A photoelectrochemical immunosensor for detection of α -fetoprotein based on Au-ZnO flower-rod heterostructures

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Abstract:

In this work, a novel label free photoelectrochemical (PEC) immunosensor has been developed for the detection of α -fetoprotein (AFP). The immunosensor was based on Au-ZnO flower-rods (FRs) heterostructure, where Au nanoparticles (NPs) were firstly electrodeposited by cyclic voltammetry methods. Scanning electron microscopy (SEM), X-ray diffraction (XRD), Mott-Schottky plot (MS), UV-vis diffuse reflectance spectrum and fluorescence emission spectrum were used for the characterizations of Au-ZnO FRs. The results demonstrated that Au NPs not only obviously enhanced the visible light absorption of ZnO FRs due to surface plasmon resonance (SPR) but also improved the separation of photo-generated electron-hole pairs. Therefore, the photocurrent of Au-ZnO FRs was increased under simulated sunlight. The photocurrent was reduced after the specific antibody-antigen immune reaction. And the photocurrent decrement was linear with the logarithm of AFP antigen concentration in the range from $0.005 \text{ ng}\cdot\text{mL}^{-1}$ to $50 \text{ ng}\cdot\text{mL}^{-1}$ with a low detection limit of $0.56 \text{ pg}\cdot\text{mL}^{-1}$ ($S/N = 3$). The PEC immunosensor also exhibited high anti-interference property and acceptable stability. This work would provide a promising photoelectrochemical strategy for the detection of other proteins in clinical diagnosis.

Keywords: ZnO flower-rods, Au nanoparticles, Surface plasmon resonance, Photoelectrochemical immunosensor, α -Fetoprotein

1. Introduction

As a sort of oncofetal proteins used for tumor marker widely, α -fetoprotein (AFP) content is selected as a key clinical indicator diagnosing primary liver cancer [1]. Normal concentration of AFP in the health human body is less than $20 \text{ ng}\cdot\text{mL}^{-1}$, while markedly increases to approximately $700 \text{ ng}\cdot\text{mL}^{-1}$ in pathological states [2,3]. Existed techniques for AFP diagnosis, such as electrochemical method [4] and chemiluminescence immunoassay [5], are energy-exhausting, time-consuming and consequently low-efficiency, though detection sensitivity are relatively acceptable [6]. Encouragingly, photoelectrochemical (PEC) sensing, as a innovatively developed technique, has attracted substantial attention because of its shorter time of diagnosis and excellent efficiency of detection.

Essentially, PEC sensing is concerning charge transfer between analyte, photoactive material and electrode under light irradiation [7]. In contrast with optical detection and electro-chemiluminescence approaches, PEC sensor shows higher sensitivity due to the separation of excitation source and detection, which can significantly remove unexpected background noise [6,8]. For the reason, PEC bioassay constructs various elegant routes for biological recognition events [9], such as the detection of DNA [10-13], protein [14-16] and small molecules [17]. Nevertheless, there are a few undesirable drawbacks in PEC technique, for instance toxic side-effect and environmental pollution triggered by usage of dyestuff and organic compounds in detecting process [8]. A problem-solving direction is using inorganic photoactive semiconductors like ZnO [18-21], TiO_2 [22-25], and SnO_2 [26] to instead of organic materials. This approach can not only improve PEC performance, but also tackle issues existing in traditional techniques mentioned above.

