A new signal amplification strategy of photoelectrochemical immunoassay for highly sensitive interleukin-6 detection based on TiO$_2$/CdS/CdSe dual co-sensitized structure

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**Abstract**

Dual co-sensitized structure of TiO$_2$/CdS/CdSe was designed to develop a novel photoelectrochemical immunoassay for highly sensitive detection of human interleukin-6 (IL-6). To construct a sensing electrode, TiO$_2$/CdS/CdSe hybrid was prepared by successive adsorption and reaction of Cd$^{+2}$ and S$^{2-}$ ions on the surface of TiO$_2$ and then was employed as matrix for immobilization of anti-IL-6 antibody, whereas CdSe QDs linked to IL-6 were used for signal amplification via the specific antibody–antigen immunoreaction between anti-IL-6 and IL-6–CdSe bioconjugate. Greatly enhanced sensitivity for IL-6 detection was derived from the new co-sensitization signal amplification strategy. First, the TiO$_2$/CdS/CdSe co-sensitized structure extended the absorption range to long wavelength of white light, which adequately utilized the light energy. Second, the TiO$_2$/CdS/CdSe co-sensitized structure possessed stepwise band-edge levels favoring ultrafast transfer of photogenerated electrons and significantly prompted the photoelectrochemical performance. Besides, the introduction of CdSe effectively prevented the recombination of photogenerated electrons in the conduction band of CdS, further causing an enhanced photocurrent. Accordingly, upon the co-sensitization strategy, a novel immunoassay based on the competitive binding of anti-IL-6 antibody with IL-6 antigen and IL-6–CdSe bioconjugate was developed, and it exhibited a wide linear range from 1.0 pg/mL to 100 ng/mL with a low detection limit of 0.38 pg/mL for IL-6 detection. The proposed co-sensitization strategy presented high sensitivity, reproducibility, specificity and stability, and also opened up a new promising platform for detection of other biomarkers.

**Keywords:**
Competitive immunoassay
Photoelectrochemistry
Co-sensitization
Human interleukin-6

1. Introduction

Highly sensitive, accurate and low-cost measurement of disease-related biomarkers holds great promise for early diagnosis, disease surveillance and corresponding drug development (Ludwig and Weinstein, 2005; Sahab et al., 2007; Shiddiky et al., 2012). In the past years, many efforts have been made on the discovery, identification and characterization of biomarkers. Human interleukin-6 (IL-6) is a pro-inflammatory cytokine that has a critical role in the inflammatory response. It has been implicated in the pathogenesis under a series of inflammatory conditions, such as psoriasis, inflammatory arthritis, cardiovascular disease and inflammatory bowel disease (Naugler and Karin, 2008; Messina et al., 2008; Peng et al., 2011). The normal level of IL-6 in serum is in the range of 10–75 pg/mL, whereas individuals with various disease states have elevated IL-6 levels in the ng/mL range (May et al., 1992). High levels of IL-6 also are associated with breast cancer and prostate cancer (Lee et al., 2005; Domingo-Domenech et al., 2006). Thus, sensitive detection of IL-6 is of great importance in early prediction for related cancers and diseases. Several methods, to date, have been developed for the detection of IL-6, such as enzyme-linked immunosorbent assay (ELISA) (Turner et al., 2004), radioimmunoassay (RIA) (Drukier et al., 2005), conductometric immunoassay (Liang et al., 2009), electrochemical immunoassay (Wang et al., 2011), chemiluminescence assay (Luo et al., 2007), fluorescent microarray technology (Wu et al., 2008), surface enhanced Raman scattering (SERS) technology (Wang et al., 2013), and so on. Despite many advances of these assays, some of them have drawbacks such as evident sample volume, complicated equipment, limited sensitivity, and clinically impractical time and expense. Accordingly, it is necessary to explore highly sensitive, simple and inexpensive techniques for the detection of both normal and elevated levels of biomarkers.

