



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

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ABSTRACT

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.

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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 nanoparticles, the

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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 $\text{Fe}_3\text{O}_4/\text{CdSe}$ 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

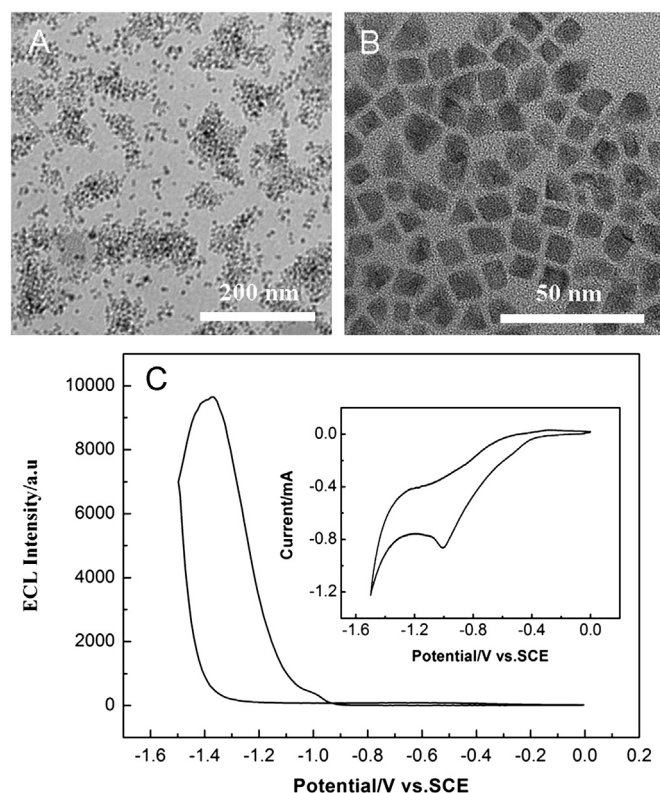


Fig. 1. Representative TEM images of (A) Fe_3O_4 nanoparticles, and (B) $\text{Fe}_3\text{O}_4/\text{CdSe}$ composite quantum dots. (C) ECL-potential curve and cyclic voltammogram (inset) of the composite quantum dots on the electrode.

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 $\text{S}_2\text{O}_8^{2-}$. There is an ECL peak at -1.38 V in the ECL-potential curve, which results from the reaction of the composite QDs with $\text{S}_2\text{O}_8^{2-}$. The possible ECL mechanisms are as follows (Myung, et al., 2002). The QDs are reduced to nanocrystalline species ($\text{QD}^{\bullet-}$), while the $\text{S}_2\text{O}_8^{2-}$ is reduced to strong oxidant $\text{SO}_4^{\bullet-}$. Then $\text{SO}_4^{\bullet-}$ reacts with $\text{QD}^{\bullet-}$ to form the excited state (QD^*) that could emit light.



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

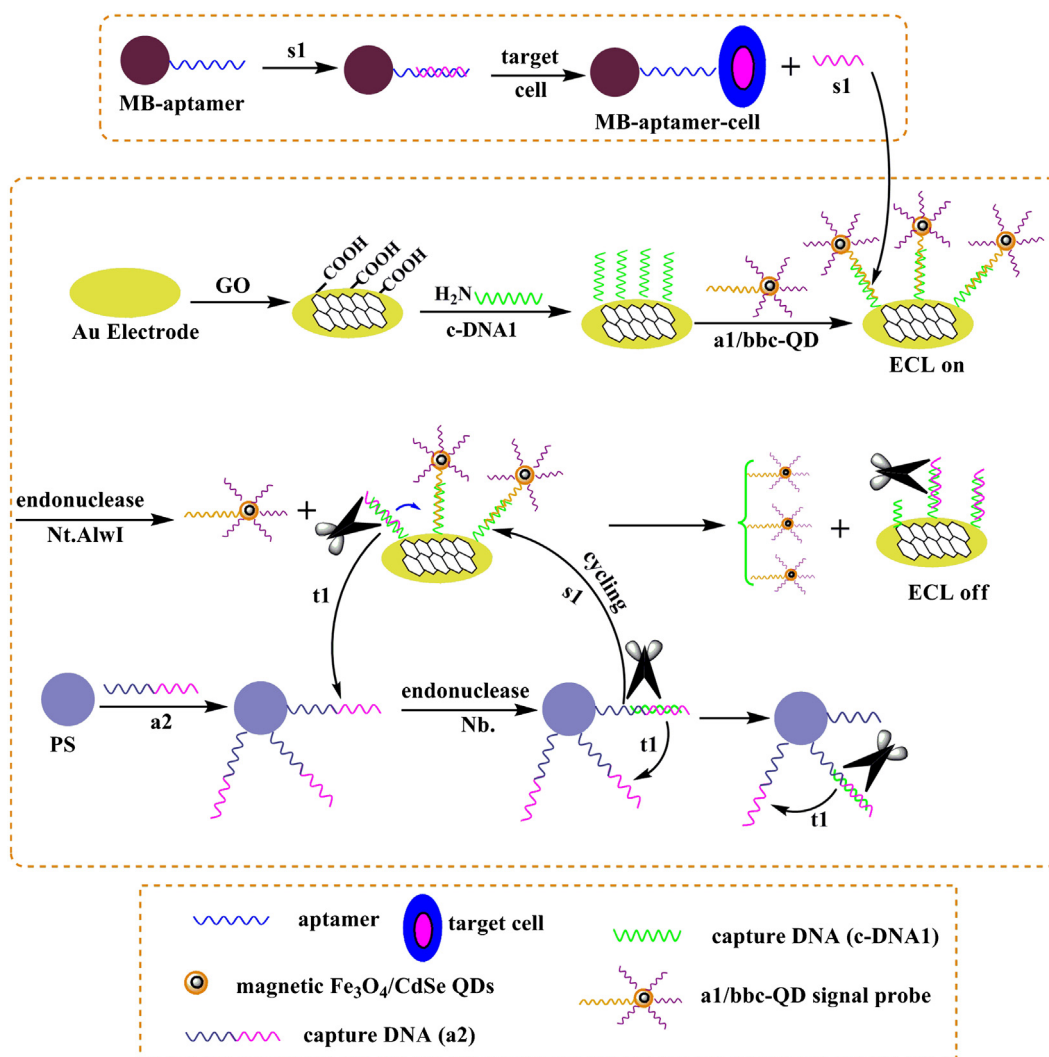
The fabrication principle for ECL detection of cancer cells by cyclic amplification technique using the $\text{Fe}_3\text{O}_4/\text{CdSe}$ composite QD

