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ORIGINAL PAPER

Study of Polycation-Capped Mn:ZnSe Quantum Dots as a Novel Fluorescent Probe for Living Cells

Xiaobo Pan · Zheng Li · Tianlong Wang · Jin Xie · Pei-Nan Wang · Ji-Yao Chen · Li Chen · Lan Mi

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Abstract Transition metal manganese ion (Mn^{2+}) doped zinc selenide quantum dots (Mn:ZnSe D-Dots) have been considered as a new material for fluorescent probes in biological labeling. However, this application is limited by the low membrane permeability of D-Dots. In this work, Mn:ZnSe D-Dots were capped with the polycation Sofast to label living cells. For the first time, the efficiency of cellular uptake in living cells is significantly enhanced. Various molar ratios of Sofast to D-Dots were explored and compared to obtain the optimal reaction conditions between Sofast and D-Dots for preparing Sofast/D-Dots nano-compound. A comparison on the fluorescence labeling ability of living cells were made between Sofast/D-Dots and pure D-Dots. Results from laser scanning confocal microscope show that Sofast/D-Dots complexes enter the cells more efficiently than pure D-Dots, even with a lower concentration and shorter incubation time. The cytotoxicities of D-Dots and Sofast/D-Dots were also studied. It was found that Sofast/D-Dots have a much lower cytotoxicity than cadmium-containing quantum dots (i.e. CdTe and CdTe/ZnS). Our results suggest that the non-heavy-metal-containing Sofast/D-Dots complexes have a great potential in the application of biological labeling, especially of long-time bioimaging in living cells.

Keywords Mn:ZnSe quantum dots · Non-heavy-metal · Polycation · Biological labeling · Confocal microscopy

X. Pan · Z. Li · T. Wang · J. Xie · P.-N. Wang · L. Mi (✉)
Key Laboratory of Micro and Nano Photonic Structures (Ministry of Education), Department of Optical Science and Engineering, Shanghai Engineering Research Center of Ultra-Precision Optical Manufacturing, Fudan University, 220 Handan Road, Shanghai 200433, People's Republic of China
e-mail: lanmi@fudan.edu.cn

J.-Y. Chen
State Key Laboratory of Surface Physics, Department of Physics, Fudan University, 220 Handan Road, Shanghai 200433, People's Republic of China

L. Chen
School of Arts and Sciences, MCPHS University, Boston, MA 02115, USA

Introduction

Semiconductor quantum dots (QDs) have been considered as promising fluorescence probes due to their excellent optical properties, such as high quantum yields, wide absorption range, and high extinction coefficient. Ever since Alivisatos' and Nie's groups reported their results in the application of QDs as biological fluorescent probes in 1998 [1, 2], great attention has been focused on the preparation and the applications of QDs [3–5]. However, most QDs reported (such as cadmium selenide (CdSe), cadmium telluride (CdTe), cadmium telluride mercury (CdTeHg), and plumbum selenide (PbSe)) contain toxic heavy metals (i.e. Cd, Pb, Hg), which limited their application in biological systems. It has been suggested that surface coating can reduce the cytotoxicity [6, 7]. However, the core/shell approach cannot fully eliminate the cytotoxicity since the release of heavy metal ions is inevitable [8, 9]. Therefore, the toxicity of QDs has become a critical issue when it comes to the applications of QDs in biological systems.

There have been some studies on using non-heavy-metal QDs in bioimaging [10, 11]. Pradhan et al. synthesized a water-soluble manganese-doped zinc selenide quantum dots (Mn:ZnSe D-Dots) and reported high photoluminescence (PL) quantum yields of 40 % [12, 13]. The overall size of Mn:ZnSe D-Dots is about 7–8 nm, which leads to excellent biological permeability. In 2010, Zeng et al. improved the preparation method of D-Dots by successfully synthesizing Mn-Doped ZnSe without pyrophoric reagents [14]. Recent

progress also include the optical properties of D-Dots [15, 16] and the D-Dots based biosensors [17–19]. However, there are few reports concerning the application of D-Dots in bioimaging, which may due to the low efficiency of cell labeling. In 2011, Liu et al. synthesized the Mn:ZnSe D-Dots and used it to label fixed cells [20]. Later on, the living cell imaging using Mn:ZnS D-Dots was achieved by Geszke et al. [21], with a long incubation time of 72 h and a high concentration of 50 μM as reported. Such long incubation time and high concentration is due to the fact that D-Dots have low efficiency of cellular uptake. In order to improve the biocompatibility of D-Dots, several groups have tried to exchange the negatively charged ligands on the surface with positively charged amine groups. Santra's group found that the zeta potential of amine modified D-Dots was close to zero, which is due to the protonation of primary amine groups resulting in severe particle aggregation [22]. And Chen's group utilized chitosan with both hydroxyl and amino groups to modify D-Dots for compromise, and obtained fluorescence images with high concentration [23].

