

Special method to prepare quantum dot probes with reduced cytotoxicity and increased optical property

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Abstract. Quantum dots (QDs) are widely used in the life sciences because of their novel physicochemical properties. However, the cytotoxicity of these nonparticles have attracted great attention recently because this has not been well resolved. Four probes were synthesized by chemical coupling and protein denaturation with CdSe/ZnS, CdTe QDs, and transferrin. Sodium dodecyl sulfate polyacrylamide gel electrophoresis and capillary electrophoresis were used to verify the conjugation of these luminescent probes. The cytotoxicity of these four luminescent probes and the original QDs were evaluated in HeLa cells. The results showed that over 92% of HeLa cells were still alive after being exposed to 3.2- μ M CdSe/ZnS QDs capped with denatured transferrin for 72 h. Furthermore, while the probe preparation was very simple, the photoluminescence quantum yield of this probe was 7% higher than the original CdSe/ZnS QDs. This provides a new way for exploiting QD probes with low cytotoxicity, which will expand applications of nanocomposite assembly in biolabeling and imaging. © 2010 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.3291999]

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1 Introduction

Quantum dots (QDs), one of the new nanomaterials, have high fluorescence intensity, photostability, low photobleaching, and simultaneous excitation of particles with different colors by one single wavelength compared to traditional organic dyes.^{1,2} In addition, they have the same surface properties, which allow a similar approach for conjugating biomolecules to QDs with different colors; thus, it is possible to monitor biological processes with different labeling molecules over the long term.^{3,4}

However, even though quantum dots have unique optical properties, as fluorescent probes in biological labeling, their cytotoxicity is of great concern. Derfus et al.⁵ found the release of free Cd²⁺ ions from the CdSe-core QDs because of surface oxidation, and cell death was correlated with the accumulative of free cadmium ions. Lovric et al.⁶ found that naked CdTe QDs could damage the plasma membrane, mitochondria, and nucleus and lead to the release of cytochrome c from mitochondria. Moreover, these QDs could induce cells apoptosis. But when the CdS shell and ZnS shell were packed on the core of the CdTe, there was noncytotoxicity to K562 cells for 48 h incubation at 3- μ M concentration,⁷ indicating that as fluorescent probes, QDs need to be prevented from releasing toxic elements in biological applications, whose key

problem is to modify the protective agents to the QDs' surface.

CdSe/ZnS QDs that have a core/shell structure are the most versatile in biology. The cores of this kind of QD are well packaged by ZnS. Usually, this kind of QD is synthesized in hydrophobic organic solvents, as solubilization of these QDs is essential for biological applications. However, the photoluminescence quantum yield (PL QY) of these water-soluble QDs is lower after solubilization, and photostability also declines—all these influence the applications of QDs in various fields.⁸ CdTe QDs, a recent arrival on the scene, are directly synthesized in a water-phase system and the synthesis method is easy. This kind of QD has also been applied to many areas of biology.^{9–13} But CdTe QDs have low photostability and a wide distribution of particle sizes, and their PL QY are not as high as that of CdSe/ZnS QDs.^{14–16} Thus, researchers have made great efforts to synthesize QDs with high PL QY and low cytotoxicity for biological applications.

The applications of QDs in cell biological and biomedical imaging are based on the special activity of the conjugated biomolecules. Covalent bonding is the normal way of conjugation bimolecular to QDs.¹⁷ Conjugation of denatured protein to the surface of QDs is another new method to prepare the nanocompounds.^{18,19} Transferrin (Tf) is an important β -globulin and the serum iron transport protein, consisting of

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a single polypeptide chain of 670 to 700 amino acids containing two structural signatures at the N-terminal and C-terminal and 19 disulphide bridges.²⁰ The relative molecular weight is 70 to 90 kDa. It is highly specific to transferrin receptors (TfR) and does not cross-react with other related proteins. It can deliver and adjust the balance of iron in the biological body and is one of the necessary factors in cell growth and propagation. As TfR is overexpressed on the surface of tumor cells, identification and diagnosis of tumors can be achieved using tagged Tf.²⁰

In this study, four nanoparticles were synthesized with CdSe/ZnS, CdTe QDs, and transferrin by covalent bonding and protein denaturation. Their optic capabilities were detected and cytotoxicity in HeLa cells was evaluated with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) viability assay. It was found that CdSe/ZnS QDs capped with denatured transferrin (dTf) present the least toxicity and superior photoluminescence. Preparation of this probe is very simple, and coupling agents are not involved in the program of synthesis, so purification processes are unnecessary. This then is a simple way to prepare nanoparticles with high PL QY and low cytotoxicity.

