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Spatial-resolved dual-signal-output electrochemiluminescent ratiometric strategy for accurate and sensitive immunoassay

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ABSTRACT

The identification of tumor markers is of great importance for clinical diagnosis but accurate detection with high sensitivity is still a great challenge. In present work, a spatial-resolved dual-signal-output electrochemiluminescent (ECL) ratiometric assay platform was constructed for sensitive detection of prostate specific antigen (PSA) on a dual-disk glassy carbon electrode. To fabricate the platform, flower-like CdS three-dimensional (3D) assemblies and $Ru(bpy)_3^{2+}$-conjugated silica nanoparticles ($Ru(bpy)_3^{2+}$@RuSi NPs), were immobilized onto the two disks as cathodic and anodic ECL emitters, respectively. After the stepwise modification of the gold nanoparticles, antibody for PSA, and bovine serum albumin onto the two disks respectively, the $Ru(bpy)_3^{2+}$@RuSi NPs-based disk were incubated with varied concentration of PSA as working electrode, whereas the flower-like CdS 3D assemblies-based disk with fixed concentration of PSA were taken as internal reference electrode. The label free assay of PSA was realized by the ratio of anodic ECL signal from working electrode to the cathodic ECL signal from the internal reference electrode ($ECL_{anode}/ECL_{cathode}$). On the basis of the spatial-resolved dual-signal-output ratiometric ECL sensor, the PSA can be detected accurately with a linear range of 0.001 – 50 ng/mL at a concentration as low as 0.34 pg/mL. Furthermore, the proposed method was applied for PSA determination in human serum samples with satisfying results. Thanks to the same modified process of the two disks, this universal design well avoids environmental errors including the interference caused in the biological recognition process, which effectively reduces the false positive or negative errors, exhibiting a greatly improved accuracy, reliability and sensitivity.

1. Introduction

Electrochemiluminescence (ECL) has become a powerful analytical technique that combines the unique advantages of electrochemistry and chemiluminescence, such as high sensitivity, good temporal/spatial control and wide dynamic range (Miao, 2008; Hu and Xu, 2010). However, the conventional ECL method used only one signal output for quantitative analysis, which could cause false positive or negative errors caused by the instrumental or environmental factors (Liu et al., 2016; Cao et al., 2018). Recently, dual-potential ECL ratiometric strategy depending on two signals output received attention because ratiometric detection based on the ratio of two signals could reduce the influence from the environmental change. For instance, Zhang et al. (Zhang et al., 2013) developed a ratiometric ECL sensing approach which employed luminol and CdS nanocrystal as two different ECL emitters on one electrode interface for measurement of mp53 oncogene. Later, the ECL ratiometric sensing strategy has been gradually applied for the detection of proteins (Feng et al., 2015; Huang et al., 2016), microRNAs (Hao et al., 2014), metal ions (Cheng et al., 2014; Lei et al., 2015) and cancer cells (Wang et al., 2016) to reduce interferences and make the detection more convincing. Although there are many advantages of ratiometric assay, sensors above used one electrode as ECL report unit for two emitting states, which may result in a cross-reaction between the two light emitting systems. Therefore, exploring a new and more accurate ECL bioanalysis method is a worth researching topic.

By physically separating the working interface for different emitters, spatial-resolved technique as an effective method has witnessed its fine performance in reducing the cross-reaction between the two light emitters. On the basis of spatial-resolved assay platform, Li et al. (2011) proposed an ECL sensing array to achieve near-simultaneous detection of three targets on different spatial areas with the aid of a single-pore-four-throw switch. Refer to the idea that the ECL signal can produce at different spatial areas, Feng et al. (2016b) designed a dual-potential ECL aptasensor array based on a screen-printed carbon electrode with

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two spatial areas for simultaneous detection of two targets, which achieved the simultaneous output of two different signals in one single scan. These spatial-resolved ECL methods provide a new way to produce two or more independent ECL signal output in one system. Very recently, Feng et al. (2016a) developed spatial-resolved ECL ratiometric aptasensor array for antibiotic chloramphenicol detection, which was based on the ratio of working signal to internal reference signal. However, the internal reference signal from the luminophor substrate may not well reduce the environmental errors caused by biological recognition. Therefore, it is eager to explore a new path for more accurate assay on the basis of spatial-resolved technology combined with dual-signal-output ECL ratiometry.