As a typical semiconductor material, ZnO with a wide band gap of 3.37 eV has been widely investigated due to its potential wide-ranging applications [27]. ZnO has broad applications including photocatalyst, solar cell, PEC sensor and nanogenerator attributing to its biocompatibility, high surface-to-volume ratio and excellent electron transfer rate which can evoke the hidden electrochemical ability of biomolecules

[28,29]. Whereas, the wider application of ZnO is restricted by its swift recombination of charge carriers, photo-corrosion and limited visible light absorbance [30]. An effective approach is loading noble metals (such as Au nanoparticles) on the surface of semiconductor nanostructures for improving the physicochemical properties [31]. Au nanoparticles (NPs), which present impressive features of high electrical conductivity, great biocompatibility and large surface area, currently draw broad interests in field of nano-scale electronics [32,33]. Furthermore, it shows SPR during PEC process, which can substantially enhance light absorption and improve PEC performance [34,35]. Xu and his team-members [36] designed a PEC system applied Au NPs functionalized with nucleic acid on the strand as novel energy-transfer nanoprobe. Ahmad and his colleagues [31] firstly proposed Au NPs functionalized flower-like ZnO hybrid nanostructures, which exhibited a great improvement in photocatalytic efficiency and lithium storage capacity compared to pure ZnO.

In this work, Au-ZnO FR heterostructures were prepared via electrodeposition, and a sensitive label free PEC immunosensor based on Au-ZnO FRs was fabricated for the determination of AFP with nontoxicity. Au NPs could enhance the light absorption of ZnO FRs in the visible spectral range due to SPR and improve the separation of photo-generated charge carriers, consequently strengthen the PEC conversion efficiency of Au-ZnO FRs [32,37,38]. The results indicated that ZnO FRs modified by Au NPs were with good photoelectrochemical activity, biocompatibility and stability, and provided protein and biomolecule detection with better strategy.

2. Experimental

2.1 Materials and reagents

Polyethylenimine (PEI, 99%) was purchased from Shanghai Aladdin biochemical Polytron Technologies Inc. $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (99%), $\text{Zn}(\text{Ac})_2 \cdot 2\text{H}_2\text{O}$ (99%), hexamethylenetetramine (HMTA, 99%) and ammonium hydroxide ($\text{NH}_3 \cdot \text{H}_2\text{O}$,

25-28%) were purchased from Sinopharm Group Chem. Re. Co., Ltd. (Shanghai, China). Albumin from bovine serum (BSA) was acquired from BBI Life Science Co. (HK, China). AFP, anti-AFP (monoclonal) and carcinoembryonic antigen (CEA) were purchased from Nanjing Sanchen Biotechnology Co., Ltd. (China). Dopamine was attained from Sigma-Aldrich Group Chem. Re. Co., Ltd. (China). ITO substrates ($15 \Omega \cdot \text{sq}^{-1}$) were obtained from Foshan Meijingyuan Glass Technology Co., Ltd. (Guangdong, China). The supporting electrolyte was 0.1 M phosphate buffer saline (PBS) containing 0.1 M KCl (pH 7.4). The washing buffer solution was 0.01 M PBS (pH 7.4) containing 0.1 M KCl. All chemical reagents without mentioned were of analytical grade and all aqueous solutions were prepared with deionized water.

2.2 Apparatus

Surface morphology of the sample was characterized with field-emission scanning electron microscopy (FE-SEM, Hitachi S4800, Japan). X-ray diffraction (XRD, X'pert, Philips, Holland) pattern of crystalline structure was measured in the range of $2\theta = 10^\circ - 80^\circ$. UV-vis diffuse reflectance spectrum (DRS) was obtained by a UV-2500 spectrophotometer. Fluorescence measurement was performed at room temperature on a Cary Eclipse fluorescence spectrometer (Agilent Technologies) in a 300 μL quartz cuvette. PEC measurement was proceeded by a home-built PEC system. Photocurrent was surveyed using the amperometric $I-t$ curve experimental technique on a CHI660D electrochemical workstation (CH Instruments, Shanghai, China) applying a potential of 0 V under simulated sunlight irradiation. A 300 W Xenon lamp (HSX-UV 300) was acted as the irradiation source. The electrochemical experiments were carried on at room temperature utilizing an electrochemical workstation (CHI660D) with a three-electrode mode: the as-prepared PEC immunosensor electrode was used as the working electrode, with Ag/AgCl as the reference electrode, and platinum wire as the counter electrode.

