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Versatile Electrochemiluminescence Assays for Cancer Cells Based on Dendrimer/CdSe−ZnS−Quantum Dot Nanoclusters

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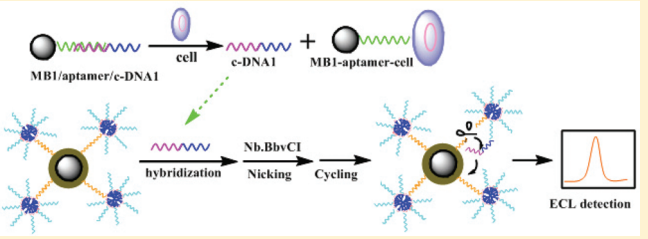
Supporting Information

ABSTRACT: In this work, a novel dendrimer/CdSe−ZnS−quantum dot nanocluster (NC) was fabricated and used as an electrochemiluminescence (ECL) probe for versatile assays of cancer cells for the first time. A large number of CdSe−ZnS−quantum dots (QDs) were labeled on the NCs due to the many functional amine groups within the NCs, which could significantly amplify the QD's ECL signal. Capture DNA was specially designed as a high-affinity aptamer to the target cell; a novel ECL biosensor for cancer cells was directly accomplished by using the biobarcode technique to avoid cross-reaction. Moreover, magnetic beads (MBs) for aptamers immobilization were combined with the dendrimer/QD NCs probe for signal-on ECL assay of cancer cells, which greatly simplified the separation procedures and favored for the sensitivity improvement. In particular, a novel cycle-amplifying technique using a DNA device on MBs was further employed in the ECL assay of cancer cells, which greatly improved the sensitivity. To the best of our knowledge, this is the first study that the novel dendrimer/QD NCs probe combined with a DNA device cycle-amplifying technique was employed in the ECL assays of cells. Excellent discrimination against target and control cells is demonstrated, indicating that the ECL assays have great potential to provide a sensitive, selective, cost-effective, and convenient approach for early and accurate detection of cancer cells.



With the development of nanobiotechnology, semiconductor nanocrystals (quantum dots, QDs) have attracted tremendous interest for their unique electronic, optical, and electrochemical properties.[1,2] Highly fluorescent QDs have shown promising applications in optical devices, biological imaging, bioconjugates, optical biosensors, etc.[3−7] QDs electrochemiluminescence (ECL) has been actively studied in recent years because of its simplified setup, low background signal, high sensitivity, and fast sample analysis.[8−10] The first water-phase QDs ECL sensor for H_2O_2 was fabricated by coating CdSe QDs on a paraffin-impregnated graphite electrode. Subsequently,[11] the QD-based ECL analytical technique has been quickly developed in many fields, and the ECL biosensors based on CdS, CdSe, ZnS, and ZnO QDs were gradually reported.[12−16] In addition, aptamers as single-stranded nucleic acid could bind specifically with protein molecular or cellular targets, QDs ECL was also applied to aptamer−target detection in recent years.[17] Nevertheless, so far, no QDs ECL has been applied to cell detection though ECL is widely used in bioassays. The main problem is that the ECL signal of QDs is lower than that of luminal or $Ru(bpy)_3^{2+}$ and that the QDs film is usually unstable in aqueous solution, which limits the applications of QDs ECL in bioassays. Thus, it is urgently needed to explore effective techniques or develop novel QDs nanostructure to enhance QDs ECL, which is of great significance for applying QDs ECL in bioassays.

In our previous works, gold nanoparticles (NPs) and carbon nanotubes were used to enhance QDs ECL.[8,14] Recently, dendrimers, the regular tree-like highly branched macromolecules, are receiving considerable attention for applications in chemical and biological areas owing to their numerous terminal groups that can be functionalized optionally.[18−21] It was reported that the polyamidoamine (PAMAM) dendrimer nanoclusters were used to amplify signal in both optical and magnetic resonance (MR) imaging for in vivo detection of tumor cells.[22] Lu et al. reported that the fourth-generation PAMAM dendrimers could enhance ECL of CdS nanocomposite membranes by electrochemical deposition method, but the ECL was not applied to bioassays.[23] In this article, the unique dendrimers/QDs nanocluster was prepared by first cross-linking fifth-generation dendrimers to larger NCs followed by labeling with large numbers of CdSe−ZnS QDs, which was further used as an amplified ECL signal probe for versatile cells assays.

In addition, DNA devices as amplification protocols have now become powerful tools due to the specificity of molecular recognition capability and their robust physicochemical properties. In various DNA devices, such as gears, walkers, logic circuits, and switches etc., movements were powered by DNAzymes,[24]

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endonucleases,[25] fuel oligonucleotides,[26] etc. DNA devices can carry out the function of circular amplification autonomously and effectively under simple operations. However, so far, a QD-based ECL method combined with a DNA device cycle-amplifying technique has not been applied to cells assays.

