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Construction of visible light-induced renewable electrode for monitoring of living cells

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ABSTRACT

Ultraviolet (UV) light-induced photocatalysts have been utilized to construct renewable electrode to solve the problem of electrode fouling and passivation. However, considering the damages of UV irradiation to environments and biosystems, it is of great significance to develop and apply visible light-induced photocatalysts for biosensing. But for intrinsic visible light photocatalysts, the high electron-hole recombination rate results in the poor photocatalytic performance. Herein, we design the poly(3,4-ethylenedioxythiophene) (PEDOT)-modified TiO_2/CdS nanocomposites electrode, which can be efficiently renewed under visible light irradiation for living cell detection. The formation of TiO_2/CdS heterojunction structure greatly enhances photocatalysis in visible light region by promoting separation of photogenerated electron–hole pairs. Additionally, the absorption in visible light of PEDOT further accelerates the electrode renewal. PEDOT coating provides a sensitive biosensing interface for electrochemical detection, and meanwhile prevents the cytotoxicity of CdS to cells. This allows electrochemical monitoring of nitric oxide release from living cells and subsequent visible light-induced electrode regeneration, demonstrating great potential of this renewable electrodes in biosensing.

1. Introduction

Electrode fouling and passivation have always been a serious problem, which is prone to cause the decrease of electrochemical performance [1–3]. Especially in the presence of proteins and secretions when cells are cultured on the electrode, this phenomenon becomes more severe [4,5]. Many methods such as mechanical polishing, flame etching, acid washing, chemical oxidation and electrochemical treatment, have been put forward to solve this problem [6–12]. These methods can realize electrode regeneration to some extent, but the harsh oxidization reactions usually destroy electrode micro/nanostructures and affect the reuse of electrode. Recently, some groups proposed to utilize photocatalysts to achieve the degradation of pollutants and renewal of electrode [13,14]. Photocatalysts can absorb photons and form photogenerated electron–hole pairs [15]. Then electron–hole pairs can directly degrade the organic pollutant molecules, or generate some reactive oxygen species such as $O_2^{-\bullet}$, OH•, H_2O_2, O_3 [16] to decompose the pollution indirectly, without destroying the micro/nanostructures on the electrode. Our previous work showed that the adsorbed proteins and secretions from cultured cells could also be efficiently degraded by photocatalytic reaction, demonstrating the capability of photocatalytically renewable electrochemical sensor in real-time monitoring of cells [17,18].

Currently most of available renewable electrodes are constructed by ultraviolet (UV) light-activated photocatalysts such as TiO_2 and ZnO, since they have high photocatalytic performance under UV light irradiation. However, UV light has unfavorable effects on the environment and causes damages to people and biosystems [19,20]. Comparing with UV light, visible light is "green" and environmentally friendly [21]. But for intrinsic visible light photocatalysts, such as CdS and CdSe, high electron–hole recombination is apt to cause the poor photocatalytic performance [22,23]. To enhance their photocatalytic performance in the visible light range, many heterojunction photocatalysts, such as semiconductor–semiconductor heterojunctions and multicomponent heterojunctions, have been designed to reduce the electron–hole recombination by separating electron–hole pairs [24]. Exemplarily, CdS has been widely used as a sensitizer of some wide band gap semiconductors, forming semiconductor–semiconductor heterojunction microstructure to increase the photocatalytic activity [25–29].

However, due to the poor conductivity and electrochemical activity of photocatalysts, they are not appropriate to be directly used for electrochemical biosensing. Furthermore, the cytotoxicity of some photocatalysts such as CdS and CdSe restricts their practicable applications in monitoring of living cells [30,31]. Recently, poly(3,4-ethylenedioxythiophene) (PEDOT), as one of the conducting polymers, has attracted considerable attention owing to its unique properties, such as high conductivity, good electrochemical activity, biocompatibility and stability [32–34]. To date, it has been widely applied in biosensing to sensitively detect physiologically important chemicals [35–40]. Additionally, because of the absorption in visible light range, PEDOT can also act as photocatalysts or photosensitizers to accelerate electrode renewal [41,42].