2.3 Fabrication of Au-ZnO flower-rod heterostructure electrode

ITO-based hierarchical ZnO architecture was synthesized in method developed by

previous works [39]. In briefly, ITO substrates ought to be cleaned stepwise with sonication in detergent, acetone, ethanol, and deionized water, and dried in a oven. Then, the substrates were seeded by spin coating (1000 rpm, 39s) with 5 mM $\text{Zn}(\text{Ac})_2 \cdot 2\text{H}_2\text{O}$ in ethanol, followed by thermal decomposition at 300 °C for 20 min. The obtained substrates were placed into growth solution, which consisted of 30 mM $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 12.3 mM HMTA, 0.6 mM PEI and 0.65 M $\text{NH}_3 \cdot \text{H}_2\text{O}$ at 85 °C for 2 h. Finally, for strengthening crystallinity, ZnO samples must be calcined at 450 °C for 30 min. The electrochemical deposition was proceeded as follows: 1) immersing the synthesized ZnO FR heterostructures electrode into 0.1 M KCl aqueous solution that contained 1 mM HAuCl_4 ; 2) depositing by cyclic voltammetry (CV) method in traditional three electrode system, in which platinum wire, saturated silver chloride electrode and prepared ZnO/ITO were as auxiliary electrode, reference electrode and working electrode, respectively. The deposited electrode essentially was pink, and dried at 60 °C for 10 min.

2.4 Immunosensor construction

Firstly, the AFP antibodies (Ab) were introduced by dropping 10 μL of 100 $\mu\text{g} \cdot \text{mL}^{-1}$ antibody onto Au-ZnO FRs electrode and then incubated at 37 °C for 2 h. The Ab/Au-ZnO electrode was obtained after rinsing for several times with washing buffer solution. Next, 15 μL of 1 wt% BSA blocking solution was introduced onto Ab/Au-ZnO FRs electrode and incubated at 37 °C for 30min to block nonspecific binding sites. After being rinsed with distilled water, the electrode (named as BSA/Ab/Au-ZnO) was served as a PEC immunosensor and incubated with 10 μL of AFP antigen (Ag) solution at 37 °C for 1 h. Before the implementation of photocurrent measurement, the last electrode (named as Ag/BSA/Ab/Au-ZnO) should be rinsed with washing buffer solution for several times. Once preparation procedures above were completed, for evaluating detection performance of PEC biosensor, the modified electrode was rinsed with 0.1 M PBS (pH 7.4) carefully, then placed into 0.1 M PBS (pH 7.4) for PEC detection on the applied potential of 0 V. Under simulated sunlight, the detection experiments were conducted at room temperature through a

conventional three electrode system where platinum wire, saturated silver chloride electrode and PEC biosensor with ITO were as auxiliary electrode, reference electrode and working electrode correspondingly.

3. Results and discussion

3.1 Mechanism of the immunosensor based on Au-ZnO flower-rod heterostructures

Scheme 1A referred to modification process of Au-ZnO/ITO electrode. As Scheme 1A shown, Au NPs were firstly electrodeposited onto ZnO FRs. Then Au-ZnO FRs were immobilized with Ab, and the obtained electrode was blocked by BSA. Consequently, PEC immunosensor was formed and used to detect AFP antigen via specific antibody-antigen immunoreactions. In Scheme 1B, Au NPs not only provided biocompatible environment for Ab in immunoassay as carrier [1], but also enhanced photocurrent and improved PEC performance as photo-sensitive materials under visible light. For ZnO FRs, the electron transfer from valence band to conduction band only worked under UV light. However, for Au-ZnO FRs, the photo-generated electron attributing to SPR directly transferred from Au NPs to conduction band of ZnO under visible light irradiation. Further, the excellent conductivity of Au NPs could strengthen electron transfer on interface. As a result, photocurrent was improved.

