



Enhanced electrochemiluminescence of CdSe quantum dots composited with CNTs and PDDA for sensitive immunoassay

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ABSTRACT

Electrochemiluminescence (ECL) of CdSe quantum dots (QDs) was greatly enhanced by the combination of carbon nanotubes (CNTs) and poly (diallyldimethylammonium chloride) (PDDA) in the CdSe QDs film, and could successfully be used to develop a sensitive ECL immunosensor for the detection of human IgG (Ag). The novel CdSe QDs–CNTs composites exhibited high ECL intensity, good biocompatibility, and high stability, which held great promise for the fabrication of the ECL biosensors with improved sensitivity. After PDDA as a binding linker was conjugated to the CdSe QDs–CNTs composite film on the electrode, the ECL signal was significantly enhanced. Subsequently, gold nanoparticles (GNPs) assembled onto the CdSe QDs–CNTs/PDDA modified electrode could amplify the ECL signal once again. After antibody (Ab) was immobilized onto the electrode through GNPs, the ECL immunosensor was successfully fabricated. It is for the first time that the unique function of PDDA for enhancing QDs ECL was explored and used to develop an ECL biosensor. The principle of ECL detection for target Ag is based on the increment of steric hindrance after immunoreaction, which resulted in the decrease of ECL intensity. The Ag concentration was determined in the linear range of 0.002–500 ng L⁻¹ with a detection limit of 0.6 pg mL⁻¹. The sensor showed good fabrication and detection reproducibility, and the assay results were in acceptable agreement with the clinical sera tests, showing a promising clinical application. This work opened the new avenues for applying QDs ECL in highly sensitive bioassays.

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1. Introduction

Semiconductor nanocrystals (NCs, also referred to as quantum dots) have generated great research interest due to their unique luminescent properties and relatively low cost (Alivisatos, 1996a,b; Qu and Peng, 2002; Burda et al., 2005) in recent years. Strongly luminescent semiconductor NCs have found potential applications in biological imaging and labeling (Bruchez et al., 1998; Chan and Nie, 1998; Han et al., 2001). Luminescent properties are usually studied by using photoexcitation (Qu and Peng, 2002), electrochemiluminescence (ECL) generated by electron injection (Ding et al., 2002), and cathodoluminescence produced by electron impact (Rodriguez-Viejo et al., 1997). ECL as the production of light from electrochemically generated reagents has been paid considerable attention due to its versatility, simplified optical setup, low background signal, good temporal and spatial control (Zhang et al., 2008a,b). ECL of II–VI QDs has been extensively studied in both organic (Myung et al., 2002, 2003; Bae et al., 2004) and aqueous media (Bae et al., 2006; Ding et al., 2006; Wang et al., 2008). Efficient and stable ECL emissions can be obtained in aqueous solution

by using H₂O₂ (Zou and Ju, 2004; Ding et al., 2006), S₂O₈²⁻ (Jie et al., 2007) and O₂ (Jiang and Ju, 2007) as coreactants. The QDs-based ECL immunosensors (Jie et al., 2007) and enzyme-based ECL biosensors for glucose (Jiang and Ju, 2007) have been successfully fabricated.

However, the reports concerning the detection of biomolecules with QDs ECL are relatively scarce, though ECL analysis has many advantages over photoluminescence due to the absence of background from unselective photoexcitation. The reasons are partially that the ECL of semiconductor NCs is weaker than that of conventional luminescent reagents such as luminal or Ru(bpy)₃²⁺, and that NCs in ECL with a high excited electrochemical potential are unstable. Thus, it is important to select effective methods to enhance NCs ECL signal for development of ECL biosensors. Thin film technique of semiconductor NCs may provide an effective way for the construction of QDs-based ECL biosensors. In recent years, carbon nanotubes (CNTs) have received particular interest due to their remarkable nanostructure, high electrical conductivity, and good chemical stability (Iijima, 1991; Iijima and Ichihashi, 1993; Wang et al., 2003). It is reported that carbon nanotubes can be used to enhance the ECL of CdS QDs film by reducing the injection barrier of electrons to the QDs (Ding et al., 2006). ECL of CdSe NCs could be enhanced by combining with carbon nanotubes–chitosan and 3-aminopropyl-triethoxysilane (Jie

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et al., 2008). In addition, poly(diallyldimethylammonium chloride) (PDDA) or CNTs–PDDA has been used in biosensing owing to the good film-forming ability and susceptibility to chemical modifications (Yao and Shiu, 2008; Srivastava et al., 2008). Conducting polymer has been shown to be a superior matrix for fluorescence detection based immunosensors (Ramanavicius et al., 2007). Gold nanoparticles (GNPs) have attracted much attention in different immunoassay due to their unique physical and chemical properties, such as easily controllable size distribution, long-term stability, and friendly biocompatibility with immunospecies. Furthermore, by using the immobilization methods, sensitivity enhancement can be achieved due to an increased electrode surface and thus a substantially improved loading of antibodies or antigens (Zhang et al., 2008a,b). Taking into consideration the above advantages, the CdSe QDs–CNTs/PDDA composites and gold nanoparticle-amplification technique can become promising candidates for fabricating the novel ECL biosensors.

In this paper, we have successfully designed a sensitive ECL immunosensor based on the enhanced QDs ECL by the combination of CNTs and PDDA in the CdSe QDs film. Herein, we pursue a novel strategy to amplify the QDs ECL signal and improve the detection sensitivity. After PDDA was conjugated to the CdSe QDs–CNTs composite film, the ECL intensity was greatly enhanced. Subsequently, GNPs were adsorbed onto the electrode and ECL was further improved. After antibody was linked to the CdSe QDs–CNT/PDDA composite film via GNPs, the electrode could be used as an ECL immunosensor. To the best of our knowledge, this is the first report that the unique function of PDDA for enhancing QDs ECL was explored and used to develop an ECL biosensor. The QDs as a potential alternative for traditional ECL emitters are reproducible through ECL process (Ding et al., 2002). Moreover, the size-dependent properties of QDs (Alivisatos, 1996a,b) would provide the emitter promising behaviors for ECL application. This work opened the new avenues for applying QDs ECL in highly sensitive bioassays, which might provide an effective candidate in clinical laboratory.