Photoelectrochemical immunoassay is a recent yet vibrantly developing technique for detection of biomolecules, which has...
the features of simple devices, low cost and easy miniaturization (Haddour et al., 2006). But even more important, it possesses the advantages of potentially higher sensitivity than those of conventional methods because of the total separation and the different energy form of the excitation source and detection signal (Wang et al., 2009b). Accordingly, photoelectrochemical immunoassay, as an ideal and promising analytical method, has received more and more attention. Up to now, a number of semiconductor nanomaterials such as TiO$_2$, ZnO, CdS, CdTe and CdSe have been used for the fabrication of photoelectrochemical biosensors (An et al., 2010; Tu et al., 2011; Willner et al., 2001; Wang et al., 2012; Zhang et al., 2011). As we all know, different semiconductors possess different energy band gaps, resulting in different optimal absorption ranges of white light. Thus, coupling of small band gap semiconductors with large band gap ones to form sensitized structures with stepwise band-edge levels cannot only increase utilization of light energy, but also facilitate charge separation, and accordingly enhance the photo-to-current conversion efficiency (Lee et al., 2010). Unfortunately, many of the previous research works used only one single photoactive material in photoelectrochemical biosensors (Wang et al., 2012; Zhang et al., 2010; Qian et al., 2010; Chen et al., 2012; Zhao et al., 2012b). However, single-sensitized structures (the basement photoactive material coupled with only one sensitizer) have been reported in some studies (Wang et al., 2009a; Shen et al., 2011; Li et al., 2012b); they cannot yet utilize the light energy sufficiently and promote charge separation efficiently. Due to the demand for ultrasensitive detection of biomarkers, it is necessary to find effective ways to further improve the photo-to-current conversion efficiency of semiconductor nanomaterials for developing photoelectrochemical immunosensors. Compared with single photoactive material as well as single-sensitized structure, multiple-sensitized or co-sensitized structure (the basement photoactive material coupled with two or more sensitizers) can make maximum use of the light energy and evidently enhance the photo-to-current conversion efficiency. For example, Lee et al. reported that TiO$_2$/CdS and TiO$_2$/CdSe electrodes can respectively reach 1.15% and 1.24% conversion efficiency under one sun illumination, whereas TiO$_2$/CdS/CdSe electrode can reach a conversion efficiency of 2.90% which is over twice that of TiO$_2$/CdS or TiO$_2$/CdSe electrode (Lee and Lo, 2009). Thus, co-sensitization strategy can realize significant signal amplification. So far, most of the highly sensitive photoelectrochemical immunoassays were enzyme-labeled immunoassays (An et al., 2010; Zhao et al., 2012a, 2012b, 2012; Li et al., 2012b). However, the introduction of enzyme clearly increased the resistance of charge transfer which hindered further enhancement of the photocurrent signal, and it also increased the cost and time of sensor preparation. In contrast, co-sensitization strategy need not introduce enzyme and can overcome these shortcomings. Due to significant signal amplification as well as saving time and cost, co-sensitization strategy would obviously be desirable in photoelectrochemical immunoassays. Surprisingly, to the best of our knowledge, this superior signal amplification strategy has not been reported in photoelectrochemical immunoassays until now.

Herein, we developed a new platform to fabricate a highly sensitive photoelectrochemical immunosensor for IL-6 detection based on TiO$_2$/CdS/CdSe dual co-sensitized structure, as shown in Scheme 1. First, TiO$_2$ was coated onto a bare ITO electrode and a compact film was formed after high temperature sintering. Then, CdS was assembled on the TiO$_2$ film by successive adsorption and reaction of Cd$^{2+}$ and S$^{2-}$ ions. Next, chitosan was coated on the surface of CdS through coordination effect of hydroxyl/amino groups with Cd$^{2+}$ ions. Subsequently, glutaraldehyde was used to link chitosan and anti-IL-6 antibody via condensation reaction between amino and carbonyl groups. At last, BSA was applied to block unbound sites and IL-6 was detected by the competitive immunoreaction of IL-6–CdSe and IL-6 with anti-IL-6 antibody immobilized on sensing electrode. The designed biosensor presented high sensitivity, reproducibility, specificity and stability. The proposed co-sensitization strategy also provided a general consideration for the design of the photoelectrochemical immunosensors for detection of other biomarkers.