In this paper, we propose a new approach of capping Mn:ZnSe D-Dots with a polycation to enhance the efficiency of cellular uptake with short incubation time and low concentration. Polycation is often used as a transfection reagent for gene delivery. It has demonstrated low cytotoxicity, excellent stability, good biocompatibility, and good affinity with cell membranes [24]. Among the commercial reagents, Sofast™ has been widely used for its high transfection efficiency, simple process, low cytotoxicity, and its high ability to form Sofast/cargo complexes with a small quantity [25–27]. In this work, we have prepared Sofast/D-Dots complex by capping D-Dots with Sofast, and studied their feasibility in labeling living cells via fluorescent imaging. Other properties of Sofast/D-Dots complex including their sizes and cytotoxity were also studied. Results were compared between Sofast/D-Dots, pure D-Dots, and Cd-containing QDs. Bright fluorescent images of living cells loaded with Sofast/D-Dots were obtained with a low concentration of D-Dots and a short incubation time.

Materials and Methods

Reagents

The water-soluble Mn:ZnSe D-Dots with 3-Mercaptopropionic acid (MPA) as ligand were purchased from NN-Labs (Fayetteville, AR, USA). Their absorption spectrum ranges from ultraviolet to 410 nm. The emission peak is at 590 nm. The polycation Sofast™ was purchased from Xiamen Sunma Biotechnology Co. Ltd, China.

Two Cd-containing QDs (CdTe/ZnS core-shell QDs and CdTe QDs) were used in this paper in order to compare their cytotoxities with D-Dots. The CdTe/ZnS (core/shell) QDs with an emission peak around 600 nm were a gift from Prof. Sungjee Kim, Department of Chemistry, Pohang University of Science and Technology (POSTECH), Korea. The CdTe QDs without the structure of shells with an emission peak at 600 nm were synthesized under the method reported previously [28].

Preparation and Characterization of Sofast/D-Dots Complexes

When D-Dots are exposed to Sofast, cationic Sofast combines with the anionic heads of MPA on the surface of D-Dots to form Sofast/D-Dots complexes. For pure D-Dots solution, all D-Dots were left in supernatant solutions after centrifuging at 16,000 rpm for 30 min, which suggests that the uncapped D-Dots can be separated from Sofast capped D-Dots (Sofast/D-Dots) by centrifugation. A set of 1 mL solutions were prepared by adding different amounts of Sofast (ranging from 1.5 to 120 μL) to D-Dots solutions (0.15 nmol). Therefore, the molar ratios of Sofast to D-Dots in 1 mL solutions ranged from 0.05:1 to 4:1. The 1 mL solutions then were centrifuged at 16,000 rpm for 30 min, after which the supernatants containing uncapped D-Dots were removed. The obtained pellets containing Sofast/D-Dots were re-suspended in deionized water as final products. The supernatant solutions obtained during the centrifugation were diluted to 1 mL and the fluorescent intensities were measured by a fluorescence spectrophotometer (Hitachi F-2500; Japan). From these fluorescent intensities, the percentages of unencapsulated D-Dots were calculated using the pure D-Dots solution (150 nM, 1 mL) as a standard.

$$\text{Percentage of uncapped D}-\text{Dots}(\%) = \frac{I_{\text{supernatant}}}{I_{\text{pure D}-\text{Dots}}}$$

The size distributions of 150 nM pure D-Dots and Sofast/D-Dots complexes were analyzed by a nanoparticle analyzer (NanoSight LM10; United Kingdom). And their zeta potential measurements were recorded with a Malvern Zetasizer Nano ZS90 (Malvern Instruments Ltd; United Kingdom). The measurements were performed in triplicate and the standard deviation was recorded.

Cell Culture

Human hepatocellular carcinoma (QGY) cells and human cervical carcinoma (HeLa) cells were procured from the Cell Bank of Shanghai Science Academy (Shanghai, China). The cells were cultured in Petri dishes in DMEM-H solution with 10 % fetal bovine serum (FBS) in a fully humidified incubator

(37 °C and 5 % CO_2) for 24 h to reach 80 % confluence with normal morphology.

We then incubated the cells with two different solutions: 150 nM Sofast/D-Dots and 2.1 μM pure D-Dots, for 1 h and for 24 h, respectively. The resulting cells then were washed with PBS for three times before the microscopic observations.

Laser Scanning Confocal Microscopy

After the treatment of either Sofast/D-Dots or pure D-Dots, the cells were examined under a laser scanning confocal microscopy (Olympus FV-300, IX71; Japan), which is equipped with a 405-nm CW semiconductor laser (Coherent; USA) as the excitation source and a 60× water-immersion objective. The fluorescence images of the intracellular D-Dots were detected with a 565 nm long-pass filter. The differential interference contrast (DIC) micrographs to exhibit the cell morphology were acquired in a transmission channel simultaneously. The three dimensional (3D) distributions of Sofast/D-Dots or pure D-Dots were depicted with the z-scan mode.

Measurements of Cell Viability

The cells were plated at a density of 80,000 cells mL^{-1} in 96-well plates and were grown in a fully humidified incubator for 12 h. The culture medium was then replaced by other different mediums: i.e. pure D-Dots, Sofast, Sofast/D-Dots, CdTe/ZnS, and CdTe QDs, with the same concentration of 600 nM. After the incubation, all mediums were again replaced by 100 μL fresh DMEM-H medium for each well. Results were obtained with incubation time of 24 h and 36 h to study the viability.

