen interaction, the photoelectric activity could be further enhanced via the interaction between the Au-TiO_2 substrate and CdSe/melamine network. Due to this amplification of PEC signals and the special structure of the label, the fabricated PEC immunosensor was applied for sensitive and specific detection of cancer biomarker carcinoembryonic antigen (CEA), and displayed a wide linear range (0.005–1000 ng mL^{-1}) and low detection limit (5 pg mL^{-1}). In addition, the immunosensor was performed with good stability and reproducibility, and the results to analyze human serum samples were satisfactory.

1. Introduction

Cancer is one of the main causes of mortality worldwide as its cure has not yet been achieved. Detection of tumor biomarkers which expresses the presence or recurrence of cancer is a powerful technique for effective early diagnosis and treatment of cancer (Limbut et al., 2006). As one of the most common cancer biomarkers, carcinoembryonic antigen (CEA) in healthy adults is at a normal level of 3–5 ng mL^{-1} (Laboria et al., 2010; Tang and Ren, 2008). Among the various methods and strategies reported for the determination of CEA, photoelectrochemical (PEC) sensing approach has received profound interest for its desirable advantages and attractive potential in future biological analysis (Liang et al., 2006). PEC bioanalysis possesses the advantages of both optical method and electrochemical method and lowers the instrumentation cost remarkably (Brown et al., 1992; Li et al., 2014, 2015; Zhao et al., 2013). Due to the different energy forms of the excitation source and the detection signal, the PEC technique possesses potentially lower background signal and higher sensitivity than the conventional electrochemical methods (Haddour et al., 2006; Li et al., 2012; Shu et al., 2015; Wang et al., 2009; Zhao et al., 2011, 2015). For improving the sensitivity of the PEC immunosensor, one the most effective methods is reducing the background signals and enhancing the photocurrent intensity (Shu et al., 2016; Yang et al., 2015).

As a relatively inexpensive semiconductor, TiO_2 is generally utilized in the photocatalysis and photoelectrochemistry due to its unique properties: nontoxicity, photostability and high electronic mobility (Kamat, 2011). However, the wide band gap of TiO_2 (~3.2 eV, anatase) only allows it to absorb the ultraviolet light less than ~387 nm, which limits the utilization of solar light (Ong et al., 2014; Wold, 1993). For increasing the UV-light activity of TiO_2, loading gold nanoparticles (Au NPs) on the surface is an effective way as we studied previously (Zhang et al., 2016). The process has been demonstrated as that electron-transfer of conduction-band electrons from TiO_2 to Au NPs enhances the charge separation and thus decreases the electron-hole (e^--h^+) pairs recombination (Chen et al., 2013). Therefore Au-TiO_2 nanosheets (NSs) were used as substrate here.

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Furthermore, as one kind of semiconductor nanocrystals, water soluble CdSe quantum dots (QDs) with unique size-dependent properties have been paid more and more attentions in the field of PEC bioassay (Hojeij et al., 2008; Yue et al., 2013). For enhancing effective photoelectric activity area of CdSe QDs, we proposed using the melamine, a star-shaped triamino molecule, for the first time for readily capturing CdSe QDs and forming a CdSe/melamine network through strong interactions between the carboxyl groups of TGA-stabilized CdSe QDs and the three amino groups of each melamine molecule.

In this work, we proposed a novel kind of signal amplification strategy based on Au-TiO_2 as substrate and CdSe/melamine network as label. The synergy effect in the designed Au-TiO_2 hybrid could hasten the spatial charge separation, retard the electron-hole recombination, and hence increase the excitation and conversion efficiency. Additionally, after the formed Ab_2-CdSe/melamine network labels were captured onto the electrode surface via the specific antibody-antigen interaction, the photoelectric activity could be further enhanced via the interaction between the Au-TiO_2 substrate and CdSe/melamine network.

