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Dual-Signal Amplification Strategy for Ultrasensitive Photoelectrochemical Immunosensing of α-Fetoprotein

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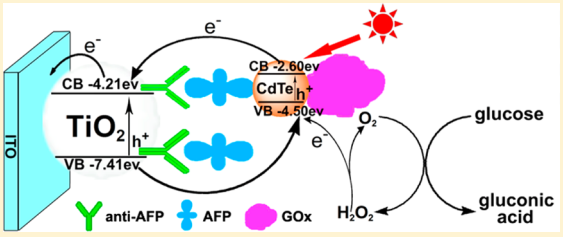
ABSTRACT: An ultrasensitive photoelectrochemical immunoassay of cancer biomarker α-fetoprotein (AFP) is proposed that uses titanium dioxide (TiO_2) coupled with AFP−CdTe−GOx bioconjugate, which featured AFP antigen and glucose oxidase (GOx) labels linked to CdTe quantum dots (QDs) for signal amplification. The synthesized CdTe QDs yielded a homogeneous and narrow size distribution, which allowed the binding of AFP and GOx on CdTe QDs. Greatly enhanced sensitivity for AFP came from a dual signal amplification strategy. First, an effective matching of energy levels between the conduction bands of CdTe QDs and TiO_2 allowed for fast electron injection from excited CdTe QDs to TiO_2 upon irradiation, which reduced the recombination process of electron−hole pairs and prompted photoelectrochemical performance. Second, GOx enzyme could catalyze glucose to produce H_2O_2, which acted as a sacrificial electron donor to scavenge the photogenerated holes in the valence band of CdTe QDs, further causing an enhanced photocurrent. Thus, on the basis of the dual signal amplification strategy, the competitive immunosensor based on the specific binding of anti-AFP antibodies to AFP and AFP−CdTe−GOx bioconjugates was achieved. This proposed biosensor for AFP possessed largely increased linear detection range from 0.5 pg/mL to 10 μg/mL with a detection limit of 0.13 pg/mL. The proposed amplification strategy shows high sensitivity, stability, and reproducibility and can become a promising platform for other protein detection.



Sensitive and accurate detection of disease-related biomarkers is critical to many areas of biomedical research and diagnosis;[1] in particular, the clinical measurement of cancer biomarkers shows great promise for early diagnosis, disease monitoring, and highly reliable prediction.[2] It also offers opportunities for understanding the fundamental biological process involved in monitoring patient response to therapy method.[3] As a tumor marker, α-fetoprotein (AFP) is an oncofetal glycoprotein with a molecular mass of approximately 70 kDa,[4] which is mainly produced by the liver, yolk sac, and gastrointestinal tract of a human fetus. However, it may also 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. Conventional immunoassays for the detection of AFP include 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] While highly accurate, some of these techniques involve disadvantages such as relatively sophisticated instruments, significant sample volume, limited sensitivity, and clinically unrealistic expense and time. Therefore, there is a real need to develop operationally simple, highly sensitive, and inexpensive methods to detect levels of the biomarkers in both normal and cancer patient sera.

As a newly developed analytical method for the detection of biomolecules, photoelectrochemical immunosensing possesses the advantages of high selectivity and sensitivity because of the separation forms of excitation source and detection signal. In addition, electronic detection instruments are simple, low-cost, and well-suited for rapid high-throughput biological assays.[13] Thus, this technique shows promising analytical applications and has attracted considerable research interest. A number of photoactive nanomaterials such as CdS,[14] CdSe,[15] ZnO,[16] and TiO_2[17] have been used in the construction of immunosensors. In particular, TiO_2-based composites have proved to be an excellent electrode material in photoelectrochemical biosensing owing to their good biocompatibility, relative conductivity, inexpensiveness, environmental safety, and chemical and thermal stability.[18] However, the wide band gap of TiO_2 (~3.2 eV, anatase) only allows it to absorb the ultraviolet light (<387 nm), which limits the utilization of solar light.[18] Besides, the strong oxidation ability of photogenerated holes formed at illuminated TiO_2 may cause destructive effects for biomolecules.[13] Hence, many efforts have been made toward the development of TiO_2-based composites in visible-light-activated photoelectrochemical biosensing, which could largely increase the efficient utilization of solar light and reduce the destructive effect of UV light and the photogenerated holes of illuminated TiO_2 to biomolecules.[19−22] Although all these