2. Experimental

2.1. Reagents

Multi-walled carbon nanotubes (CNTs, CVD method, purity >95%, diameter 30–60 nm, length 0.5–15 μm) were purchased from Nanoport. Co. Ltd. (Shenzhen, China). Poly(diallyldimethylammonium chloride) (PDDA, 20%, w/w, in water, MW = 200,000–350,000), and Poly(allylamin hydrochloride) (PAH) were from Sigma–Aldrich. Chloroauric acid (HAuCl_4), and trisodium citrate were obtained from Shanghai Reagent Company (Shanghai, China). Gold nanoparticles (GNPs) were prepared by citrate reduction of HAuCl_4 in aqueous solution (Enustun and Turkevich, 1963). Human IgG (Ag) and goat anti-human IgG (Ab) were purchased from Ningbo Xinzhi Biochemical Reagents (Ningbo, China). Low-density lipoprotein (6.7 mg mL^{-1}) was obtained from Nanjing Huibiao Biological Technology Co. Bovine serum albumin (BSA, 96–99%) was obtained from Sigma (St. Louis, MO, USA). All other reagents were of analytical reagent grade and used without further purification. 0.1 M phosphate buffer solution (PBS) with various pH was prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4 , and then adjusting the pH with 0.1 M NaOH and H_3PO_4 . 0.1 M PBS (pH 7.4) containing 0.1 M $\text{K}_2\text{S}_2\text{O}_8$ and 0.1 M KCl was used as the electrolyte. Doubly distilled water was used throughout the experiments.

2.2. Apparatus

The electrochemical measurements were carried out on a CHI 812 electrochemical working station (Shanghai CH Instruments

Co., China). All experiments were carried out with a conventional three-electrode system. The electrodes were a 4-mm-diameter Au disk working electrode modified with CdSe QDs composite film, a saturated calomel reference electrode (SCE), and a Pt counter electrode. The ECL emission was detected with a Model MPI-A Electrochemiluminescence Analyzer (Xi'An Remax Electronic Science & Technology Co. Ltd., Xi'An, China) at room temperature. The spectral width of the photomultiplier tube (PMT) was 200–800 nm and the voltage of the PMT was 500–800 V in the detection process. Electrochemical impedance spectroscopy (EIS) was carried out with a CHI 660A electrochemical working station (Shanghai CH Instruments Co., China), using the same three-electrode system as that in the ECL detection. UV absorption spectra were acquired with a Ruili 1200 photospectrometer (Peking Analytical Instrument Co., Peking, China). Photoluminescence (PL) spectra were obtained on an RF-540 spectrophotometer (Shimadzu). Field-emission scanning electron microscopy (FESEM, JEOL JSM-6340 F) was used to characterize the sensor. High-resolution TEM (HRTEM) images were taken using a JEOL 2010 electron microscope at an accelerating voltage of 200 kV. Samples for HRTEM were prepared by placing two drops of the dispersed samples solution on a carbon film coated copper grid (400 mesh) and then drying under air.

2.3. Carbon nanotubes (CNTs) functionalization with PAH (CNT–PAH)

The multi-walled carbon nanotubes (CNTs) were functionalized by polymer wrapping with poly(allylamin hydrochloride) (PAH) (Olek et al., 2006). 50 mg of CNTs was dispersed in a 0.5 wt% PAH (Mw 70,000) salt solution (0.5 M NaCl, 500 mL), after sonicating for 3 h, the solution was stirred overnight at 80 °C, and again left in an ultrasonic bath for 2 h. Excess polymer was removed by repeated centrifugation and redispersion in water, until a stable, homogeneous CNTs suspension was obtained. Amine functionalities on the CNTs surface (CNT–PAH) ensure good separation and stability due to electrostatic interactions (repulsions) in aqueous solution.

2.4. Preparation of the CNTs–CdSe QDs composites

2.4.1. Preparation of the CdSe quantum dots

The sodium selenosulfate stock solution (Na_2SeSO_3) (0.1 M) was prepared by stirring sodium sulfite (0.5 M) and elemental selenium (0.2 M) at ca. 70 °C for 24 h, using distilled water as solvent. Mercaptoacetic acid–capped CdSe QDs (CdSe/TGA) were synthesized using a slightly modified procedure reported previously (Gaponik et al., 2002). After 20 mL of 5 mM CdCl_2 was mixed with 20 μL of TGA; 1 M NaOH was added to adjust its pH to 10. The clear solution was diluted to 50 mL and bubbled with highly pure N_2 for 30 min. Then 0.5 mL of 0.1 M Na_2SeSO_3 was injected into this mixture to obtain a clear light yellow solution of CdSe/TGA QDs. The final molar ratio of $\text{Cd}^{2+}/\text{TGA}/\text{Se}^{2-}$ was 1:2.5:0.5. The sizes of the obtained NCs could be tuned by simply varying the reflux time.

2.4.2. Preparation of the CdSe QDs–CNTs composites

In a typical experiment, 3 mL CdSe QDs solution was added into a 2 mL dispersion of functionalized CNTs (2.5 mg mL^{-1}). The reaction mixture was briefly sonicated and then stirred for 15 min. Excess particles were removed by subsequent centrifugation and redispersion in water. This procedure results in homogeneous coating of the CNTs surface with nanocrystals, and the CdSe QDs–CNTs composite solution was obtained.