Preferred position for Scheme 1

3.2 Structure analysis and optical performance of Au-ZnO FRs

Morphological analyses of ZnO FRs and Au-ZnO FRs were shown in Fig. 1A and 1B, respectively. Fig. 1A exhibited some large flowers covering the surface of multinumber of ZnO nanorods. For Au-ZnO FRs (Fig. 1B), large amount of Au NPs spreaded on the surface of ZnO, and they were in a dispersive situation. The satisfactory dispersity was due to the higher Fermi level of ZnO, which can lead greater electronic interaction with Au particles and inhibit Au agglomeration during electrodeposition process. Fig. 1C depicted the crystallinity characterization of Au-ZnO FRs. Three peaks at 31.8° , 34.4° and 36.3° stood for (1 0 0), (0 0 2) and (1 0 1) planes of hexagonal phase ZnO (JCPDS 36-1451), respectively.

Four peaks at 38.2°, 44.4°, 64.6° and 77.5° assigned to Au (JCPDS 04-0784) were clearly observed for Au-ZnO FRs, which revealed the presence of metallic Au particles on ZnO. XRD pattern of crystalline structures manifested that Au-ZnO FRs were successfully synthesized by electrodeposition. With respect to photovoltaic effect of Au-ZnO FRs, Mott-Schottky (MS) analysis was conducted, for which was scanned in a 1 M KOH solution and proceeded from positive to negative voltage, with step of 50 mV·s⁻¹ and frequency of 3 kHz. Flat-band potential (E_{FB}) was estimated from the following MS equation [40]:

$$\frac{1}{C^2} = \frac{2}{q\epsilon\epsilon_0N_d} \left(E - E_{FB} - \frac{kT}{q} \right)$$

By plotting $1/C^2$ versus E , the flat-band potential of an n -type semiconductor photoanode could be obtained from the slope $2/(q\epsilon\epsilon_0N_d)$ and intercept at $C = 0$. Fig. 1D depicted the $1/C^2$ versus E plots for ZnO FRs and Au-ZnO FRs. First, ZnO FRs and Au-ZnO FRs exhibited positive slopes when a negative potential was applied, indicating an n type conductivity [40,41]. Second, donor density (N_d) value of Au-ZnO FRs ($N_d = 1.61 \times 10^{18}$) was much smaller than that of ZnO FRs ($N_d = 2.41 \times 10^{18}$), where N_d was calculated by extrapolating a straight line, namely, the slope and y-intercept. Third, The flat band potential was found to shift from -0.51 V for the ZnO FRs to -0.75 V for the Au-ZnO FRs. The more negative potential shift demonstrated that the separation efficiency of charge carrier was improved [41].

Preferred position for Fig.1

Fig. 2A displayed UV-vis diffuse reflectance spectra for pristine ZnO FRs and Au-ZnO FRs. There was not obvious difference between ZnO FRs and Au-ZnO FRs in UV. However, Au-ZnO FRs exhibited a broad absorption within visible light. And it showed a surface plasmon at 540 nm (curve a). In Fig. 2B, the fluorescence emission spectra of ZnO FRs (a) and Au-ZnO FRs (b) composites were displayed. With excitation of 375 nm, the fluorescence intensity of obtained Au-ZnO FRs was notably weaker than that of ZnO FRs. The result suggested that electrodeposited Au NPs enhanced charge separation considerably, since Au NPs could extract electrons from the conduction band of ZnO and acted as a sink which

could store and shuttle photo-generated electrons. Hence, PEC performance of ZnO was improved by the marriage between Au and ZnO FRs.