2. Experimental

2.1. Materials and reagents

TiO$_2$ powder (P25) was purchased from the Degussa Co. (Germany). Cadmium nitrate (Cd(NO$_3$)$_2$·4H$_2$O) was obtained from Shanghai Jinhai Jinting Chemical Plant (China). Sodium sulfide (Na$_2$S·9H$_2$O) and methanol were obtained from Nanjing Chemical Reagent Co., Ltd. (China). Cadmium chloride (CdCl$_2$·2.5H$_2$O) and sodium hydroxide (NaOH) were purchased from Shanghai Chemical Reagent Co., Ltd. (China). Selenium powder (Se), sodium borohydride (NaBH$_4$), thiolglycolic acid (TGA), chitosan powder (CS, from crab shells, 85% deacetylation) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were all obtained from Sigma-Aldrich (USA). Ascorbic acid (AA) and glutaraldehyde (GLD, 25% aqueous solution) were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Bovine serum albumin (BSA) was purchased from Nanjing.

![Scheme 1. Fabrication procedure of the photoelectrochemical immunosensor.](image-url)
2. Preparation of ITO/TiO2/Cds electrode

Before preparation, ITO slices (type JH52, Nanjing Zhongjingkeyi Technology Co., Ltd., China, ITO coating 30 ± 5 nm, sheet resistance ≤ 10 Ω square⁻¹) were cleaned by ultrasonic treatment for 10 min in acetone, 1 M NaOH of water/ethanol mixture (1:1, v/v), and water, respectively, and then dried at 100 °C for 6 h.

A certain amount of TiO2 powder was ultrasonically dispersed in DI water, and then 20 μL of the homogeneous suspension was applied onto a piece of ITO slice with fixed area of 0.25 cm². After drying in air, the film was sintered at 450 °C for 30 min in air atmosphere and finally cooled down to room temperature. In order to acquire different thicknesses of TiO2 film, the concentrations of TiO2 suspension were varied to 0.5, 0.75, 1.0, 1.25, 1.5 and 2.0 mg/mL. The deposition of CdS on ITO/TiO2 electrode referred to the successive ionic layer adsorption and reaction (SILAR) technique with some modification (Li et al., 2012a). The ITO/TiO2 electrode was dipped into 0.1 M Cd(NO3)2 methanol solution for 1 min and rinsed with methanol, then followed by dipping into 0.1 M Na2S methanol/water mixture (1:1, v/v) for 1 min and again rinsed with methanol. The two-step procedure is termed as “one coating”. This coating procedure was repeated several times as needed. And the resulting thin film is referred to as the ITO/TiO2/Cds electrode.

2.5. Fabrication of the immunosensor and IL-6 analysis

CS solution (0.08 wt%) was prepared by dissolving chitosan powder in 1% acetic acid. 12 μL CS solution was dropped on ITO/TiO2/Cds electrode and dried at 50 °C. After the electrode was washed with 0.1 M NaOH and DI water several times, 20 μL of 5% GD solution was dropped onto the electrode and kept at room temperature for 30 min. Then the electrode was rinsed with DI water several times to remove physically adsorbed GD thoroughly. Anti-IL-6 antibodies were introduced onto the GLD-activated electrode by dropping 20 μL of 80 μg/mL antibodies dissolved in 10 mM PBS (pH 7.4) and incubating at 4 °C for at least 12 h. Subsequently, it was rinsed with a washing buffer solution and then incubated with 20 μL of BSA blocking buffer solution at 37 °C for 30 min to block unbound sites. After being rinsed with washing buffer solution, the resulting electrode was employed as a photoelectrochemical immunosensor.

For IL-6 analysis, the fabricated immunosensor was incubated with a 20 μL of mixture containing 10 μL of IL-6–Cds bioconjugates and 10 μL of different concentrations of IL-6 antigens at 37 °C for 1 h. Then, the electrode was rinsed with washing buffer solution and applied in photocurrent measurement.

2.6. Photoelectrochemical detection

Photoelectrochemical detection was carried out in PBS (pH 7.4, 0.1 M) containing 0.1 M AA, which served as a sacrificial electron donor during the photocurrent measurement. White light produced by the Xe lamp, with a spectral range of 200–2500 nm, was utilized as excitation light and was switched on and off every 10 s. The applied potential was 0.0 V. The AA electrolyte was deaerated by pumping pure nitrogen for 10 min before photocurrent measurement.