In this paper, a novel dendrimers/CdSe−ZnS−QD nanocluster as amplified signal probe was introduced to versatile ECL assays of cancer cells for the first time. Large numbers of QDs loaded on the dendrimer NCs greatly amplified the QDs ECL signal. High specificity of aptamers to target cells much favored for the selectivity improvement of the ECL assays. Taking advantages of the magnetic microbeads (MBs) for aptamers immobilization and the biobarcode technique to avoid cross-reaction in the ECL assays, the separation procedures were greatly simplified and the sensitivity was improved. In particular, this is the first study that the novel dendrimers/QD NC as an ECL signal probe was combined with the DNA cycle-amplifying technique for signal-on ECL assays of cells, which has promising applications in cancers diagnosis due to its high sensitivity, simplicity, and low cost.

EXPERIMENTAL SECTION

Chemicals and Materials. Cadmium oxide (CdO, 99.99%), zinc acetate ($Zn(CH_3COO)_2 \cdot 2H_2O$, 99.9%, powder), selenium (99.9%, powder), sulfur (99.9%, powder), trioctylphosphine (TOP, 90%), oleic acid (OA, 90%), 1-octadecene (ODE, 90%), and mercaptopropionic acid (MPA, 99.8%) were purchased from Aldrich. Fifth-generation PAMAM dendrimers (ethylenediamine core, generation 5, 5 wt % in methanol, 0.797 g/mL) were purchased from Sigma-Aldrich (Shanghai) Trading Co., Ltd. Homobifunctional amine-reactive cross-linker with poly(ethylene glycol) (PEG) spacer arms $BS(PEG)_5$ (cat. 21581, MW 532.50) was purchased from Pierce. The endonuclease (Nb.BbvCI) was purchased from the New England Biolabs (NEB). All the DNA sequences were synthesized and purified by SBS Genetech Co. Ltd. (China), and the sequences of this work are listed in Supporting Information Tables S1 and S2. 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and imidazole were obtained from Sigma. Magnetic microbeads (MBs) modified with carboxyl or thiol groups (450−500 nm, 10 mg/mL) were obtained from BaseLine Chrom-Tech Research Centre (Tianjin, China). The 0.1 M PBS (pH 7.4), 0.1 M imidazole−HCl buffer (pH 7.0) was prepared according to the standard methods. Chloroauric acid ($HAuCl_4$) and trisodium citrate were obtained from Shanghai Reagent Company (Shanghai, China). Multiwalled carbon nanotubes (CNTs, diameter, 30−50 nm) were purchased from Nanoport. Co. Ltd. (Shenzhen, China). Poly(diallyldimethylammonium chloride) (PDDA, 20%, w/w in water, MW = 200 000−350 000) was from Sigma-Aldrich. All other reagents were of analytical grade. Fe_3O_4@Au MBs were prepared as follows: First, 1.0 mL of thiol-modified Fe_3O_4 MBs was transferred into a 1.5 mL Eppendorf tube and washed three times with PBS buffer. Second, the Fe_3O_4 MBs were resuspended in PBS buffer and added dropwise into a 10 mL aqueous solution of Au particles. After reaction for 16 h under shaking condition at room temperature, the reddish-brown Au−Fe_3O_4 NPs were separated magnetically and washed to remove the unbound Au particles.

Ramos cells (CRL-1596, B-cell, human Burkitt's lymphoma) and CEM cells were obtained from the Chinese Academy of Medical Sciences. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 100 IU/mL penicillin−streptomycin. The cell density was determined using a hemocytometer. After which, $\sim 1 \times 10^6$ cells dispersed in RPMI 1640 cell media buffer were centrifuged at 3000 rpm for 5 min and redispersed in 1 mL of cell media buffer. During all experiments, the cells were kept in an ice bath at 4 °C.