2 Materials and Methods

2.1 Materials

CdSe/ZnS QDs and CdTe QDs were synthesized according to previous reports.^{16,21} Sodium dodecyl sulfate (SDS) and NaBH₄ (96%) were purchased from Sinopharm Chemical Reagent Co. 3-mercaptopropionic acid, sodium thioglycolate, N-hydrocylsulfo-succinimide (Sulfo-NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), MTT, and transferrin were purchased from Sigma-Aldrich Fine Chemicals (St. Louis, Missouri). All other chemicals and materials used in the experiments were of analytical grade, and water was deionized.

2.2 Instruments

The following equipment was purchased from the companies indicated in parentheses: Luminescence spectrometer (LS-55, PerkinElmer, Waltham, Massachusetts), fiber-optic spectrometer (QE65000, Ocean Optics, Dunedin, Florida), micro-plate reader (ELX808™, Biotek, Winooski, Vermont), inverted fluorescence microscope (IX71, Olympus, Nagano, Japan), cooled color charge-coupled device (CCD, Pixera Penguin 150CL, San Jose, California), circular dichroism spectrometer (J-810, Jasco, Tokyo, Japan), vertical electrophoresis system (DYY-6C, Beijing Liuyi Instrument Factory, China), Gbox-M Biosens Gel Documentation System (Syngene, Cambridge, England), high-voltage power (0 to 30 kV, Shanghai Nuclear Research Institute, China).

2.3 Modification of Water-Soluble CdSe/ZnS QDs

500 μL CdSe/ZnS-chloroform solution (1.75×10^{-4} M) was added by 200 mg sodium thioglycolate powder in an eppendorf (EP) tube. After stirring for 12 h, 100 μL of distilled water was added to the tube, following by stirring and incubation. The supernatant layer of the solution was dissociated for the next precipitation process with acetone. The whole process was repeated more than three times to remove

the free sodium thioglycolate. Last, the precipitate was dissolved in deionized water to get water-soluble CdSe/ZnS QDs.

2.4 Preparation of QD Probes

The conjugation process of CdSe/ZnS-Tf included three steps. First, 10 μL EDC phosphate buffered saline (PBS) solution (5 mg/mL, pH 7.4) was added to 40 μL water-soluble CdSe/ZnS QDs solution (1.5×10^{-4} M). Then the mixture was added to 100 μL PBS (pH 7.2), followed by 15 min shaking. 75 μL transferrin PBS solution (2 mg/mL) was added into the mixture, and the solution was stirred for 2 h at room temperature. The resulting mixture was filtered, and CdSe/ZnS-Tf was obtained.

80 μL NHS (0.1 mg/ml pH 7.4) PBS solution was added to 50 μL CdTe solution with 3-mercaptopropionic acid as the stabilizer. After 10 min incubation at room temperature, 75 μL PBS and 50 μL transferring aqueous solution (2 mg/ml) were added, and the mixture was shaken at room temperature for 30 min to allow the conjugation reaction yielding CdTe-Tf.

Transferrin was denatured by chemically treating with NaBH₄. The process was as follows: transferrin (10 mg) and NaBH₄ (0.254 mg) were dissolved in 3 mL deionized water under stirring. The reaction proceeded at room temperature for 1 h. After that, the reagent was incubated in a constant temperature water bath at 70 °C for 20 to 30 min until no more H₂ was generated. Then the denatured transferrin was obtained.

QDs were precipitated with acetone and redissolved in a measured amount of dTf solution, and the admixture was incubated at 70 °C in a constant temperature water bath for 15 min. After being cooled and diluted in PBS (pH 7.2) to the needed concentration, denatured transferrin was coupled with QDs. The preparation of CdTe-dTf and CdSe/ZnS-dTf were finished.

2.5 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

10% resolving gel and 4% stacking gel were used in SDS-PAGE electrophoresis. A 15 μL aliquot of the sample was mixed with 15 μL sample buffer, and the mixture was then injected into the gel wells. After running for 3 h at 100 V, 40 M, the gel was fixed in 12% acetum for 1 h. The fluorescence of samples was observed before staining in 0.01% Coomassie blue R250. The image of dyed protein was obtained after the gel being discolored.

2.6 Procedure of Capillary Electrophoresis

Capillary electrophoresis (CE) analyses were carried out on a home-built system. A capillary was fixed on the detecting platform of an inverted fluorescence microscope, while a mercury lamp was used as excitation source, and the excited fluorescence signal of QDs was collected using a fiber-optic spectrometer. CE experiments were all performed in 75 μm ID × 60-cm-long fused-silica capillaries. The effective length (length from injection to the detection window) was 35 cm. Hydrodynamic injection was performed by siphoning at 15-cm height differences for 60 s at the anode. Water-soluble polymer solutions were used as sieving media. A solution of

25 mM $\text{Na}_2\text{B}_4\text{O}_7$ (pH 9.20) was used as CE separation buffer. The separation was achieved at room temperature. Between runs, the capillary was washed with 0.10 M NaOH, pure water, and running buffer for 10 min to ensure the reproducibility.