3. Results and discussion

3.1. Characterization of TGA-capped Cds QDs

Fig. 1A and B displays the UV–vis absorption spectrum and high resolution transmission electron microscopy (HRTEM) image of the synthesized TGA-capped Cds QDs, respectively. The UV–vis
absorption spectrum exhibited a broad absorption range below 600 nm, and a sharp absorption peak located at 518 nm. The size of CdSe QDs was evaluated to be 2.54 nm from UV–vis spectrum according to Peng’s empirical formula (Yu et al., 2003). The lattice fringes of the prepared CdSe QDs were clearly shown in HRTEM image and the average size of 2.81 nm of CdSe QD was obtained according to the size distribution in the inset of Fig. 1B.

3.2. Optimum preparation conditions of ITO/TiO2/CdS electrode

Because TiO2 is a wide energy band gap (~3.2 eV) semiconductor, it can absorb only ultraviolet light, leading to great limitation on the utilization of white light. Compared with TiO2, CdS has a lower energy band gap (~2.4 eV), which can absorb longer-wavelength light. Furthermore, CdS has a higher conduction band edge respected to that of TiO2, which is advantageous to the injection of excited electrons from CdS to TiO2 (Wang et al., 2009a). Thus, coupling of TiO2 with CdS can evidently extend the absorption range, increase the utilization of light energy and improve the photocurrent intensity.

As the area of the modified electrode (0.25 cm²) and the applied volume of the TiO2 suspension (20 μL) were fixed, the thickness of TiO2 film could be adjusted by the concentration of TiO2 suspension. In order to optimize the thickness of TiO2 film, different concentrations of TiO2 suspension were used to prepare ITO/TiO2/CdS electrode and four coating numbers of CdS were fixed. As shown in Fig. 2A, the photocurrent intensity of TiO2/CdS modified electrode increased with increasing concentration of TiO2 suspension from 0.5 to 1.0 mg/mL, and then the photocurrent intensity decreased upon further increase of concentration. As a result, the electrode fabricated by 1.0 mg/mL TiO2 suspension exhibited the highest photocurrent intensity. Increasing the concentration of TiO2 suspension could increase the thickness of TiO2 film, which offered more amount of TiO2 and enlarged the surface area for deposition of more amount of CdS (Park et al., 2000). Consequently, more light absorption occurred on the TiO2/CdS single-sensitized structure to induce the increase of the photocurrent. Yet, the diffusion resistance for electron motion increased evidently with further increase in the thickness of TiO2 film, leading to gradually decreased photocurrent due to more and more surface recombination centers on excess TiO2 (Kuang et al., 2006). Hence, the optimal concentration of 1.0 mg/mL TiO2 suspension was adopted for fabricating the electrode in the following experiments.

To optimize the deposition of CdS, different coating numbers of CdS were explored to prepare ITO/TiO2/CdS electrode and 1.0 mg/mL TiO2 suspension was fixed. Fig. 2B shows the photocurrent intensity of TiO2/CdS modified electrodes with different coating numbers of CdS. As the coating number increases, the deposition of CdS on the surface of TiO2 film gradually increased, resulting in more light absorption (Chi et al., 2008). Meanwhile, the direct contact area of bare TiO2 to the electrolyte gradually decreased, leading to less recombination of injected electrons from TiO2 to AA electrolyte (Chi et al., 2008). As a result, two reasons above can be taken into account for the photocurrent increase. As can be seen, TiO2/CdS modified electrode with four coating numbers of CdS
possessed the highest photocurrent intensity. After the coating number was further increased, excessive amount of CdS deposited on the surface of TiO₂ film. As the extra CdS increased the diffusion resistance for electron transfer and offered more and more surface recombination centers, the photocurrent gradually decreased (Vogel et al., 1994). Thus, four coating numbers of CdS were selected to fabricate the electrode in the following experiments.