as signal probe was shown in Scheme 1. The aptamers for cancer cells were firstly linked to the magnetic microbeads (MBs), and then the complementary DNA (s1) was added to hybridize with the aptamers. Meanwhile, GO was firstly assembled onto the Au electrode surface, and the amino-group functionalized capture DNA (c1) was covalently linked to the GO with carboxyl groups, then the prepared composite QDs/DNA signal probe (a1) was added to hybridize with the capture DNA (c1). In addition, the capture-DNA (a2) was previously conjugated to the polystyrene (PS) sphere. In the presence of target cells, s1 was released due to the specific recognition of cancer cells to aptamers, and then the released s1 was bound to c1 to displace signal probe (a1) owing to the competition hybridization. Subsequently, the formation of double-stranded recognition region of endonuclease in s1/c1 hybrids led to scission of strand c1 under the effect of endonuclease1 (Nt.AlwI). Target s1 and the fragment t1 spontaneously dissociated from s1/c1 hybrids because of instability of hybridization structure. Then the single-stranded DNA s1 hybridized to other strands c1 to continue the cyclic competition hybridization and scission, resulting in massive release of a1 and t1 from the hybrids. Then t1 hybridized with a2 on the PS sphere and triggered a series of scission reactions under the effect of endonuclease2 (Nb.BbvCI), and released a number of s1 fragments, which then recycled to the headstream. As the above DNA devices and cleavage reactions could be cycled for multiple rounds, large

numbers of signal probe (a1) were dissociated from the electrode, which led to greatly amplified decrease of ECL signal. As more concentration of target cells released more s1 products, resulting in scission of more QDs/DNA signal probe (a1), thus more decrease of ECL signal was obtained. The change of ECL signal was related to the concentration of target cells, which could be applied to the detection of cells.

Fig. 2A showed the ECL signals for detecting different concentrations of target cells. In the absence of target cells, the composite QDs signal probe on the electrode displayed high ECL signal (curve a). When the target cells were present, the ECL peak signal gradually decreased with increasing cell concentrations (curve b to k), indicating that the proposed strategy could be used to detect the cells concentrations.

The relationship between the changes of ECL signal (ΔI) and the concentrations of target cells was shown in Fig. 2B, it was found that ΔI gradually increased with increasing cell concentrations. Inset was the standard calibration curve for cells detection, ΔI was proportional to the cells concentrations in the range from 300 to 9000 cells mL^{-1} ($R=0.993$) with a detection limit of 98 cells mL^{-1} at 3σ . As 200 μL of cells in PBS was used in our experiment detection, thus this detection limit was equivalent to 20 cells/200 μL , suggesting that this strategy is highly sensitive and has great potential for early and accurate detection of cancer cells. A series of three duplicate measurements of 2000 cells mL^{-1} were



Scheme 1. Schematic representation for the strategy of cell detection by DNA cyclic amplification technique using the $\text{Fe}_3\text{O}_4/\text{CdSe}$ composite QDs as signal probe.

