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CdSe quantum dot-functionalized TiO_2 nanohybrids as a visible light induced photoelectrochemical platform for the detection of proprotein convertase subtilisin/kexin type 6

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ABSTRACT

Proprotein convertase subtilisin/kexin type 6 (PCSK6) plays a major role in promoting the progression of rheumatoid arthritis to a higher aggressive status. A novel highly sensitive photoelectrochemical platform was developed for the detection of PCSK6 by using CdSe quantum dots (QDs)-functionalized TiO_2 nanoparticles (NPs) nanohybrids (TiO_2@CdSe) as the photo-to-electron conversion medium. TiO_2@CdSe showed excellent visible-light absorbency, and much higher photoelectrochemical activity than both CdSe QDs and TiO_2 NPs. The 5′ and 3′ primers of PCSK6 ssDNA acted as capture probes to realize the detection of PCSK6 ssDNA by the specific recognition. The capture probes can be fixed by poly-L-lysine (PLL) through positively strong electrostatic attraction and the carboxyl group of TiO_2@CdSe nanohybrids. PLL was electropolymerized on ITO electrode by cyclic voltammetry (CV). Simultaneously, the amino group of PLL can interact with the carboxyl group of TiO_2@CdSe nanohybrids to enhance the stability of the photoelectrochemical signal. The fabricated aptsensor exhibited excellent performance towards PCSK6 with a wide linear range (0.5 pg/mL to 80.0 ng/mL) and a detection limit of 0.1 fg/mL. This work opens up a new detection platform for PCSK6 with good sensitivity, reproducibility and stability.

1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of the synovium and hyperplasia of synovial fibroblasts. The inflammation can erode adjacent cartilage and bone, causing subsequent joint destruction (Sokka, 2003). Fibroblast-like synoviocytes (FLS) from patients with RA can attach and invade normal cartilage in a severe combined immunodeficiency mouse coimplementation model (MullerLadner et al., 1996). Further, FLS are potent players in all aspects of the pathogenesis of RA (Tolboom et al., 2005). FLS are the matrix metalloproteinase (MMP), which are inactive precursors and must be processed by proprotein convertases (PC) to produce biologically active proteins. At present, 9 PC have been identified, and PCSK6 is one of them. And now, it has been identified that PCSK6 may play an important role in the hyperplasia and erosion capacity of synovial fibroblasts (Wang et al., 2014a, 2014b, 2014c). PCSK6 exacerbated inflammation by regulating the expression or secretion of cytokines (Wang et al., 2014a, 2014b, 2014c). PCSK6 correlated positively with typical markers of inflammation and apoptosis (Chang and Han, 2006). Thus, PCSK6 is now a sensitive gene of RA. So the detection of PCSK6 is very important for the early warning of RA. The inhibitor of PCSK6 is now a product in research of Pfizer Inc., PFE. However, no work has been done for the detection of PCSK6, thus we propose a platform for PCSK6 detection based on photoelectrochemical (PEC) method in this work. The aptsensor proposed in our report will be helpful in the early detection. It will certainly provide the better effect for patients.

Genetic testing requires developing the simple methods with low cost, miniaturization, easy-construction and fast-detection. Traditional methods for detecting DNA are slow and labor consuming, such as polymerase chain reaction (PCR), RT-PCR and electrophoresis. The DNA PEC biosensor offers a promising alternative method for the faster, cheaper and simpler nucleic acid assay. PEC assay is a newly developed analytical method with high sensitivity and low background current due to the separation of excitation source and detection signal. Various PEC sensors have been fabricated. They have been used in the detection of DNA (Okamoto et al., 2005; Wang et al., 2014a, 2014b, 2014c; Zhang

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et al., 2013, 2011), DNA damage (Wu et al., 2013; Zhang et al., 2012a, 2012b), enzyme, protein, small molecule (Swaminathan et al., 2011; Yan et al., 2014) and metal ion (Hai et al., 2014; Zhang and Guo, 2012).

Important semiconductor materials have the wide band gap. They possess many useful properties, such as piezoelectricity, optical absorption, emission, high voltage–current nonlinearity, chemical agents and catalytic activity. Considerable efforts have been devoted to prepare various TiO_2 morphologies, such as nanowires (Li et al., 2014; Nikhil et al., 2014; Rao et al., 2014), nanofibers (Liu et al., 2014), nanospheres (Coughlin et al., 2013; Liu et al., 2014; Zhang et al., 2014), nanotubes (Lai et al., 2012; Li et al., 2014; Xiao et al., 2014; Yang et al., 2012; Zhang et al., 2012a, 2012b), nanorods (Feng et al., 2014) and nanoparticles (Kim et al., 2014; Zhou et al., 2014). However, the weak visible light absorption ability limits the application of TiO_2 because of the wide band gap. Sensitizing TiO_2 with narrow band gap semiconductors is a promising approach to increase the visible light absorption, such as CdS–TiO_2 (Nagao et al., 2014), ZnS–TiO_2 (Nagao et al., 2014) and alpyridine ruthenium complexes–TiO_2 (Lahav et al., 2000; Torres et al., 2000). CdSe has a high optical absorption efficiency with narrow band gap of ~1.8 eV in bulk phase (Kuang et al., 2006; Mastai et al., 1999; Peng et al., 2005) and ~2.2 eV for quantum dot (QD) phase (Wang et al., 2014a, 2014b, 2014c). The doped system can enhance the separation efficiency of photogenerated e^-/h^+ pairs and improve the PEC performance of semiconductors (Wang et al., 2009a, 2009b). What is more, it has been proved that semiconductor nano-materials can generate multiple charge carriers with a single photon and improve the efficiency accordingly. Thus, TiO_2@CdSe nanohybrids will possess great potential in the photoelectrical application. However, most of the reports focus on their applications in photovoltaics (Yang et al., 2012), opto-electronics and as-photoanodes (Rao et al., 2014) of the solar cells (Jin et al., 2012; Kim et al., 2014; Li et al., 2014; Zhou et al., 2014).