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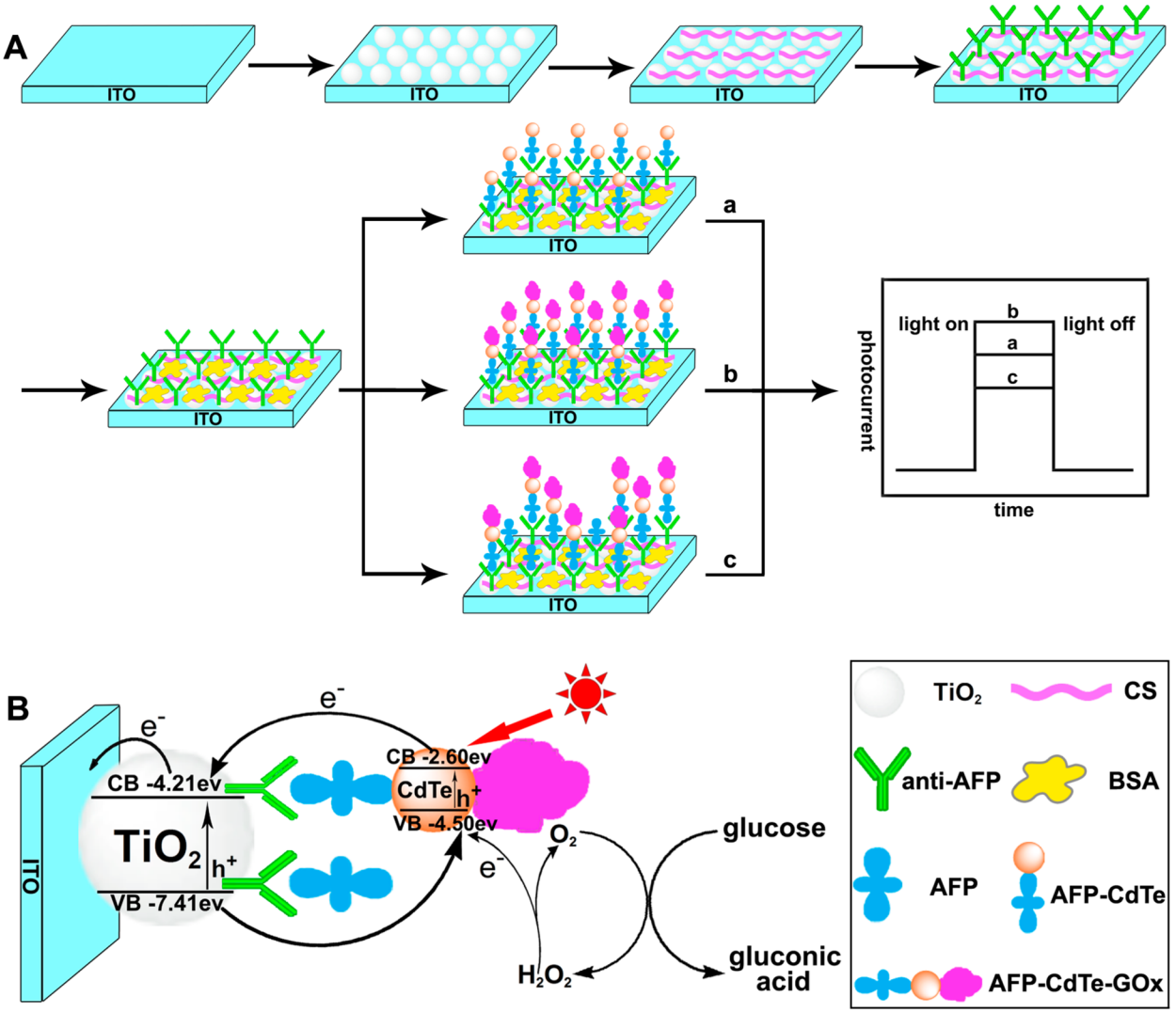
Scheme 1. (A) Fabrication Process of Photoelectrochemical Immunosensor and (B) Corresponding Electron-Transfer Mechanism



reports optimized TiO_2-based sensing materials to a certain extent, the increasing demand for early, accurate, and ultrasensitive detection of cancer biomarkers is further pushing the enhancement of detection sensitivity by signal amplification strategy.[23−25]

Herein, we describe the construction of a novel competitive photoelectrochemical immunosensor for AFP detection based on TiO_2 electrode with enhanced detection sensitivity. Chitosan as a biocompatible matrix with high permeability and good susceptibility to chemical modifications was coated on the surface of TiO_2 electrode for further immobilization of anti-AFP antibody. CdTe quantum dots (QDs) were modified with AFP and glucose oxidase (GOx) to form AFP−CdTe−GOx bioconjugate, which were used to enhance detection sensitivity by dual signal amplification via the competitive immunoreaction of AFP−CdTe−GOx and AFP with the antibodies immobilized on TiO_2 electrode. Primarily, utilization of CdTe QDs broadens the absorption of solar light to significantly reduce the destructive effect of UV light. Furthermore, effective matching of energy levels between TiO_2 and CdTe facilitates charge separation due to quick electron transfer, which amplifies the photocurrent response by the photosensitizing effect of CdTe QDs. Additionally, when the immunosensor was exposed to a solution containing glucose; the GOx can catalyze the conversion of glucose into gluconic acid and H_2O_2. The latter product acts as a sacrificial electron donor to scavenge photogenerated holes in the valence band of CdTe QDs, thereby further improving the accumulation of electrons to lead to an enhanced photocurrent. All these characteristics promote an excellent photoelectrochemical biosensor for AFP detection with high selectivity and sensitivity. Moreover, the established method can provide an approach for the design of photoelectrochemical immunosensors in the detection of other significant proteins.