The cell viability assays were determined by a modified MTT method using WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H tetrazolium, monosodium salt] (Beyotime; Jiangsu, China). Each well was added with 10 μL of the WST-8 reagent solution. The cells were then incubated for 2 h. Absorbance measurements were conducted at 450 nm using a microplate reader (BIO-TEK Synergy™ HT; USA). The untreated cells were used as the control groups. The absorbance values of the samples before adding WST-8 dyes were also measured at 450 nm as a reference to avoid any influence from the different QDs. The experiments were conducted and measured independently for three times. Each time, we average our measurements from three wells.

Results and Discussions

Optimal Ratio of Sofast to D-Dots for Sofast/D-Dots

Figure 1 gives the fluorescent intensities of uncapped D-Dots in the supernatants from Sofast/D-Dots solutions with various molar ratios ranging from 0.05:1 to 4:1. The fluorescence spectrum of pure D-Dots solution with 150 nM was also shown as a reference, with its peak intensity normalized to 1. As shown in Fig. 1, with increasing amount of cationic polymer Sofast added to the D-Dots solution, the fluorescent intensities of uncapped D-Dots in supernatants decreased successively, which indicates that the percentage of unencapsulated D-Dots decreased gradually as the ratio of Sofast to D-Dots increases. When the molar ratio of Sofast to D-Dots is 0.05:1, the percentage of unencapsulated D-Dots in the solution is around 53 %. While the ratio reaches 1:1, no D-Dots peak can be found in the spectra. As the ratio continues to increase and exceeds 1:1 (i.e. 2:1 and 4:1), no peaks are spotted in the spectra. Therefore, it is safe to conclude that the optimal molar ratio of Sofast to D-Dots for preparing the Sofast/D-Dots complexes is 1:1. This ratio was used in this paper for further studies in material characterization and cell experiments.

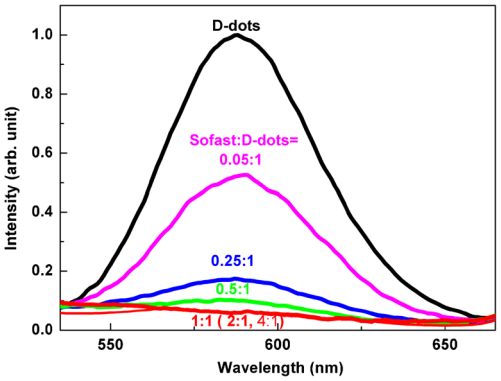

Fig. 1 The fluorescent intensities of uncapped D-Dots in the supernatants of the prepared Sofast/D-Dots solutions under various molar ratios of Sofast to D-Dots. The intensity from pure D-Dots was normalized to 1

It is worth pointing out that the fluorescence method is much more sensitive and accurate for obtaining the optimal ratio of Sofast to D-Dots as compared with the widely used zeta potential method [26, 29].

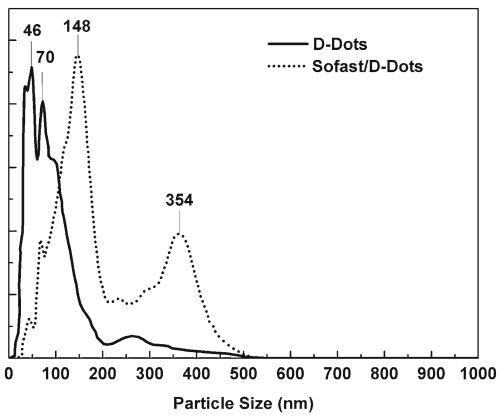

Fig. 2 Size distributions of pure D-Dots and Sofast/D-Dots complexes

The fluorescent intensity of Sofast/D-Dots obtained with the optimal reaction molar ratio was measured and found a 5.3 ±2.0 % decrease comparing with the intensity of pure D-Dots at the same molar concentration. Thus, the fluorescence of D-Dots decreased a little after capped with Sofast.

Particle Sizes and Zeta Potential of Sofast/D-Dots

A nanoparticle analyzer was used to measure the particle sizes of Sofast/D-Dots and pure D-Dots under the same concentration. The average sizes were then calculated by the Nanoparticle Tracking Analysis software [30]. The calculated average size for Sofast/D-Dots was found to be 214 nm and for pure D-Dots, 104 nm. The difference in the size is due to the successful encapsulation of Sofast. The size distribution graph is shown in Fig. 2. Pure D-Dots nanoparticles are mostly distributed between 30 and 90 nm, with two peaks at 46 and 70 nm. While for Sofast/D-Dots, two broader distribution peaks can be found at 148 and 354 nm. This two-peak distribution character of the Sofast/D-Dots could be due to branched form of polycation [31], where D-Dots were encapsulated with different patterns.