Preferred position for Fig. 2

3.3 Electrochemical properties of the PEC immunosensor based on Au-ZnO FRs

Cyclic voltammetry (CV) was employed as a effective approach for analyzing behavior change of the modified electrode. CV was performed by a three-electrode system in 50 mM PBS buffer containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ with scan step of 50 $\text{mV}\cdot\text{s}^{-1}$. As Fig. 3A shown, a pair of well-defined redox anodic (E_{pa}) and cathodic (E_{pc}) peaks at 0.38 V and 0.09 V was observed for the bare Au-ZnO FRs electrode (curve a). Then the peak current of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ was decreased after Ab of AFP combined with Au-ZnO FRs (curve b). The decrease of peak current was due to the combination between Ab of AFP and Au-ZnO FRs which restricted the diffusion of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ towards electrode surface. And the result also indicated that Ab co-immobilized with Au NPs successfully. Subsequently, after blocking electrode with BSA (curve c) and incubating with AFP antigen (Ag, 50 $\text{ng}\cdot\text{mL}^{-1}$) (curve d), further decreased peak current and greatly increased ΔE_p were observed. The changed peak current and ΔE_p before and after incubating with Ag indicated that the immunological reaction indeed occurred. In summary, the results suggested that Au NPs combined with protein triumphantly and the specific immunity response was successfully complicated on Au-ZnO FRs substrate.

Electrochemical impedance spectroscopy (EIS) effectively illustrated the electrochemical features of modified electrodes and provided further information of impedance change in modification process. The semicircle diameter could represent the electron transfer resistance, R_{ct} , which dominated the electrode interface. Nyquist plots were recorded over the frequency varied from 1 Hz to 100 kHz in 0.1 M KCl solution containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as redox species at 0.18 V. In Fig. 3B, a small semicircle presented for bare Au-ZnO FRs electrode, indicating a fast electron transfer process of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (curve a). R_{ct} was magnified after combining with Ab (curve b). Moreover, the electrode was blocked by BSA (curve c) and Ab was

immunoreacted with Ag of different concentrations ($0.005 \text{ ng}\cdot\text{mL}^{-1}$, $0.5 \text{ ng}\cdot\text{mL}^{-1}$, $50 \text{ ng}\cdot\text{mL}^{-1}$) (curve d-f). As a result, R_{ct} fortified continuously. The explanation was that the protein existing as insulative inert layer increased R_{ct} via blocking electron transfer between electrolyte and electrode. The results of EIS shown in Fig. 3B were in good agreement with that of cycle voltammetry shown in Fig. 3A, and suggested that the sensing interface was fabricated completely.

Preferred position for Fig. 3

3.4 Photoelectrochemical performance of PEC immunosensor

PEC detection was taken under simulated sunlight at the applied potential of 0 V and the working area was $1 \times 1 \text{ cm}^2$. Fig. 4 depicted the photocurrent response of different electrodes. It showed that the photocurrent of the Au-ZnO electrode (curve a) in the same experimental condition was stronger than that of ZnO (curve b), because charge separation and PEC performance were both significantly reinforced by SPR and excellent conductivity of Au NPs. After bedecking Ab of AFP, the photocurrent of Ab/Au-ZnO was decreased (curve c). And it fell more after the electrode blocked by BSA (curve d). These could be explained that inert materials like insulated protein hindered electron transfer. Finally, the photocurrent of Ag/BSA/Ab/Au-ZnO was descended again when the fabricated immunosensor incubated with corresponding antigen of $50 \text{ ng}\cdot\text{mL}^{-1}$ (curve e), for which the increase of steric hindrance due to the combination of antigen should be responsible. Impressively, we noticed that photo-response was absent under irradiation of simulated sunlight in pure ITO electrode for lacking of photoelectrochemical active species (curve f) on ITO surface. It manifested that bare ITO electrode exerted little disturbance on our experimental findings. The results presented that the PEC immunosensor was complicated and the specific immunity reaction was finished effectively.