In the dual-signal-output ECL ratiometry, screening the ECL emitter pair to generate two distinguishable ECL signal in one single scan is a key factor. CdS as one of the extensively used ECL emitter has been used for the bioanalysis and exhibits high stability. It has been reported that CdS could be excited at negative potential and produce an ECL in the presence of $S_2O_8^{2-}$ in the aqueous solution (Jie et al., 2007; Wang et al., 2013). As a conventional ECL reagent, $Ru(bpy)_3^{2+}$ could be excited at a positive potential and generate a strong ECL in the presence of tripropyl amine (TPA) (Leland and Powell, 1990; Bozorgzadeh et al., 2015; Wang et al., 2015; Yang et al., 2016). Given the fact that ECL reactions of CdS and $Ru(bpy)_3^{2+}$ occur under different potentials, it is possible to achieve dual-signal-output ratiometric strategy based on the ratio of two signals excited by both of the above ECL tags in one single scan.

Inspired by the aforementioned perspectives, herein, a new, accurate and sensitive biosensor based on spatial-resolved dual-signal-output ratiometric strategy was successfully assembled for PSA assay using a dual-disk glassy carbon electrode (DDCE). flower-like CdS three-dimensional (3D) assemblies and $Ru(bpy)_3^{2+}$-conjugated silica ($Ru(bpy)_3^{2+}$@RuSi) NPs, selected as ECL signal tags, were coated onto the spatial-resolved areas (disk I and the disk II of DDCE), respectively. After gold nanoparticles (Au NPs), antibody of PSA (Ab1) and bovine serum albumin (BSA) were modified by the self-assembly method, PSA with fixed concentrations was immobilized on CdS modified disk I of DDCE, whereas PSA with varied concentrations was immobilized onto $Ru(bpy)_3^{2+}$@RuSi NPs modified disk II of DDCE. Taking the changed ECL intensities from disk II at positive potential as working signal and the constant ECL intensities from disk I at negative potential as reference signal, the concentration of target protein can be quantified in a label free manner by the ratio of ECL intensities at two excitation potentials ($ECL_{anode}/ECL_{cathode}$). Different from the previous reported ECL ratiometric assay (Feng et al., 2016b; Takeuchi et al., 2010; Branchini et al., 2011), in this work, the environmental interference caused by process of biological recognition was considered for the first time, which would develop a more accurate strategy for identification of tumor markers.

2. Experimental

2.1. Materials and reagents

Tris(2,2′-bipyridyl) ruthenium(II) chloride hexahydrate ($Ru(bpy)_3Cl_2{\cdot}6H_2O$), (3-Aminopropyl) triethoxysilane (APTES), and tripropylamine (TPA) were purchased from Sigma-Aldrich (USA). Thiourea and cadmium chloride hydrate ($CdCl_2{\cdot}2H_2O$) were bought from Shanghai Chemical Reagent Corporation (Shanghai, China). Triton X-100, tetraethyl orthosilicate (TEOS), ammonia solution (NH_4OH, 25%), 1-hexanol, and cyclohexane were obtained from Aladdin Industrial Co., Ltd. (Shanghai, China). Prostate specific antigen (PSA, (L2C001)) standards and mouse monoclonal antibodies to PSA, clone L1C00401 (primary anti-PSA antibody, Ab1) were from Shanghai Linc-Bio Science Co., Ltd. (Shanghai, China). Human serum albumin (HSA), human immunoglobulin G (hIgG) and bovine serum albumin (BSA, Mr = 67,000) were from Shanghai Solarbio Bioscience & Technology Co., Ltd. (Seebio Biotechnology). 0.1 mol/L phosphate buffered saline (PBS, pH 7.4) contained 87.2 mmol/L Na_2HPO_4, 14.1 mmol/L KH_2PO_4, 2.7 mmol/L KCl, and 137 mmol/L NaCl. All chemicals were at least analytical grade, and the water used in all experiments was purified by a pure water system (Kangning Water Treatment Solution Provider, China).