2.5. Preparation of the ECL immunosensor

A gold disk electrode with 4 mm diameter was polished carefully with 1.0, 0.3 and 0.05 μm $\alpha\text{-Al}_2\text{O}_3$ powder on fine abrasive

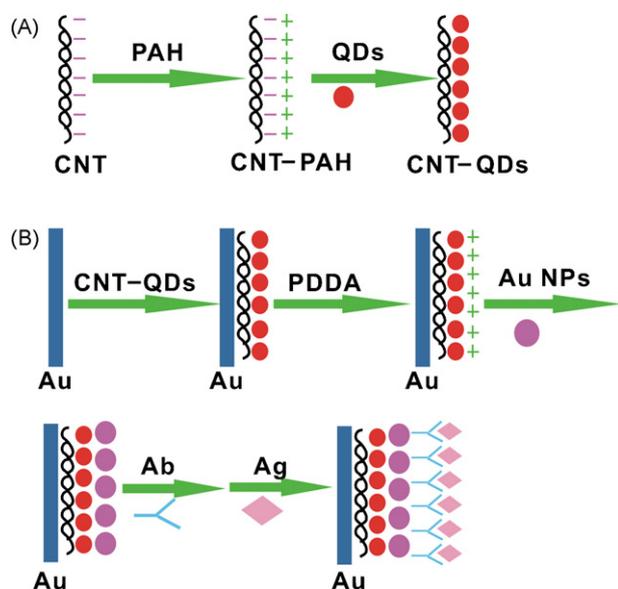


Fig. 1. The fabricating procedures for CdSe QDs-CNTs conjugates (A), and the ECL immunosensor (B).

paper and washed ultrasonically with water. Before modification, the bare electrode was scanned in 0.5 M H_2SO_4 between -0.2 and 1.5 V until a reproducible cyclic voltammogram (CV) was obtained. After the electrode was rinsed thoroughly with doubly distilled water and allowed to dry at room temperature, $10 \mu\text{L}$ of CdSe QDs/CNT-PAH composite solution was dropped on the electrode and dried in the air, followed by dropping $10 \mu\text{L}$ of 0.5% PDDA aqueous solution containing 0.5 M NaCl on the electrode. After the electrode was dried for 2 h, it was immersed into the gold nanoparticles (GNPs) solution for 8 h. Then the electrode was rinsed with redistilled water and dipped in 0.5 mg mL^{-1} antibody solution (50 mM PBS, pH 6.0) at 4°C for at least 12 h. Finally, it was rinsed with pH 7.4 PBS and incubated in $20 \mu\text{L}$ of 2 wt% BSA at 37°C for 1 h to block nonspecific binding sites. After rinsed with

pH 7.4 PBS, the electrode was used as an ECL immunosensor, which was incubated in $40 \mu\text{L}$ of HlgG (Ag) samples at 37°C for 50 min.

Fig. 1 outlines the fabricating procedures for the CdSe QDs-CNTs conjugates (A) and the ECL immunosensor (B). In Fig. 1A, CNTs was first functionalized by polymer wrapping with PAH, then the negatively charged CdSe QDs were electrostatically absorbed to the CNTs surface modified with PAH. Fig. 1B includes the formation of the CdSe QDs-CNTs composite film on the Au electrode, the linkage of PDDA to the film, the conjugation of GNPs to PDDA, the immobilization of antibody (Ab) on the electrode with GNPs, and the specific immunoreaction.

2.6. ECL detection

The modified electrode above was in contact with 0.1 M PBS (pH 7.4) containing 0.1 M $\text{K}_2\text{S}_2\text{O}_8$ and 0.1 M KCl, and scanned from 0 V to -1.5 V. ECL signals related to the HlgG concentrations could be measured.

3. Results and discussion

3.1. Characterization of GNPs, CdSe QDs and CNTs

Fig. 2A and C shows the absorption spectra and transmission electron microscopy (TEM) of GNPs, the absorption peak was at 519 nm and the size of GNPs was ca. 16–18 nm.

The photoluminescence (PL) and absorption spectra (inset) of the CdSe QDs was shown in Fig. 2B, the PL emission peak at 566 nm ($\lambda_{\text{ex}} = 402$ nm) and absorption maximum at 480 nm indicated the consequence of quantum confinement (Alivisatos, 1996a,b). The size of the CdSe QDs, estimated from the first adsorption peak in UV-visible spectra and the empirical equations (Yu et al., 2003) is about 2.1 nm.

Fig. 2D and E shows the TEM images of CNTs and CdSe QDs-CNTs, respectively. The diameter of the CNTs was about 30–40 nm, and the CNTs surface was densely coated with CdSe nanoparticles.