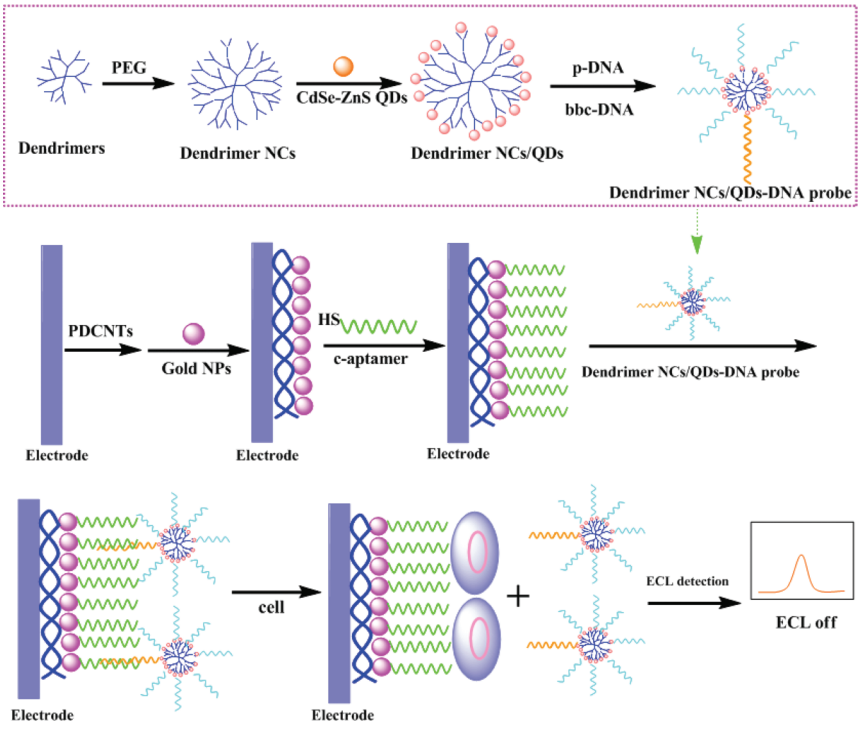
Apparatus. Transmission electron microscopy (TEM) images were recorded using a JEOL JSM-6700F instrument (Hitachi). Photoluminescence (PL) spectra were obtained on an RF-540 spectrophotometer (Shimadzu). Field-emission scanning electron microscopy (FESEM) was carried out on a JEOL JSM-6340 F instrument. Electrochemical and ECL measurements were carried out on an MPI-A ECL analyzer (Xi'An Remax Electronic Science & Technology, Xi'An, China) using a three-electrode system. The electrodes were a magnetic Au disk working electrode, a saturated calomel reference electrode, and a Pt counter electrode.

Preparation of the PAMAM Dendrimer NCs/CdSe−ZnS QD−DNA Probe. The fabrication procedure of the dendrimers NCs/QDs−DNA probe is shown in Scheme 1. Dendrimer nanoclusters (NCs) were prepared according to the literature.[22] To control the size of the dendrimer NCs, different molar ratios between dendrimers and NHS−PEG−NHS were used. It was found that a molar ratio of 50:1 $[NH_2]/[NHS]$ was appropriate to obtain the NCs with an average diameter of about 150 nm as determined by SEM images. Then 0.40 mg of dendrimers was dispersed in 1.0 mL of PBS and cross-linked by adding 70 μL of the homobifunctional amine-reactive cross-linking agent NHS−PEG−NHS (250 μM in DMSO) for 16 h. Un-cross-linked dendrimers were removed by multiple washes on centrifugal filter devices (Amicon ultrafree-CL, 0.1 μm, Millipore Corp.) and the precipitate was redispersed in 1.0 mL of PBS. Then 0.1 M EDC (100 μL) and 0.025 M NHS (100 μL) were added to 400 μL of QDs solution (pH 7.4 PBS) for 1 h to active the QDs, then the QDs solution were mixed with 400 μL of the above dendrimer NCs and reacted for at least 2 h, followed by multiple washes on centrifugal filter devices (Amicon ultrafree-CL, 0.1 μm, Millipore Corp.). After the unlinked QDs were removed, the precipitate was redispersed in 1.0 mL of PBS, followed by adding 0.1 M EDC (200 μL) and 0.025 M NHS (200 μL) to activate the dendrimer NCs/CdSe−ZnS QDs. Then, 500 μL of 1.0×10^{-5} M biobarcode (bbc)-DNA and 50 μL of 1.0×10^{-5} M probe-DNA (p-DNA) were added to 100 μL of the mixture and incubated at 37 °C overnight with gentle mixing, and then centrifuged at 10 000 rpm for 30 min at 4 °C to remove unbound oligonucleotides.

Preparation of the ECL Biosensor for Signal-Off Assay of Cells. The fabrication procedure of the ECL biosensor for signal-off assay of cells is illustrated in Scheme 1. Briefly, gold disk electrodes were polished carefully with α-Al_2O_3 powder on fine abrasive paper and washed ultrasonically with water. After the electrodes were dried, 6 μL of PDCNTs was dropped on the electrodes and dried. Then the electrodes were immersed in the GNPs solution for 30 min.

For cell detection, the PDCNTs/GNPs electrodes (see the Supporting Information) were first modified with 1.0×10^{-6} M thiolated aptamer at room temperature overnight, followed by immersing them in 1 mM MCH for 1.5 h to block the uncovered electrode surface. After hybridization in the dendrimer NCs−QDs−probe at 37 °C for 1 h, the electrodes were incubated with 100 μL of PBS containing different numbers of

Scheme 1. Fabrication Steps of the Dendrimer NCs/QDs−DNA Probe and ECL Biosensor for Signal-Off Detection of Cells



cells at room temperature for 50 min. Then the electrodes were rinsed with 0.1 M PBS buffer and the ECL measurements were performed.

