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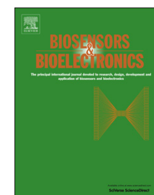

Electrochemiluminescence immunosensor for tumor markers based on biological barcode mode with conductive nanospheres

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ABSTRACT

A novel sandwich-type electrochemiluminescence (ECL) immunosensor was developed for highly sensitive and selective determination of tumor markers based on biological barcode mode. *N*-(4-aminobutyl)-*N*-ethylisoluminol (ABEI) and the second antibody (Ab_2) were simultaneously immobilized on conductive nanospheres to construct ABEI/Ab_2-CNSs probes, which could form sandwich immunocomplex by Ab_2 and emit ECL signals by ABEI. The gold layer coated on the surface of the conductive nanospheres could extend the outer Helmholtz plane (OHP) of the ECL immunosensor effectively. Benefited from it, all ABEI molecules immobilized on conductive nanospheres would act as biological barcode to give in-situ ECL signals without interfering with the activity of the second antibody. In such a case, the sensitivity of the ECL immunosensor would be greatly improved because an antigen molecule would correspond to ECL signals of thousands of ABEI molecules. Using prostate specific antigen (PSA) as a model tumor marker, the ECL intensity was found to increase with the logarithm of PSA concentration with a wide linear range from 0.04 to 10 fg/mL. In addition, specificity, stability, reproducibility, regeneration and application were satisfactory. Therefore, this developed ECL immunosensor has a potential for practical detection of disease-related proteins besides tumor markers in the clinical diagnostics.

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1. Introduction

Tumor markers are biochemical substances which are produced by either the tumor itself or the surrounding normal tissue as a response to tumor cells (Freedland, 2011; Wickström et al., 2011). Since the presence of tumor markers in serum or other body fluids in response to precancerous or cancerous conditions induces an array of biochemical processes, they are often used for evaluating disease process, recurrence, metastasis and prognosis (Marta et al., 2013; Liu and Ma, 2013; Qu et al., 2013; Xiang and Lu, 2012), and the low concentrations of tumor markers may be related to the early stage of cancerous conditions. Therefore, it is crucial to develop diagnostic tools for the detection of very low concentrations of these markers in healthy humans, sub-healthy humans or patients to identify the early stage.

Lots of methods and strategies have been developed for the detection of tumor markers, including radioimmunoassay (RIA) (Tyan et al., 2013; Su et al., 2011), chemiluminescence immunoassay (CLIA) (Tian et al., 2010; Yang et al., 2010b), enzyme-linked immunosorbent assay (ELISA) (Darwish et al., 2013; Chalupova et al., 2013; Saadi et al., 2013), chemiluminescent enzyme immunoassay (CLEIA) (Dong et al., 2012; Xiao et al., 2009) and time-resolved fluorescence immunoassay (TRFIA) (Hou et al., 2012; Lu et al., 2012; Niu et al., 2011), etc. However, there are still some problems challenging their application: (1) the sensitivity is not high enough to determine tumor markers at low level, (2) radioactive or toxic markers are needed, (3) experimental procedures are complex, (4) detection time is long, and (5) instruments used are expensive, and so on.

Electrochemiluminescence (ECL) is a valuable detection method, which has been applied extensively due to its acknowledged advantages such as versatility, simplified optical setup, very low background signal, and good temporal and spatial control (Richter, 2004). $Ru(bpy)_3^{2+}$ and luminol are the most widely used ECL systems. Besides, *N*-(4-aminobutyl)-*N*-ethylisoluminol (ABEI), a derivative of isoluminol with similar ECL mechanism as luminol, is brought to our attention because of its relatively high ECL efficiency as bioassay label compared with luminol (Tian et al., 2009; Yang et al., 2002). Based on ECL, electrochemiluminescence immunoassay (ECLIA) has gained much attention in recent years

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due to its wide dynamic range, high sensitivity, low background, environmentally friendly labels, simple instrumentation and easy methodology. In many cases, a sandwich-type is the commonly used mode of ECLIA. In most of the previous related research work focused on labeling materials and labeling methods such as: using $Ru(bpy)_3^{2+}$-encapsulated silica nanosphere (Yang et al., 2010a; Qian et al., 2010), quantum dots functionalized graphene sheet (Liu et al., 2013), dendrimer multiply labeled luminol on Fe_3O_4 nanoparticles (Li et al., 2013a), *N*-(aminobutyl)-*N*-(ethylisoluminol)-functionalized gold nanoparticles (Shen et al., 2011; Tian et al., 2009), Au-MSN-HRP-Ab2 composites (Wei et al., 2010) and Ru-AuNPs/graphene (Li et al., 2013b) as labels, basing on energy transfer between quantum dots and quantum dots (Guo et al., 2012), between $Ru(bpy)_3^{2+}$ and quantum dots (Hao et al., 2012) and between quantum dots and gold nanoparticles (Qian et al., 2013), using quantum dots as labels and graphene as conducting bridge (Guo et al., 2013), etc. However, using conductive nanospheres multi-functionalized by the second antibody and luminophore as labels was seldom reported.

Biological barcode technology was first reported by Mirkin et al. (Thaxton et al., 2009; Nam et al., 2002; Oh et al., 2006), in which gold nanoparticles multi-functionalized with specific probes can identify target analyte specifically and a large number of oligonucleotide strands. Those oligonucleotide strands with identical sequences playing a role of surrogate target to amplify the detection sensitivity effectively are termed as barcode. Due to the efficient amplification, the detection of proteins and DNA by bio-barcode assay can reach the attomolar level (Goluch et al., 2006; Nam et al., 2004). However, this ultrasensitive method suffers from cumbersome steps greatly.