EXPERIMENTAL SECTION

Materials and Reagents. Cadmium chloride ($CdCl_2·2.5H_2O$) and sodium hydroxide were purchased from Shanghai Chemical Reagent Co. (China). 3-Mercaptopropionic acid (MPA), Chitosan powder (CS, from crab cells, 85% deacetylation), glucose oxidase (GOx, EC 1.1.3.4, 158.9 units/mg, from *Aspergillus niger*), glucose, and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were all obtained from Sigma−Aldrich. TiO_2 nanoparticles (P25) were purchased from the Degussa Co. (Germany). Glutaraldehyde (GLD, 25%

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aqueous solution) was purchased from Sinopharm Chemical Reagent Co., Ltd.. Bovine serum albumin (BSA) was purchased from Nanjing Bookman Biotechnology Co. Ltd. Mouse anti-human α-fetoprotein monoclonal antibody, human α-fetoprotein, carcinoembryonic antigen (CEA), prostate-specific antigen (PSA), interleukin-6 (IL-6), and human IgG (HIgG) were obtained from Shanghai Linc-Bio Science Co. Ltd. (China). All other reagents were of analytical grade and were used as received. All aqueous solutions were prepared with deionized water (DI water, 18 MΩ/cm), which was obtained from a Milli-Q water purification system. Phosphate buffer (pH 7.2, 10 mM) was used for preparation of the antibody and antigen solution, washing buffer solution, and blocking buffer solution which contained 1% (w/v) BSA.

Apparatus. The UV−visible (UV−vis) spectrum was recorded by a Shimadzu UV-3600 UV−visible spectrophotometer. Electrochemical impedance spectroscopy (EIS) was performed on an Autolab potentiostat/galvanostat (PGSTAT 30, Eco Chemie B.V., Utrecht, The Netherlands) with a three-electrode system in KCl solution (0.1 M) containing $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (5 mM, 1:1) mixture as a redox probe, and recorded at an open circuit potential of 192 mV with an amplitude of 5 mV over a frequency range of 0.01 Hz−100 kHz. Photoelectrochemical measurements were performed with a homemade photoelectrochemical system. A 500 W Xe lamp, with a spectral range of 200−2500 nm, was used as the irradiation source with light intensity of 400 $\mu W \cdot cm^{-2}$ estimated by a radiometer (Photoelectric Instrument Factory, Beijing Normal University). Photocurrent was measured on a CHI 660D electrochemical workstation (Shanghai Chenhua Apparatus Corp.) with a conventional three-electrode system, where the modified indium tin oxide (ITO) electrode with an area of 0.25 cm^2 was employed as the working electrode, a Pt wire served as counterelectrode, and a saturated Ag/AgCl electrode was used as reference electrode. All photocurrent measurements were carried out at a constant potential of −0.4 V (vs saturated Ag/AgCl electrode) in phosphate buffer solution (pH 7.4, 0.1 M) containing glucose (60 mM) at room temperature. Thermostatic shaker and water bath were used to control the temperatures during the sample preparation and biosensor fabrication processes, respectively.

Synthesis of CdTe Quantum Dots, AFP−CdTe, and AFP−CdTe−GOx. CdTe quantum dots (QDs) functionalized with MPA were prepared according to the previous protocol.[26] The 5 mL of resulting colloid was centrifuged in a Millipore ultrafiltration centrifuge tube (MWCO = 3000) at 5000 rpm for 30 min twice, and the final fixed volume was 1 mL. The CdTe QDs were first activated by adding 10 mg of EDC into 1 mL of CdTe QDs solution at room temperature for 15 min. Subsequently, 10 μL of AFP solution (0.84 mg/mL) was added into the above solution and mixed gently at 30 °C for 3 h, followed by stored at 4 °C in the refrigerator overnight. The bioconjugate AFP−CdTe was finally obtained via centrifugation of the above solution at 6000 rpm for 15 min in a Millipore ultrafiltration centrifuge tube (MWCO = 100 000) and dilution to 1 mL with 10 mM phosphate buffer (pH 7.2). The bioconjugate AFP−CdTe−GOx was freshly prepared by adding 10 mg of EDC into 1 mL of AFP−CdTe solution, with gentle mixing at room temperature for 15 min, followed by addition of 100 μL of GOx solution (6 mg/mL). The mixture was incubated at 30 °C for 3 h and left overnight at 4 °C in the refrigerator. Finally, it was centrifuged at 6000 rpm for 15 min in a Millipore ultrafiltration centrifuge tube (MWCO = 100 000) and diluted to 1 mL with 10 mM phosphate buffer (pH 7.2). The resulting AFP−CdTe and AFP−CdTe−GOx were stored at 4 °C when not in use.