Preparation of the DNA Device Cycle-Amplifying Technique for ECL Detection of Cancer Cells. The fabrication procedure of the DNA device cycle-amplifying technique for ECL detection of cancer cells is illustrated in Scheme 2. First, 40 μL of carboxylated magnetic microbeads (MB1) was placed in a 1.5 mL Eppendorf tube (EP tube) and washed three times with 0.1 M imidazol−HCl buffer (pH 7.0). Then 50 μL of 0.1 M imidazol−HCl buffer and 100 μL of 0.2 M EDC were added to the EP tube, and the mixture was incubated at 37 °C for 1 h to activate the carboxyl groups on the MB. After being washed with 0.01 M PBS buffer (pH 7.4), the activated MB was incubated with 200 μL of 1.0×10^{-6} M amino group modified aptamer at 25 °C for 12 h. Excess reagents were removed by magnetic force, followed by adding 500 μL of 1.0×10^{-6} M complementary DNA (cDNA1) solution and incubating at 37 °C for 1 h to obtain the MB1−aptamer−cDNA1 biocomplex. Then the MB1−aptamer−cDNA1 biocomplex was incubated with 100 μL of PBS containing different numbers of cells at room temperature for 50 min. Then the cDNA1 released from the MB-probe biocomplex was separated from the solution with a magnetic field (Scheme 2).

After the dendrimer NCs/QDs−DNA probe was prepared as above, the Fe_3O_4@Au MB2 were washed and resuspended in pH 7.4 PBS buffer, then 50 μL of 1.0×10^{-6} M dendrimer NCs/QDs−DNA probe was added to the MB2 buffer. After shaking gently for 16 h at room temperature, excess reagents were removed by magnetic force, and the obtained MB2/dendrimer NCs/QDs−DNA conjugates were resuspended in pH 7.4 PBS buffer. Then, 40 μL of the above MB2 conjugates was added to capture the released cDNA1, followed by adding 20 μL of NEB buffer 2 containing 0.5 U/μL nicking endonuclease to perform the cycling DNA hybridization and cleavage reaction by incubation at 37 °C for 70 min. (Scheme 2).

ECL Detection. After the above cycling reaction was accomplished and magnetic separation, the PDCNTs/cDNA2/electrode was immersed in the released dendrimer NCs/QDs−DNA probe solution at 37 °C for 1 h, and then the ECL measurements were performed (Scheme 2).

The ECL emission was detected with a model MPI-A electrochemiluminescence analyzer using a three-electrode system at room temperature. The electrodes were a modified Au disk working electrode (4 mm diameter), a saturated calomel reference electrode, and a Pt counter electrode. The modified electrodes above were in contact with 0.1 M PBS (pH 7.4) containing 0.05 M $K_2S_2O_8$ and 0.1 M KCl and scanned from 0 to −1.5 V. The spectral width of the photomultiplier tube (PMT) was 200−800 nm, and the voltage of the PMT was −600 V ∼ −800 V in the detection process. ECL signals related to the cell concentrations could be measured.

In addition, for studying ECL behavior of the pure CdSe−ZnS QDs and dendrimer NCs/QDs, 6 μL of QDs (or dendrimer NCs/QDs) was dropped onto the electrode and dried in air, then the ECL measurements were performed as above.

RESULTS AND DISCUSSION

Characterization of the Dendrimer Nanoclusters/CdSe−ZnS QDs. Figure 1A shows the TEM image of the CdSe−ZnS QDs; the average diameter of the CdSe−ZnS nanoparticles is about 10 nm and their size distribution is relatively uniform. Figure 1B shows the photoluminescence (PL) spectra of the CdSe−ZnS QDs. The PL emission peak at 601 nm (λ_{ex} = 450 nm) indicated the consequence of quantum confinement.[27]

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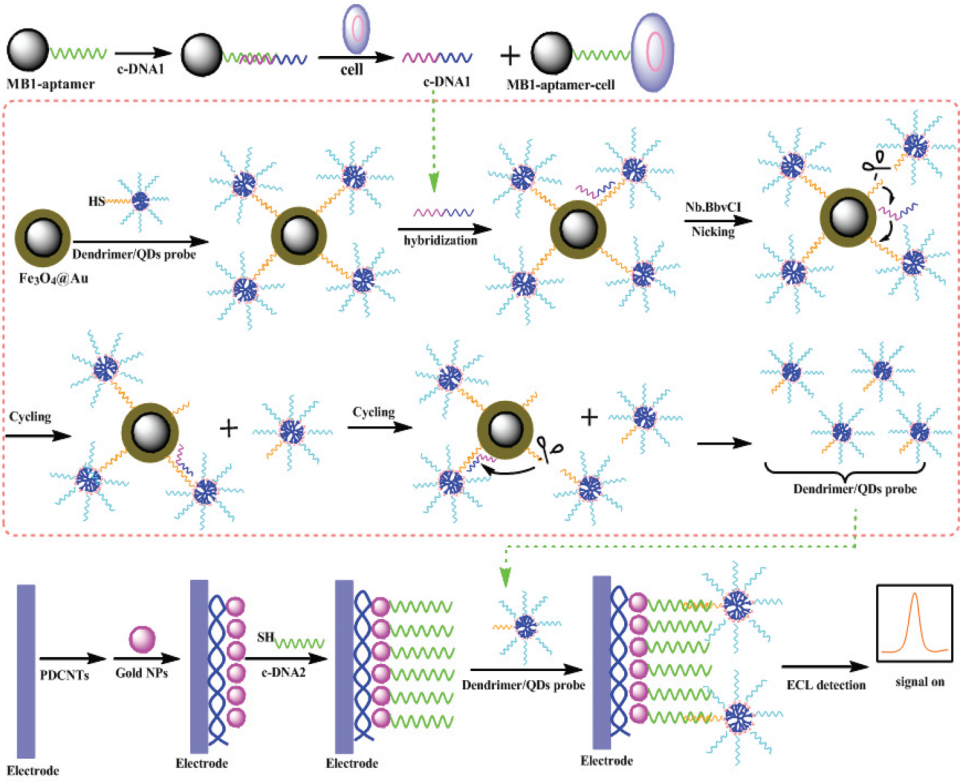
Scheme 2. Schematic Representation of the Strategy for Cell Assay Based on the MB1−Aptamer Biocomplex and DNA Cycle-Amplifying Technique and ECL Detection Based on the Dendrimer NCs/QDs−DNA Signal Probe