### 3.3. SEM and AFM characterization of electrode surfaces

The surface topography of ITO/TiO₂/CdS/CS electrode for each fabrication step was observed by SEM. Fig. 3A–D shows SEM images of bare ITO, ITO/TiO₂, ITO/TiO₂/CdS and ITO/TiO₂/CdS/CS electrode surfaces in order. It could be seen from Fig. 3A that the bare ITO electrode was covered with a mass of indium tin oxide nanoclusters. When TiO₂ film formed on the ITO electrode surface, a large number of TiO₂ nanoparticles with the size range of 22–28 nm could be observed (Fig. 3B). The as-prepared TiO₂ mesoporous film was beneficial to deposit more amount of CdS and increase the photocurrent (Crossland et al., 2013). After CdS deposition, the size of the particles on electrode surface increased to 32–40 nm, which confirmed that CdS had been successfully coated on the surface of TiO₂ film (Fig. 3C). After CS was subsequently coated, as shown in Fig. 3D, the outline of TiO₂/CdS hybrid particles became blurred, which was attributed to gel-like CS covering. Additionally, the insets in Fig. 3A–D shows the photograph images of bare ITO, ITO/TiO₂, ITO/TiO₂/CdS and ITO/TiO₂/CdS/CS electrodes in order. According to color changes of the electrode surfaces, it also suggested the successful preparation of ITO/TiO₂/CdS/CS electrode.

Fig. 4A shows AFM image of the ITO/TiO₂/CdS/CS electrode surface. TiO₂/CdS hybrid particles were clearly observed and the rough surface favored loading more anti-IL-6 antibodies. Fig. 4B shows the AFM image of ITO/TiO₂/CdS/CS/anti-IL-6 electrode surface. It can be seen that the size of the surface covering is smaller than that of Fig. 4A. This is because the size of anti-IL-6 protein molecule is not more than a dozen nanometers, whereas the particle size of TiO₂/CdS hybrid is about 32–40 nm. Besides, the evident decline in surface roughness also suggested the successful immobilization of anti-IL-6. Fig. 4C shows surface morphology of the ITO/TiO₂/CdS/CS/anti-IL-6/BSA electrode after incubation with IL-6. Making a comparison between Fig. 4B and C, it was found that the surface morphology in Fig. 4C was smoother and more compact, which could be attributed to the formation of immune complex and the blocking of BSA in interstitial places (Zhao et al., 2012b). Fig. 4D shows the surface morphology of ITO/TiO₂/CdS/CS/anti-IL-6/BSA electrode after incubation with IL-6–CdSe bioconjugate. Compared with Fig. 4C, it could be easily found that the size of the surface covering in Fig. 4D became larger and the surface roughness significantly enhanced, which was because the surface of a single CdSe QD can link with several IL-6 protein molecules to form large-sized IL-6–CdSe bioconjugate. Thus, the SEM and AFM characterization suggested the successful construction of the immunosensor.

![Fig. 3. SEM images of (A) bare ITO, (B) ITO/TiO₂, (C) ITO/TiO₂/CdS and (D) ITO/TiO₂/CdS/CS electrode surfaces. Insets: photograph images of the corresponding electrodes.](image-url)
3.4. EIS and photoelectrochemical characterization

As an effective method for characterizing the interface properties of electrodes, EIS was applied to monitor the fabrication procedure of the immunosensor. Fig. 5A exhibits the impedance spectra of the electrodes formed in different construction steps, and the inset shows the applied equivalent circuit. The impedance spectrum includes a semicircle and a linear part, and the semicircle diameter equals the electron transfer resistance ($R_{et}$). For the bare ITO electrode, the impedance spectrum exhibited a very small semicircle, indicating a very small $R_{et}$ (curve a). After TiO$_2$, CdS, CS, anti-IL-6 and BSA were modified onto the surface of bare ITO electrode step by step, gradually increased $R_{et}$ was observed owing to low conductivity of semiconductors or insulating effect of organic molecules (curve b–f), demonstrating that the layer-by-layer assembled immunosensor was successfully fabricated. After the as-prepared immunosensor was separately incubated with IL-6 antigens and IL-6–CdSe bioconjugates, the $R_{et}$ increased further (curves g and h), demonstrating that the immunocomplex between anti-IL-6 and IL-6 antigen or anti-IL-6 and IL-6–CdSe bioconjugate was successfully formed on the surface of the sensing electrode.