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In this work, CdS-sensitized TiO_2 is combined with PEDOT to construct a kind of visible light-induced renewable electrode. The CdS-sensitized TiO_2 heterojunction structure reduces the recombination of electron–hole pairs and thus enhancing the photocatalysis in visible light region greatly, and the narrow bandgap of PEDOT renders the absorption in visible light range and further facilitates the electrode renewal. PEDOT coating endows the sensor with excellent electrochemical properties and biocompatibility. These features allow real-time monitoring of nitric oxide (NO) release from living cells cultured on the electrode as well as subsequent visible light-induced regeneration of fouled electrode for reutilization.

2. Experiment

2.1. Materials

ITO conductive glass (film thickness: 180 nm, conductivity: 10 Ω/sq) was purchased from Crystal Great Technology Co., Ltd. (Shenzhen, China). Commercial TiO_2 NPs (P25) were obtained from Evonik Degussa. 3,4-ethylenedioxythiophene (EDOT), 5-Hydroxytryptamine (5-HT), poly-L-lysine (PLL), L-arginine (L-Arg) and NOS inhibitor Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) were purchased from Sigma. The cell culture medium (CCM) RPMI 1640, L-glutamine and HEPES were purchased from GIBCO. Calcein-AM and PI for live and dead cell staining were obtained from Dojindo laboratory (USA). The human umbilical vein endothelical cells (HUVECs) lines were obtained from CHI Scientific, Inc. (Shanghai, China). Cadmium nitrate tetrahydrate ($Cd(NO_3)_2 \cdot 4H_2O$), Sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$), poly(styrenesulfonate) (PSS, MW = 70,000) and other chemical reagents were reagent grade and used without further purification.

2.2. Apparatus

SEM images were obtained by using the field-emission scanning electron microscope (Zeiss SIGMA). The HRTEM and TEM images were acquired on a JEM-2100 transmission electron microscope. EDX spectroscopy was recorded by using FEI quanta 200 SEM with EDAX Genesis unit (AMETEK, USA). UV–Vis diffuse reflectance spectrum (DRS) absorption was measured by Shimadzu UV-3100. The powder X-ray diffraction (XRD) was recorded with a Bruker D8-Advance using Cu Kα radiation. All electrochemical measurements were carried out on a CHI 660A electrochemical workstation (CH Instruments, Shanghai, China). A three-electrode system was used in the experiment including a bare or modified ITO (0.6 cm × 0.6 cm) working electrode, Ag/AgCl reference electrode and Pt counter electrode. In order to facilitate cell culture and detection, a PDMS frame was fabricated around the electrode (1.0 cm × 1.0 cm). Inverted fluorescent microscopes (AxioObserver Z1 and Axiovert 200M, Zeiss, Germany) were used for cell culture and observation.

2.3. Fabrication of PEDOT@CdS/TiO_2/ITO Electrode

First, before TiO_2 NPs were decorated to the ITO electrode by electrophoretic deposition, amino-functioned TiO_2 NPs were synthesized according to a previous report [43]. Briefly, 0.04 g commercial TiO_2 NPs (P25) were dispersed in 20 mL ethanol by sonication for 30 min. Then 1 mL 3-aminopropyltriethoxysilane (APTES) was added, stirred, and refluxed at 90 °C for 4 h. APTES-treated TiO_2 NPs were sufficiently rinsed with ethanol. Next, the APTES-treated TiO_2 NPs obtained were dispersed in water and adjusted the pH to 4 by 0.1 $mol \cdot L^{-1}$ HCl. The solution was used to deposit TiO_2 NPs at −1.0 V for 1200 s.

Second, CdS quantum dots (QDs) were assembled on the surface of TiO_2/ITO electrode by successive ionic layer adsorption and reaction (SILAR). Concretely, electrode was immersed into a 0.5 $mol \cdot L^{-1}$ $Cd(NO_3)_2$ ethanol solution for 5 min, rinsed with ethanol, and then immersed into a 0.5 $mol \cdot L^{-1}$ Na_2S methanol solution for another 5 min and rinsed again with methanol. The two-step procedure was a cycle, and this cycle was repeated four times.