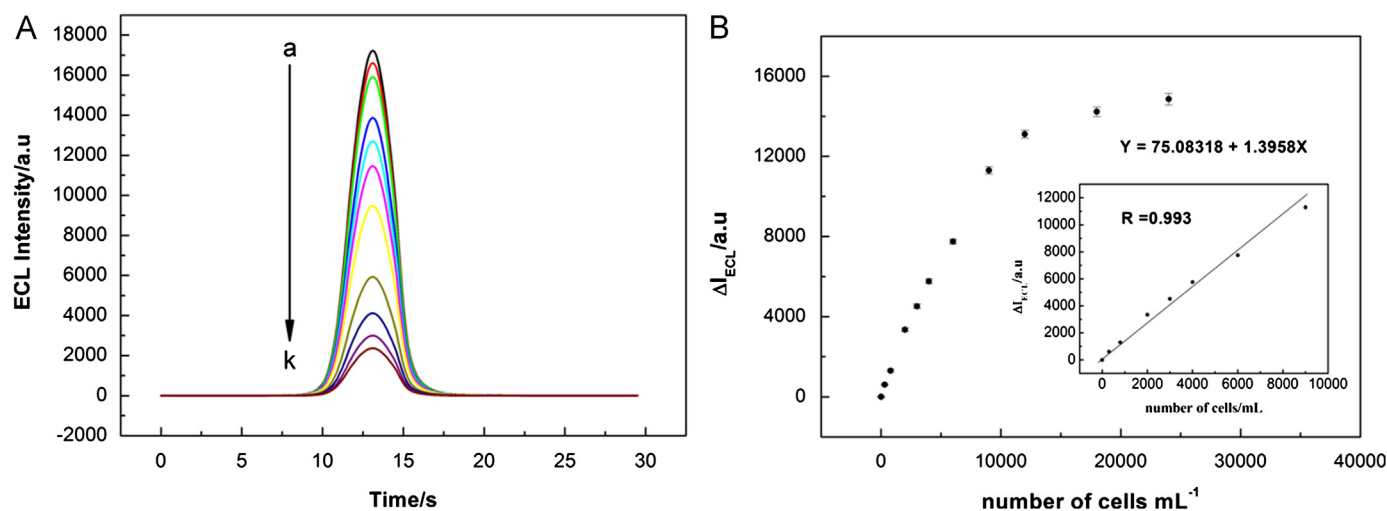


Fig. 2. (A) ECL signal responses for detection of different concentrations of target cells. The concentrations of the cells: (a) 0; (b) 300; (c) 800; (d) 2000; (e) 3000; (f) 4000; (g) 6000; (h) 9000; (i) 12000; (j) 18000 and (k) 24000 cells mL^{-1} . (B) Relationship between ΔI and cells concentrations from 300 to 24000 cells mL^{-1} . Inset is the detection linear range from 300 to 9000 cells mL^{-1} for target cells.

used for estimating the precision, and the relative standard deviation (RSD) was 5.7%, showing good reproducibility. In addition, to demonstrate that the cyclic amplification reaction is functional, we design the control experiment in the absence of polystyrene beads. Fig. S2 showed the detection linear range for target cells without the cyclic amplification on the polystyrene beads. It was observed that the changes of ECL signal were lower than that achieved with cyclic amplification in the presence of polystyrene beads, and the detection limit was 256 cells mL^{-1} at 3σ , indicating that the cyclic amplification by using polystyrene beads and endonuclease2 was functional, thus the higher detection sensitivity was obtained.

In order to evaluate the analytical reliability and application potential of the proposed method for early clinical diagnosis of cancer, target and control cells at the same concentration (2000 cells mL^{-1}) were spiked into two different clinical samples and then measured using the ECL method, which was shown in Fig. S3(a, b). In addition, the ECL signals of the same amount of cells in cell media were also measured (Fig. S3c and d). The assay results of clinical samples were compared with those of the cell media using the proposed method. The results show that the ECL signal of the target cells in clinical sample was almost the same as that of the target cells in cell media, and the target cells in both sample and cell media obviously showed greatly higher signals than the control cells, suggesting that the proposed ECL assay have wide applications in early clinical diagnosis of cancer.

4. Conclusions

In conclusion, a sensitive electrochemiluminescence biosensor for detection of cancer cells was developed by cyclic amplification technique using a new bifunctional quantum dot as signal probe. The magnetic composite QDs allowed rapid and convenient biolabeling and separation. The promising graphene oxide nano-amplified platform displayed good stability, high biocompatibility and more surface area for the ECL biosensor. In particular, the DNA devices were cycled multiple rounds, which greatly improved the detection sensitivity. The present work opened a new promising way for designing the magnetic QD-based ECL assays for cancer cells by cyclic amplification strategy, which has great potential in clinical applications.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.bios.2013.06.048>.

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