The fabrication procedure of the immunosensor could also be monitored by photocurrent responses, as shown in Fig. 5B. After TiO$_2$ was coated onto a bare ITO electrode, the photocurrent intensity increased properly ($I=12.03 \mu A$), because TiO$_2$ can absorb only ultraviolet light, leading to low photo-to-current conversion efficiency. While CdS was subsequently coated on, the photocurrent intensity ($I=40.94 \mu A$) increased to 3.4 times higher than that of ITO/TiO$_2$ electrode thanks to the sensitization effect of CdS (Wang et al., 2009a). Later on, the photocurrent intensity decreased gradually after the modification of CS, anti-IL-6 and BSA. This could be attributed to the fact that the immobilization of those organics partly obstructed AA to the surface of CdS for reaction with the photogenerated holes. Therefore, the photocurrent responses also proved the successful fabrication of the immunosensor. Herein, IL-6 detection is based on the competitive interaction of anti-IL-6 antibody with IL-6 and IL-6–CdSe. When the constructed immunosensor was incubated with IL-6, the photocurrent intensity further decreased ($I=8.26 \mu A$). While the immunosensor was incubated with IL-6–CdSe, the photocurrent intensity ($I=21.95 \mu A$) was nearly 2.7 times higher than that with IL-6 incubation, and the reason can be illustrated by Scheme 2. After the immunosensor was incubated with IL-6, as
shown in Scheme 2A, the photogenerated electrons in the conduction band of CdS could be partly injected in AA electrolyte to reduce AA\(^{+}\), although TiO\(_2\)/CdS single-sensitized structure enhanced the photocurrent evidently. When the immunosensor was incubated with IL-6–CdSe, CdSe was immobilized on the electrode via specific antibody–antigen immunoreaction, resulting in the formation of TiO\(_2\)/CdS/CdSe dual co-sensitized structure. According to Fermi-level alignment (Lee and Lo, 2009), this dual co-sensitized structure possessed stepwise band-edge levels, as shown in Scheme 2B, favoring ultrafast transfer of photogenerated electrons. Moreover, the introduction of CdSe not only further broadened the absorption to longer-wavelength light, but also effectively prevented the photogenerated electrons in the conduction band of CdS from being injected in AA electrolyte. Therefore, based on TiO\(_2\)/CdS/CdSe dual co-sensitized structure, a highly sensitive signal amplification strategy for IL-6 detection was achieved.

3.5. Photoelectrochemical detection for IL-6

IL-6 detection is based upon the competitive interaction of anti-IL-6 antibody with IL-6 antigens and IL-6–CdSe bioconjugates. All the incubation mixtures had the same volume consisting of a fixed concentration of IL-6–CdSe bioconjugates and different concentrations of IL-6 antigens. Fig. 6A shows the photocurrent responses of the immunosensor after incubation in the presence of different concentrations of IL-6. Compared to large-sized IL-6–CdSe bioconjugate, small-sized IL-6 could be more easily bound on the sensing electrode. Therefore, more IL-6 was bound on the immunosensor as the concentration of IL-6 increased, which resulted in gradually decreased photocurrent. As shown in Fig. 6B, the photocurrent linearly decreased with increase of logarithm of IL-6 concentrations in the range 1.0 pg/mL–100 ng/mL. The regression equation was 

\[
I = \frac{13.97}{C_{IL-6}}^{2.14 \log C_{IL-6}} \quad \text{(ng/mL)}
\]

with the correlation coefficient of 0.9973. The detection limit (\(S/N = 3\)) for IL-6 concentration was estimated to be 0.38 pg/mL. As shown in the inset of Fig. 6B, the actual photocurrent was about 20.68 \(\mu\)A with 0.38 pg/mL IL-6 incubation (curve a), whereas the photocurrent was 21.95 \(\mu\)A without IL-6 incubation (curve b). Thus the evident photocurrent difference of 1.27 \(\mu\)A could be observed to demonstrate the low detection limit. Compared with those of previous reports shown in Table 1, the well-designed photoelectrochemical immunoassay exhibited a lower detection limit as well as a wider linear range. Moreover, it also verified that the proposed co-sensitization strategy realized high sensitivity and was particularly promising for the detection of biomarkers for early diagnosis and disease surveillance.
3.6. Reproducibility, specificity and stability of the immunosensor

