Biosensors and Bioelectronics 50 (2013) 368–372

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Amplified electrochemiluminescence detection of cancer cells using a new bifunctional quantum dot as signal probe

Guifen Jie *, Yanbin Zhao, Shuyan Niu

Key Laboratory of Biochemical Analysis, Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, China

A R T I C L E I N F O

Article history:
Received 2 May 2013
Received in revised form
18 June 2013
Accepted 24 June 2013
Available online 8 July 2013

Keywords:
Composite quantum dot
Electrochemiluminescence
Cyclic amplification
Biosensor

A B S T R A C T

In this work, we prepared a new electrochemiluminescent signal probe using a small bifunctional composite quantum dot (QD) with intense electrochemiluminescence (ECL) and excellent magnetic property, and developed a sensitive ECL biosensor for detection of cancer cells via DNA cyclic amplification technique. The graphene oxide (GO) with unique electrical properties was used as nano-amplified platform to immobilize a large number of capture DNA (c-DNA1). The endonuclease-assisted amplification technique was applied to amplify the ECL signal change induced by target cells. Specifically, the bifunctional composite QDs with excellent magnetic property can be conveniently labeled, separated, and developed the ECL signal probe, thus an ECL method for rapid and sensitive detection of cancer cells was developed. So far, it is for the first time that the small magnetic electrochemiluminescent QDs were applied to the assays of cancer cells by using amplification strategy, which is expected to have great potential for early clinical diagnosis of cancer.

1. Introduction

The optical properties of semiconductor quantum dots (QDs) are attractive to many researchers because of their potential applications (Goldman et al., 2002; 2004; Oh et al., 2005). Compared to fluorescent proteins and organic dyes, QDs offer greater brightness and photostability, tunable emission from visible to infrared wavelengths, and large absorption coefficients across a broad spectral range (Bruchez et al., 1998; Chan and Nie, 1998). This allows for highly specific, quantitative optical imaging and multiplex assays (Bagalkot and Gao, 2011; Smith et al., 2008). There is a growing interest in using QDs as addressable fluorescent probes for biosensing events (Medintz et al., 2005; Chan et al., 2002). Electrochemiluminescence (ECL) is a powerful detecting tool with excellent controllability, low background signal, high sensitivity, and fast sample analysis (Jie et al., 2009; Liu et al., 2007). The ECL properties of semiconductor nanomaterials have attracted more and more attention, especially II–VI nanocrystals containing CdS, CdSe and CdTe have become the most popular ECL emitters in aqueous systems (Jie et al., 2010; 2011; Cheng et al., 2010). Recently, the biosensors and bioassays by virtue of intrinsic ECL of a variety of QDs nanostructures are extensively developed for diagnostic purposes, due to the efficient ECL emission and facile bioconjugation, as well as nano-enhanced signal amplification (Lei and Ju, 2011; Huang et al., 2011). A mass of methods involving immunoassays (Jie et al., 2010; Lin et al., 2011; Li et al., 2011) aptamer (Wang et al., 2011; Jie et al., 2012) or DNA (Divsar and Ju, 2011) binding, and cytosensing (Jie et al., 2011; Han et al., 2011) have been developed for sensitive detection of biological targets using cathodic ECL of nanostructures.

However, the immobilization, biolabeling and separation processes of biomolecules using pure QDs or their nanostructures are complicated, the ECL signal and stability of pure QDs are not high, which limits the wide applications of QDs in ECL bioassays. Magnetic quantum dots combine magnetic nanocrystals and QDs together, they are possible to simultaneously show magnetic and optical properties, therefore, have revealed some novel applications in biomedical fields (Gu et al., 2004). Thus, it is significant to develop the new magnetic electrochemiluminescent QDs; they cannot only be rapidly and conveniently separated in biolabeling process, but also be easily immobilized on the magnetic electrode, which has more promising applications in ECL biosensing or bioassays. In our previous work, the magnetic electrochemiluminescent nanocomposites were fabricated using polyelectrolyte, and applied to ECL immunoassay (Jie et al., 2012), but the nanocomposite diameter was very large (300–350 nm) and not appropriate for direct biolabeling. In addition, the magnetic electrochemiluminescent composite QDs were applied to ECL detection of thrombin (Jie, et al., 2012b). But this work was based on ECL quenching of QDs film by gold nanoparticels, the

* Corresponding author. Tel./fax: +86 532 84022750.
E-mail address: guifenjie@126.com (G. Jie).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.06.048

composite QDs were not directly labeled and used as signal probe. Furthermore, so far, the small magnetic electrochemiluminescent composite QDs have not been applied to cells assays by amplification technique.

Nicking enzyme signaling amplification (NESA) has been reported to have distinct specificity for DNA detection and offers high sensitivity (Li et al., 2011; Xu et al., 2009). Additionally, taking advantage of the nicking enzyme, reactions can be performed in an isothermal condition without specialized instrumentation and have the potential to be widespread for routine bioanalysis (Connolly and Trau, 2010).

Recently, graphene has attracted significant attention in scientific research because of its unique and outstanding properties (i.e., high active surface area, exceptional electrical, mechanical, thermal, optical, and chemical properties). Graphene oxide (GO) has been widely used for sensitive and selective detection of various biomolecules, including small molecules (Lu et al., 2009; Chang et al., 2010; Li et al., 2010), nucleic acids (Guo et al., 2011), and proteins (Pu et al., 2011), both in solution and in living cells (Wang et al., 2010).

