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Ultrasensitive electrochemiluminescence immunoassay for tumor marker based on quantum dots coated carbon nanospheres

Long Li [a], Yan Zhang [a], Shuai Li [a], Xiu Wang [a], Chen Li [a], Shenguang Ge [b], Jinghua Yu [a,*], Mei Yan [a], Xianrang Song [c]

[a] Key Laboratory of Chemical Sensing & Analysis in Universities of Shandong, School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, P. R. China
[b] Shandong Provincial Key Laboratory of Fluorine Chemistry and Chemical Materials, University of Jinan, Jinan 250022, PR China
[c] Cancer Research Center, Shandong Tumor Hospital, Jinan 250012, PR China

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ABSTRACT

In this work, a novel electrochemiluminescence (ECL) immunosensor based on CdTe quantum dots (QDs) coated carbon nanosphere (CN/QDs) for the detection of carcinoembryonic antigen (CEA) was developed. The carbon nanospheres (CNs) with good monodispersity and uniform structure were synthetized by a hydrothermal method using glucose as raw material. Then QDs functionized CNs were prepared and employed for signal amplification to improve the sensitivity and the detection limit of immunosensor. For this proposed immunosensor, chitosan was firstly deposited on the pretreated indium tin oxide (ITO) electrode surface, which promoted the electron transfer. Subsequently, gold nanoparticles (AuNPs) were assembled onto chitosan film modified electrode to improve the absorption capacity of antibodies. Then, primary antibodies were immobilized onto the electrode through the reaction between AuNPs and amino. At last bovine serum albumin (BSA) was employed to block the nonspecific binding sites. As a result, a novel ECL immunosensor was obtained on the prepared CN/QDs. The CEA was determined in the range of 0.005–200 ng mL^{-1}, with a low detection limit of 1.2 pg mL^{-1} ($S/N=3$). The proposed ECL immunosensor provides a rapid, simple, and sensitive immunoassay protocol for protein detection, which could be applied in more bioanalytical systems.

1. Introduction

Carcinoembryonic antigen (CEA) is one of the most widely used tumor makers responsible for clinical diagnosis of colorectal, pancreatic, breast, and cervical carcinomas [1–3]. Thus, the determination of CEA level is very useful to clinical tumor diagnoses. In immunoassay systems, many immunoassay techniques, such as fluorescence, chemiluminescence, surface plasmon, and mass spectrometric immunoassays have been devoted into realizing the ultrasensitive detection [4–7]. These methods are well established and now commercially available. Though with good sensitivity, these methods need the sample to be labeled, which made the analysis complex, time consuming and expensive. Thus, the development of sensitive and selective immunosensors has attracted much attention.

As an alternative, electrochemiluminescence (ECL) analysis has received considerable attention due to its versatility, low-cost, low background signals, applicability to a wide range of analytes, and high sensitivity [8,9]. For point-of-care applications, the sensors need to be highly sensitive to address the levels of biomarkers including low-abundance proteins. With the increasing demand for early-disease screening and diagnosis, numerous immunoassays have been developed for the enhancement of detection sensitivity by signal amplification or employment of different detection technologies [10,11]. Fortunately, due to their versatile properties, nanomaterial-based signal-amplification strategies hold a very large application prospect in realizing ultrasensitive detection [12–14]. A lot of nanomaterials, including metal nanoparticles, magnetic nanoparticles and carbon-based nanostructures [15–17], have been used for carriers or tracers to obtain the amplified electrochemical detection signal. Among these nanomaterials, carbon-based nanostructures have attracted much attention among scientists in various disciplines. Carbon material comprises carbon nanotubes (CNTs) [18–21] and CNs. Compared with CNTs, CNs possesses homogeneity of particle size, better tenability, and porous nanostructure for large loading of guest molecules [22–26]. In addition, the synthesis method of homogeneous CNs with good biocompatibility and surface-functional groups under hydrothermal conditions is "green" and cost-effective [27,28]. Such CNs can be good candidates for developing ultrasensitive immunosensor as carriers.

* Corresponding author. Tel.: +86 531 82767161; fax: +86 531 82765969.
E-mail addresses: ujn.yujh@gmail.com, 306375767@qq.com (J. Yu).

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Since ECL detection is performed based on the measurement of light emission from luminophores, it is superior to other luminescent methods, because of its low background and controllable procedure. Three types of luminophores, $Ru(bpy)_3^{2+}$, luminol, and QDs, have been applied in ECL detection [29–32]. With the additional advantages of low cost and easy preparation, ECL detection with QDs has attracted much attention in bioanalytical field. ECL based on QDs, such as CdTe, CdSe and CdS [33–35], has been extensively investigated for immunoassays [36], ECL mechanism study [37], and DNA detection [38–40]. One hurdle in these works is that QDs can diffuse into the electrolyte solution during oxidative corrosion at high voltage, which is essentially involved in ECL detection. To address this problem, in recent years, several methods have been demonstrated for stable ECL emission to composite QDs with nanomaterial scaffolds or to develop surface chemistry for QD attachment to the electrode surface [41–43].

Herein, we prepared a novel ECL immunsensor using CdTe QDs functionalized CNs as labels for protein detection. CNs with good monodispersity was employed as carriers for immobilization of QDs and antibodies. Here, the second antibodies were covalently bound to CdTe QDs on the surface of CNs. Enhanced sensitivity could be achieved by the increase of CdTe QD loading per sandwich immunoreaction [44]. To further expand this strategy with different detection technologies for other protein detections, herein, we present a detailed investigation on synthetic strategy of CN/QDs labels and their versatile applications in immunoassays. To obtain immunosensors with good regenerative performance, Au–chitosan film was constructed on ITO surface for the immobilization of anti-CEA antibodies. The CN/QDs labels were brought to the Au–chitosan/ITO electrode surface through a subsequent "sandwich" immunoreaction, which allowed sensitive detection of CEA, with detection limits of 1.2 pg mL^{-1} for ECL measurements.

