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Visible-light driven biofuel cell based on hierarchically branched titanium dioxide nanorods photoanode for tumor marker detection

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ABSTRACT

In this work, a novel sensing platform based on visible light driven biofuel cell (BFC) has been facilely designed for sensitive detection of prostate-specific antigen (PSA) with the photo-response bioanode, realizing the dual route energy conversion of light energy and chemical energy to electricity. The hierarchical branched TiO_2 nanorods (B-TiO_2 NRs) decorated with CdS quantum dots (QDs) act as the substrate to confine glucose dehydrogenase (GDH) for the visible light driven glucose oxidation at the bioanode. Three dimensional flowers like hierarchical carbon/gold nanoparticles/bilirubin oxidase (3D FCM/AuNPs/BOD) bioconjugate served as biocatalyst for O_2 reduction at the biocathode. With an increase in the concentration of PSA, the amount of BOD labels on biocathode increases, thus leading to the higher current output of the as-proposed visible light driven BFC. Based on this, this sensing platform provide great performance in sensitivity and specificity, increasing linear detection range from 0.3 pg mL^{-1} to 7 μg mL^{-1} with a detection limit of 0.1 pg mL^{-1}. Most importantly, our new sensing strategy provided a simple and inexpensive sensing platform for tumor markers detection, suggesting its wide potential applications for clinical diagnostics.

1. Introduction

Highly sensitive and selective determination of tumor markers with portable and short assay time is significant in early cancer screening, because their levels in blood or tissue provide essential information about the stages of tumors (Arya et al., 2011; Joo et al., 2012; Wu et al., 2007). Prostate-specific antigen (PSA), a sense protease secreted by prostate glandular cells, has been considered as the most reliable tumor marker for the early detection of prostate cancer (Zhang et al., 2014). Therefore, it is highly desired to develop a sensitive and reliable appraisal system for early diagnosis of PSA. To date, various methods for the detection of PSA have been explored, such as electrochemical (He et al., 2015; Kavosi et al., 2015; Triroj et al., 2011), photoelectrochemical immunoassay (Shu et al., 2016), and electrogenerated chemiluminescence (ECL) immunoassay (Xu et al., 2011a, 2011b). Despite much advances of above-mentioned assays, these methods struggled with the problems that high cost and sophisticated instrumentation.

As renewable energy conversion technology, biofuel cells (BFCs) have spurred enormous interest due to their moderate operation condition, environmentally benign and low expenditure (Yu et al., 2016; Moehlenbrock et al., 2008). Recently, some reports about BFCs based sensors have been well demonstrated in developing different kinds of sensors for glucose, ethanol, and fructose (Gao et al., 2010; Gao et al., 2015; Pan et al., 2011; Liu et al., 2012). However, the relatively low sensitivity and stability in existing BFCs based sensors cannot meet the needs for higher detection sensitivity. Therefore, it is great desire to develop more promising sensing systems. Visible light driven BFCs (BFC) which could convert both light and chemical energy into electricity was proposed. Very recently, Dong's group developed a visible light enhanced BFC employing a photo-responsive cathode, representing another proof-of-concept advance in dual-directional energy conversion (Zhang et al., 2014). In addition, our group fabricated a paper-based analytical devices relying on visible light enhanced BFC with a visible responsive photocathode (Wu et al., 2015). Inspired by previous work, we demonstrated a new concept that the visible light driven BFCs were utilized to develop the highly sensing system for the detection of PSA.

Recently, hierarchically branched titanium dioxide nanorods have attracted dramatically increasing interest due to its large surface area, excellent light harvesting and a highly conductive pathway for charge carrier collection (Wang et al., 2011; Yu et al., 2015; Cho et al., 2011). Nevertheless, its large band gap limits light

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absorption in the visible region. Regarding this issue, various modification strategies have been widely investigated, such as by surface dye sensitization (Tian et al., 2009), nonmetal doping (Liu et al., 2008; Li et al., 2009), and forming composites (Xu et al., 2011a, 2011b). CdS with narrow band gap semiconductor, which is capable of harvesting photons in the visible, have been intensively studied as popular visible light-active materials (Wang et al., 2009). Then, compositing with CdS is a promising and effective strategy to promote the visible light-response of TiO_2. Herein, B-TiO_2 NRs sensitized with CdS QDs and further immobilized glucose dehydrogenase (GDH) served as visible light driven bioanode, realizing a dual ways of energy conversion.

Three-dimensional (3D) flower-like hierarchically carbon material (3D FCM), as a novel carbon nanomaterial with large surface area, may have advantages to conjugate more enzymes. In present work, 3D FCM modified with AuNPs (3D FCM/AuNPs) was chosen as the carrier to load a higher amount of BOD to realize efficient for O_2 reduction. Meanwhile, The AuNPs/FTO was served as host matrix for the immobilization of Ab_1. A "sandwich" configuration between 3D FCM/AuNPs/BOD/mAb_2 and AuNPs/Ab_1 in the presence of PSA was used to fabricate the biocathode.

Herein, we demonstrated a new concept that a sensing platform based on visible light driven BFC was utilized to develop the highly sensitive immnuosensor for detection of PSA, in which the 3D FCM/AuNPs/BOD bioconjugate served as a biocatalyst for enhancing O_2 reduction, B-TiO_2 NRs/CdS/GDH was fabricated and served as bioanode to oxide glucose. Under optimal conditions, the proposed immunosensor based on visible light driven BFC exhibited wide detection range and low detection limit. Additionally, the results demonstrated that the developed sensing platform supplied a sensitive, selective, and convenient method for tumor markers detection.