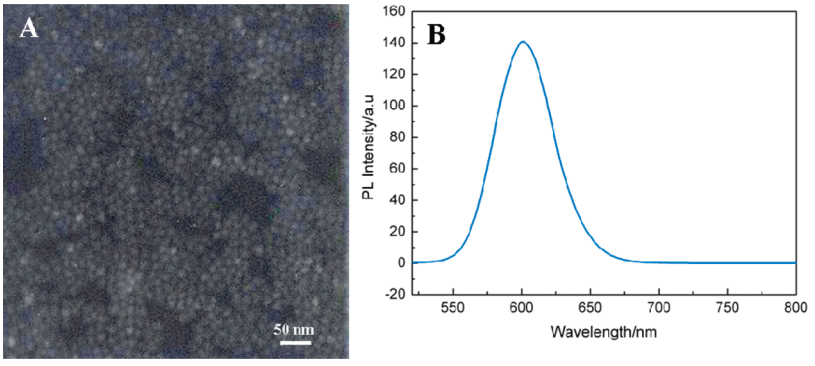


Figure 1. (A) TEM image and (B) photoluminescence (PL) of the CdSe−ZnS QDs.

The fabrication procedure of the dendrimers NCs/QDs−DNA probe is shown in Scheme 1. First, the dendrimer NCs were prepared by cross-linking dendrimers with the homobifunctional amine-reactive cross-linking agent NHS−PEG−NHS,[22] and the resulting NC has an average diameter of about 150 nm (Supporting Information Figure S1A, SEM image). Then the CdSe−ZnS QDs were assembled upon the NCs through covalent interactions. Finally, the amino group functionalized probe DNA and bbc DNA were covalently linked to the dendrimers NCs/QDs.

In addition, ζ-potential was used to investigate the surface charge of the hybrid nanostructure in the fabrication process. Figure 2 shows the ζ-potential values of pure dendrimer NCs (Figure 2a), pure QDs (Figure 2b), and dendrimer NCs/QDs probe (Figure 2c), respectively. The pure dendrimer NCs were positively charged with a ζ-potential of +8 mV due to the amino groups. After the loading of negatively charged CdSe−ZnS QDs on dendrimer NCs, the ζ-potential shifted to −4 mV. The results suggest that the dendrimer NCs/QDs probe has been successfully fabricated.

ECL Behavior of the Dendrimer NCs/CdSe−ZnS QDs. Figure 3 shows the ECL−potential curves of the pure CdSe−ZnS QDs (Figure 3, curve a) and the dendrimer NCs/QDs (Figure 3, curve b), respectively. The inset is the cyclic voltammogram (CV) of the dendrimer NCs/QDs on the electrode. In the CV, two cathodic peaks were observed at

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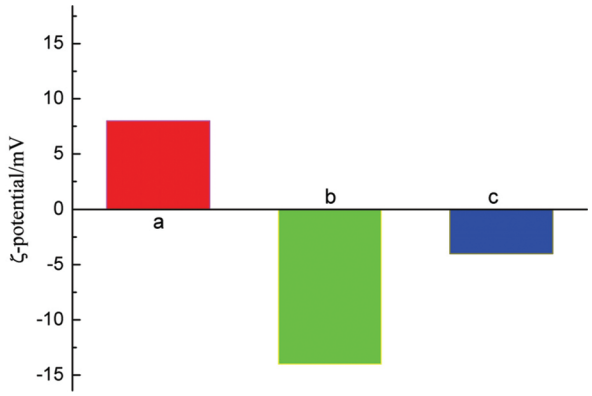

Figure 2. Value of ζ-potentials during the dendrimer NCs/QDs assembly process. The bars represent (a) pure dendrimer NCs, (b) pure QDs, and (c) dendrimer NCs/QDs probe.