The charge characteristics of uncapped D-Dots and Sofast/D-Dots were studied by zeta potential measurements. The average zeta potential of pure D-Dots was −17.6±1.8 mV, and that of Sofast/D-Dots was 3.49± 0.61 mV. With polycation Sofast modified, surface charge of D-Dots changed from negative to positive, which was similar to other polycation encapsulated nanoparticles [32].

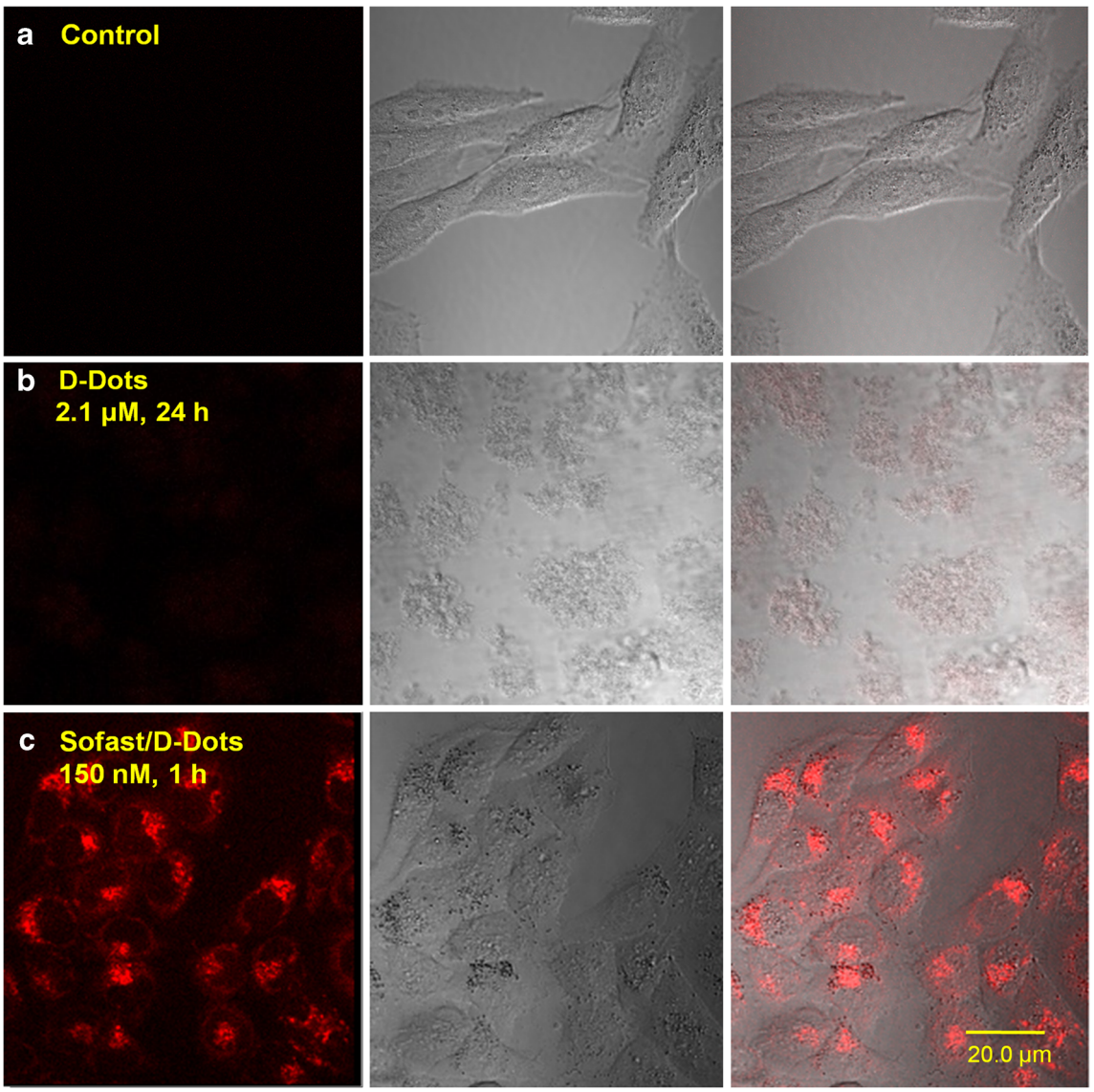

Fig. 3 Fluorescence images of QGY cells **a** in PBS solutions for 1 h as the control group, **b** incubated with 2.1 μM pure D-Dots for 24 h, and **c** incubated with 150 nM Sofast/D-Dots for 1 h

Comparison in Labeling Efficiencies Between D-Dots and Sofast/D-Dots Complexes for Living Cells

In order to compare the labeling efficiency between pure D-Dots and Sofast/D-Dots, we incubated the cells in D-Dots solution for 24 h and in Sofast/D-Dots solution for 1 h. The distributions of D-Dots in the cells were then observed by a laser scanning confocal microscopy.

Figure 3 gives the distributions of pure D-Dots and Sofast/D-Dots in QGY cells, with the untreated cells as a control group.

