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Ultrasensitive photoelectrochemical immunoassay for CA19-9 detection based on CdSe@ZnS quantum dots sensitized TiO_2NWs/Au hybrid structure amplified by quenching effect of $Ab_2@V^{2+}$ conjugates

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ABSTRACT

A novel, enhanced photoelectrochemical immunoassay was established for sensitive and specific detection of carbohydrate antigen 19-9 (CA19-9, Ag). In this protocol, TiO_2 nanowires (TiO_2NWs) were first decorated with Au nanoparticles to form TiO_2NWs/Au hybrid structure, and then coated with CdSe@ZnS quantum dots (QDs) via the layer-by-layer method, producing TiO_2NWs/Au/CdSe@ZnS sensitized structure, which was employed as the photoelectrochemical matrix to immobilize capture CA19-9 antibodies (Ab_1); whereas, bipyridinium (V^{2+}) molecules were labeled on signal CA19-9 antibodies (Ab_2) to form $Ab_2@V^{2+}$ conjugates, which were used as signal amplification elements. The TiO_2NWs/Au/CdSe@ZnS sensitized structure could adequately absorb light energy and dramatically depress electron–hole recombination, resulting in evidently enhanced photocurrent intensity of the immunosensing electrode. While target Ag were detected, the $Ab_2@V^{2+}$ conjugates could significantly decrease the photocurrent detection signal because of strong electron-withdrawing property of V^{2+} coupled with evident steric hindrance of Ab_2. Thanks to synergy effect of TiO_2NWs/Au/CdSe@ZnS sensitized structure and quenching effect of $Ab_2@V^{2+}$ conjugates, the well-established photoelectrochemical immunoassay exhibited a low detection limit of 0.0039 U/mL with a wide linear range from 0.01 U/mL to 200 U/mL for target Ag detection. This proposed photoelectrochemical protocol also showed good reproducibility, specificity and stability, and might be applied to detect other important biomarkers.

1. Introduction

Sensitive and accurate detection of disease-related targets is critical to many areas of life and medical sciences, from food safety, environmental monitoring to clinical diagnosis. Especially, highly sensitive detection of cancer biomarkers shows great promise for early diagnosis and disease monitoring (Kitano, 2002; Srinivas et al., 2001). Carbohydrate antigen 19-9 (CA19-9), a Lewis antigen of the cell surface associated mucin 1 (MUC1) protein with an average molecular weight of 1000 kDa, is a gold standard for pancreatic cancer diagnosis (Gui et al., 2013; Gold et al., 2006). Elevated levels of CA19-9 also are associated with gastric, urothelial, and colorectal carcinomas (Xiao et al., 2014; Jha et al., 2013; Narita et al., 2014). Thus, sensitive detection of CA19-9 is of great importance in early prediction for related cancers and diseases. To date, a variety of methods have been developed for CA19-9 detection, such as enzyme-linked immunoassay (Heidari et al., 2014), photoluminescence (Gu et al., 2011), chemiluminescence immunoassay (Shi et al., 2014; Lin and Ju, 2005), and electrochemical immunoassay (Tang et al., 2013; Yang et al., 2015). Despite many advances of these assays, some of them have drawbacks such as evident sample volume, complicated equipment, limited sensitivity, difficult automation and high cost. Thus, development of highly sensitive, simple and inexpensive techniques for CA19-9 detection is very desirable.

Photoelectrochemical analysis is a newly emerged yet dynamically developing technique for the detection of various biological molecules. Recently, it has aroused a great research interest because of the features of simple devices, low cost and easy miniaturization than optical methods such as chemiluminescence