Fabrication of Photoelectrochemical Immunosensor. The photoelectrochemical immunosensor was fabricated on an ITO slice (type JH52, Beijing Zhongjingkeyi Technology Co., Ltd., Nanjing, China; ITO coating 30 ± 5 nm, sheet resistance ≤10 $\Omega \cdot square^{-1}$). Briefly, the ITO electrodes were sonicated in acetone, NaOH (1 M) in ethanol/water mixture (1:1, v/v), and water, respectively, for 15 min each. The fabrication procedure of the immunosensor is shown in Scheme 1A. First, a certain amount of TiO_2 powder was ultrasonically dispersed in water, and then 20 μL of the homogeneous suspension was applied onto a piece of ITO slice. After drying in air, the film was sintered at 450 °C for 30 min in air and finally cooled down to room temperature. In order to obtain TiO_2 modified electrode with different thickness, the concentrations of TiO_2 suspension were varied between 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/mL. CS solution (0.05 wt %) was prepared by dissolving chitosan powder in 1% acetic acid. Twenty microliters of CS solution was dropped on ITO/TiO_2 electrode and dried at room temperature. After the ITO/TiO_2/CS electrode was washed with 1 M NaOH and deionized (DI) water, respectively, 20 μL of GLD (5%) solution was dropped onto the electrode and remained at room temperature for 30 min. Then the electrode was rinsed with DI water thoroughly to remove physically adsorbed GLD. The AFP antibodies (anti-AFP) were introduced onto the ITO/TiO_2/CS/GLD-activated electrode by dropping 25 μL of antibody (Ab, 50 μg/mL) dissolved in phosphate buffer solution (10 mM, pH 7.2) onto the electrode and allowing it to incubate at 4 °C for at least 12 h. Finally, it was rinsed with a washing buffer solution and then incubated with 20 μL of blocking buffer solution at 37 °C for 30 min to block nonspecific binding sites. Subsequently, the electrode was rinsed with washing buffer solution and used as a photoelectrochemical immunosensor, and incubated in a 20 μL of mixture containing 10 μL of AFP−CdTe−GOx and 10 μL of different concentrations of AFP at 37 °C for 40 min. Finally the electrode was rinsed with washing buffer solution and ready to be used.

Preparation of Clinical Samples. Serum specimens from healthy persons and clinically diagnosed patients with liver carcinoma were provided by the Affiliated Drum Tower Hospital of Nanjing University, and measured by the same procedure applied for AFP detection above. In order to cover the wide linear detection range of the proposed immunosensor, the sera were used either as received or diluted appropriately with 10 mM phosphate buffer solution (pH 7.2) before assay.

RESULTS AND DISCUSSION

Photoelectrochemical Properties of ITO/TiO_2 Electrode. As an n-type, wide-band-gap, and environmentally friendly semiconductor, TiO_2 possesses good biocompatibility and stability. Furthermore, owing to its large surface area and unique chemical, physical, and electronic properties, TiO_2 is suitable for constructing sensing devices, especially electrochemical sensors and biosensors.[18] Due to availability of nanomaterials, tremendous improvements have been achieved in terms of the sensitivity, selectivity, and reproducibility of the biosensors based on TiO_2.[18] Given the potential of photoelectrochemistry as a sensitive analytical method, coupling with the selectivity of immunoreactions could bring a new exciting detection technique to the analysis of biomolecules.[13,17] A well-

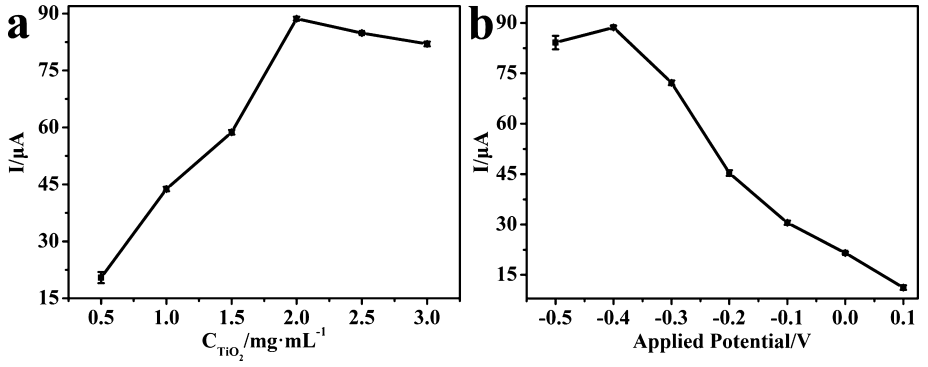

Figure 1. Effects of (a) varied concentrations of titanium dioxide suspension and (b) applied potentials on photocurrent responses of ITO/TiO_2 electrode measured in 0.1 M phosphate buffer solution (pH 7.4) containing 60 mM glucose.

established relationship between the photocurrent response of photoactive materials and the concentration of analyte makes the construction of photoelectrochemical biosensors possible.[19−22] For applications of TiO_2 in photoelectrochemical biosensing, optimal photocurrent intensity will be desirable. In order to optimize the thickness of TiO_2 film on the surface of ITO electrode, different concentrations of TiO_2 suspensions were used to prepare TiO_2 films. As shown in Figure 1a, the photocurrent of TiO_2-modified electrode increased with increasing concentrations of TiO_2 suspension from 0.5 to 2.0 mg/mL, and then a slightly decreasing photocurrent was observed upon further increase of its concentration. TiO_2-modified electrode made by 2.0 mg/mL suspension showed the highest photocurrent intensity. Increasing the concentration of TiO_2 suspension could increase the thickness of the TiO_2 film, which would result in more photoactive materials and light absorption. As a result, photocurrent was increased. However, the diffusion resistance for electron motion also increased in thicker TiO_2 films, which made the photocurrent decrease with increasing TiO_2 film thickness.[13] Thus, the optimal concentration of 2.0 mg/mL TiO_2 suspension was used for preparing the biosensor in the following experiments.