The three pictures on the left are fluorescence images of D-Dots (red). The middle three are DIC images exhibiting the cell morphology. The three on the right are merged images of the fluorescence and DIC images. The fluorescence images of pure D-Dots incubated cells are shown in Fig. 3b, where we hardly see any red fluorescence signals of D-Dots. Notice that these cells are incubated with D-Dots solution under a high concentration of 2.1 μM for a long incubation time of 24 h. The fact that we still don't detect much fluorescence signals indicates that the D-Dots could hardly pass into the cell membrane. On the other hand, brighter fluorescence images from living cells were obtained in Fig. 3c with Sofast/D-Dots incubated cells, which was achieved after a much lower concentration (150 nM) for much shorter incubation time (1 h) as compared to pure D-Dots treatment.

Figure 4 gives the 3D distribution of Sofast/D-Dots in QGY cells obtained under the Z-scan mode of microscope. The distribution on the x-y plane is shown in the middle left.

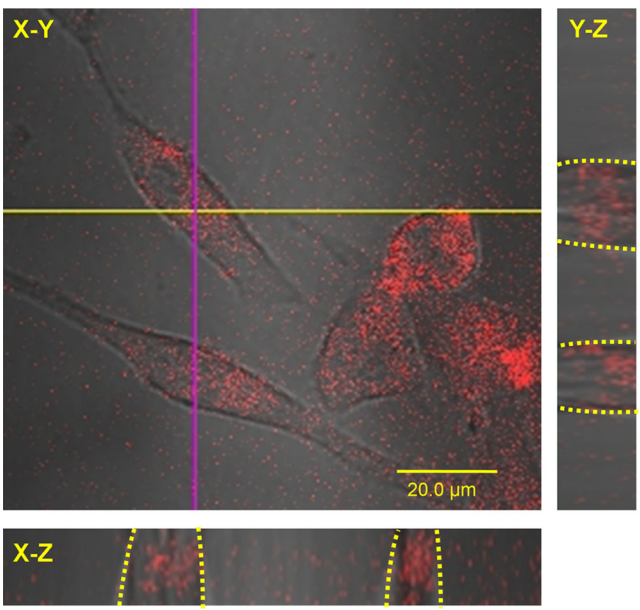

Fig. 4 Three dimensional fluorescence images of QGY cells incubated with 150 nM Sofast/D-Dots. *Mid-Left*: distribution on the x-y plane. *Bottom*: distribution on the x-z plane along the *yellow marked*. *Right*: distribution on the y-z plane along the *red marked line*. The *dashed lines* indicate the edges of cells

The distribution on the x-z plane along the yellow marked line is shown at the bottom while the distribution on the y-z plane along the red marked line is shown at the right. From the figures, we clearly see that the red fluorescence signals are distributed all over the cytoplasm, with some near the nuclei. This indicates that D-Dots are not just absorbed on the membranes, but rather scatter around in the cytoplasm after the Sofast/D-Dots complexes entered the cells. This result is similar to our previous report where CdTe QDs were used to label cells [33].

The uptake efficiencies of pure D-Dots and Sofast/D-Dots in HeLa cells were also investigated under the same approach. Figure 5 gives the resulting fluorescence signals of D-Dots on HeLa cells. The cell images treated under pure D-Dots with high concentration and long incubation time are shown in Fig. 5b while the cells treated under Sofast/D-Dots are shown in Fig. 5c. A clear improvement in the D-Dots uptake can be found in Fig. 5c, where the cells are treated under Sofast/D-Dots complex for less time (1 h) and lower concentration (150 nM). Comparing Fig. 5b and c, we clearly see the advantage of Sofast/D-Dots complexes in labeling living cells. The reason of such advantage lies in the charge distribution of Sofast and D-Dots. Pure D-Dots with MPA as ligands are negatively charged. So are cell membranes [34]. Thus, pure D-Dots would repel to cell membranes and in consequences reduce the uptake efficiency when they attempt to enter the cell. On the other hand, when D-Dots are capped with positively charged Sofast, the positive Sofast/D-Dots complex would easily be attracted to the cell membrane by electrostatic attraction and then penetrate the cell membranes due to endocytosis effect [31], resulting in a significant improvement on the labeling efficiency. This also explains the fact that successful labeling can be achieved even with low concentration and short incubation time. Indeed, as compared to some of the previous reports, the incubation concentration of CdTe or CdSe QDs for cell labeling was usually taken to be 600 nM [33, 35], which was four times higher than what we used for Sofast/D-Dots.

Comparison of Cytotoxicities Among D-Dots, Sofast/D-Dots, CdTe/ZnS and CdTe QDs

No remarkable morphological change of the cells was found under microscope after the incubation of D-Dots for 24 h and Sofast/D-Dots for 1 h. In order to investigate the cytotoxicities of our labeling solutions, we incubated QGY and HeLa cells in various solutions including D-Dots, Sofast, Sofast/D-Dots, and two Cd-containing solutions (CdTe/ZnS and CdTe QDs). All solutions have a concentration of 600 nM. The cell viability assay results were studied under an incubation time of 24 h and 36 h with the viability of control group scaled to 100 %, as shown in Fig. 6. For both QGY and HeLa cells, when treated with D-Dots, Sofast, and Sofast/D-Dots, the cell