2.7 Circular Dichroism Spectrometer

The circular dichroism (CD) spectrum was detected by a CD spectrometer. The spectrum diameter was 1 mm. The scanning speed was 100 nm/min, and the scanning range was 190 to 400 nm. The time constant was 1 s, and the resolution was 0.2 nm. Samples were dissolved in PBS (pH 7.2) at 1 mg/mL. The results were averaged from three scans.

2.8 Cell Culture Conditions and Treatments

2.8.1 MTT Assay

The cytotoxicity of QDs was evaluated by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] viability assay. HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum, 100 mg/mL streptomycin, and 100 U/mL penicillin at 37 °C in a humidified atmosphere with 5% CO_2 . For MTT assays, cells were dispensed in 96-well plates at a density of 1000 cells/well. After incubating for 16 to 18 h, the cells were washed with fresh medium. The treated cells were added by different concentrations (from 0.1 to 3.2 μM) of the nanoparticles and incubated for 2 h, 12 h, 24 h, 48 h, and 72 h, respectively. At different incubation time, 20 μL MTT stock solution (5 mg/mL) was added to each well, and cells were cultured for another 4 h at 37 °C in the dark. The media was then removed, and 150 μL dimethyl sulfoxide (DMSO) was put in each well. Plates were shaken gently for 10 min while the cells were lysed with DMSO. The absorbance at 490 nm was measured with a micro-plate reader. In this study, the data were from three or four independent experiments, with the same treatment repeated in triplicate. The cell viability was normalized to 100% for the control well containing no QDs and the same treatments.

2.8.2 Cell Images

For imaging, the cells were treated as described earlier and exposed to QD probes for defined time intervals. After being incubated for the different time periods (2, 12, 24, 48, and 72 h), the cell morphology and metabolic activity were detected by an IX71 inverted fluorescence microscope. Before and after the QD probes being washed out, fluorescence and transparent images were taken, and the spectrum was recorded from four parallel wells for each concentration of the probes.

3 Results and Discussion

3.1 Characterization of QD Probes

The photofluorescence (PL) spectra of these QDs probes and original QDs were measured. As shown in Fig. 1, compared to the original CdSe/ZnS, CdTe QDs [Figs. 1(a) and 1(b), curve a, respectively], the dTf-coated CdSe/ZnS and CdTe QDs (Figs. 1(a) and 1(b), curve b, respectively) showed a higher PL QY and PL peak positions shifted to blue. But the Tf conjugated, CdSe/ZnS, and CdTe QDs displayed contrary re-

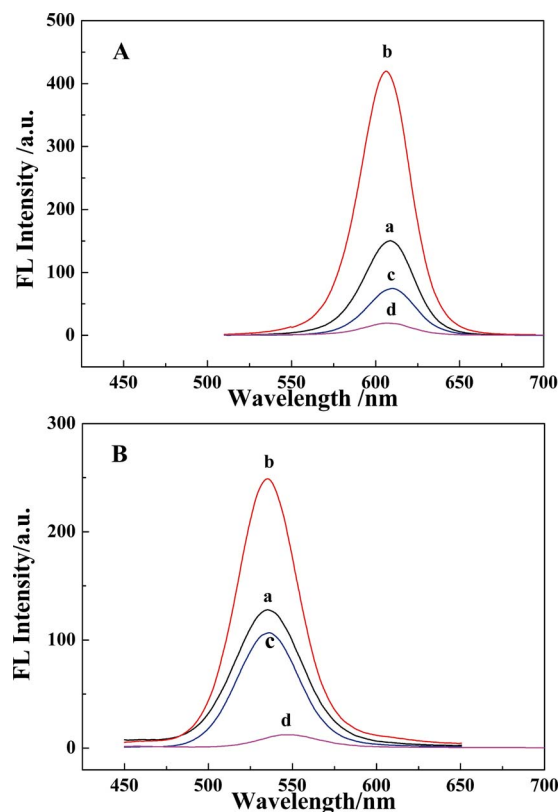


Fig. 1 Photoluminescence (PL) spectra of QDs and QD probes. (a) PL spectra of original of CdSe/ZnS (curve a), CdSe/ZnS-dTf (curve b), CdSe/ZnS-Tf (curve c), and CdSe/ZnS after being heated 15 min at 70 °C for (curve d). (b) PL spectra of original CdTe (curve a), CdTe-dTf (curve b), CdTe-Tf (curve c), and CdTe after being heated 15 min at 70 °C for curve d.