The applied potential is an important parameter for producing the photocurrent. As shown in Figure 1b, when the concentration of TiO_2 suspension was 2.0 mg/mL, the photocurrent was enhanced sharply with the decrease of applied potential from 0.1 to −0.4 V, and then it decreased slightly with more negative applied potential. Because the energy level of conduction band of TiO_2 is around −0.5 V versus normal hydrogen electrode (NHE),[27] the intensity of resulting anodic photocurrent can be controlled by the applied potentials.[28] The more negative the potentials, the less energy difference between the conduction band of TiO_2 and the electrode, which makes the electron transfer much faster and easier. This indicated that the photogenerated electrons were effectively driven to the counterelectrode by the negative potential, which could be beneficial to the photogenerated electron−hole separation, leading to increased lifetime of photogenerated electrons and thus finally an enhanced photocurrent response. However, much more negative applied potential could not further increase the photocurrent intensity due to the energy level of the conduction band of TiO_2. Therefore, −0.4 V was selected as the applied potential for photoelectrochemical experiments to obtain relatively larger photocurrent and consequently better sensitivity of the proposed biosensor.

Characterization of AFP−CdTe−GOx Bioconjugate. The formation of AFP−CdTe and AFP−CdTe−GOx bioconjugates was demonstrated with UV−visible absorption spectroscopy. Figure 2 shows the absorption spectra of CdTe

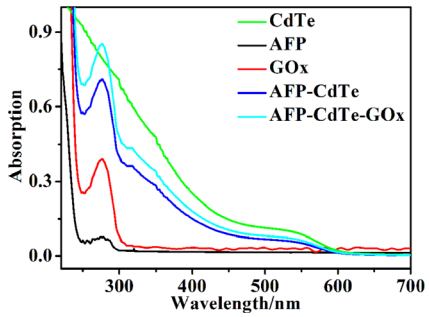

Figure 2. UV−visible adsorption spectra of CdTe quantum dots, AFP, GOx, AFP−CdTe, and AFP−CdTe−GOx bioconjugates.

QDs, AFP, GOx, AFP−CdTe, and AFP−CdTe−GOx bioconjugates. CdTe QDs exhibited a broad absorption from 450 to 600 nm. The characteristic peak of AFP and GOx appeared at 280 nm, which corresponds to $\pi-\pi^*$ transition in the tryptophan and tyrosine residues of the proteins.[29] After CdTe QDs were modified with AFP without or with GOx, two absorption peaks were simultaneously observed in comparison with those of pure CdTe QDs and proteins. All these results confirmed that AFP−CdTe and AFP−CdTe−GOx were successfully obtained.

Optimization of Experimental Conditions. In the competitive immunoassays, incubation temperature and time for the antibody−antigen interaction greatly influence the sensitivity of the developed immunosensor.[13,23] Figure 3a shows the effect of incubation temperature on the photocurrent responses for the AFP−CdTe−GOx bioconjugate, which was examined at different temperatures ranging from 20 to 50 °C. The photocurrent responses increased with increasing temperature up to 37 °C, which is attributed to the increasing immunoreaction rate between anti-AFP antibodies and AFP−CdTe−GOx bioconjugate. The enhanced photocurrent responses could be ascribed to more AFP−CdTe−GOx bioconjugate immobilized onto electrode. As shown in Scheme 1B, a greater amount of CdTe can lead to much more efficient light absorption and consequently increase the photocurrent response by more electron injection from the excited CdTe to the conduction band of TiO_2, due to a perfect matching of energy levels between the two semiconductors. Besides, when

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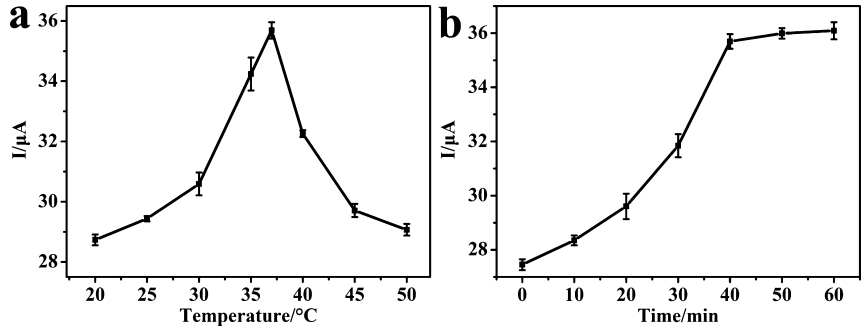

Figure 3. Effects of (a) incubation temperature and (b) incubation time on photocurrent responses of ITO/TiO_2/CS/anti-AFP/BSA electrode in the presence of a mixture containing 10 μL of AFP−CdTe−GOx and 10 μL of 10 mM phosphate buffer solution (pH 7.2), which were carried out in 0.1 M phosphate buffer solution (pH 7.4) containing 60 mM glucose.