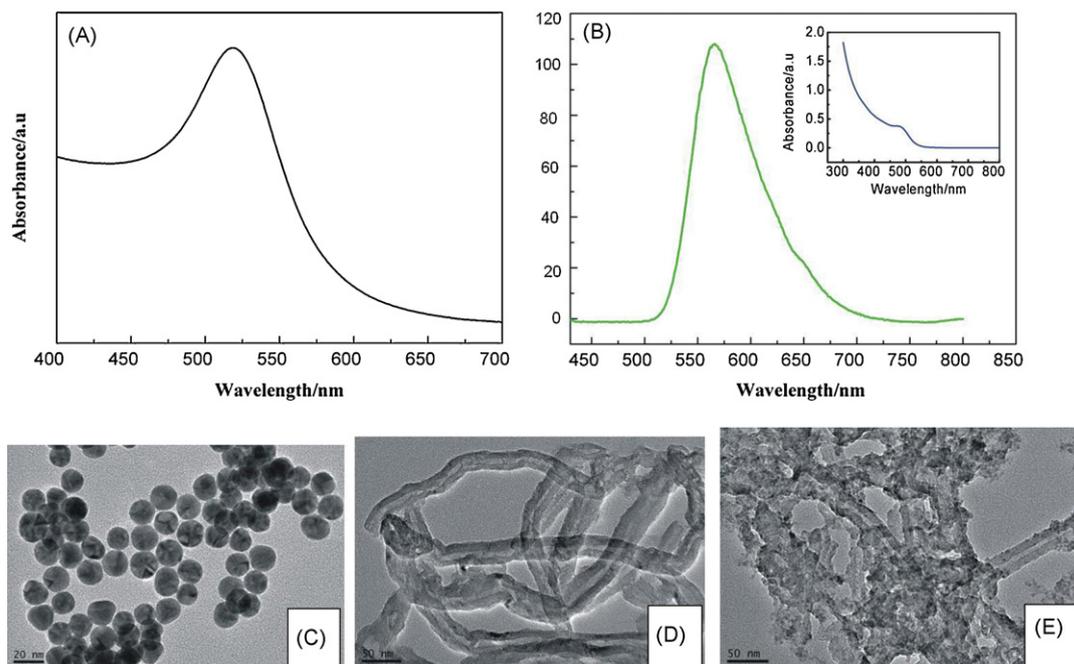


Fig. 2. (A) Absorption spectra of the gold nanoparticles (GNPs), (B) Photoluminescence (PL) and absorption (inset) spectra of the CdSe QDs in aqueous solution (Excitation wavelength: 402 nm), (C) Transmission electron microscopy (TEM) of the GNPs, (D) TEM of the CNTs, (E) TEM of the CdSe QDs-CNTs.

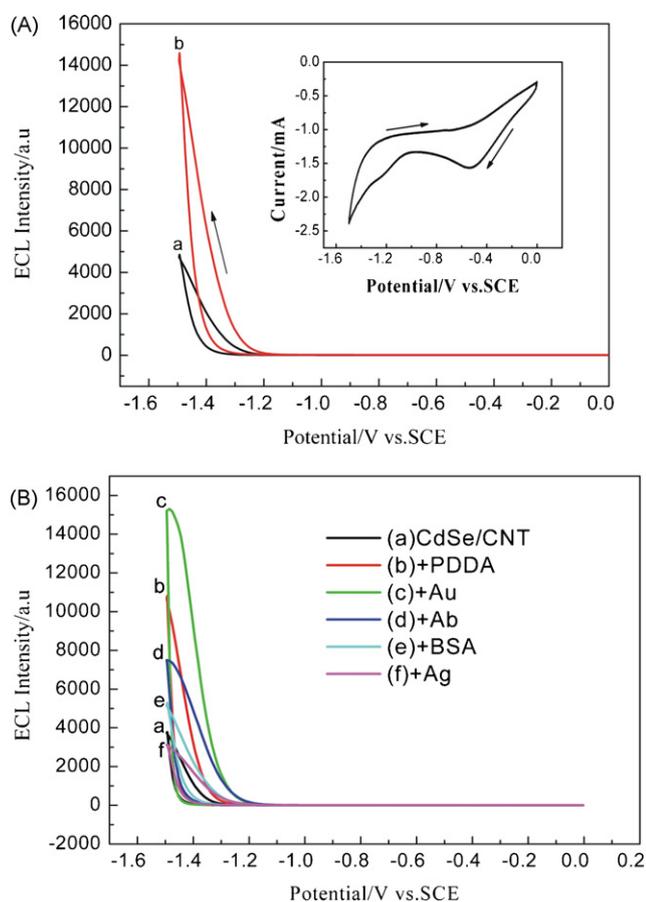


Fig. 3. (A) ECL–potential curves of (a) the CdSe QDs modified Au electrode and (b) the CdSe QDs–CNTs modified Au electrode in 0.1 M PBS (pH 7.4) containing 0.1 M KCl and 0.1 M $K_2S_2O_8$. Inset: cyclic voltammogram of the CdSe QDs–CNTs modified Au electrode. Scan rate: 100 mV s^{-1} . The voltage of the photomultiplier tube was set at 700 V. (B) ECL–potential curves of (a) CdSe QDs–CNTs, (b) (a)+PDDA, (c) (b)+GNPs, (d) (c)+Ab, (e) (d)+BSA, and (f) (e)+Ag modified Au electrodes in 0.1 M PBS (pH 7.4) containing 0.1 M KCl and 0.1 M $K_2S_2O_8$ (the voltage of the photomultiplier tube was set at 500 V). Scan rate: 100 mV s^{-1} .

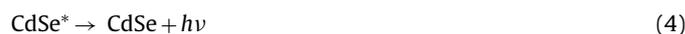
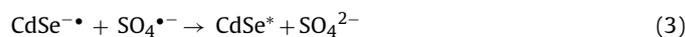
3.2. Electrochemical and ECL behaviors of the CdSe QDs–CNTs composite film

The curve (inset) in Fig. 3A shows the cyclic voltammograms (CVs) of CdSe QDs–CNTs composite film on the Au electrode. One cathodic peak was observed at -0.52 V , corresponding to the reduction of $S_2O_8^{2-}$ (Jie et al., 2007).

Fig. 3A (a) and (b) show the ECL–potential curves of the pure CdSe QDs film and the CdSe QDs–CNTs composite film modified electrode, respectively. The quantities of the CdSe QDs are equal in both films. One ECL peak was observed in both curves, resulting from the reaction between the CdSe QDs and $S_2O_8^{2-}$. The ECL intensity from the CdSe QDs–CNTs composite film is about 3 fold higher than that observed from the pure CdSe QDs film. The results indicated the more porous structure, larger surface area, and better conductivity of the CdSe QDs–CNTs composite film facilitated the ECL reaction. Therefore, the CdSe QDs–CNTs composite film was promising for the construction of the ECL biosensor.