sults. Their PL QY was lower and peak positions shifted to red compared with the original QDs (Figs. 1(a) and 1(b), curve c, respectively). The quantum yields were measured by the optically dilute measurement method using rhodamine 6 G (whose PL QY is assumed to be 95% in ethanol) as in previous reports.²² PL QYs were 26%, 33%, and 18%, respectively, for CdSe/ZnS QDs, CdSe/ZnS-dTf, and CdSe/ZnS-Tf, while those of CdTe QDs, CdTe-dTf, and CdTe-Tf were 21%, 35%, and 16%, respectively. All these illuminated that dTf directly conjugated to the surface of QDs. Moreover, the PL QYs of dTf-modified QDs increased, while that of Tf-modified QDs decreased.

3.2 Proof of Conjugation by SDS-PAGE

SDS-PAGE was carried out on CdSe/ZnS QDs coated with dTf to prove the conjugate formation. The concentration of QDs was kept stable, while the concentration of dTf increased. Figure 2 shows the electrophoresis results for the different concentration ratios of dTf-coated CdSe/ZnS QDs (wells I, II, III), pure dTf (well IV), pure Tf (well V), 70 °C heated CdSe/ZnS QDs (well VI), and original CdSe/ZnS QDs (well VII). The evidence displayed that the color of bands "a" in wells I, II, III became deeper with increased concentration of dTf. At the same time, the luminescent image of the gel is shown in Fig. 2(b), where the bands "a" of dTf-

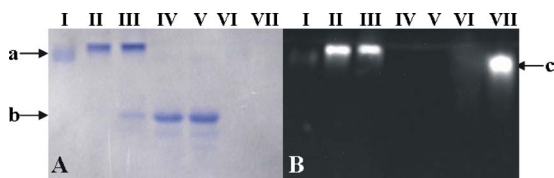


Fig. 2 SDS-PAGE electrophoresis of CdSe/ZnS QDs, Tf, dTf, QDs-dTf. (a) SDS-PAGE image of gel plate stained by Coomassie Blue. (b) SDS-PAGE luminescence image. Wells are shown as I: CdSe/ZnS-dTf (CdSe/ZnS: dTf=6:1); II: CdSe/ZnS-dTf (CdSe/ZnS: dTf=6:3); III: CdSe/ZnS-dTf (CdSe/ZnS: dTf=6:6); IV: dTf; V: Tf; VI: CdSe/ZnS QDs after heating; VII: CdSe/ZnS QDs. (Color online only.)

coated CdSe/ZnS showed strong luminescence in well II and III; these were possibly due to the change of charge of protein after denatured transferrin coating, resistance was augmented, and the protein band shifted from position b (Fig. 2(a), well IV) to band a. By comparing the two bands in well II and well III in Fig. 2(a), it is found that band b of dTf also appeared in well III, suggested that there was excessive dTf in the ratio of 6:6 (dTf: CdSe/ZnS). However, it is puzzling that well I had faint luminescence as well as the same concentration with well II and well III. The reason was due to the sample being heated in the preparation. The same treatment of CdSe/ZnS was carried out, and the results showed that the original CdSe/ZnS QDs had a wide band with strong fluorescence (Fig. 2(b), well VII), while after heating, the fluorescence intensity of QDs was enormously decreased (Fig. 2(b), well VI). This was confirmed in the photoluminescence spectra as well. The fluorescence intensity of QDs under heat sharply dropped (Fig. 1(a), curve d). In our previous work, the photoluminescence of water-soluble QDs presented sensitive temperature dependence.^{16,23} The fluorescence intensity of QDs has irreversible decreased, which is the reason for faint light in well I, Fig. 1(b). Thus, it was probable that in well I, dTf could not package CdSe/ZnS QDs absolutely because of the small ratio. Therefore, the fluorescence intensity was greatly reduced after heating. With the increase of dTf, QDs were coated completely and hold back the effect of heat, so it exhibited high fluorescence. The result was the same as Fig. 1(a) (curve b). Thus, it is easy to determine why bright bands were in well II and III.