2. Experimental

2.1. Synthesis of TiO_2 NRs, B-TiO_2 NRs and CdS/B-TiO_2 NRs on FTO

The TiO_2 NRs was directly grown on the FTO substrate with a previously reported hydrothermal method (Liu et al., 2009). Generally, FTO glass substrate was degreased by sonicating in acetone, ethanol and ultrapure water, respectively. Then, 10 mL ultrapure water was mixed with 10 mL of 12 M HCl with stirring for 10 min After that 0.4 mL titanium butoxide was added into the above solution for 30 min and then the mixture was transferred into a Teflon-lined stainless steel autoclave, followed by immersion of FTO substrate into the solution with the conducting side facing down. The autoclave was sealed and heated in an oven at 150 °C for 4 h, which was then allowed to cool to room temperature naturally. Then the product was rinsed thoroughly with ultrapure water, drying in ambient air at 60 °C.

In addition, the branched was grown on TiO_2 NRs by another hydrothermal method. Briefly, 20 mL ultrapure water was well mixed with 0.25 mL 12 M HCl. Then 0.1 mL $TiCl_3$ solution was added to this solution dropwise to prepare a branch forming solution. After that, the TiO_2 NRs/FTO was placed at an angle against the wall of the breaker, heating to 80 °C for 1 h in the oven. Then the sample was rinsed with ultrapure water and allowed to dry at 60 °C in ambient air. All the samples (rod and branched TiO_2) were subsequently annealed at 450 °C for 2 h.

Finally, CdS QDs were deposited on the surface of the B-TiO_2 NRs electrode with a chemical bath deposition (Li et al., 2012). Typically, 1.0 M ammonia solution, 1.0 mM $CdSO_4$ and 5.0 mM thiourea were thoroughly dissolved to obtain the CdS precursor solution. Then B-TiO_2 NRs electrode was completely immersed into the above solution and heated in an oil bath at 60 °C for 20 min to form CdS/B-TiO_2 NRs electrode. After that, the electrode was washed thoroughly with ultrapure water and then annealed in a N_2 flow at 450 °C for 2 h.

2.2. Synthesis of three-dimensional (3D) flower like hierarchical carbon (3D FCM) and 3D FCM/AuNPs

The 3D FCM was synthesized according to the previous report with slight modification (Wang et al., 2014). The detail experiment procedures were in Supplementary Material.

The procedure of preparation 3D FCM/AuNPs could be described as below. Firstly, 2.0 mg 3D FCM was carboxyl functionalized by sonicating in a mixture of concentrated HNO_3 and H_2SO_4 (v/v, 1:3) for 3 h followed by thorough washing with ultrapure water until the pH reached about 7.4. Then, 0.6 mL of 25 mM $HAuCl_4$ aqueous solution was added into the 3D FCM and incubated for 2 h with slightly stirring, followed by drop wise addition of 1.0 mL freshly prepared $NaBH_4$ (0.1 M). After centrifugation and washing by ultrapure water, the obtained 3D FCM/AuNPs nanocomposites were stored at 4 °C before use.

2.3. Electrode preparation and visible light driven BFC based sensing platform assembly

The construction of the as-proposed sensing platform based on visible light driven BFC was shown in Scheme S1. For the bioanode, the GDH was immobilized onto the CdS through PDDA (Tang et al., 2008). Briefly, the CdS/B-TiO_2 NRs electrode was immersed into PDDA solution (10 μg mL^{-1}) and kept for 30 min, followed by washing with ultrapure water to remove excess PDDA. Then the PDDA modified CdS/B-TiO_2 NRs electrode was immersed into a solution containing (10 μg mL^{-1}) GDH, which was assembled onto the surface of positively charged PDDA/CdS/B-TiO_2 NRs electrode via the negative-positive charge interaction and dried at 4 °C to form GDH/CdS/B-TiO_2 NRs electrode. For the biocathode, the FTO was sonicated with acetone, absolute ethanol, and ultrapure water for about 10 min, respectively. Then the end of FTO was immersed into the solution: 0.3 mL 1 mM $HAuCl_4 \cdot 3H_2O$ solution, 0.5 mL PBS and ultrapure water in a total of 5.0 mL. The electrodeposition experiment was carried out in the water bath at 50 °C. The AuNPs modified FTO electrode was prepared in the potential range of −0.5 to −2.0 V for 20 or 40 s at a potential scan rate of 50 mV s^{-1} under nitrogen atmosphere.

The biocathode was constructed by immobilizing the Ab_1 on the AuNPs layer through the interaction between amino on Ab_1 and AuNPs. Briefly, 70 μL Ab_1 (0.1 mg mL^{-1}) was dropped onto the AuNPs/FTO electrode and incubated at room temperature for 1 h. Afterwards, the Ab1/AuNPs/FTO electrode were rinsed with PBS to remove physically absorbed Ab_1 and the BSA was utilized to block possible remaining active sites against nonspecific adsorption, followed by washing thoroughly.

2.4. Assay procedure of the visible light driven BFC based sensing platform

The detail assay procedures of this immunosensor are described as below. Briefly, the AuNPs/Ab_1 electrode was immersed into sample solutions contained different concentrations of PSA and allowed to incubate for 35 min at room temperature followed by washing thoroughly with PBS. Thereafter, the modified electrode was immersed into 3D FCM/AuNPs/BOD/mAb_2 bioconjugate and allowed to incubate for 35 min at room temperature, followed by washing with PBS. Finally, the prepared bioanode and biocathode were placed in parallel into 40 mL supporting electrolyte containing 10 mM NAD^+/NADH and 40 mM glucose under air-saturated atmosphere. The light source with the intensity of 30 mW cm^{-2} was