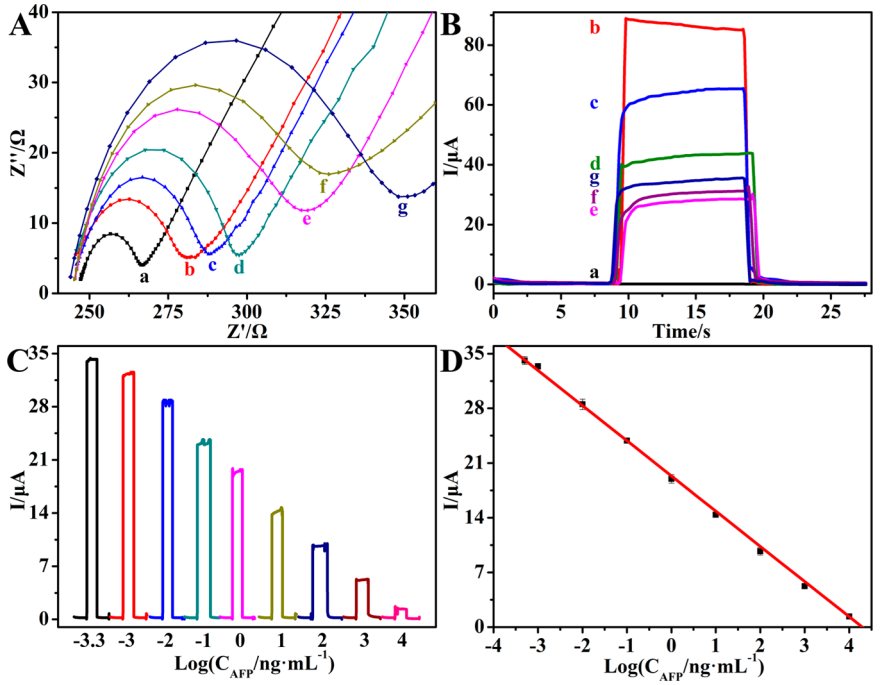

Figure 4. (A) Nyquist plots of electrochemical impedance spectroscopy (EIS) and (B) corresponding photocurrent responses of the modified ITO electrode: (a) ITO, (b) ITO/TiO_2, (c) ITO/TiO_2/CS, (d) ITO/TiO_2/CS/anti-AFP, (e) ITO/TiO_2/CS/anti-AFP/BSA, and (f, g) ITO/TiO_2/CS/anti-AFP/BSA electrode after incubation with 20 μL of (f) AFP−CdTe or (g) AFP−CdTe−GOx bioconjugate (5.11 μg/mL vs AFP). (C) Photocurrent responses and (D) corresponding calibration curve of ITO/TiO_2/CS/anti-AFP/BSA electrode in the presence of mixture containing 10 μL of AFP−CdTe−GOx bioconjugates and 10 μL of different concentrations of AFP, which were measured in 0.1 M phosphate buffer solution (pH 7.4) containing 60 mM glucose.

the immunosensor was exposed to the glucose solution, the GOx could catalyze the conversion of glucose into gluconic acid and H_2O_2.[23] The latter product acted as a sacrificial electron donor to deplete the photogenerated holes located at the valence band of CdTe and improved the efficiency of charge separation. The increasing amount of GOx immobilized on the electrode resulted in a higher yield of electron donor H_2O_2. Subsequently, the scavenging of photogenerated holes was enhanced, and thus the photocurrent was further increased. However, when temperature was over 37 °C, the photocurrent response decreased. The reason may be that the high temperature caused an irreversible denaturation of glucose oxidase. In addition, temperatures lower than 37 °C could reduce the immunoreaction rate; thus the incubation time should be prolonged. In the following experiments, 37 °C was employed as the optimal incubation temperature for further studies.

As shown in Figure 3b, the photocurrent responses increased with incubation time and reached a plateau at 40 min. An incubation time longer than 40 min did not sharply improve the response. Consequently, 40 min was chosen as the incubation time for determination of AFP.

Construction of Photoelectrochemical Immunosensor. As an effective tool for characterizing the interface properties of electrodes, electrochemical impedance spectroscopy (EIS) was used to monitor the biosensor fabrication process.[23] Figure 4A displays the Nyquist diagrams of each electrode involved during every construction step of the photoelectrochemical biosensor. The electron-transfer resistance (R_{et}) which equals the semicircle diameter, reflects the restricted diffusion of the redox probe through the multilayer system related directly to film permeability. The probe showed a low R_{et} at a bare ITO electrode. After TiO_2 was drop-cast onto the surface of ITO electrode, R_{et} got much bigger,

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indicating that the compact film hindered the access of the redox probe to the electrode surface. Subsequently, chitosan, anti-AFP antibodies, and BSA were constructed on the TiO_2 film step by step, and the R_{et} of each related electrode increased correspondingly. Given the semiconductor nature of CdTe and the insulating property of protein, R_{et} increased gradually after AFP−CdTe and AFP−CdTe−GOx bioconjugates were immobilized separately on the photoelectrochemical biosensor. Due to the elevating hindrance effect and insulating effect introduced by GOx progressively, the larger R_{et} of AFP−CdTe−GOx bioconjugates suggested a successful formation of AFP−CdTe−GOx bioconjugates and stepwise fabrication of the proposed biosensor.