3.3 Capillary Electrophoresis Analysis

To choose the optimal ratio of dTf to CdSe/ZnS QDs, capillary electrophoresis was used. First, the pure CdSe/ZnS QDs were measured and an electrophoresis peak appeared at retention time about 727 s (Fig. 3, curve a). After conjugation with the ratio 6:1 (QDs: dTf), two electrophoresis peaks were observed at about 350 s and 430 s (Fig. 3, curve b). It was speculated that the conjugation of dTf to QDs was not homogeneous in this proportion and the amount of QDs coated by dTf was different. However, at ratio 6:3 (QDs: dTf), only one electrophoresis peak appeared (Fig. 3, curve c). It was confirmed that the electrophoresis peak was caused by the CdSe/ZnS-dTf. When the ratio of CdSe/ZnS QDs to dTf was 6:6, there was still only one peak. Therefore, it was affirmed that dTf completely coated on QDs with ratio 6:3 (QDs: dTf). The results were coherent with the SDS-PAGE. A band of protein emerged in well III (Fig. 2(a), band b), indi-

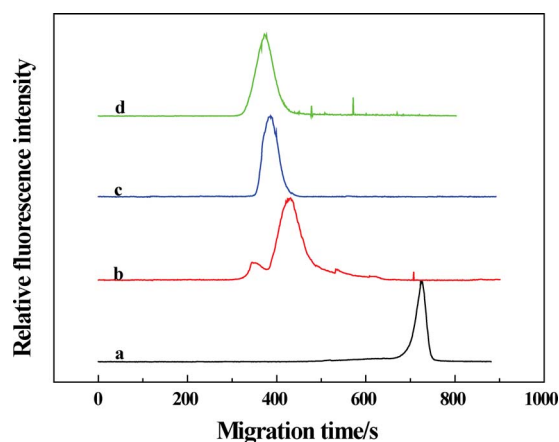


Fig. 3 Capillary electrophoresis of a series of aqueous solutions with the same CdSe/ZnS QDs but different denatured transferrin (dTf)-to-QDs ratio. Curve a: the pure CdSe/ZnS QDs; curve b: the ratio 6:1 of QDs dTf; curve c: the ratio 6:3 of QDs dTf; curve d: the ratio 6:6 of QDs dTf.

cating that the dTf was in excess. The ratio 6:3 of QDs:dTf was the optimal ratio for conjugation of dTf and QDs.

3.4 Cytotoxicity of CdTe-dTf, CdSe/ZnS-dTf, CdTe-Tf, CdSe/ZnS-Tf, CdSe/ZnS, and CdTe QDs for the HeLa Cells

3.4.1 CdSe/ZnS-dTf

Based on the preceding results, to evaluate the effect of the concentration and incubative time on the cytotoxicity, six kinds of QDs were compared at five concentrations in HeLa cells. As shown in Fig. 4, it was found that 3.2- μ M CdSe/CdS-dTf were almost nontoxic when they were exposed to HeLa cells for different incubation times. There was almost no change in the morphology of cells incubated with this probe, and the cells were growing well. Cells were seldom seen in suspension. At the same time, the amount of cells increased gradually. At the incubation time of 72 h, the density was obviously augmented, indicating that the proliferation of cells has been occurring. The fluorescence imaging showed the contour of HeLa cells too. Simultaneously, QD probes were seen gathered in the cells. The position of the PL peak was detected always at 605 nm from 2 to 72 h by spectrometer (Fig. 5). The cytotoxicity of CdSe/CdS-dTf evalu-

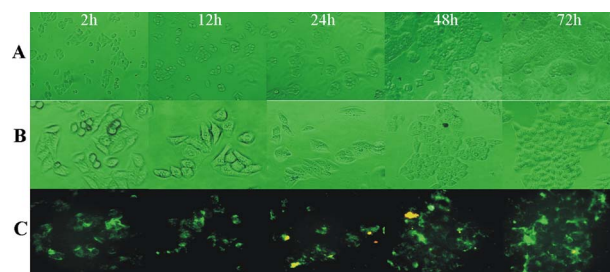


Fig. 4 Morphology of HeLa cells after incubation with 3.2 μ M CdSe/ZnS-dTf QDs for 2, 12, 24, 48, and 72 h. (a) Before and (b) after washing off CdSe/ZnS-dTf; (c) fluorescence images of cells of (b).

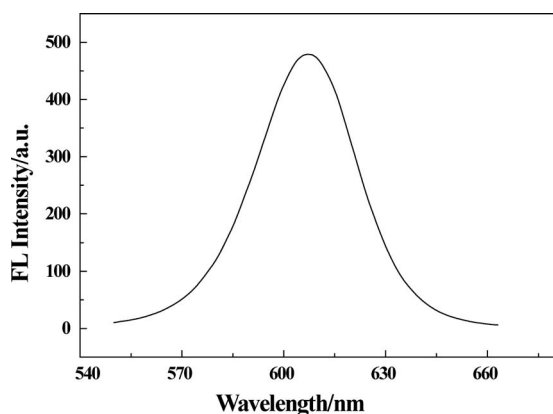


Fig. 5 Fluorescence spectra of HeLa cells after incubating with CdSe/ZnS-dTf at 37 °C for 72 h.

ated with the MTT viability assay is shown in Fig. 6. It was found that this kind of QD probe was almost nontoxic to HeLa cells; even at 3.2 μM and incubated for 72 h, cell viability was still at 92%.

