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An ultrasensitive electrochemiluminescence immunosensor based on zeolitic imidazolate frameworks encapsulating spherical graphite crystals

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ABSTRACT

Here, an ultrasensitive electrochemiluminescence (ECL) immunosensor for the detection of cardiac troponin I (cTnI) was developed by using zeolitic imidazolate frameworks encapsulating spherical graphite crystals (CQDs@ZIF-8) as luminophore. Firstly, luminescent spherical graphite crystals (CQDs) were encapsulated in the zeolitic imidazolate framework (ZIF-8) nanocrystals. To improve the poor conductivity of CQDs@ZIF-8, Au nanoparticles were synthesized on the surface of CQDs@ZIF-8 to form AuNPs/CQDs@ZIF-8. Furthermore, L-cysteine (L-cys) as the coreactant of CQDs was immobilized on the AuNPs to enhance the ECL signal of CQDs. Then, the obtained L-cys/AuNPs/CQDs@ZIF-8 was introduced to construct an ultrasensitive ECL immunosensor for the detection of cTnI. As a result, the proposed immunosensor displayed excellent analytical performances for the detection of cTnI with a linear range from 1 fg μL^{-1} to 10 pg μL^{-1} and a detection limit of 0.33 fg μL^{-1}.

1. Introduction

Cardiac troponin I (cTnI), as a specialized myocardial regulatory protein, has been acknowledged as an important principle diagnostic biomarker to monitor acute myocardial infarction (AMI) which is a serious threat to the public health [1–6]. However, the traditional detection methods are quietly limited to expensive instrument, long detecting time or low sensitivity. Therefore, it is very meaningful to develop an ultrasensitive determination method of cTnI for early assessment of the severity and progression rate of AMI.

Electrochemiluminescence (ECL), as a powerful detection tool combined the merits of electrochemistry and chemiluminescence, has attracted great attentions due to its high sensitivity, simple format and rapid response in recent years [7–11]. However, traditional ECL luminophores were hard to be immobilized and the luminescence efficiency of traditional ECL luminophores was not enough for ultrasensitive detection. Therefore, the key points to enhance the sensitivity of ECL system are developing new luminophores with high luminescence efficiency and immobilizing these well performed luminophores efficiently. Up to now, several kinds of novel luminophores have been introduced into the constructions of ECL systems, such as noble-metal nanoclusters [12], iridium complex [13], semiconductor quantum dots [14–16] and spherical graphite crystals [17–19]. Among these luminophores, spherical graphite crystals with good biological compatibility, high luminescence efficiency and low cost have been widely reported in the researches of ECL analytical technologies. However, spherical graphite crystals are difficult to be immobilized on the electrode, which limits its application in ECL biosensor. Pillai's group has reported a strategy based on zeolitic imidazolate framework (ZIF-8) nanocrystals encapsulating fluorescent spherical graphite crystals (CQDs@ZIF-8) [20]. However, the poor conductivity limited the application of CQDs@ZIF-8 in ECL system. Based on this strategy, Au nanoparticles (AuNPs) were synthesized on the surface of the CQDs@ZIF-8 to form AuNPs/CQDs@ZIF-8 with improved electric conductivity.

Inspired by all of these perspectives, an ultrasensitive ECL immunosensor for the detection of cTnI was developed based on CQDs@ZIF-8. Firstly, AuNPs were synthesized on the surface of the CQDs@ZIF-8 nanocomposite. Then, L-cysteine (L-cys) as the coreactant of CQDs was immobilized on the AuNPs to enhance the ECL signal of CQDs. Subsequently, the detection antibodies (Ab_2) were immobilized on the AuNPs through Au—N bonds to form Ab_2 bioconjugates (Ab_2/L-cys/AuNPs/CQDs@ZIF-8). At the same time, AuNPs were electrodeposited on the glass carbon electrode (GCE) forming a porous AuNPs film (DpAu) to fix the capture antibodies (Ab_1). Finally, through special sandwiched immunoreactions with cTnI, the obtained Ab_2 bioconjugates could be modified on the electrode surface through sandwiched immunoreactions with cTnI and provide a significantly enhanced ECL signal. In the construction of this ECL immunosensor, L-cys could increase the ECL intensity of the CQDs and improve the sensitivity of the