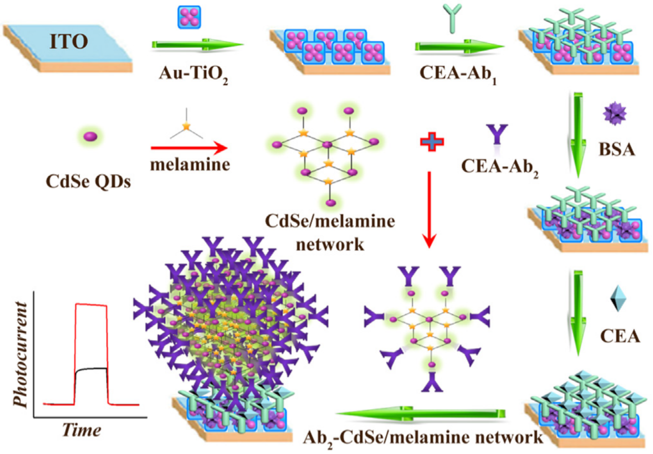
2. Experimental

2.1. Materials and reagents

Indium-tin-oxide (ITO) glasses (resistivity $\leq 10\ \Omega$/sq) were purchased from Zhuhai Kaivo Optoelectronic Technology Electronic Components Co., Ltd., China. All chemicals were used as-received without further processing. CEA, CEA primary antibody (CEA-Ab_1) and CEA secondary antibody (CEA-Ab_2) were purchased from Beijing Dingguo-Changsheng biotechnology Co., Ltd. (China). $HAuCl_4 \cdot 3H_2O$, $AgNO_3$ and melamine were obtained from Shanghai Reagent Co. (China). Cetyltrimethylammonium bromide (CTAB), ascorbic acid (AA), bovine serum albumin (BSA, 96–99%), Na_2HPO_4 and KH_2PO_4 were obtained from Sinopharm Chemical Reagent Beijing Co., Ltd. (China). Phosphate buffered solution (PBS, 0.1 mol L^{-1}, pH 7.4) which obtained by mixing 0.1 mol L^{-1} Na_2HPO_4 solution with 0.1 mol/L KH_2PO_4 solution was used as electrolyte through the experiment. Thioglycolic acid (TGA) was obtained from Tianjin Kermel Chemical Reagent Co., Ltd. (China). EDC/NHS solution was ultrapure water containing 0.01 mol L^{-1} of EDC and 0.002 mol L^{-1} of NHS. Ultrapure water was prepared by a Millipore Milli-Q system and used throughout.

2.2. Apparatus

Photocurrent was measured on a PEC workstation (Zahner-Zennium PP211, Germany) at a bias voltage of 0.1 V with light intensity of 150 W m^{-2}. The distance between the light source and the electrode was fixed at 10 cm. Electrochemical impedance spectroscopy (EIS) was performed on an autolab impedance measurement unit (IM6e, Zahner elektrik, Germany). All experiments were carried out at room temperature and all the photocurrent measurements were performed at a constant potential. The UV–vis absorption spectra was recorded on Shimadzu UV3600 UV–vis–NIR spectrophotometer (Lumerical Solutions, Inc.). XRD patterns of the prepared samples were acquired with a Rigaku D/MAX 2200 X-ray diffractometer (Tokyo, Japan) (Braggequation $2d \sin \theta = n\lambda$, $n=1$, $\lambda=0.154$ nm). Transmission electron microscopy (TEM, JEOL JEM 1200EX working at 100 kV) were utilized to characterize morphology and interfacial lattice details. Scanning electron microscope (SEM) images were obtained using a field emission SEM (Zeiss, Germany). Energy Dispersive X-Ray Spectroscopy (EDS) were recorded by JEOL JSM 6700F microscope (Japan).



Scheme 1. Fabrication process of the PEC immunosensor.

2.3. Fabrication of the PEC immunosensor

After Au NPs and TiO_2 NSs were synthesized and TGA-stabilized CdSe QDs and Ab_2-CdSe/melamine network were prepared, which were expressed in Supporting Information (SI), the immunosensor was fabricated as shown in Scheme 1. First, 3 mg of TiO_2 NSs powder was ultrasonically dispersed in 1 mL of distilled water, and then 10 µL of the homogeneous suspension was dropped onto a piece of ITO slice.

After drying in air, the electrode was sintered at 450 °C for 30 min in a muffle furnace to obtain desired film stability and photocurrent intensity and finally cooled down to room temperature. Then, 10 µL of obtained Au NRs sol was dropped on TiO_2 NSs film and dried at room temperature. After washed with water, the Au-TiO_2 modified ITO electrode was obtained successfully. After that, 6 µL of 10 µg mL^{-1} CEA-Ab_1 were introduced to the Au-TiO_2/ITO electrode surface at 4 °C in a moisture atmosphere to avoid evaporation of solvent and followed by rinsing with distilled water to remove the physically adsorbed CEA-Ab_1 after incubation for 1 h. Subsequently, 6 µL 1% BSA was incubated on the modified electrode for 1 h at 4 °C to block the nonspecific binding sites and then washed with the washing buffer thoroughly. Next, 6 µL of CEA solution with different concentrations was dropped onto the electrodes and incubated for 1 h at 4 °C followed by washing with washing buffer. After the binding reaction between Ab_1 and Ag, the electrodes were allowed for modification with 6 µL of Ab_2-CdSe/melamine network for 1 h at 4 °C and then washed thoroughly with washing buffer solution. The resulting electrodes were finally employed as a sandwich PEC immunosensor for CEA analysis.

2.4. PEC detection

The performance of the PEC sensor was tested by its photocurrent-time response in pH 7.4 of 0.1 mol L^{-1} PBS containing 0.20 mol L^{-1} of AA, which served as a sacrificial electron donor during the photocurrent measurement. For CEA detection, the original photocurrent signal (I_0) of PEC sensor was first measured after the CEA antigen joined. When the modified electrode was incubated with Ab_2-CdSe/melamine network, the residual photocurrent signal (I_1) was recorded at the same condition as the original one. The photocurrent increase factor (ΔI) was calculated as follows: $\Delta I(\mu A) = I_1 - I_0$. The PEC response of modified electrode to light irradiation could be analyzed in terms of the incident photon to charge carrier conversion efficiency (IPCE), also referred to as external quantum efficiency (Zheng et al., 2011). IPCE was determined from short circuit photocurrents Jsc (A/cm^2) monitored