3.4.2 CdSe/ZnS-Tf

Compared with CdSe/ZnS-dTf, the cell density decreased slightly after incubation with 3.2 μM CdSe/ZnS-Tf for 72 h; meanwhile, the proliferation was slower [Fig. 7(a)]. During incubation, a PL peak at 608 nm was also obtained (data not shown here). The cell viability decreased to 86.7% at 72 h, as shown in the MTT viability assay [Fig. 8(a)], while the viability of cells declined with prolonged incubation time.

3.4.3 CdTe-dTf

CdTe-dTf of 3.2 μM was more highly toxic for HeLa cells than CdSe/ZnS QD probes. Live cells were greatly reduced after 48-h incubation, and suspended cells increased. A few fixed cells were observed in wells after washing CdTe-dTf at 72 h [Fig. 7(b)]. As shown in Fig. 8(b), the cytotoxicity of CdTe-dTf was time and concentration dependent. Cell viability

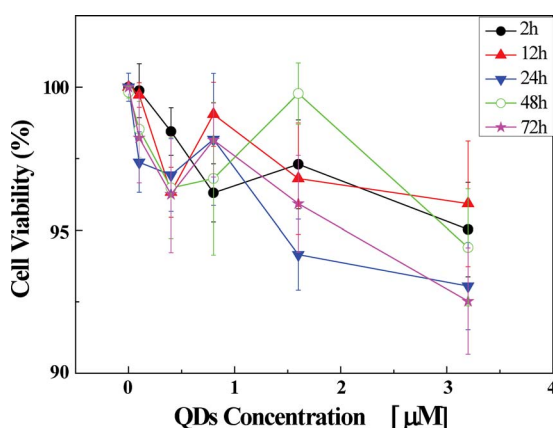


Fig. 6 Viability of HeLa cells after treatment with CdSe/ZnS-dTf QDs with different concentrations and incubation time.

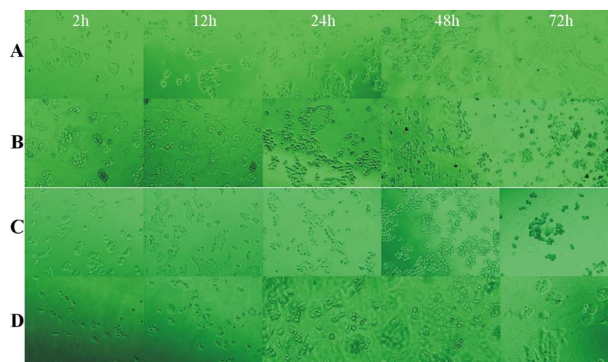


Fig. 7 Morphology of HeLa cells after incubation with 3.2 μM CdSe/ZnS-Tf (a), CdTe-dTf (b), CdTe-Tf (c), and CdSe/ZnS QDs (d) for 2, 12, 24, 48, and 72 h.

ity was depressed by prolonging time and augmenting dose. 22.5% cells were alive after 72 h at 3.2 μM concentration [Fig. 8(b)].

3.4.4 CdTe-Tf

For CdTe-Tf, the morphology of HeLa cell labeling with 3.2 μM concentration for 48 h was absolutely globose, and nearly no fixed cells existed [Fig. 7(c)]. With the MTT viability assay, 0.8 μM of CdTe-Tf resulted in the decrease of cell viability up to 60% in 48 h, and only 14.2% cells were alive with 3.2 μM for 72 h incubation [Fig. 8(c)].

3.4.5 CdSe/ZnS and CdTe QDs

The pure CdSe/ZnS and CdTe QDs were used to evaluate the cytotoxicity in HeLa cells. When the QDs were washed out after 2-h incubation, no fluorescence in the cells could be detected in the cells. With prolonged incubation time, a few scattered fluorescent groups were detected. However, the fluorescence was visually cluttered and the outline of cells were not visible, while the location of labeling was uncertain too [Fig. 9(c)]. The probable reason was that with prolongation of the incubation, a few QDs were adsorbed on the surface of the cells nonspecifically and could not be washed out. Comparing the images of these two QDs, CdTe QDs (Fig. 9) have higher cytotoxicity than CdSe/ZnS core-shell QDs [Fig. 8(d)]. In this case, the proliferation of cells was slow; there were only a few cells with 24-h incubation after washing off the CdTe QDs, and nearly all cells were dead after 72 h. However, a few proliferous cells were observed when 3.2 μM of CdSe/ZnS QDs were exposed to HeLa cells for 72 h [Fig. 7(d)]. The result was approved by the MTT viability assay. The CdSe/ZnS and CdTe QDs led to the decrease of cell viability up to 58.8% [Fig. 8(d)] and 3.2% (Fig. 10) with 72-h incubation and 3.2- μM concentration.