Preferred position for Fig. 4

Fig. 5A showed the photocurrent responses of Ag/BSA/Ab/Au-ZnO electrodes after the specific immunity reaction with different concentrations of AFP antigen. It was found that the photocurrent was decreased with the increase of Ag concentration. The

photocurrent change (ΔI) between Ag/BSA/Ab/Au-ZnO and BSA/Ab/Au-ZnO was proportional to the logarithm of Ag concentration in the range of 0.005 ng·mL⁻¹ to 50 ng·mL⁻¹. The linear regression equation was expressed as ΔI (μA) = 1.091 log c (pg·mL⁻¹) + 7.1776 ($R^2 = 0.993$) with the detection limit of 0.56 pg·mL⁻¹ (S/N = 3) (Fig. 5B). The detection limit was low enough to measure AFP in human sera. The detection limit of 0.56 pg·mL⁻¹ (S/N = 3) was lower than 38 pg·mL⁻¹ of CdTe QDs modified electrode [42] and 20 pg·mL⁻¹ of CdS/TiO₂ modified electrode [43]. Table 1 presented comparison of the results derived from the proposed immunosensor and those of other teams in details. The results displayed that although the detection limit of the as-prepared PEC biosensor was not the lowest, it was with enough sensitivity.

Preferred position for Fig. 5

Preferred position for Table 1

3.4 Optimization of AFP antibody concentration

For PEC immunosensor, the concentration of AFP antibody (Ab) would greatly influence the detection sensitivity. Thus, the concentration of AFP antibody was optimized. We have monitored the photocurrent of Ab/Au-ZnO electrode with different concentrations of Ab. In Fig. 6, $\Delta I = I_0 - I$, where I_0 and I were the photocurrents of bare Au-ZnO FRs and Ab/Au-ZnO with different concentrations of Ab, respectively. Fig. 6 displayed that the photocurrent change increased with the concentration of Ab from 20 μg·mL⁻¹ to 100 μg·mL⁻¹. However, the photocurrent change decreased slightly with the further increasing concentration of Ab. Therefore, 100 μg·mL⁻¹ was selected as optimized concentration for Ab in our experiment.

Preferred position for Fig. 6

3.5 Stability and selectivity of PEC immunosensor

The stability of the fabricated immunosensor was assessed and presented in Fig. 7A. No remarkable change of photocurrent response was recorded with irradiation light on and off, which repeated for 15 times with the interval of 10 s. The result manifested

the excellent stability of the developed PEC immunosensor.

For evaluating selectivity of the proposed sensing platform, we choose carcinoembryonic antigen (CEA) and dopamine (DA) as interference sources. CEA and DA with similar physical and chemical features to AFP can at most exclude disturbance from material difference [44]. Fig. 7B presented the photocurrent changes for CEA ($20 \text{ ng}\cdot\text{mL}^{-1}$), DA ($20 \text{ ng}\cdot\text{mL}^{-1}$), AFP ($20 \text{ ng}\cdot\text{mL}^{-1}$), the mixture of CEA ($20 \text{ ng}\cdot\text{mL}^{-1}$) and AFP ($20 \text{ ng}\cdot\text{mL}^{-1}$), and the mixture of DA ($20 \text{ ng}\cdot\text{mL}^{-1}$) and AFP ($20 \text{ ng}\cdot\text{mL}^{-1}$) at the same experimental conditions. The results showed that the photocurrent change of the sample containing $20 \text{ ng}\cdot\text{mL}^{-1}$ AFP was as 2.4 and 2.1 greater as that of the sample containing $20 \text{ ng}\cdot\text{mL}^{-1}$ CEA and DA correspondingly, and single interfering protein had no obvious effect on the PEC response of AFP ($20 \text{ ng}\cdot\text{mL}^{-1}$). What's more, with the addition of CEA and DA into the antigen and antibody of AFP system, the varieties of photocurrent were hardly observed. Variation of photocurrent triggered by specific immunity reaction demonstrated the significant selectivity of our proposed PEC immunosensor.

Preferred position for Fig.7

4. Conclusions

In this work, we proposed a new sort of PEC immunosensor based on Au-ZnO flower-rod heterostructures for α -fetoprotein detection. Attributing to surface plasmon resonance, Au NPs obviously enhanced the light absorption of ZnO FRs in the visible spectral range and improved the separation of photo-generated charge carriers. Therefore, the PEC efficiency of Au-ZnO FRs was increased. The as-prepared PEC immunosensor possessed a large linear detection range from $0.005 \text{ ng}\cdot\text{mL}^{-1}$ to $50 \text{ ng}\cdot\text{mL}^{-1}$ with a detection limit of $0.56 \text{ pg}\cdot\text{mL}^{-1}$. The PEC immunosensor fabricated by the proposed method showed advantages in performance, sensitivity, cost-cut, and efficiency. It would provide a promising strategy for the detection of other proteins in clinical diagnosis using PEC sensing.