The fabrication of the immunosensor could also be monitored by photoelectrochemical experiments[13] and showed in Figure 4B. After TiO_2 was drop-cast onto a bare ITO surface, the photocurrent intensity increased significantly, demonstrating that TiO_2 is a great choice for the construction of a photoelectrochemical biosensor. Subsequently, the photocurrent intensity decreased gradually after immobilization of chitosan, anti-AFP antibodies, and BSA. This could be explained by the fact that the immobilization of these organic materials on the ITO/TiO_2 electrode hindered the diffusion of glucose to the surface of TiO_2 for reaction with the photogenerated holes and resulted in decreased photocurrent intensity. After blocking with BSA, the as-obtained immunosensor was incubated with AFP−CdTe bioconjugates, and the photocurrent response increased slightly. This could be ascribed to the fact that, after immobilization of AFP−CdTe bioconjugates on the immunosensor through specific antibody−antigen immunoreactions, CdTe absorbed more light, which led to separation of photogenerated electron−hole pairs followed by fast electron injection from the excited CdTe to the conduction band of TiO_2. Because the nonconductive properties of organic biomolecules immobilized previously would obstruct electron transfer, the photocurrent intensity did not increase significantly. However, the photocurrent intensity still increased to a certain extent as a result of the photosensitizing effect of CdTe QDs, causing the first signal amplification. As a proof-of-concept, the AFP−CdTe−GOx bioconjugates were also immobilized on the developed immunosensor through specific antibody−antigen immunoreactions after blocking with BSA. It was found that the photocurrent intensity further increased, which could be attributed to the fact that GOx could sufficiently catalyze glucose in the detection solution into gluconic acid and H_2O_2. The latter product acted as a much more effective electron donor than glucose to deplete the photogenerated holes located at the valence band of excited CdTe and facilitated the charge separation, thus further increasing the photocurrent intensity, resulting in the second signal amplification. Therefore, a dual signal amplification strategy for ultrasensitive detection of AFP was achieved by the synergistic reaction of CdTe and GOx, and on the other hand this indicated a successful synthesis of AFP−CdTe and AFP−CdTe−GOx bioconjugates, respectively.

Quantitative Determination of AFP. AFP detection is based on the competitive interaction of anti-AFP antibodies with AFP and AFP−CdTe−GOx bioconjugates. Hence, by tracking the photocurrent related to the amount of AFP, a novel competitive immunoassay can be accomplished based on the proposed dual signal amplification strategy. The experiment was conducted by mixing equal volumes of certain concentrations of AFP and a fixed concentration of AFP−CdTe−GOx bioconjugates together, and then this mixture was applied to the developed immunosensor after blocking with BSA. Figure 4C displays the typical photocurrent responses of the photoelectrochemical biosensor in the presence of different concentrations of AFP, whereas Figure 4D shows the derived calibration curve. Since AFP is much smaller than the AFP−CdTe−GOx bioconjugate, more AFP was immobilized on the biosensor with increasing concentrations, leading to decreased photocurrent intensity. The photocurrent decrement was proportional to the logarithmic value of AFP concentration, ranging from 0.5 pg/mL to 10 μg/mL with a correlation coefficient of 0.9997. The regression equation was $I = -4.60 \log C_{AFP} + 19.2$, where I was the photocurrent of ITO/TiO_2/CS/anti-AFP/BSA electrode incubated with a mixture containing 10 μL of AFP−CdTe−GOx and 10 μL of different concentrations of AFP. The detection limit for AFP concentration is 0.13 pg/mL, which is much lower than those of previous methods such as ELISA (12.8 ng/mL),[7] electrochemical (0.8 ng/mL),[8] ECL (3.3 pg/mL),[9] mass spectrometric (0.2 ng/mL),[10] and photoelectrochemical (40 pg/mL)[13] immunoassays. The proposed competitive photoelectrochemical immunosensor showed a much wider linear range as well as lower detection limit. This demonstrates that, by integrating the sensitivity of photoelectrochemistry and the specificity of immunoreactions, this novel dual signal amplification strategy would bring a new insightful prospect to photoelectrochemical biosensors.

Reproducibility, Specificity, Stability, and Application in Real Sample. The reproducibility of an assay was expressed in terms of values for a within-batch (intra-assay) and a between-batch (interassay) relative standard deviation (RSD). Analyzed from the experimental results, the intra-assay RSDs were 1.4%, 1.6%, and 2.0% at AFP concentrations of 0.1, 10, and 100 ng/mL, respectively. The interassay RSDs of 1.0%, 0.8%, and 1.5% were obtained by measuring the same samples with five electrodes prepared independently under identical experimental conditions. The results indicated a satisfactory precision and reproducibility of the proposed protocol.