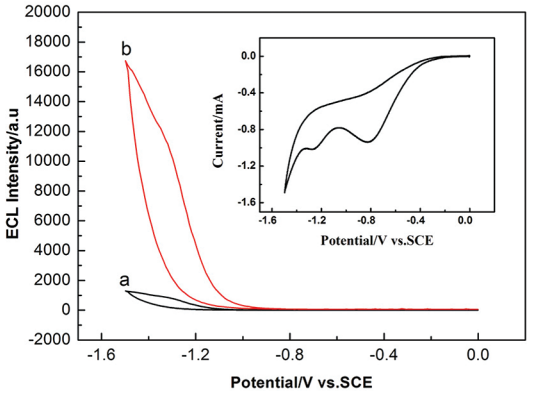

Figure 3. ECL−potential curves of (a) pure CdSe−ZnS−QDs, (b) dendrimer NCs/CdSe−ZnS QDs, and cyclic voltammogram (inset) of dendrimer NCs/CdSe−ZnS QDs on the electrode.

−0.83 V (C1) and −1.27 V (C2), respectively, in pH 7.4 PBS containing 0.1 M KCl and 0.05 M $K_2S_2O_8$. The peak C1 was assigned to the reduction of $S_2O_8^{2-}$, because it was not found in the absence of $S_2O_8^{2-}$. C2 appeared in both the dendrimer NCs/QDs/electrode and bare electrode in PBS without $K_2S_2O_8$; perhaps it was related to the reduction of hydrogen ion in PBS.

One ECL peak appeared at −1.49 V in both ECL curves in the cathodic process, resulting from the reaction of CdSe−ZnS QDs with $S_2O_8^{2-}$. In comparison with the pure QDs (Figure 3, curve a), a 13-fold enhancement in ECL signal (Figure 3, curve b) was observed using the dendrimer NCs/QDs nanostructure, indicating that the novel dendrimer NCs could greatly amplify the QDs ECL signal. The reasons may be that a larger number of QDs were assembled on the NCs and the surface area of the nanostructure significantly increased, which favored for the electron transfer in the ECL reaction; the other is that amine functional groups in NCs also facilitate the radical generation and accelerate the electron-transfer process during the ECL reaction.[23] Therefore, the unique dendrimer NCs/QDs nanostructure would become an ideal candidate for ECL bioassays. The possible ECL mechanisms are as follows.[28]

$$\mathrm{CdSe-ZnS} + e^- \rightarrow (\mathrm{CdSe-ZnS})^{-\bullet} \quad (1)$$

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

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

$$(\mathrm{CdSe-ZnS})^* \rightarrow \mathrm{CdSe-ZnS} + h\nu \quad (4)$$

ECL Biosensor for Signal-Off Cell Detection Based on the Dendrimers/QDs NCs Probe. The fabrication principle for ECL biosensing of target cells is shown in Scheme 1. PDCNTs were first assembled onto the electrode surface, then the negatively charged Au NPs were absorbed onto the electrode. After the thiol-modified aptamer was immobilized on the electrode surface, the dendrimer NCs/QDs−DNA biocomplex was added to hybridize with the aptamers. With the recognition of target cells to aptamers, the double-strand aptamer/probe DNA dehybridized, and the dendrimer NCs/QDs−DNA probe was released, which resulted in the decrease of ECL signal. The change of ECL signal was proportional to the concentration of target cells, which could be used for cell detection.

Figure 4B shows the ECL−potential curve of the electrode after the dendrimer NCs/QDs−DNA biocomplex was assembled onto the electrode via aptamer−DNA hybridization. The ECL peak at −1.49 V was consistent with that in Figure 3, corresponding to the reaction of QDs with $S_2O_8^{2-}$.

Figure 4A shows the ECL signals that were responsive to different cell concentrations. The ECL peak intensity gradually decreased with increasing cell concentrations due to the specific binding of cells with aptamers on the electrode. The results suggested that the cells concentration could be determined with the ECL biosensor. This ECL biosensor for cells is fast, convenient, and cost-effective, which has great potential in applications for early and accurate detection of cancer cells.

The standard calibration curve for cell detection is shown in the inset of Figure 4B. The changes of ECL peak intensity increased linearly with the cell concentrations in the range of 400−10 000 cells mL^{-1}, and the detection limit was calculated to be 210 cells mL^{-1} at 3σ. The regression equation could be expressed as $\Delta I_{ECL} = 2769.6 \log C - 5868.3$ (ΔI represents the change of ECL peak height; C represents the concentration of cells, cells mL^{-1}; $R^2 = 0.996$). According to the linear equation, we could detect cell concentration quantitatively.

In order to evaluate the potential application of the proposed method, the assay was performed using artificial complex samples by mixing equal amounts of Ramos target cells and CEM control cells; the ECL responses are shown in Supporting Information (Figure S2). The control cells produced no noticeable difference in the sample versus pure target cells, and the ECL response to the blank PBS was very low. The results suggest the assay was able to collect the Ramos cells in a complex sample, showing good selectivity and actual application of the assay.