According to previous reports, the electrochemically reduced and oxidized Si NCs (Ding et al., 2002) or CdSe NCs (Myung et al., 2002) can react with the coreactants to produce ECL. In this case, upon the potential scan with an initial negative direction, the CdSe QDs immobilized on the electrode were reduced to nanocrystalline species ($CdSe^{\bullet-}$) (Myung et al., 2002), while the coreactant $S_2O_8^{2-}$ was reduced to the strong oxidant $SO_4^{\bullet-}$. Then $SO_4^{\bullet-}$ could

react with the negatively charged $CdSe^{\bullet-}$ through electron transfer, which produced the excited state ($CdSe^*$) to emit light. The possible ECL mechanisms are as follows (Myung et al., 2002).



3.3. Preparation and characterization of the ECL immunosensor

As we know, CNTs functionalized with carboxylic acid groups are negatively charged while PAH is a cationic polyelectrolyte. When CNTs and PAH were mixed by sonication, PAH was adsorbed on the surface of the CNTs by electrostatic interaction. Then CNT–PAH was mixed with the QDs, and the QDs were covalently bound to the amine functionalities on the surface of CNTs. On the other hand, as the QDs are negatively charged, this could accelerate the adsorption of QDs through the electrostatic interaction. For the preparation of the immunosensor, the CdSe QDs–CNTs composite film was firstly formed on the electrode surface, and then PDDA with positive charge was electrostatically adsorbed to the CdSe QDs–CNTs/electrode. After GNPs were assembled onto the CdSe QDs–CNTs/PDDA/electrode surface, Ab was immobilized onto the electrode through the interaction between GNPs and mercapto or primary amine groups in biomolecules (Ab).

3.3.1. Electrochemiluminescence (ECL)

In order to characterize the fabrication process of the ECL immunosensor, ECL signals at each immobilization steps were recorded. As shown in Fig. 3B, when PDDA was conjugated to the CdSe QDs–CNTs composite film (curve a), the ECL intensity was greatly enhanced (curve b). According to our previous studies (Jie et al., 2008), it may be inferred that the amine groups of PDDA play a key role in the catalysis of ECL reaction. Then, when GNPs were assembled onto the electrode through PDDA, the ECL signal was further improved (curve c). The reason may be that the GNPs play an important role similar to a conducting wire, which makes it easier for the electron transfer in ECL reaction. Finally, after antibody (Ab) was immobilized onto the electrode with GNPs (curve d), and BSA was used to block the nonspecific binding sites (curve e), the immunoreaction occurred (curve f), which resulted in the gradual decrease in ECL intensity. The reason is that the antigen–antibody complex and the BSA protein on the electrode acted as the inert electron and mass-transfer blocking layer, and hindered the diffusion of ECL reagents toward the electrode surface significantly which could have influence on the decrease of ECL signal.

3.3.2. Scanning electron microscopy (SEM) images

Fig. 4A, B, and C displays the typical SEM images of the CdSe QDs–CNTs/PDDA, CdSe QDs–CNTs/PDDA/GNPs, and CdSe QDs–CNTs/PDDA/GNPs/Ab assembled on the electrode surface, respectively. It could be found that a thick film of CdSe QDs–CNTs/PDDA was homogeneously formed on the electrode, and the ends of carbon nanotubes could be observed (Fig. 4A). By comparison, the well-dispersed GNPs attached to the CdSe QDs–CNT/PDDA spread over the electrode more uniformly (Fig. 4B). This indicates that PDDA plays a key role in the attachment, it acts as a bridge to connect GNPs with QDs–CNTs. As shown in Fig. 4C, after Ab was immobilized onto the electrode, the composite film became much thicker and rougher, and the structure of film was completely changed, confirming that the immunosensor was fabricated.