2. Materials and methods

2.1. Reagents

ITO (thickness of ITO layer: 150 nm; resistance < 15 Ω/square; thickness of glass: 1.1 mm) was obtained from Xiamen ITO Photoelectricity Industry (Xiamen China). H_2O_2 (Shanghai Chemical Plant, China) was of analytical grade and used as received. Chitosan solution (1%, wt%) was prepared by ultrasonically dissolving chitosan powder in 1% acetic acid. CEA were purchased from Shanghai Linc-Bio Science Co., Ltd. (Shanghai, China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), (3-aminopropyl)-triethoxysilane (APTS), bovine serum albumin (BSA) and thioglycolic acid were purchased from Sigma-Aldrich Chemicals Co. All other chemicals were of analytical grade and were used as received. The phosphate buffer solutions (PBS) were prepared by mixing Na_2HPO_4 and KH_2PO_4. Twice-distilled water was used throughout the study.

2.2. Apparatus

The scanning electron microscope (SEM) images were taken by FEI Sirion-200 field emission scanning electron microscope (HITACHI S-4800, Japan). The morphology and size of CNs and CN/QDs were analyzed with a transmission electron microscope (TEM, JEOL JEM-1400 microscope, Japan). The electrochemical and ECL measurements were carried out on an MPI-E multifunctional electrochemiluminescent analytical system (Xi'an Remex Analyze Instrument Co., Ltd., China). All ECL measurements were performed in a 5 mL glass cell composed of an ITO working electrode, a platinum counter electrode, and an Ag/AgCl (saturated KCl solution) reference electrode. The photoluminescence (PL) spectra were obtained on a RF-5301 spectrofluorometer (P.C. Shimadzu, Japan). UV–vis absorption spectra were recorded on a UV-3101 spectrophotometer (Shimadzu, Japan). Energy dispersive spectrometer (EDS) was obtained on a JSM-6700F microscope (Japan).

2.3. Synthesis of gold nanoparticles

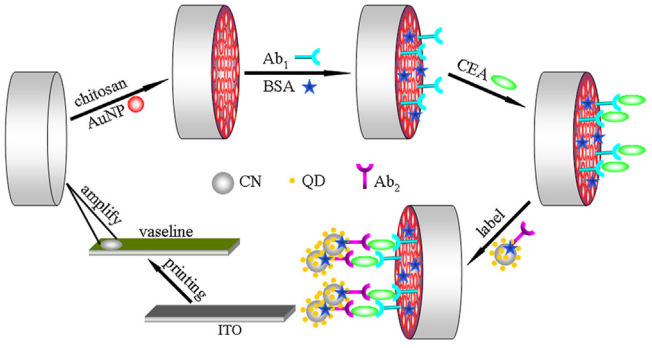
Gold nanoparticles (AuNPs) were prepared from $HAuCl_4$ solution by reduction reaction according to the reported literatures with a slight modification [45,46]. Prior to experiment, all glassware used in the following procedures was cleaned in a bath of freshly prepared HNO_3/HCl (3:1) solution, and dried in air after rinsed thoroughly with twice-distilled water. Then, 1.0 mL of 1% trisodium citrate was added into 100 mL of boiling 0.01% $HAuCl_4$ solution and stirred for 10 min in the boiling case. The solution was then left stirring and cooling down after turned wine red which indicated the formation of AuNPs. Prepared AuNPs were stored in a glass bottle at 4 °C.

2.4. Preparation of Au–chitosan/ITO electrode

A sheet of ITO glass was cut into slides (8 × 25 mm) and cleaned by sequential ultrasonic cleaning in acetone (10 min), dichloromethane (10 min), and twice-distilled water (2 min), as reported previously [47,48]. These clean slides were placed in a small beaker where a mixture of H_2O:H_2O_2 (30%):NH_3 (25%) (5:1:1) was added and soaked for 1 h at room temperature. Then the slides were washed with plenty of twice-distilled water and dried in vacuum at 100 °C for 4 h. The preparation of the ITO electrodes also includes designing the pattern for vaseline-printing stencil in order to make sure each of the ITO modified the same area. The fabrication detail was shown in Scheme 1, the pattern for vaseline-printing stencil was prepared using Adobe illustrator, and the stencil was generated by traditional photolithography technique. The stencil was placed onto the cleaned ITO glass slides, and vaseline was smeared on the ITO electrodes and washed with twice-distilled water for several times. Then 10 μL of 1% chitosan acetate solution was deposited on the pretreated ITO electrode which was without vaseline and dried for 2 h at room temperature. The chitosan membrane-modified ITO was immersed in prepared colloidal gold solution for 30 min to form Au–chitosan assembly and washed with plenty of twice-distilled water. The prepared Au–chitosan/ITO electrode was stored at 4 °C prior to use.

2.5. Preparation of the CN/QDs/Ab_2 labels

The preparation of the water-soluble CdTe QDs was reported previously [49]. In brief, 0.1028 g of $CdCl_2 \cdot 2.5H_2O$ was dispersed in 35 mL of twice-distilled water, and then 80 μL of thioglycolic



Scheme 1. Schematic diagram of the sandwich type immunoassay with CN/QDs/Ab_2 as labels.