Signal-On ECL Detection of Cancer Cells Based on Fe_3O_4@Au−Aptamer and Dendrimer NCs/QDs Probe. The fabrication principle for ECL detection of cells is shown in Supporting Information (Scheme S1). The thiol-modified aptamers were first attached on the surface of the core−shell magnetic nanoparticles (Fe_3O_4@Au), then the dendrimer NCs/QDs−DNA biocomplex was added to hybridize with the aptamers. With the recognition of target cells to aptamers, the double-strand aptamer/probe DNA dehybridized, and the dendrimer NCs/QDs−DNA probe was released and separated with a magnetic field. The gold electrode modified with PDCNTs/GNPs/capture DNA was incubated in the released dendrimer NCs/QDs−DNA solution for 1 h at 37 °C to form ds-DNA and detected by the ECL method.

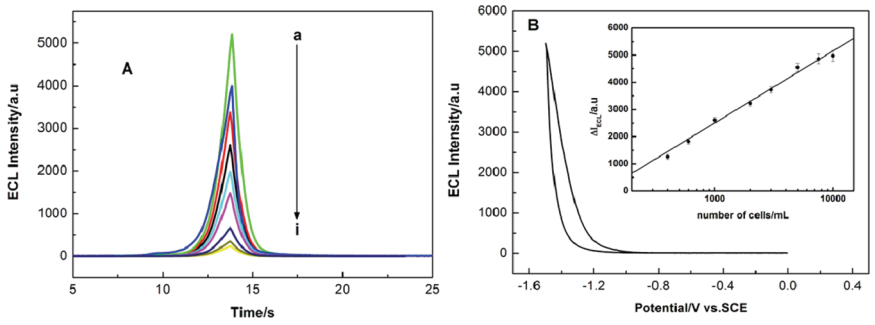

Figure 4. (A) ECL responses of the biosensor for detection of cells. The concentrations of cells (cells mL^{-1}) were as follows: (a) 0, (b) 400, (c) 600, (d) 1000, (e) 2000, (f) 3000, (g) 5000, (h) 7500, and (i) 10 000. (B) ECL−potential curve of the electrode after the dendrimer NCs/QDs−DNA biocomplex was assembled onto the electrode. The inset is the calibration curve of the changes in ECL peak intensity vs the concentration of target cells plotted on a logarithm scale: 400−10 000 cells mL^{-1}.

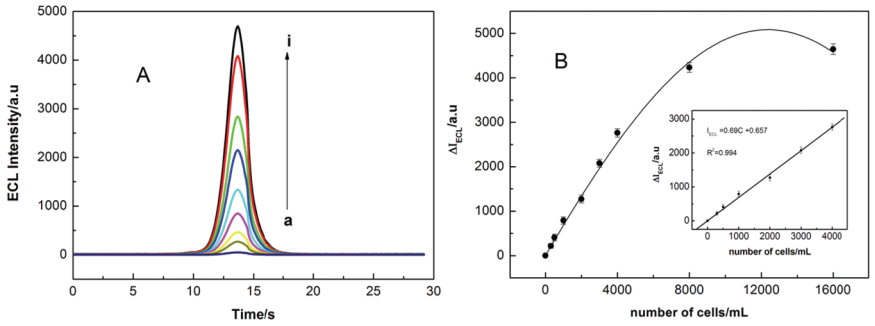

Figure 5. (A) ECL signals for detection of different concentrations of target cells. The concentrations of cells were as follows: (a) 0, (b) 300, (c) 500, (d) 1000, (e) 2000, (f) 3000, (g) 4000, (h) 8000, and (i) 16 000 cells mL^{-1}. (B) Calibration curves for detection of cells from 300 to 16 000 cells mL^{-1}. The inset is the amplification of the linear range from 300 to 4000 cells mL^{-1} for target cells determination. (The blank was deducted.)

Figure 5A shows the ECL signals that were responsive to changes in target cell concentration; the ECL peak intensity gradually increased with increasing cell concentrations. The standard calibration curve for cell detection is shown in Figure 5B; the ECL signal was proportional to the cell concentration in the range from 300 to 4000 cells mL^{-1}. A series of five duplicate measurements of 500 cells mL^{-1} were used for estimating the precision, and the relative standard deviation (RSD) was 6.3%, showing good reproducibility. The detection limit for cell concentration was calculated to be 162 cells mL^{-1} at 3σ.

As shown in Supporting Information (Figure S3), the feasibility of the ECL assay for actual applications was investigated. Target and control cells at the same concentration (1000 cells mL^{-1}) were spiked into two different samples and then incubated with the Fe_3O_4@Au−aptamer; the released dendrimer NCs/QDs were measured using the ECL method. For comparison, the signals of the same amount of cells in cell media were also measured. The target cells in both sample and media clearly show a significantly higher signal than the control cells, indicating that the assay has wide applications in diagnosis.

