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Label-free and high-throughput biosensing of multiple tumor markers on a single light-addressable photoelectrochemical sensor

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ABSTRACT

The sensitive and label-free detection of multiple biomarkers on a single electrode by photoelectrochemical (PEC) sensors based on light addressing strategies is very attractive for developing portable and high-throughput biosensing systems. The essential prerequisite of this proposal is the employment of uniform photovoltaic material modified electrodes with high conversion efficiency. Herein, a novel two-step constant potential deposition method for the rapid fabrication of bismuth sulfide film modified ITO electrodes (Bi_2S_3/ITO) was established. The produced Bi_2S_3/ITO, with excellent uniformity and high conversion efficiency in visible light ranges, was further modified with gold nanoparticles (AuNPs) and then divided into separated identical sensing zones by insulative paints. The adsorption-based immobilization of antibodies of three tumor markers, i.e., a-fetoprotein (AFP), carcinoembryonic antigen (CEA) and cancer antigen 19-9 (CA19-9), onto different sensing zones of the electrode and the further blocking with BSA established a label-free and light-addressable PEC sensor (LF-LAPECS), which can achieve the rapid and sensitive detection of these biomarkers with wide linear ranges, low detection limits and self-calibration ability. Moreover, the detection throughput can be conveniently improved by enlarging the size of the substrate electrode and increasing the number of separated sensing zones. The present work thus demonstrates the promising applications of PEC techniques for developing sensitive, time-saving, cost-effective and high-throughput biosensing methods.

1. Introduction

In recent years, photoelectrochemical (PEC) sensing techniques have gained tremendously popularity for the detection of biomarkers owing to their high sensitivity, low cost and simple instrumentation (Zhang et al., 2014a; Zhao et al., 2015). And, the demands of multi-target detection have attracted continuous research interest in the field of PEC biosensing. As is well known, a single-marker detection usually causes a high rate of false positives or negatives in clinical analysis (Kong et al., 2013), while multiplexed assays which quantitatively detect a panel of targets can effectively improve the diagnostic accuracy, shorten analytical time and reduce detection cost (Jia et al., 2014; Kong et al., 2013). To date, a series of PEC biosensors have been developed for multiplexed bioanalysis, of which the detection modes can be generally divided into two types according to the configuration of substrate electrodes, i.e., the electrode array mode (Sun et al., 2015; Wang et al., 2013; Zhang et al., 2014b) and the single electrode mode (Wang et al., 2015a; Zhang et al., 2016; Zheng et al., 2016). For the former mode, intricate fabrication of electrode arrays or employment of multi-channel electrochemical analysis equipment (Xiao et al., 2012) usually leads to high cost or complicated instrument. As for the latter mode, special strategies like multiple enzyme tags (Zhang et al., 2016), wavelength-selective photoactive materials (Zheng et al., 2016) and light addressing detection (Wang et al., 2015a) have been employed for multiplexed detection on a single electrode. However, expensive equipment, special signal tags or complicated probe labeling procedures applied in these sensors are limited factors for high-throughput detection.

Label-free PEC sensors are a kind of rapid assay by combining the advantages of both label-free assays and PEC sensing methods, which can avoid complicated and time-consuming procedures of marker labeling and multiple incubation (Li et al., 2015b). Theoretically, the combination of label-free PEC sensing principles with light addressing detection strategies should enable the construction of label-free and light-addressable photoelectrochemical sensors (LF-LAPECS) for the rapid and high-throughput detection of multiple targets on a single

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electrode. However, currently label-free PEC sensors are generally used for single-target detection, e.g., biomolecules (Haddour et al., 2006; Li et al., 2015a), toxins (Liu et al., 2015a, 2015b) and ions (Han et al., 2015). So far, only one label-free PEC sensor has been developed by Yu et al. for multiple-target detection using electrode arrays (Zhang et al., 2014b).

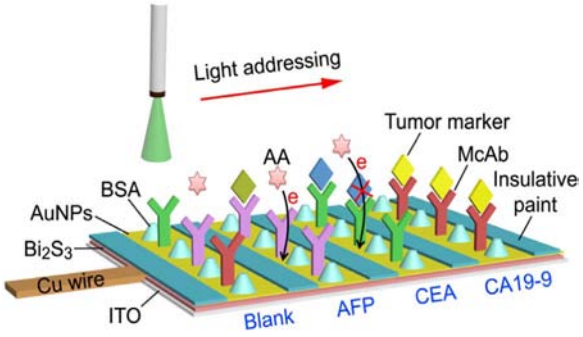
Considering the work principle of LF-LAPECS, the deposition of highly uniform photovoltaic materials on substrate electrodes is vital to label-free and multiplexed PEC detection on a single electrode. Currently, the widely used deposition methods of photovoltaic materials are layer-by-layer assembly (LBL) (Zhang et al., 2011) and successive ionic layer adsorption and reaction (SILAR) (Nguyen Van et al., 2015). However, there may be certain limitations for these methods, e.g., the LBL method requires good solution dispersion stability of photovoltaic materials while the SILAR method usually involves laborious adsorption and washing cycles. In this regard, electrodeposition methods might be a promising alternative because of their merits of simplicity, labor saving and ease of film thickness controlling (Li et al., 2014). On the other hand, the employment of photovoltaic materials with narrow band gaps should be also favorable to LF-LAPECS, because this kind of PEC sensing materials can use light sources with small beam, high power density and low damage to accommodate more divided sensing arrays on area-fixed substrate electrodes but still possessing high sensitivity. Therefore, bismuth sulfide (Bi_2S_3), which can be synthesized by electrodeposition method with narrow band gaps (1.3–1.7 eV) (Yin et al., 2014a) and possesses excellent PEC sensing properties (Sun et al., 2014; Zhou et al., 2015), should be an ideal material for the development of label-free and high-throughput PEC sensors.