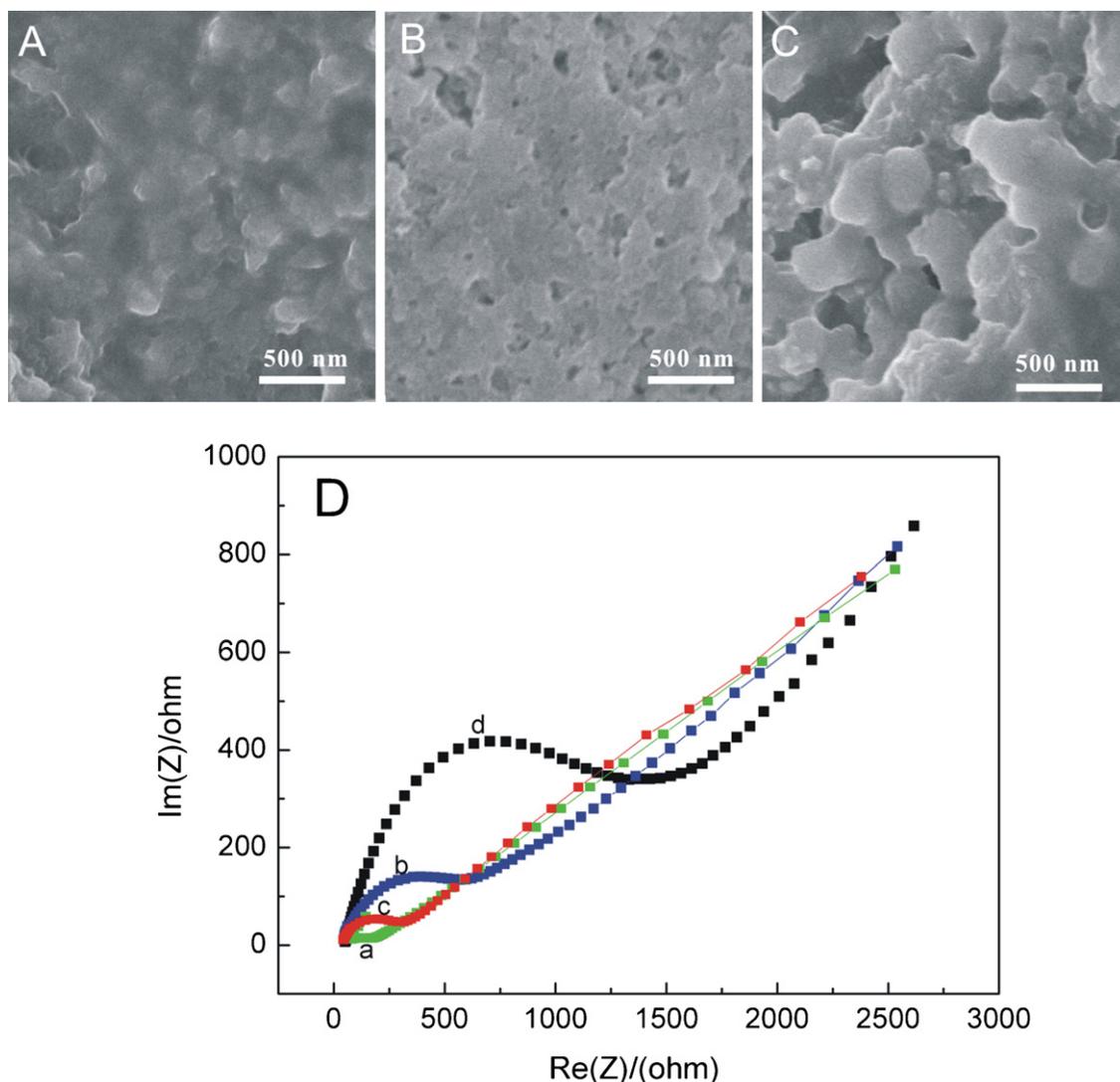


Fig. 4. Representative SEM images of (A) CdSe QDs–CNTs/PDDA, (B) (A)+GNPs, (C) (B)+Ab; (D) Electrochemical impedance spectroscopy (EIS) of (a) bare, (b) CdSe QDs–CNTs/PDDA, (c) CdSe QDs–CNTs/PDDA/GNPs, (d) CdSe QDs–CNTs/PDDA/GNPs/Ab modified Au electrodes in 10 mM PBS (2.5 mM $\text{Fe}(\text{CN})_6^{4-/3-}$ + 0.1 M KCl, pH 7.4). The frequency range is between 0.01 and 100,000 Hz with signal amplitude of 5 mV.

3.3.3. Electrochemical impedance spectroscopy (EIS)

EIS is an effective method for probing the features of surface-modified electrodes. The impedance spectra include a semicircle portion and a linear portion. The semicircle diameter at higher frequencies corresponds to the electron-transfer resistance (R_{et}), and the linear part at lower frequencies corresponds to the diffusion process. Fig. 4D shows the EIS of the electrode at different stages. It was observed that the EIS of the bare Au electrode displayed an almost straight line (curve a), which was characteristic of a mass diffusion limiting process. After the electrode was modified with CdSe QDs–CNTs/PDDA composite film, the EIS showed an electron transfer resistance of about 600Ω (curve b). Subsequently, GNPs were assembled onto the electrode through PDDA, the resistance for the redox probe obviously decreased (curve c), implying that the GNPs were excellent electric conducting materials and accelerated the electron transfer. Finally, when Ab was immobilized onto the electrode with GNPs, the EIS showed a large increase in diameter (curve d), suggesting that the antibody formed an additional barrier and further prevented the redox probe to the electrode surface.

3.4. Optimization of experimental conditions

3.4.1. Choice of conditions for the preparation of ECL immunosensor

First, the ECL performance of the immunosensor mainly depended on the amounts of CdSe QDs–CNTs dropped on the electrode. According to the ECL experimental results, $10 \mu\text{L}$ of CdSe QDs–CNTs solution was selected as the optimal amount. Then, the effects of both the amount and concentration of PDDA on ECL were also studied and $10 \mu\text{L}$ of 0.5% PDDA aqueous solution containing 0.5 M NaCl was the optimal condition.

3.4.2. Choice of conditions for the ECL detection

The effects of incubation temperature, incubation time, and pH value on the ECL intensity of the immunosensor were investigated as follows.

The effect of incubation temperature on the immunoreaction was studied in the range from 20°C to 45°C . As shown in Fig. S1(A) (Supplemental Information), the ECL intensity of the immunosensor reached a minimum value at 37°C ,