DNA Device Cycle-Amplifying Technique for ECL Detection of Cancer Cells. The fabrication principle for ECL detection of cancer cells based on the DNA device cycle-amplifying technique is shown in Scheme 2. The aptamers were first linked to the magnetic nanoparticles, then the cDNA (c-DNA1) was added to hybridize with the aptamers. In the presence of target cells, c-DNA1 was released due to the recognition of cells to aptamers. After the dendrimer NCs/QDs−DNA signal probe was conjugated to MB2, the released c-DNA1 hybridized with the DNA signal probe to form the double-stranded DNA, followed by specific cleavage of DNA signal probe by nicking endonuclease. Then, the DNA signal probe detached from MB2, and the released c-DNA1 entered the following cycling steps to hybridize with another DNA signal probe. After the cycled cleavage of DNA signal probe from MB2, the dendrimer NCs/QDs−DNA signal probe was captured by the modified electrode for ECL measurement (Scheme 2), and the ECL signal was significantly amplified, which could be used to detect the cell concentrations.

Figure 6A shows the ECL signals that were responsive to changes in target cell concentrations; the ECL peak intensity gradually increased with increasing cell concentrations. The standard calibration curve for cell detection is shown in Figure 6B; the ECL signal was proportional to the cell concentration in the range from 100 to 4000 cells mL^{-1}. A series of five duplicate measurements of 800 cells mL^{-1} were used for estimating the precision, and the relative standard deviation (RSD) was 5.1%, showing good reproducibility. The detection limit for cell concentration was calculated to be 68 cells mL^{-1} at 3σ, which was much lower than the above ECL methods without the DNA cycle-amplifying technique, suggesting that this strategy is highly sensitive and has great potential for early and accurate detection of cancer cells.

In order to truly evaluate this assay for target cells, the same experiment was repeated with the CEM control cells at the same concentration (2000 cells mL^{-1}) (Supporting Information Figure S4A, graph b); the ECL response was almost the same with the blank without cells (Supporting Information

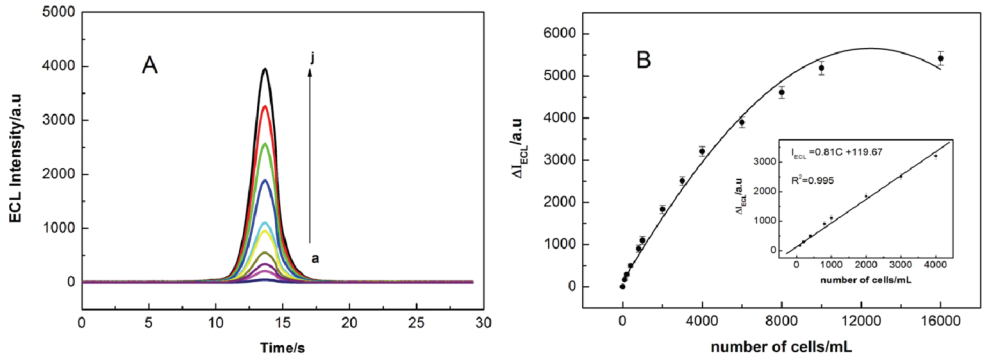

Figure 6. (A) ECL responses to different concentrations of target cells. The concentrations of the cells were as follows: (a) 0, (b) 100, (c) 200, (d) 400, (e) 800, (f) 1000, (g) 2000, (h) 3000, (i) 4000, and (j) 6000 cells mL^{-1}. (B) The calibration curve of ECL peak heights vs the concentrations of target cells from 100 to 16 000 cells mL^{-1}. The inset is the amplification of the linear range from 100 to 4000 cells mL^{-1} for target cells determination. (The blank was deducted.)

Figure S4A, graph a). In addition, the nontarget aptamer sequence (random DNA) was also used for recognition of both target and control cells; the sequence had no specificity for either cell type and produced no noticeable change in ECL signals versus blank solution (Supporting Information Figure S4A, graph c). This indicates that the method has good selectivity for assay of target Ramos cells (Supporting Information Figure S4A, graph d). Furthermore, two different actual samples were analyzed with the method. By comparison, the control cells produced no noticeable difference in the complex sample (Supporting Information Figure S4B, graph b) versus the pure target cells (Supporting Information Figure S4B, graph a) at the same concentration, suggesting good application of the ECL method in actual assay.

CONCLUSIONS

In summary, a novel dendrimers/CdSe−ZnS−QD nanocluster was newly explored and used as an amplified ECL signal probe for versatile assays of cancer cells for the first time. This study has several significant advantages. First, a large number of CdSe−ZnS QDs were assembled onto the novel dendrimer NCs due to the many functional amine groups of NCs, which could greatly amplify the QDs ECL signals. Second, a novel DNA device cycle-amplifying technique on magnetic microbeads was employed in the ECL assays, which significantly improved the sensitivity and simplified the separation procedures. Third, the high specificity of aptamers to target cells and the biobarcode technique to avoid cross-reaction were used in the assays, which much favored for the improvement of selectivity and sensitivity. To the best of our knowledge, this is the first report that the dendrimer/QDs NCs as an ECL signal probe combined with the cycle-amplifying technique was applied in the assays of cells, which has promising applications for the early and accurate detection of cancer cells.

ASSOCIATED CONTENT

Supporting Information. Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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