In this work, we constructed for the first time a label-free and light-addressable PEC sensor for the rapid and sensitive detection of multiple tumor markers on a single electrode. Inspired by the electrodeposition of Bi_2S_3 films on bismuth rods (Grubač and Metikoš-Huković 2002), we developed a novel two-step constant potential deposition method to produce compact and uniform Bi_2S_3 nanodendrite (Bi_2S_3 ND) films on ITO with high photo-to-electron conversion efficiency within a short period of 5 min. The modification of Bi_2S_3/ITO with gold nanoparticles (AuNPs) by adsorption and the division of the electrode into different zones by an insulative and hydrophobic paint allowed the facile construction of separated label-free immunosensing arrays on a single Bi_2S_3/ITO. By using an inexpensive and portable green laser pen as the light source (Hu et al., 2013), LF-LAPECS can be used for the sensitive detection of AFP, CEA and CA19-9, which are usually over-expressed in many carcinomas such as hepatoma (Jia et al., 2012), gallbladder cancer (Ono et al., 1996) and gastric cancer (Choi et al., 2006), through a simple one-step sample incubation process. That's to say, after the incubation of the sensor with samples for only 1 h, the concentration of target markers can be readily detected one by one by simply moving the light source from one sensing area to another, on the basis of the repression of photo-induced electron transfer due to steric effects during the formation of immune complexes (Scheme 1). Moreover, the detection throughput of LF-LAPECS can be conveniently improved by simply enlarging the size of the substrate electrode and increasing the number of separated sensing zones. Particularly, its single-electrode response mode created a chance for developing self-calibration PEC sensors when one of the separated sensing areas was employed as a reference.

2. Experimental section

2.1. Chemicals

Ascorbic acid (AA), chloroauric acid ($HAuCl_4{\cdot}4H_2O$), trisodium citrate dehydrate (cit), sodium borohydride ($NaBH_4$), disodium hydrogen phosphate ($Na_2HPO_4{\cdot}12H_2O$), sodium dihydrogen phosphate ($NaH_2PO_4{\cdot}2H_2O$), potassium ferricyanide ($K_3Fe(CN)_6$), potassium ferrocyanide ($K_4Fe(CN)_6$), potassium chloride (KCl), hydrochloric acid (HCl), sodium hydroxide (NaOH), bismuth nitrate ($Bi(NO_3)_3{\cdot}5H_2O$) and sodium sulfide ($Na_2S{\cdot}9H_2O$) were purchased from Sinopharm Chemical Reagent Co. Ltd., China. The three tumor markers (i.e., CA19-9, CEA, and AFP) and their monoclonal antibodies (CA19-9 McAb, CEA McAb, AFP McAb) were obtained from Linc-Bio Science Co. Ltd (Shanghai, China). Albumin from bovine serum (BSA) was obtained from Sigma-Aldrich, Shanghai. Indium tin oxide (ITO) sheets (resistance $< 7\ \Omega$/sq) were purchased from South China Science and Technology Co., Shenzhen, China. Poly-(dimethylsiloxane) (PDMS) was obtained from RTV615 GE Toshiba Silicones Co., Ltd. All chemicals were of analytical grade and used without further purification. All aqueous solutions were prepared using ultrapure deionized water (> 18 MΩ·cm) produced on Heal Force, Nison Instrument Ltd., Shanghai, China. The electrolyte solution for photocurrent measurements was 0.1 M PBS (pH 7.4) containing 0.1 M AA. All the buffer solutions were adjusted to suitable pH values with 1.0 M HCl or NaOH by a pH meter (PB-10, Sartorius).



Scheme 1. Schematic representation for the rapid detection of multiple biomarkers on a single LF-LAPECS.

2.2. Apparatus

All the electrochemical measurements were performed on a CHI 830 analyzer (CH Instruments, Shanghai, China). Scanning electron microscopy (SEM) images were collected on a field emission scanning electron microscope (Zeiss, Germany). Transmission electron microscopy (TEM) images were characterized by high resolution transmission electron microscope (HRTEM) (FEI G2 tecnai F30, American). UV–vis spectra were collected on a UV 2550 spectrophotometer. X-ray powder diffraction (XRD) measurements were carried out on XRD-2000X (Shimadzu, Japan). X-ray photoelectron spectroscopy (XPS) analysis was carried out on an Escalab 250 X-ray Photoelectron Spectroscope (Thermofisher, American) using Al (mono) Kα radiation. The electrode system contained a modified ITO working electrode, a platinum plate counter electrode (10 mm×20 mm) and a potassium chloride (KCl) saturated calomel reference electrode (SCE). All the photocurrent responses were collected at open circuit potentials. A green laser pen of 50 mW at 525 nm with a diameter of ~2.5 mm for the illumination area was used as the light source.

Construction of LF-LAPECS for tumor markers detection. Construction of LF-LAPECS for the detection of single and multiple tumor markers was performed according to procedures shown in Fig. S1 and S2. The corresponding experimental details are also presented in Supporting Information.

3. Results and discussion

3.1. Characterizations of electrodeposited Bi_2S_3 films

The formation of Bi_2S_3 films on ITO was achieved by a simple two-step constant potential deposition method, which produced uniform