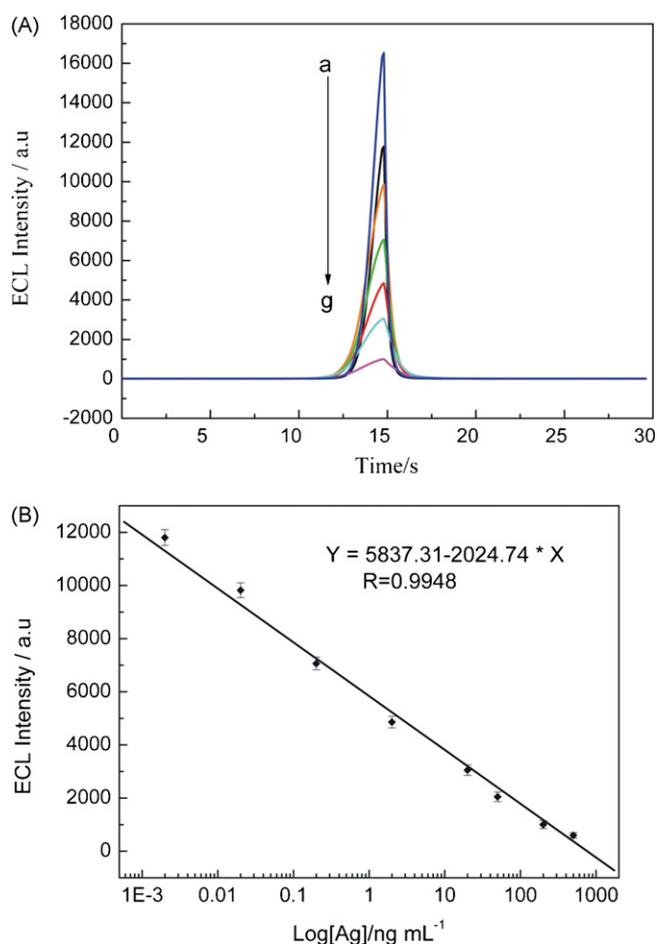


Fig. 5. (A) ECL profiles of the immunosensor in the absence (a) and presence (b–g) of different concentrations of Ag in pH 7.4 PBS containing 0.1 M KCl and 0.1 M $K_2S_2O_8$. Ag concentration ($ng\ mL^{-1}$): (a) 0, (b) 0.002, (c) 0.02, (d) 0.2, (e) 2, (f) 20, (g) 200 (the voltage of the photomultiplier tube was set at 600 V). (B) Calibration curve for Ag determination. Scan rate: $100\ mV\ s^{-1}$.

indicating the maximum immunoreaction occurred at the temperature.

In addition, the effect of incubation time on the immunoreaction was shown in Fig. S1(B). With the increase of time, the ECL intensity decreased, and then reached a constant value after 50 min, suggesting that the immunoreaction reached an equilibrium state. Thus, the incubation temperature of $37\ ^\circ C$ and the incubation time of 50 min were selected for the ECL immunosensor.

The study of pH influence on the ECL detection was conducted in the range of 6.4–9.0. As shown in Fig. S2, the ECL intensity of the immunosensor showed great dependence on the pH value of the solution. The ECL intensity increased from 6.4 to 8.0, and the maximum was observed at pH 7.4–8.0. Since the optimal pH value for the biological systems was 7.4, the ECL detection was performed in pH 7.4 PBS containing 0.1 M KCl and 0.1 M $K_2S_2O_8$.

3.5. Detection of HlgG (Ag)

Fig. 5A shows the ECL responses of the immunosensor before (a) and after (b–g) reacting with different concentrations of HlgG (Ag). As can be seen in Fig. 5A, the ECL peak intensity in the presence of Ag was lower than that in the absence of Ag, and decreased gradually with increasing concentrations of Ag (b–g). The reason was that the immunocomplex after immunoreaction inhibited the ECL reaction between CdSe QDs and $K_2S_2O_8$, and thus decreased the ECL intensity. On the one hand, the immunocomplex increased the steric

hindrance, which slowed down the electron transfer speed in ECL reaction. On the other hand, the immunocomplex may inhibit the transfer of $K_2S_2O_8$ to the surface of the CdSe QDs–electrode, which also decreased the ECL reaction. The results suggested that the Ag concentration could be determined with the ECL immunosensor.

The standard calibration curve for Ag detection was shown in Fig. 5B. The ECL intensity decreased linearly with the Ag concentrations in the range from 0.002 to $500\ ng\ L^{-1}$, and the detection limit was $0.6\ pg\ mL^{-1}$. According to the linear equation, we could detect Ag concentration quantitatively. Higher serum HlgG levels could be detected by an appropriate dilution with pH 7.4 PBS.

3.6. Specificity, stability, reproducibility, and regeneration of the immunosensor

To investigate the specificity of the immunosensor, we mixed $2\ ng\ mL^{-1}$ human IgG, $200\ ng\ mL^{-1}$ goat IgG, and $200\ ng\ mL^{-1}$ low-density lipoprotein (LDL), and then detected the ECL response of the mixture. Compared with the ECL response of the immunosensor in $2\ ng\ mL^{-1}$ pure human IgG, no significant difference (R.S.D = 8.4%) was observed, indicating that the goat IgG and LDL could not cause the observable interference. The results suggest that the immunosensor displays good specificity for the determination of human IgG.

After the immunosensor was stored in pH 7.4 PBS at $4\ ^\circ C$ over 30 days, it was used to detect the same HlgG concentration, the analytical performances did not show an obvious decline, demonstrating that the immunosensor had good stability.

The reproducibility of the immunosensor was estimated by determining $20\ ng\ mL^{-1}$ IgG with four immunosensors made at the same electrode. Four measurements from the batch resulted in a relative standard deviation of 8.3%, indicating good reproducibility of the fabrication protocol.

Regeneration is of interest to immunoassay. After the immunosensor was used to detect HlgG, the electrode was treated with 0.2 M glycine–hydrochloric acid (Gly–HCl) buffer solution (pH 2.8) for 9 min to break the antibody–antigen linkage. The intra-assay variation coefficient (CV) was the difference among four determinations of one sample on the same electrode after the immunosensor was regenerated. The intra-assay CV obtained at HlgG concentration of $20\ ng\ mL^{-1}$ was 9.8%. The results demonstrated that the immunosensor could be regenerated and used again.

3.7. Application of the immunosensor in human IgG levels

The feasibility of the immunoassay system for clinical applications was investigated by analyzing several real samples. In comparison with the ELISA method, these serum samples were diluted to different concentrations with PBS of pH 7.4.

Table 1 describes the correlation between the results obtained by the proposed ECL immunosensor and ELISA method. It obviously indicates that there is no significant difference between the results and ELISA method. Thus, the developed immunosensor could be satisfactorily applied to the clinical determination of IgG levels in human serum.