2.2. Apparatus

The images of scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were observed under a S-4800 (Hitachi, Tokyo, Japan) and a Tecnai G2 F20 S-TWIN TEM (FEI Co., Ltd., USA), respectively. ECL signals were measured with a MPI-B electrochemiluminescence analyzer (Xi'An Remax Electronic Science & Technology Co., Ltd., Xi'An, China). The UV–vis absorption spectra were recorded on a Shimadzu UV-3600 UV–visible-NIR photo spectrometer (Shimadzu Co., Tokyo, Japan). A three-electrode system was used with a dual-disk glassy carbon electrode (DDCE) (3 mm in diameter) as the working electrode, an Ag/AgCl as the reference electrode and a platinum electrode as the auxiliary electrode.

2.3. Synthesis of flower-like CdS 3D assemblies

The flower-like CdS 3D assemblies were prepared using a solvothermal process as previously described with minor modifications (Guo et al., 2011). First, $CdCl_2{\cdot}2H_2O$ power and thiourea power were respectively dissolved into ethanol/water mixed solvent (2:3, *v/v*) under vigorous stirring for 30 min to form two different solutions. The thiourea solution was then slowly added to the above $CdCl_2$ solution and the pH of the mixture was adjusted by NaOH to 5.4. The mixture solution was transferred into a Teflon-lined stainless-steel autoclave and maintained at 180 °C for 9 h. Afterwards, the product was collected by centrifugation at the speed of 9000 rpm for 15 min and then washed with water and ethanol several times, allowing it to dry in an oven at 60 °C. For electrode modification, the obtained yellow power was dispersed in 0.025 mg/mL of chitosan (CS) solution by sonication, and the final concentration of CdS solution was 0.4 mg/mL.

2.4. Preparation of $Ru(bpy)_3^{2+}$@RuSi NPs

The $Ru(bpy)_3^{2+}$@RuSi NPs were prepared following the published method (Wu et al., 2012). The micro-emulsion was firstly prepared by mixing 1.77 mL of Triton X-100, 1.8 mL of 1-hexanol, 7.5 mL of cyclohexane, and 340 μL of $Ru(bpy)_3^{2+}$ (40 mmol/L). After vigorously stirring for 20 min, 100 μL of TEOS and 60 μL of NH_4OH were added into the above prepared cocktail. The reaction was kept for 24 h at ambient temperature under stirring. Thereafter, acetone was added to destroy the emulsion. Followed by centrifuging and washing with ethanol and water, respectively, the orange-colored RuSi NPs were obtained. To make the surface of RuSi NPs containing amino groups, 60 μL of APTES was injected into 1.0 mL of as-prepared RuSi NPs solution (2.0 mg/mL suspended in ethanol). After stirring for 30 min, the mixture was centrifuged and washed with ethanol two times (10,000 rpm, 10 min) and dispersed in 500 μL of water. Subsequently, 500 μL of 2.0 mg/mL $Ru(bpy)_3^{2+}$-NHS was mixed with 500 μL of APTES-modified RuSi NPs and stirred vigorously for 24 h. After centrifugation and washed several times with water to remove excess $Ru(bpy)_3^{2+}$, the resulted $Ru(bpy)_3^{2+}$@RuSi NPs drop was then suspended in 500 μL of water.

2.5. Fabrication of the spatial-resolved dual-signal-output ratiometric ECL sensor

The fabrication process of the spatial-resolved dual-signal-output ratiometric ECL sensor is illustrated in Scheme 1. The DDCE was separated into two spatial-resolved areas spaced 2 mm apart to avoid the cross-talk of the two areas. Before the fabrication, the DDCE was