Usually, nonspecific adsorption is a major problem in immunosensing, since it cannot be distinguished from specific adsorption and consequently influences the sensitivity. In order to prove that the as-observed photocurrent changes arise from specific interaction between the antibody and the antigen, controllable experiments were performed. The photocurrent responses of BSA-blocked photoelectrode in the presence of a mixture containing AFP−CdTe−GOx and 10 ng/mL AFP without or with 100 ng/mL carcinoembryonic antigen (CEA), prostate-specific antigen (PSA), interleukin 6 (IL-6), and human IgG (HIgG) are shown in Figure 5. As can be seen, compared to the result obtained in the presence of only AFP−CdTe−GOx and AFP, no significant photocurrent differences were found with the single interfering protein, although its concentration is 10 times of that of AFP. The specificity of the proposed protocol was further evaluated by measuring the photocurrent response of mixture containing AFP−CdTe−GOx, 10 ng/mL AFP, and 100 ng/mL CEA, PSA, IL-6, and HIgG. No obvious photocurrent difference could be observed when the mixed sample contained four different interfering proteins and each concentration was 10-fold that of AFP. All these results demonstrate that the observed photocurrent is mainly due to the specific binding without obvious interference from nonspecific adsorption, and as a result, the high sensitivity of this dual signal amplification strategy is revealed.

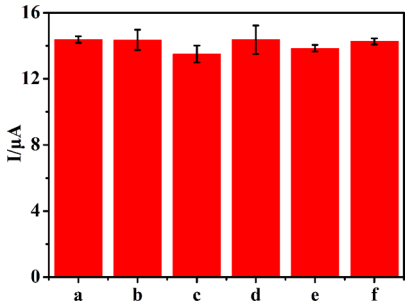

Figure 5. Selectivity of the proposed immunoassay in the presence of a mixture containing AFP−CdTe−GOx and 10 ng/mL AFP (a) alone or with (b) 100 ng/mL carcinoembryonic antigen (CEA) (c) prostate-specific antigen (PSA), (d) interleukin 6 (IL-6), or (e) human IgG (HIgG), and (f) all six substances mixed together. Error bars show the standard deviation of five replicate determinations.

The long-term stability of the competitive immunosensor was also examined. When the anti-AFP antibody-modified immunosensor was stored in a dark and moisturizing environment at 4 °C over 2 weeks, no apparent change in photocurrent response in the detection of 10 ng/mL AFP was found, illustrating its good long-term storage stability.

The feasibility of this dual signal amplification strategy for clinical application was investigated on several real samples of human serum. In order to cover the wide linear detection range of the proposed protocol, these serum samples were used either as-received or diluted to a specific concentration with 10 mM phosphate buffer solution, pH 7.2. The comparison results for 0.00200, 0.0200, 0.200, 3.90, 47.7, and 217.8 ng/mL AFP are listed in Table 1. By comparison with the ELISA method, the

Table 1. Comparison Assay Results of Clinical Serum Samples for ELISA and the Proposed Method

serum sample	ELISA (ng/mL)	proposed method (ng/mL)	rel deviation (%)
1	0.00200	0.00203	1.5
2	0.0200	0.0198	−1.0
3	0.200	0.206	3.0
4	3.90	3.76	−3.6
5	47.7	47.6	−0.2
6	217.8	216.6	−0.6

RSDs obtained from the developed method showed acceptable feasibility to detect AFP in human serum. Satisfactory results were achieved, proving the potentiality of this dual signal amplification strategy based on a synergistic effect of CdTe and GOx for practical applications with good sensitivity and selectivity.

CONCLUSIONS

An ultrasensitive photoelectrochemical immunosensor of cancer biomarker α-fetoprotein (AFP) was developed on titanium dioxide (TiO_2) electrode. AFP and glucose oxidase (GOx) could be easily bound to CdTe quantum dots (QDs) to yield AFP−CdTe−GOx bioconjugate. By introducing AFP−CdTe−GOx biocongujate in a competitive immunoreaction of AFP antibody and AFP antigen, a dual signal amplification strategy was achieved, based on the synergistic effect of CdTe and GOx. The greatly enhanced sensitivity was obtained first by the fast electron migration from excited CdTe QDs to TiO_2 upon irradiation due to the effective matching of energy levels between TiO_2 and CdTe QDs. In addition, the GOx-catalyzed reaction of glucose depleted the photogenerated holes by the reaction product of H_2O_2 and further prompted the photocurrent response, causing an excellent photoelectrochemical performance. The resulting immunosensor for quantitative detection of AFP possesses high sensitivity, good reproducibility, and long-term stability. This proposed method can be expanded readily for detecting other cancer biomarkers and has the potential for reliable diagnosis of cancer and other diseases.

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Notes

The authors declare no competing financial interest.

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