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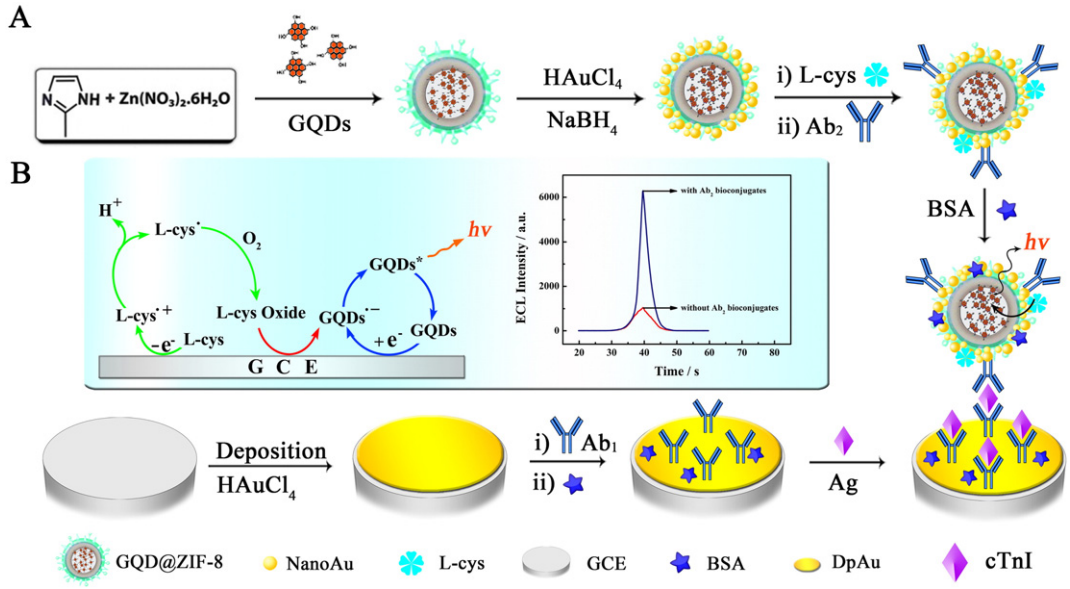

Scheme 1. Illustration of the preparation of Ab_2/L-cys/AuNPs/CQDs@ZIF-8 bioconjugates (A); the fabrication of the proposed ECL immunosensor and the reacted mechanism (B).

proposed ECL immunosensor. Therefore, the preparation of CQDs@ZIF-8 boarded the application of CQDs in ECL system and proposed an effective way for the immobilization of CQDs. And the proposed immunosensor might hold a new promise tool for sensitive cTnI detection.

2. Experimental

2.1. Reagents

Potassium persulfate ($K_2S_2O_8$) and zinc nitrate ($Zn(NO_3)_2 \cdot 6H_2O$) were purchased from Kelong Chemical Company (Chengdu, China). Gold chloride ($HAuCl_4$) was purchased from Kangda Company (Shanghai, China). L-cysteine (L-cys), sodium borohydride ($NaBH_4$), citric acid (CA), 2-methylimidazole, bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Hemoglobin (Hb), cTnI antibody (anti-cTnI), cTnI, and α-fetoprotein (AFP) were bought from Biocell Company (Zhengzhou, China). Phosphate buffered solution (PBS) (pH 7.4, 0.1 M) was prepared with 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4 and 0.1 M KCl. All chemicals were of analytical grade and used without further purification. All solutions were prepared with ultrapure water (18.2 MΩ) and stored in the refrigerator (4 °C) when no use.

2.2. Apparatus

The ECL emission was monitored with a model MPI-A electrochemiluminescence analyzer (Xi'an Remax Electronic Science & Technology Co. Ltd., Xi'an, China) with the voltage of the photomultiplier tube (PMT) at 800 V and the potential range from −2.0 to 0 V with a scan rate of 100 mV s^{-1}. Electrochemical impedance spectroscopy (EIS) measurements were performed with a CHI 660 A electrochemical workstation (Shanghai Chenhua Instrument, China). All the ECL and electrochemical experiments were carried out with a three-electrode system: an Ag/AgCl (sat. KCl) as reference electrode, a platinum wire as counter electrode, and the modified glassy carbon electrode (GCE) as working electrode, respectively. The morphologies of the nanomaterials were characterized by the transmission electron microscope (TEM, H600, Hitachi Instrument, Japan).

2.3. Preparation of CQDs

The preparation of CQDs was according to a previous literature with a little modification [21]. Firstly, 4 g CA was added into a 10 mL beaker and heated to 200 °C. When the CA was liquated, the color of the liquid was changed from colorless to pale yellow, and then became orange after about 30 min, indicating the successful synthesis of CQDs. The obtained orange liquid was diluted to 100 mL with ultrapure water and neutralized to pH 7.0 with NaOH (0.25 M).

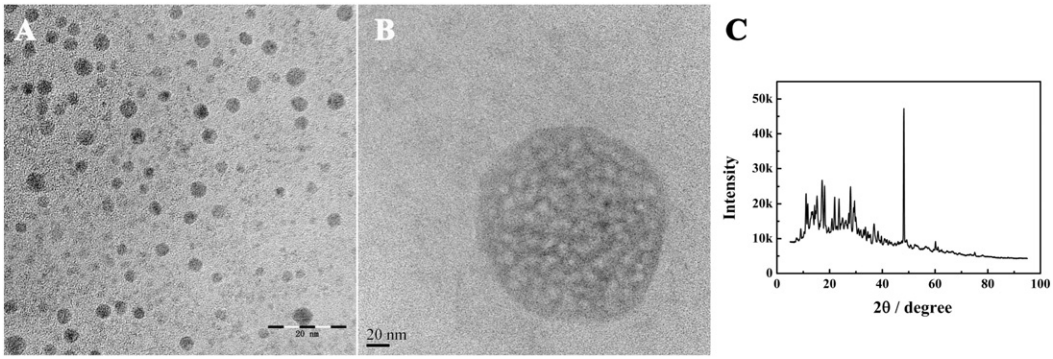

Fig. 1. TEM image of CQDs (A) and CQDs@ZIF-8 nanocomposites (B) and XRD image of CQDs@ZIF-8 nanocomposites (C).