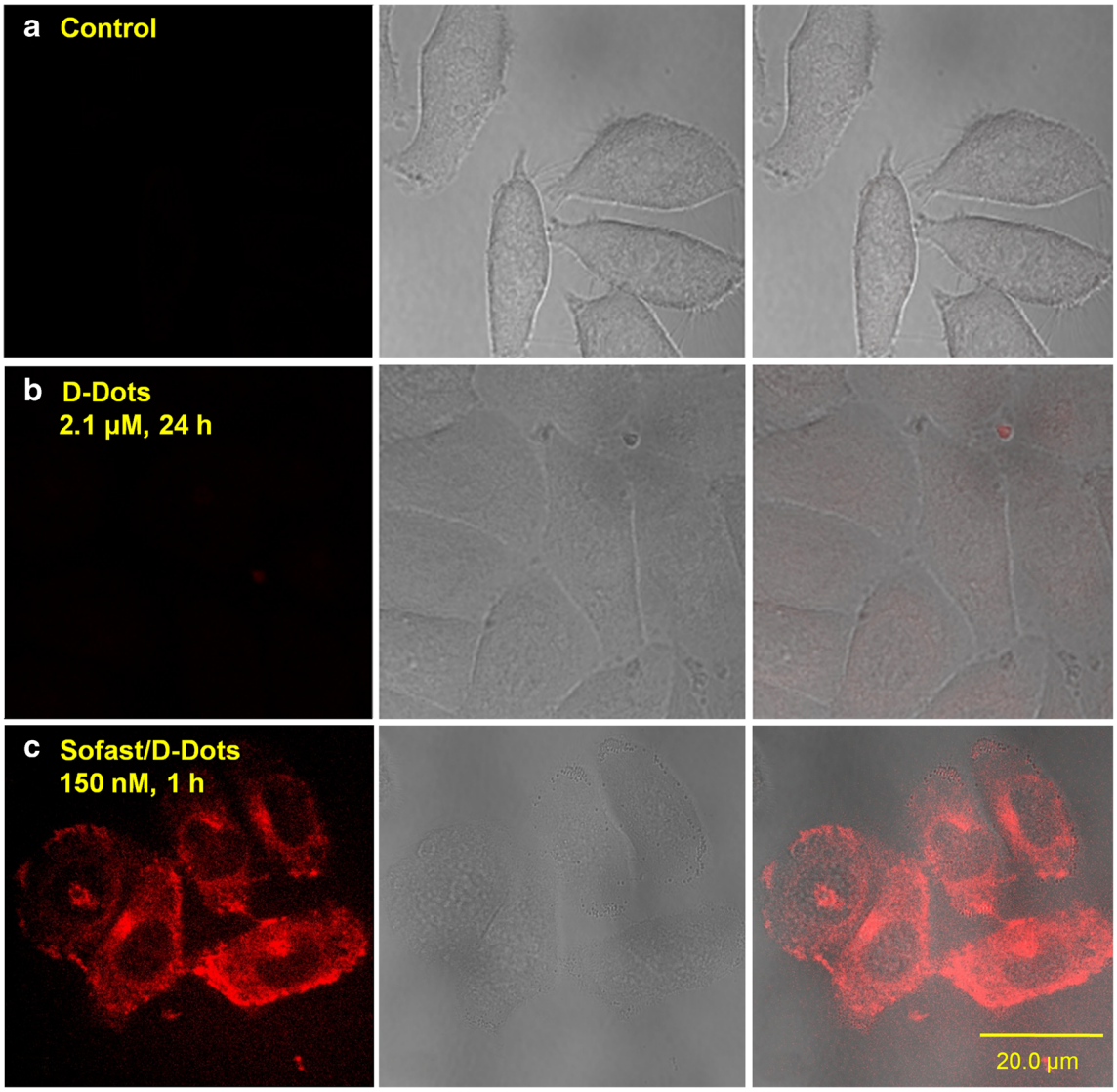

Fig. 5 Fluorescence images of HeLa cells **a** in PBS solutions for 1 h as the control group, **b** incubated with 2.1 μM pure D-Dots for 24 h, and **c** incubated with 150 nM Sofast/D-Dots for 1 h

viability was measured to be greater than 90 %. No significant change was found when the incubation time was increased from 24 h to 36 h, which suggests a low cytotoxicity of D-Dots, Sofast, and Sofast/D-Dots. However, the viability of QGY cells significantly dropped under the treatment of CdTe/ZnS and CdTe QDs, as shown in Fig. 6a. The cell