L-lysine is an important α-amino acid with some basic properties. The electrode modified by L-lysine can perform stable signal and positive surface (Anson et al., 1983). PLL was formed on the electrode by *in-situ* electropolymerization of L-lysine and it can play the same role as L-lysine. The immobilization in the biologic field using polymer film is very simple and the covalent binding can be simultaneously applied for the bio-immobilization (Wang, 2000). Owing to its versatility and easiness of the preparation, PLL has been used for the electrode modifying, electrochemical analysis, and immobilization suspension cells by electropolymerization (Huang et al., 2014). And PLL was also used to construct sensors for the detection of glucose, lactose, etc (Hua et al., 2012; Huang et al., 2014; Li et al., 2013; Mizutani et al., 1995).

In this work, the PEC behavior of CdSe QDs-functionalized TiO_2 nanohybrids (TiO_2@CdSe) was studied and a visible light-induced PEC aptsensor was proposed by using TiO_2@CdSe as PEC signal medium (Scheme 1(1)). The key is the immobilization of DNA on different kinds of surfaces for fabricating the aptsensor. It is the significant factor for improving the stability, reproducibility and sensitivity of the aptsensor. So the first step was the electropolymerization of L-lysine to anchor the primer probe by the strong electrostatic interaction. After that, TiO_2@CdSe nanohybrids were modified on the ITO surface to produce the photoelectrochemical signal. CdSe QDs were synthesized to composite with TiO_2 NPs for amplifying the PEC signal. CdSe QDs could be stimulated under visible light irradiation and the energy levels of TiO_2 NPs and CdSe QDs were aligned with electrolyte solution. In order to capture the target PCSK6 accurately, 3′ primer and 5′ primer were fixed by the amino groups of PLL by the positively strong electrostatic attraction and fixed by the carboxyl groups of TiO_2@CdSe nanohybrids. 3′ primer and 5′ primer could recognize PCSK6 target by specific hybridization. The fabricated aptsensor showed a sensitive photocurrent response, a relatively wide linear range (from 0.0005 to 80 ng/mL) and a low detection limit (0.1 pg/mL).

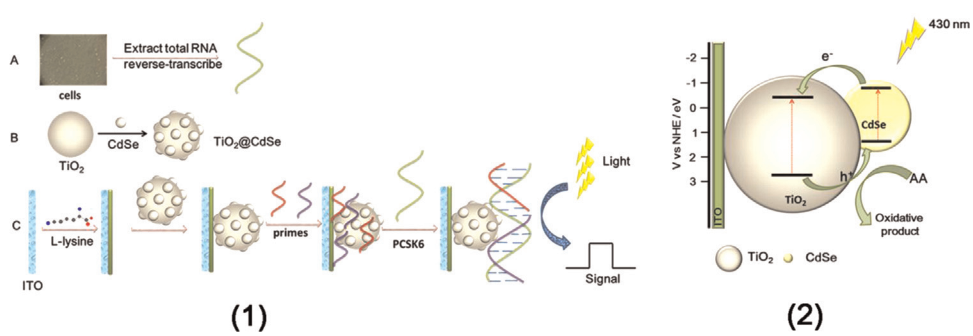
2. Experimental

2.1. Regents and apparatus

Phosphate buffered solutions (PBS, 1/15 mol/L KH_2PO_4 and 1/15 mol/L Na_2HPO_4) were used as the electrolyte for all electrochemistry measurements. All other chemical reagents were analytical reagent grade and directly used without further purification. The ultrapure water (resistivity of 18.25 MΩ cm) and pipette tips were put into a LDZX-30KBS pressure steam sterilizer (Shanghai Shenan Medical Instrument) and sterilized at 121 °C for 40 min. After cooled to room temperature, they were stored in a 4 °C refrigerator.

A conventional three-electrode system was used in all the experiments. ITO electrode (ITO conductive glass was cut into 1.0 cm × 2.5 cm rectangle electrode, washed orderly with acetone, 95% ethanol and ultrapure water by ultrasonication for 30 min and dried with pure nitrogen) was the working electrode. A platinum wire was the auxiliary electrode. A KCl-saturated calomel electrode (SCE) was the reference electrode. All of the CV tests were carried out on CHI760E electrochemical workstation (HuakePutian Co., Ltd., Beijing, China.). All of the electrochemical impedance spectroscopy (EIS) and PEC tests were carried out on IM6x electrochemical workstation (Zahner, Germany).

Scanning electron microscope (SEM) images were obtained from JSM-6700F microscope (JEOL, Japan). Transmission electron microscope (TEM) images were recorded by a JEM-2100F microscope (JEOL, Japan). Fourier transform infrared (FT-IR) spectra were obtained with Perkin-Elmer 580B spectrophotometer (Perkin-Elmer, United States).



Scheme 1. (1) Preparation of PCSK6 (A) preparation of TiO_2@CdSe nanohybrids (B) and the fabrication of PCSK6 aptsensor (C); (2) schematic illustration of the decreased photocurrent mechanism of TiO_2@CdSe nanohybrids.