In addition, cancer is today's most pressing health concern. It has been reported that very sensitive monitoring of cancer cells could provide an easier and more effective way to monitor progression of the disease (Paterlini-Brechot and Benali, 2007). Consequently, the research of accurate and sensitive recognition and detection of cancer cells is extremely important for cancer diagnosis and therapy. Worth noticing is that aptamer-based specific recognition of tumor cells and detection technique has become an attractive field in recent years (Chen et al., 2009).

Herein, a small bifunctional composite quantum dot was used to develop a novel ECL signal probe for sensitive detection of cancer cells by cyclic amplification technique. The graphene oxide with unique electrical and chemical properties was used as nano-amplified platform to immobilize much more DNA capture probes (c-DNA1). The magnetic quantum dots display intense ECL, excellent magnetism, and good biocompatibility, which offer promising advantages for ECL biosensing. This novel method is expected to have great potential applications for early clinical detection of cancer cells.

2. Experimental section

For detail please refer the Supporting information.

3. Results and discussion

3.1. Characterization of the magnetic composite QDs

Fig. 1A showed the transmission electron microscopy (TEM) image of the Fe_3O_4 nanoparticles, the nanoparticles possessed spherical shape with uniform size, and the average diameter was about 6 nm. After the magnetic composite Fe_3O_4/CdSe QDs were prepared, it was observed that the shape of the composite QDs was quadrate (Fig. 1B), and the average size was about 10 nm. The resulting composite QDs exhibit unique magnetism and high quality optical properties, suggesting that composite QDs can be easily labeled, separated, and developed ECL signal probe, which showed many promising advantages for ECL biosensing.

3.2. Fluorescence of the composite QDs

To study the fluorescence property of the composite QDs, Fig. S1 showed the confocal microscopy images of the pure composite QDs and Ramos cells after incubation with the

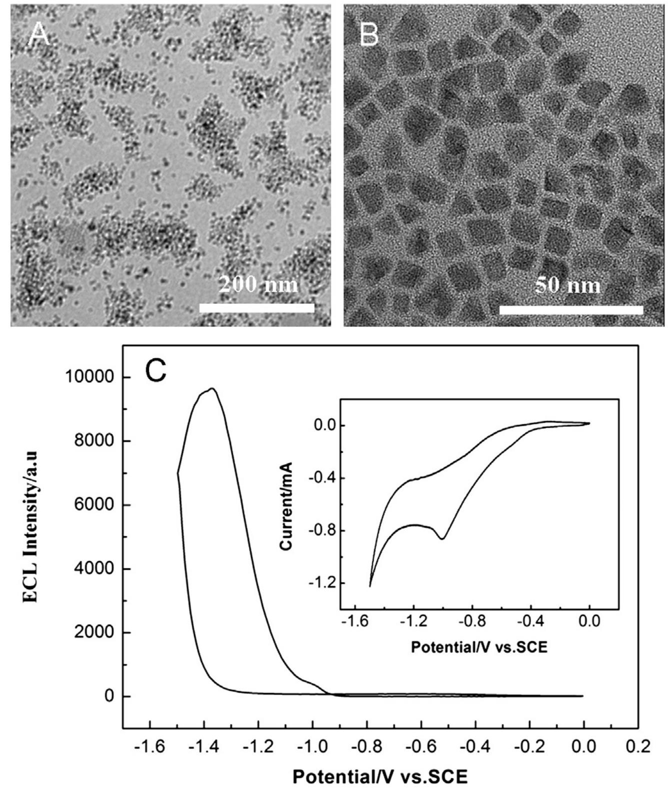

Fig. 1. Representative TEM images of (A) Fe_3O_4 nanoparticles, and (B) Fe_3O_4/CdSe composite quantum dots. (C) ECL–potential curve and cyclic voltammogram (inset) of the composite quantum dots on the electrode.

composite QDs. It was found that the composite QDs exhibited intense fluorescence (Fig. S1A). When the Ramos cells were labeled with the composite QDs via cells aptamers (Fig. S1B–D), the cells exhibited bright fluorescent signal, indicating the promising optical imaging ability of the composite QDs for in vitro and in vivo detection of tumor cells.

3.3. ECL behavior of the composite quantum dots

The ECL–potential curve and cyclic voltammogram (CV, inset) of the composite quantum dots were shown in Fig. 1C. A cathodic peak at −1.95 V is observed in the CV response, corresponding to the reduction of $S_2O_8{}^{2-}$. There is an ECL peak at −1.38 V in the ECL–potential curve, which results from the reaction of the composite QDs with $S_2O_8{}^{2-}$. The possible ECL mechanisms are as follows (Myung, et al., 2002). The QDs are reduced to nanocrystalline species ($QD^{-\bullet}$), while the $S_2O_8{}^{2-}$ is reduced to strong oxidant $SO_4{}^{-\bullet}$. Then $SO_4{}^{-\bullet}$ reacts with $QD^{-\bullet}$ to form the excited state (QD*) that could emit light.

$$QD + e^- \rightarrow QD^{-\bullet} \quad (1)$$

$$S_2O_8^{2-} + e^- \rightarrow SO_4^{2-} + SO_4^{-\bullet} \quad (2)$$

$$QD^{-\bullet} + SO_4^{-\bullet} \rightarrow QD^* + SO_4^{2-} \quad (3)$$

$$QD^* \rightarrow QD + h\nu \quad (4)$$

3.4. ECL detection of cancer cells by cyclic amplification technique using the composite QDs as signal probe

The fabrication principle for ECL detection of cancer cells by cyclic amplification technique using the Fe_3O_4/CdSe composite QD