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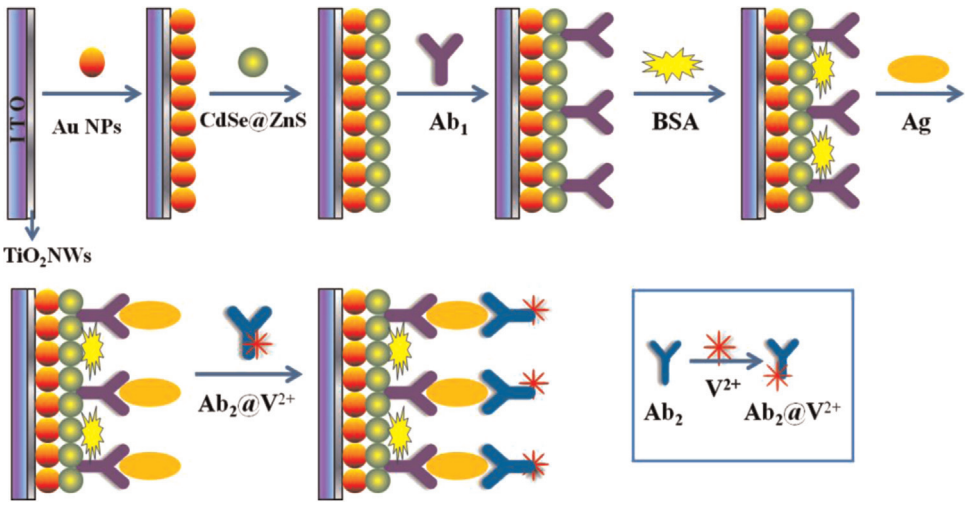
(Kang et al., 2009; Zhao et al., 2015), fluorescence (Sheng et al., 2009; Zhu et al., 2011), and Raman scattering (Ko et al., 2013; Wang et al., 2015). Moreover, photoelectrochemical assays own potentially higher selectivity than traditional electrochemical methods, due to reduced background signals originating from different energy forms of excitation source and detection signal (Haddour et al., 2006; Wang et al., 2009). To date, semiconductor nanomaterials have proved to be the most popular photoactive materials to construct photoelectrochemical biosensors. Thereinto, TiO_2 is an excellent substrate photoactive material owing to its photoelectric activity, good biocompatibility, inexpensiveness, environmental safety, and chemical and physical stability (Qiu et al., 2011; Sano et al., 2012). Recently, one-dimensional TiO_2 crystalline films including nanowires, nanotubes and nanorods have been caught particular attention since they have enlarged surface area and can provide direct pathway for photogenerated electron transfer, which accordingly leads to evident enhancement of charge separation, and effective prohibition of charge recombination (Shankar et al., 2009; Mor et al., 2006; Zhu et al., 2001; Liu and Aydil, 2009; Wu and Yu, 2004; Chen et al., 2010). However, as a wide energy band gap semiconductor (3.2 eV), TiO_2 can only absorb the ultraviolet light (<387 nm), leading to great limitation to utilization of light energy (Qiu et al., 2011). As a result, many efforts have been poured on the exploitation of TiO_2-based hybrid structures to develop visible-light-motivated photoelectrochemical biosensors, which could adequately increase the light absorption efficiency, significantly enhance the photocurrent conversion efficiency and evidently promote the sensitivity of the related biosensors (Fan et al., 2014a, 2014b; Li et al., 2012).

According to signal changes for detection, photoelectrochemical immunoassays can be divided into two types: signal-on and signal-off. Currently, most of the developed photoelectrochemical immunoassays belong to the latter type, because steric hindrance generated by immunized recognition between antibody and antigen would apparently obstacle electron transfer, leading to photocurrent decrease. In order to enhance the sensitivity of photoelectrochemical immunoassays, enzymes are often employed for signal amplification (An et al., 2010; Zhao et al., 2012; Li et al., 2012). However, the introduction of enzyme not only increased the cost of sensor preparation but also made the testing process more complicated. Hence, establishing other simple, low cost and effective photoelectrochemical protocols for signal amplification would be highly expected. Inspired by several researches conducted by Willner and his co-workers, N-(2-carboxymethyl)-N′-methyl-4,4′-bipyridinium (V^{2+}) possesses strong electron-withdrawing capability due to evident electron deficiency of its structure (Tel-Vered et al., 2008; Sheeney-Haj-Ichia et al., 2002a, 2002b). Specifically, when semiconductor nanomaterials such as CdS or CdSe connected with V^{2+} molecules, the photocurrent intensity was obviously lower than that of CdS or CdSe alone, because V^{2+} molecules acted as traps for the conduction-band electrons (Tel-Vered et al., 2008; Zhang et al., 2012). Accordingly, V^{2+} can be well used as signal-off labels linking with signal antibodies (Ab_2) to form $Ab_2@V^{2+}$ conjugates for signal amplification. As both V^{2+} and Ab_2 jointly facilitate decrease of photocurrent signal, using $Ab_2@V^{2+}$ conjugates as signal amplification elements can contribute to an excellent sensitivity for signal-off photoelectrochemical immunoassays. However, to be of our knowledge, this kind of signal amplification protocol has not appeared in photoelectrochemical immunoassays.

Herein, we presented an enhanced, promising platform to construct an ultrasensitive photoelectrochemical immunoassay for CA19-9 (antigen, Ag) detection based on TiO_2NWs/Au/CdSe@ZnS sensitized structure and signal amplification of $Ab_2@V^{2+}$ conjugates, as illustrated in Scheme 1. Firstly, TiO_2NWs were synthesized by a hydrothermal growth method, and then were modified onto a bare ITO (indium tin oxide) electrode. Next, the ITO/TiO_2NWs electrode was coated with Au nanoparticles, and then was modified with CdSe@ZnS film via layer-by-layer assembling oppositely charged polyelectrolyte and CdSe@ZnS QDs, forming TiO_2NWs/Au/CdSe@ZnS sensitized structure to significantly enhance the photocurrent intensity. Afterwards, Ab_1 was immobilized on the electrode by EDC coupling reaction between carbonyl and amino groups. After BSA blocked unbound sites on the electrode surface, the immunosensing electrode was ready. For target CA19-9 determination, different concentrations of Ag were first bound on the sensing electrode by specific immunoreaction between Ag and Ab_1, and then the fixed concentration of $Ab_2@V^{2+}$ conjugates as signal amplification elements were further immobilized through specific immunoreaction between Ag and Ab_2, which led to significantly reduced photocurrent. The proposed photoelectrochemical protocol exhibited ultrahigh sensitivity, reproducibility, specificity, and stability.



Scheme 1. Construction process of the photoelectrochemical immunoassay for CA19-9 detection.