The reproducibility of the competitive immunoassay was appraised by both intra-assay (within-batch) and inter-assay (between-batch) relative standard deviation (RSD). Analyzed from the experimental results of five replicate determinations, the intra-assay RSDs were 3.2%, 2.7%, and 2.4% towards 0.1, 1, and 10 ng/mL of IL-6, respectively. The inter-assay RSDs of 4.4%, 3.6%, and 3.7% were acquired via measuring the same samples with five electrodes fabricated independently under identical experimental conditions. The results suggested a satisfactory precision and reproducibility of this immunoassay.

Specificity is an important criterion for immunoassay, since the nonspecific adsorption can influence the sensitivity. To demonstrate that the photocurrent response originated from specific binding, we selected some representative interfering proteins involved in human Interleukin-8 (IL-8), human IgG (HlgG), carcinoembryonic antigen (CEA), and prostate-specific antigen (PSA) for the interference test. The result demonstrated that the photocurrent response of the immunosensor was not affected by IL-8, HlgG, CEA, PSA and their mixture, as shown in Fig. S1. Compared to the photocurrent detected in the absence of interfering protein, the relative deviations of the photocurrents tested in the presence of single interfering protein or their mixture were well within 4.6% for the measurement of 10 ng/mL IL-6. And the relative standard deviation (RSD) of five replicate determinations for each interference test was within 4.0%. All these results indicated that the proposed immunoassay possessed a satisfactory specificity without obvious interference from nonspecific adsorption.

The stability of the designed immunosensor was also evaluated. After the immunosensor was stored in PBS (pH 7.4, 10 mM) at 4 °C in a refrigerator for over 2 weeks, no obvious change was found in photocurrent response for the detection of 1 ng/mL IL-6, demonstrating its good storage stability.

3.7. Comparison with sandwich immunoassay

Alternatively, a sandwich immunoassay can also be designed according to the proposed co-sensitization strategy (the construction detail of the sandwich immunoassay is described in Supplementary material). As illustrated in Scheme S1, the ITO/TiO2/CdS/CdSe/anti-IL-6/BSA photoelectrochemical electrode is the same as the proposed competitive immunoassay. After different concentrations of IL-6 antigens were immobilized on the photoelectrochemical electrode, CdSe QDs labeled IL-6 secondary antibodies were subsequently immobilized via the specific antibody–antigen immunoreaction for signal amplification. The photoelectrochemical test revealed that the sandwich immunoassay for human IL-6 detection exhibited a narrow linear range from 100 pg/mL to 100 ng/mL and with the detection limit of 66 pg/mL, which demonstrated that the signal amplification and sensitivity of the sandwich immunoassay was much poor than that of the competitive immunoassay (see Fig. S2). This was because the introduction of secondary antibodies further enlarged both the resistance and distance between CdSe QDs and TiO2/CdS hybrid on the sensing electrode, which resulted in the increase of electron–hole recombination and the reduction of the photocurrent.

4. Conclusions

In summary, a new promising platform on photoelectrochemical immunoassay for highly sensitive detection of biomarkers based on TiO2/CdS/CdSe dual co-sensitized structure was established. To illustrate the promising platform, competitive immunoassay for detection of human IL-6 was designed and it demonstrated that the new co-sensitization signal amplification strategy was very effective. Compared to the results reported previously, the well-designed immunoassay exhibited a lower
detected limit of 0.38 pg/mL as well as a wider linear range from 1.0 pg/mL to 100 ng/mL for IL-6 detection. The greatly enhanced sensitivity was attributed to (i) adequate utilization of light energy, (ii) ultrafast electron transfer and (iii) effective inhibition of charge recombination for the TiO2/CdS/CdSe dual co-sensitized structure. Due to easy preparation and significant signal amplification, this co-sensitization strategy can be applied also for detecting other biomarkers and has the potential for reliable prediction of cancer and other diseases.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2014.03.011.

References