Third, conducting polymer PEDOT was electrodeposited to the electrode from a solution containing 10 $mmol \cdot L^{-1}$ EDOT in 0.1 $mol \cdot L^{-1}$ PSS solution at +1.0 V for 10 s. Ultimately, PEDOT@CdS/TiO_2/ITO electrode was fabricated successfully.

2.4. Characterization of Renewable Performance of the Electrode in Visible Light

5-Hydroxytryptamine (5-HT), collagen, poly-L-lysine (PLL) and cell culture medium (CCM) were chosen to passivate electrode. For 5-HT passivation, electrode was in 0.5 $mmol \cdot L^{-1}$ 5-HT solution at +0.6 V for 600 s. For collagen, PLL and CCM, electrodes were immersed in 0.05 wt.% PLL solution, 0.05 wt.% collagen and CCM for 96 h, respectively. After passivation, the electrode was renewed by visible light irradiation at room temperature for 2 h. A 200 W tungsten lamp (primary wavelength: 400–780 nm) was used as the visible light source to renew the electrode.

2.5. HUVECs Culture and NO Release Detection

Human umbilical vein endothelical cells (HUVECs) were routinely cultured using RPMI 1640 culture medium with 4.766 mg/mL HEPES, 0.292 mg/mL L-glutamine, 0.85 mg/mL $NaHCO_3$, 12% fetal bovine serum, penicillin and streptomycin (100 U) in culture flask at 37 °C in a humidified incubator (95% air with 5% CO_2). For NO release detection, electrode was sterilized by exposed to UV light, followed by seeding HUVECs with an approximate density of 1×10^6 cells/cm^2. After being cultured 12 h on electrode, the cells were used for NO release detection.

3. Results and Discussion

3.1. Fabrication and Characterization of the PEDOT@CdS/TiO_2/ITO Electrode

The fabrication process of PEDOT@CdS/TiO_2/ITO electrode is illustrated in Scheme 1. Amino-functioned TiO_2 NPs were firstly synthesized and electrophoretically deposited onto ITO electrode. SEM image, TEM image and Ti peak of EDX (Fig. S1A–C) indicated that the TiO_2 NPs were successfully deposited, and XRD pattern (Fig. 1E) indicated that TiO_2NPs were composed of anatase and rutile [44]. Then, CdS QDs were assembled onto TiO_2/ITO by SILAR. SEM and TEM images showed that CdS QDs with diameter about 5–10 nm were formed on TiO_2 successfully (Fig. S1D–E), which was confirmed by the Cd peak and S peak shown in EDX result (Fig. S1F). HRTEM result further demonstrated that CdS with 0.204 nm lattice fringe spacing were deposited successfully on TiO_2 with 0.315 nm lattice fringe spacing (Fig. 1A). After CdS QDs deposition, the intensity of TiO_2 NPs decreased and a weak peak of CdS (43.8°) was observed in XRD pattern (Fig. 1E) [45], which also confirmed that CdS QDs were deposited on TiO_2 NPs successfully. Finally, PEDOT@CdS/TiO_2/ITO electrode was prepared by electrodepositing EDOT onto the CdS/TiO_2/ITO electrode (Fig. 1B). HRTEM result (Fig. 1C) showed that CdS QDs and TiO_2 NPs were covered by PEDOT membrane with *ca.* 2 nm thickness, which was supported by the C peak of PEDOT displayed in EDX spectrum (Fig. 1D). The weakened XRD peak of TiO_2 NPs in PEDOT@CdS/TiO_2 (Fig. 1E) also indicated that PEDOT was formed successfully.

UV–Vis DRS was used to test the absorption properties of the three kinds of materials. As shown in Fig. 1F, compared to the ultraviolet absorption edge of TiO_2, the absorption edge of CdS/TiO_2 reached about 600 nm due to the sensitization of CdS. After PEDOT electrodeposition, the absorption of PEDOT@CdS/TiO_2 was extended to the whole visible light range because of the narrow bandgap of PEDOT (about 2.0 eV) [46,47], indicating that PEDOT@CdS/TiO_2 nanocomposites have visible light photocatalytic performance.