Herein, we present a novel sandwich-type electrochemiluminescence (ECL) immunosensor for the ultrasensitive detection of tumor markers based on biological barcode mode, using conductive nanospheres multi-functionalized with the second antibody and luminophore ABEI. Thousands of ABEI molecules were labeled as barcode, and nearly all of them could emit ECL signals efficiently with the help of conductive nanospheres. Therefore, the detection sensitivity was improved greatly, with a detection range of 0.04 to 10 fg/mL using prostate specific antigen (PSA) as model analyte.

2. Experimental

2.1. Apparatus

A laboratory-built ECL detection system, as described previously (Guo and Gai, 2011) was used in this study. A three-electrode system, including bare or modified gold electrode (Φ=3 mm), platinum wire electrode and Ag/AgCl electrode as working electrode, counter electrode and reference electrode, respectively was used. Electrochemical impedance spectroscopy (EIS) experiment was performed with a CHI 660B electrochemistry workstation (Chenhua Instrument Company, Shanghai, China). The morphology of the nanospheres used was characterized using a SU70 scanning electron microscope (SEM, Hitachi, Toyko, Japan).

2.2. Reagents and materials

N-(4-aminobutyl)-*N*-ethylisoluminol (ABEI), bovine serum albumin (BSA), tetraethoxysilane (TEOS), glutaraldehyde (GLD) and 2-aminoethanethiol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chloroauric acid ($HAuCl_4 \cdot 4H_2O$), (3-aminopropyl)-triethoxysilane (APS), sodium citrate, hydroxylammonium chloride were obtained from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). Prostate specific antigen (PSA), the first antibody anti-PSA (Ab_1) and the second antibody anti-PSA (Ab_2) were purchased from Zhengzhou Biocell Biotechnology Company (Zhengzhou, China), and stored at −20 °C before use. Carbonate buffer solution (CBS, pH 9.6) containing 0.015 mol/L sodium carbonate and 0.035 mol/L sodium bicarbonate, and 1 mmol/L H_2O_2, was used as the working solution for the ECL measurement. All other reagents were of analytical grade. Ultra-pure water (18 MΩ cm), obtained from a Heal Force PW ultrapure water system (Heal Force Bio-Meditech Holdings Limited, Hong Kong, China), was used in the experiment throughout.

2.3. Synthesis of conductive nanospheres (CNSs)

Amino-functionalized SiO_2 nanospheres were prepared as described previously (Stober and Fink, 1968; Pan et al., 2012; Jiao et al., 2012) with some modifications. Briefly, ethanol, water and concentrated ammonia–water were mixed to about 100 mL with a volume ratio of 88:8:1, and kept stirred. Then 4.5 mL of TEOS was added and allowed to react for 10 h to obtain SiO_2 nanospheres. After centrifugal washing with water, the precipitate was dispersed in anhydrous ethanol to form a 50 mg/mL suspension. Finally, amino-functionalized SiO_2 nanospheres were obtained by adding 2 mL of APS into 20 mL of the above suspension and refluxing for 4 h at 80 °C in 80 mL of anhydrous ethanol with stirring, the morphology of which was monitored by SEM, as shown in Fig. 1A.

Conductive nanospheres (CNSs) were synthesized according to the literatures (Hu et al., 2005; Wei et al., 2010). A 400 mL solution containing 0.0125 mg/mL gold nanoparticles (AuNPs) with a diameter of 13 nm, prepared as described previously (Polte et al., 2010), and 0.8 mg/mL of amino-functionalized SiO_2 nanospheres was stirred for 12 h, washed until no AuNPs could be found in the

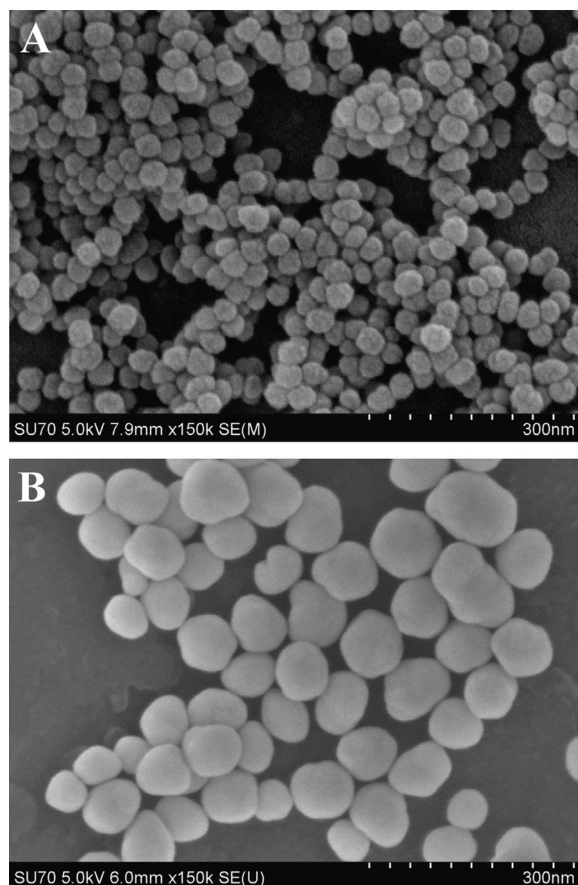

Fig. 1. The SEM image of (A) SiO_2 nanospheres and (B) SiO_2/Au conductive nanospheres (CNSs).

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