These results demonstrated that the order of cytotoxicity of QDs in HeLa cells from high to low was CdSe/ZnS-dTf, CdSe/ZnS-Tf, CdSe/ZnS, CdTe-dTf, CdTe-Tf, and CdTe. MTT viability assay showed the same result. For 72-h incubation and 3.2- μM concentration, the cell viability was up to 92% with the lowest cytotoxicity CdSe/ZnS-dTf, while down to 3.2% with the highest cytotoxicity CdTe QDs.

The toxicity of QDs depends on various factors that come from both inherent physicochemical properties and environ-

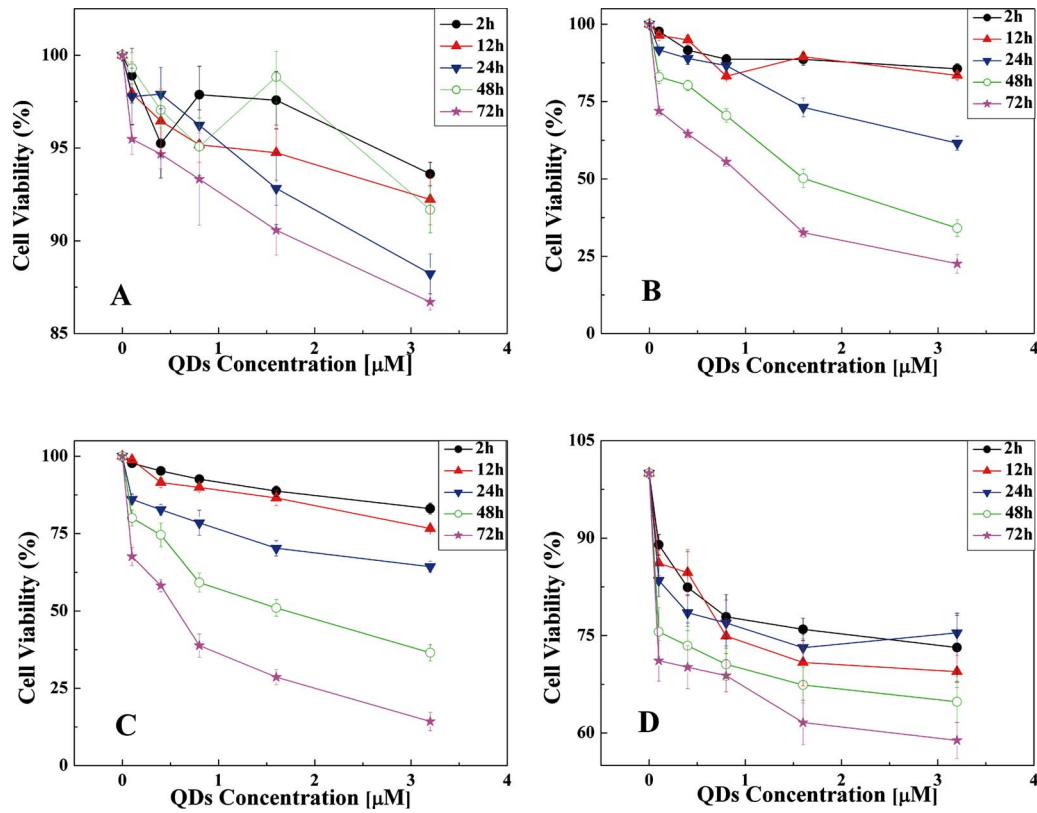


Fig. 8 Viability of HeLa cells after treatment with CdSe/ZnS-Tf (a), CdTe-dTf (b), CdTe-Tf (c), and CdSe/ZnS QDs (d) with different concentrations and incubation time.