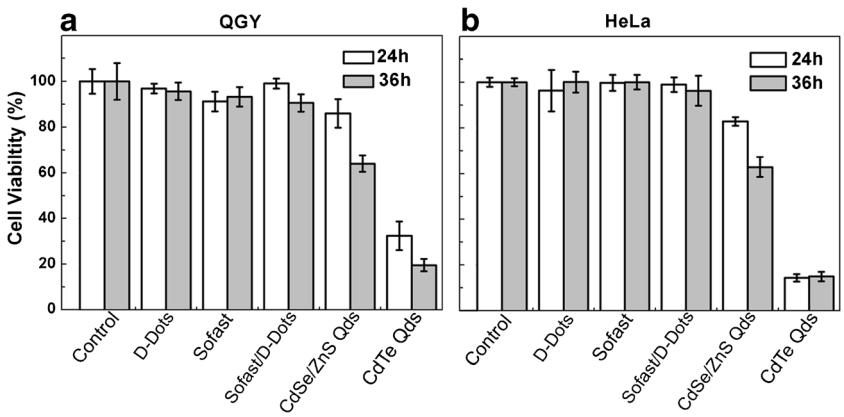

Fig. 6 Comparison on the cytotoxity measurements of different nanoparticles under a concentration of 600 nM on **a** QGY cells and **b** HeLa cells after 24 and 36 h incubations. The cell viability was shown as mean ± SD of three measurements

viability was found to be 82 % for CdTe/ZnS and 30 % CdTe QDs after incubation time of 24 h. The viability continues to drop (to 60 % and 20 % respectively) as the incubation time increases to 36 h. Similar results were found for HeLa cells where CdTe QDs result in the highest toxicity, as shown in Fig. 6b. The high cytotoxicity of CdTe QDs comes from the release of Cd^{2+} ions, which also explains the remarkable reduce on the toxicity when CdTe QDs were capped with ZnS as the shell, as reported previously [28, 36]. However, none of these two Cd-containing compounds has a higher viability when comparing to pure D-Dots and Sofast/D-Dots. It is also worth mentioning that the concentration of Sofast/D-Dots and pure D-Dots used in the cytotoxicity experiments is 600 nM, which is four times the concentration we have used in labeling. Thus, the toxicity of 150 nM Sofast/D-Dots used in previous labeling process should be expected to be even less. Based on our experimental results, we conclude that, with high labeling efficiency and low cytotoxicity, Sofast/D-Dots show great potential in the applications as bio-probes, especially for long-time imaging and tracking. In fact, a broader application of Sofast/D-Dots complexes in cancer diagnosis and therapy can be achieved when Sofast is further conjugated with targeted molecules or anti-cancer drugs.

Conclusion

Sofast capped D-Dots were prepared for high-efficiency labeling in living cells. The optimal molar ratio of Sofast to D-Dots was found to be 1:1. Successful labeling was achieved with Sofast/D-Dots complex with a low concentration of 150 nM for a short incubation time of 1 h. The positively charged Sofast makes it much easier for D-Dots compound to penetrate the membranes of living cells, which results in higher uptake efficiency when compared to pure D-Dots. The cytotoxicity of Sofast/D-Dots was also investigated and compared with cadmium-containing QDs. High cell viability of over 90 % was found with Sofast/D-Dots with no significant reduction under longer incubation time and higher concentration. Our results suggest that with the high labeling efficiency and low cytotoxicity, the Sofast/D-Dots complexes have great advantages for long-time biological labeling and tracking.