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Figure Captions

Scheme 1 Schematic representation of the fabrication of the proposed biosensor.

Fig. 1 (A) SEM image of ZnO FRs, (B) SEM image of Au-ZnO FRs, (C) XRD patterns and (D) Mott-Schottky plots of ZnO FRs and Au-ZnO FRs.

Fig. 2 (A) UV-vis diffuse reflectance spectra and (B) fluorescence emission spectra of ZnO FRs (a) and Au-ZnO FRs (b).

Fig. 3 (A) Cyclic voltammetry at $50 \text{ mV}\cdot\text{s}^{-1}$ in 50 mM PBS buffer containing 5 mM $[\text{Fe}(\text{CN})_6^{3-/4-}]$ redox indicator, Au-ZnO FRs (a), Ab/Au-ZnO (b), BSA/Ab/Au-ZnO (c), Ag (50 $\text{ng}\cdot\text{mL}^{-1}$) /BSA/Ab/Au-ZnO (d). (B) Nyquist plot obtained in 0.1 M KCl solution containing 5 mM $[\text{Fe}(\text{CN})_6^{3-/4-}]$, Au-ZnO FRs (a), Ab/Au-ZnO (b), BSA/Ab/Au-ZnO (c), BSA/Ab/Au-ZnO incubated with different concentrations of AFP antigen (d-f): 0.005 $\text{ng}\cdot\text{mL}^{-1}$, 0.5 $\text{ng}\cdot\text{mL}^{-1}$, 50 $\text{ng}\cdot\text{mL}^{-1}$.

Fig. 4 Photocurrent response of the modified ITO electrodes: Au-ZnO FRs (a), ZnO FRs (b), Ab/Au-ZnO (c), BSA/Ab/Au-ZnO (d), Ag/BSA/Ab/Au-ZnO: 50 $\text{ng}\cdot\text{mL}^{-1}$ (e) and bare ITO (f).

Fig. 5 (A) Photocurrent response of the modified ITO electrodes after the specific immunity reaction with different concentrations of AFP antigen: 0.005 $\text{ng}\cdot\text{mL}^{-1}$ (a), 0.05 $\text{ng}\cdot\text{mL}^{-1}$ (b), 0.5 $\text{ng}\cdot\text{mL}^{-1}$ (c), 5 $\text{ng}\cdot\text{mL}^{-1}$ (d), 50 $\text{ng}\cdot\text{mL}^{-1}$ (e). (B) The linear relationship between the photocurrent and the logarithm of antigen concentration.

Fig. 6 The effect of AFP antibody concentration on the PEC immunosensor.

Fig. 7 (A) The stability of time-dependent photocurrent responses of the PEC immunosensor with light on and off. (B) Comparison of photocurrent change with

different interference factors: dopamine (DA) and carcino embryonie antigen (CEA).

Error bars showed the standard deviation of repeated measurements.

Scheme 1

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Table 1
Comparison of several immunosensors for determination of AFP.

Electrode materials	Detection range ($\text{ng}\cdot\text{mL}^{-1}$)	LOD ($\text{pg}\cdot\text{mL}^{-1}$)	Ref.
ZnO	0.1 - 500	10	[6]
TiO ₂ /CNHs	0.0001 - 160	0.03	[25]
Au NPs/Silica	0.5 - 100	100	[38]
CdTe QDs	0.05 - 500	38	[43]
CdS/TiO ₂	0.05 - 20	20	[44]
Au-ZnO FRs	0.005 - 50	0.56	This work