mental conditions. It was reported that QD size, concentration, outer coating functional groups and materials, mechanical stability, the species of cells, and exposure times have been included as determining factors in QD cytotoxicity.^{5,11,24-31} The QD probes we synthesized exhibited different degrees of cytotoxicity in HeLa cells. CdSe/ZnS-dTf was almost nontoxic and CdTe-Tf was highly cytotoxic. CdSe/ZnS-dTf appeared highly biocompatible, possibly because CdSe/ZnS had inherent core-shell structure and the outer-coated ZnS inhibited the release of Cd ion. On the other hand, dTf directly wrapped on the surface of QDs could be seen as another “shell” and enhanced the stability of QDs. CdSe inside the structure was not easy to disassemble, and dTf provided a highly stable layer for CdSe/ZnS QDs. All these led to reducing the influence from environmental conditions. While the QD probes prepared by coupling agent

have another protection layer compared with the original QDs, the cytotoxicity is a little depressed. But if Tf was not coated on the QDs directly, the QD probes were barely affected by the outer environment, and stability was not as good as dTf-coated QDs. As a result, the damage by these probes was bigger. For uncoated QDs, no biomolecule was covered on their surface, and they lacked effective coating; thus, QDs were severely toxic to cells. Furthermore, Cd²⁺ ion was more easily released from non-core/shell structure CdTe QDs and led to enormous toxicity.

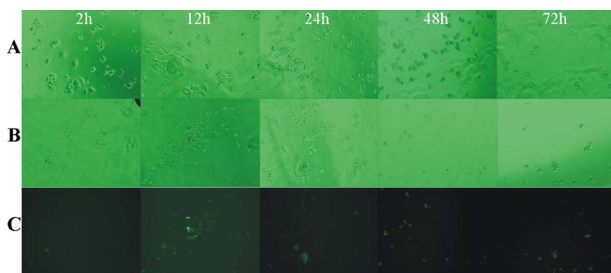


Fig. 9 Morphology of HeLa cells after incubation with 3.2 μM CdTe QDs for 2, 12, 24, 48, and 72 h.

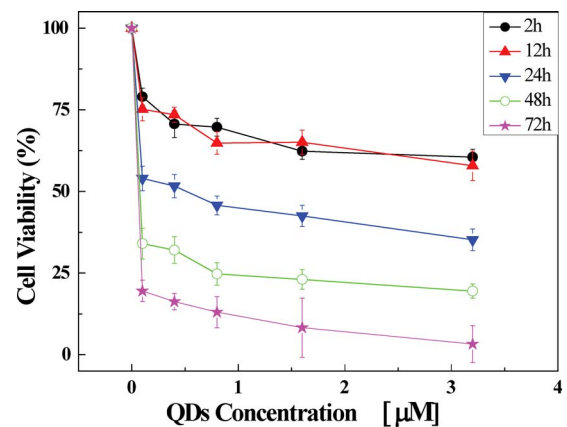


Fig. 10 Viability of HeLa cells after treatment with CdTe QDs with different concentrations and incubation time.

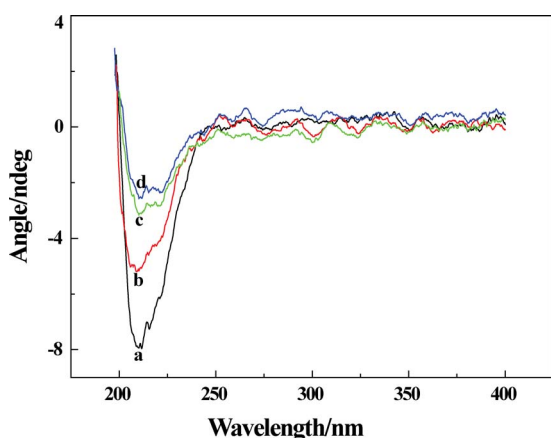


Fig. 11 CD spectra of (curve a) dTf, (curve b) CdSe/ZnS-dTf, (curve c) Tf, (curve d) CdSe/ZnS-Tf.

The cell images revealed that CdSe/ZnS-dTf had lower cytotoxicity and could be used to label live cells, indicating that dTf maintained their biological activities after coating onto QDs. CD spectra were employed to verify the result too. This showed that compared with Tf (Fig. 11, curve c), little change of dTf's peak and a small blue shift of the negative peak occurred (Fig. 11 curve a). Also there was little influence on the peak after dTf was coated onto QDs (Fig. 11, curve b). These data show that the structure of transferrin remained mostly intact after denaturalization. Partial allostery possibly happened and dTf maintained partial biological activities.

4 Conclusion

The study of quantum dots has become increasingly popular; but cytotoxicity is one of the key problems for their prevalent application. In this paper, denatured transferrin was adopted to modify the quantum dots to prepare quantum dot probes. The results showed that this special method could reduce the cytotoxicity and increase the optical properties of quantum dot probes over covalent conjugations. The PL QY of this probe was 7% higher than original CdSe/ZnS QDs, and after exposure to a 3.2- μ M probe for 72 h, over 92% of HeLa cells were still alive. This work presents a method for preparation of QD bioprobes with high optical properties and low cytotoxicity and offers profitable help for biological application of QDs.

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