Table 1
Comparison of serum HlgG levels determined using two methods.

	Serum samples		
	1	2	3
Immunosensor (ng/mL) ^a	0.032	0.40	24.4
ELISA ^a	0.03	0.45	23.0
Relative deviation (%)	4.56	–8.3	4.2

^a The average value of five successive determinations.

4. Conclusion

In this work, a novel nanocomposite film consisting of the CdSe QDs–CNTs and PDDA has been designed to construct an electrochemiluminescence immunosensor for a rapid and sensitive immunoassay. The QDs–CNTs composites exhibited high ECL intensity, good biocompatibility, and high stability, which were demonstrated to be well-competent for the development of ECL immunosensors. After PDDA as a cross-linker was conjugated to the QDs–CNTs composite film, the ECL intensity was significantly enhanced. To the best of our knowledge, this is the first report that PDDA was used to enhance QDs ECL and develop an ECL biosensor. Moreover, gold nanoparticles were assembled onto the QDs–CNTs/PDDA electrode for antibody immobilization, which could also improve the ECL signal and adsorption capacity of Ab, and thus enhanced the detection sensitivity. The developed immunosensor integrated the advantages of sensitive ECL detection, specific immunoreaction with nanoparticle amplification technique, which could provide a promising tool for protein detection in clinical application. In particular, this highly enhanced ECL from the QDs composite film would open new avenues to apply QDs ECL in analytical systems and ECL biosensors.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.bios.2009.04.039](https://doi.org/10.1016/j.bios.2009.04.039).

References

- Alivisatos, A.P., 1996a. *J. Phys. Chem.* 100, 13226–13239.
- Alivisatos, A.P., 1996b. *Science* 271, 933–937.
- Bae, Y.J., Lee, D.C., Rhogojina, E.V., Turbergs, D.C., Korgel, B.A., Bard, A.J., 2006. *Nanotechnology* 17, 3791–3797.
- Bae, Y.J., Myung, N., Bard, A.J., 2004. *Nano Lett* 4, 1153–1161.
- Burda, C., Chen, X.B., Narayanan, R., El-Sayed, M.A., 2005. *Chem. Rev.* 105, 1025–1102.
- Bruchez, M., Moronne, M., Gin, P., Weiss, S., Alivisatos, A.P., 1998. *Science* 281, 2013–2016.
- Chan, W.C.W., Nie, S.M., 1998. *Science* 281, 2016–2018.
- Ding, S.N., Xu, J.J., Chen, H.Y., 2006. *Chem. Commun.*, 3631–3633.
- Ding, Z., Quinn, B.M., Haram, S.K., Pell, L.E., Korgel, B.A., Bard, A.J., 2002. *Science* 296, 1293–1297.
- Enustun, B.V., Turkevich, J.J., 1963. *Am. Chem. Soc.* 85, 3317–3328.
- Gaponik, N., Talapin, D.V., Rogach, A.L., Hoppe, K., Shevchenko, E.V., Kornowski, A., Eychmuller, A., Weller, H., 2002. *J. Phys. Chem. B* 106, 7177–7185.
- Han, M., Gao, X., Su, J.Z., Nie, S., 2001. *Nat. Biotechnol.* 19, 631–635.
- Iijima, S., Ichihashi, T., 1993. *Nature* 363, 603–605.
- Iijima, S., 1991. *Nature* 354, 56–58.
- Jiang, H., Ju, H.X., 2007. *Chem. Commun.*, 404–406.
- Jie, G.F., Liu, B., Pan, H.C., Zhu, J.J., Chen, H.Y., 2007. *Anal. Chem.* 79, 5574–5581.
- Jie, G.F., Zhang, J.J., Wang, D.C., Cheng, C., Chen, H.Y., Zhu, J.J., 2008. *Anal. Chem.* 80, 4033–4039.
- Wang, J., Musameh, M., Lin, Y., 2003. *J. Am. Chem. Soc.* 125, 2408–2409.
- Myung, N., Bae, Y., Bard, A.J., 2003. *Nano Lett.* 3, 1053–1055.
- Myung, N., Ding, Z.F., Bard, A.J., 2002. *Nano Lett.* 2, 1315–1319.
- Olek, M., Bu1sgen, T., Hilgendorff, M., Giersig, M., 2006. *J. Phys. Chem. B* 110, 12901–12904.
- Qu, L., Peng, X.J., 2002. *Am. Chem. Soc.* 124, 2049–2055.
- Rodriguez-Viejo, J., Jensen, K.F., Mattoussi, H., Michel, J., Dabbousi, B.O., Bawendi, M.G., 1997. *Appl. Phys. Lett.* 70, 2132–2134.
- Srivastava, S., Ball, V., Podsiadlo, P., Lee, J., Ho, P., Kotov, N.A., 2008. *J. Am. Chem. Soc.* 130, 3748–3749.
- Wang, X.F., Xu, J.J., Chen, H.Y., 2008. *J. Phys. Chem. C* 112, 7151–7157.
- Yu, W.W., Qu, L.H., Guo, W.Z., Peng, X.G., 2003. *Chem. Mater.* 15, 2854–2860.
- Yao, Y.L., Shiu, K.K., 2008. *Electroanalysis* 20, 1542–1548.
- Zhang, L.H., Zou, X.Q., Ying, E., Dong, S., 2008a. *J. Phys. Chem. C* 112, 4451–4454.
- Zhang, S., Zheng, F., Wu, Z., Shen, G., Yu, R., 2008b. *Biosens. Bioelectron.* 24, 129–135.
- Zou, G.Z., Ju, H.X., 2004. *Anal. Chem.* 76, 6871–6876.