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References

1. Bruchez M, Moronne M, Gin P, Weiss S, Alivisatos AP (1998) Semiconductor nanocrystals as fluorescent biological labels. Science 281:2013–2016
2. Chan WCW, Nie S (1998) Quantum dot bioconjugates for ultrasensitive nonisotopic detection. Science 281:2016–2018
3. Medintz IL, Uyeda HT, Goldman ER, Mattoussi H (2005) Quantum dot bioconjugates for imaging, labelling and sensing. Nat Mater 4: 435–446
4. Chang B, Yang X, Wang F, Wang Y, Yang R, Zhang N, Wang B (2013) Water soluble fluorescence quantum dot probe labeling liver cancer cells. J Mater Sci-Mater M 24:2505–2508
5. Delehanty J, Mattoussi H, Medintz I (2009) Delivering quantum dots into cells: strategies, progress and remaining issues. Anal Bioanal Chem 393:1091–1105
6. Law W-C, Yong K-T, Roy I, Ding H, Hu R, Zhao W, Prasad PN (2009) Aqueous-phase synthesis of highly luminescent CdTe/ZnTe core/shell quantum dots optimized for targeted bioimaging. Small 5: 1302–1310
7. Reiss P, Protière M, Li L (2009) Core/shell semiconductor nanocrystals. Small 5:154–168
8. Su Y, Hu M, Fan C, He Y, Li Q, Li W, L-h W, Shen P, Huang Q (2010) The cytotoxicity of CdTe quantum dots and the relative contributions from released cadmium ions and nanoparticle properties. Biomaterials 31:4829–4834
9. Kamruzzaman Selim KM, Xing Z-C, Guo H, Kang I-K (2009) Immobilization of lactobionic acid on the surface of cadmium sulfide nanoparticles and their interaction with hepatocytes. J Mater Sci Mater Med 20:1945–1953
10. Wu P, Yan X-P (2013) Doped quantum dots for chemo/biosensing and bioimaging. Chem Soc Rev 42:5489–5521
11. Koyakutty M, Seby J, Deepa T, Sonali S, Deepthy M, Shantikumar N (2009) Bio-conjugated luminescent quantum dots of doped ZnS: a cyto-friendly system for targeted cancer imaging. Nanotechnology 20:065102
12. Pradhan N, Goorskey D, Thessing J, Peng X (2005) An alternative of CdSe nanocrystal emitters: pure and tunable impurity emissions in ZnSe nanocrystals. J Am Chem Soc 127:17586–17587
13. Pradhan N, Battaglia DM, Liu Y, Peng X (2007) Efficient, stable, small, and water-soluble doped ZnSe nanocrystal emitters as non-cadmium biomedical labels. Nano Lett 7:312–317
14. Zeng R, Rutherford M, Xie R, Zou B, Peng X (2010) Synthesis of highly emissive Mn-Doped ZnSe nanocrystals without pyrophoric reagents. Chem Mater 22:2107–2113
15. Zhang Y, Gan C, Muhammad J, Battaglia D, Peng X, Xiao M (2008) Enhanced fluorescence intermittency in Mn-Doped single ZnSe quantum dots. J Phys Chem C 112:20200–20205
16. Gan C, Zhang Y, Battaglia D, Peng X, Xiao M (2008) Fluorescence lifetime of Mn-doped ZnSe quantum dots with size dependence. Appl Phys Lett 92:241111–241113
17. Ma Q, Yu W, Huang H, Su X (2011) Determination of L-tyrosine based on luminescence quenching of Mn-Doped ZnSe quantum dots in enzyme catalysis system. J Fluoresc 21:125–131
18. Xu Z, Li B, Tang W, Chen T, Zhang H, Wang Q (2011) Glycopolypeptide-encapsulated Mn-doped ZnS quantum dots for drug delivery: fabrication, characterization, and in vitro assessment. Colloids Surf B 88:51–57
19. Gao X, Niu L, Su X (2012) Detection of DNA via the fluorescence quenching of Mn-doped ZnSe D-dots/doxorubicin/DNA ternary complexes system. J Fluoresc 22:103–109
20. Liu J, Wei X, Qu Y, Cao J, Chen C, Jiang H (2011) Aqueous synthesis and bio-imaging application of highly luminescent and low cytotoxicity Mn^{2+}-doped ZnSe nanocrystals. Mater Lett 65: 2139–2141

21. Geszke M, Murias M, Balan L, Medjahdi G, Korczynski J, Moritz M, Lulek J, Schneider R (2011) Folic acid-conjugated core/shell ZnS:Mn/ZnS quantum dots as targeted probes for two photon fluorescence imaging of cancer cells. Acta Biomater 7:1327–1338
22. Santra S, Yang H, Holloway PH, Stanley JT, Mericle RA (2005) Synthesis of water-dispersible fluorescent, radio-opaque, and paramagnetic CdS:Mn/ZnS quantum dots: a multifunctional probe for bioimaging. J Am Chem Soc 127:1656–1657
23. Chang S-Q, Kang B, Dai Y-D, Zhang H-X, Chen D (2011) One-step fabrication of biocompatible chitosan-coated ZnS and ZnS:Mn2+ quantum dots via a γ-radiation route. Nanoscale Res Lett 6:591
24. Reschel T, Koňák Č, Oupický D, Seymour LW, Ulbrich K (2002) Physical properties and in vitro transfection efficiency of gene delivery vectors based on complexes of DNA with synthetic polycations. J Control Release 81:201–217
25. He X, Huo H, Wang K, Tan W, Gong P, Ge J (2007) Plasmid DNA isolation using amino-silica coated magnetic nanoparticles (ASMNPs). Talanta 73:764–769
26. Liu D, Qiao W, Li Z, Chen Y, Cui X, Li K, Yu L, Yan K, Zhu L, Guo Y, Cheng L (2008) Structure–function relationship research of glycerol backbone-based cationic lipids for gene delivery. Chem Biol Drug Des 71:336–344
27. Mukhtar MM, Li S, Li W, Wan T, Mu Y, Wei W, Kang L, Rasool ST, Xiao Y, Zhu Y, Wu J (2009) Single-chain intracellular antibodies inhibit influenza virus replication by disrupting interaction of proteins involved in viral replication and transcription. Int J Biochem Cell Biol 41:554–560
28. Mao W, Guo J, Yang W, Wang C, He J, Chen J-Y (2007) Synthesis of high-quality near-infrared-emitting CdTeS alloyed quantum dots via the hydrothermal method. Nanotechnology 18:485611
29. Adamczak M, Hoel HJ, Gaudernack G, Barbasz J, Szczepanowicz K, Warszyński P (2012) Polyelectrolyte multilayer capsules with quantum dots for biomedical applications. Colloids Surf B 90:211–216
30. Filipe V, Hawe A, Jiskoot W (2010) Critical evaluation of Nanoparticle Tracking Analysis (NTA) by NanoSight for the measurement of nanoparticles and protein aggregates. Pharm Res 27: 796–810
31. Cho YW, Kim JD, Park K (2003) Polycation gene delivery systems: escape from endosomes to cytosol. J Pharm Pharmacol 55:721–734
32. Zhang M, Xu R, Xia X, Yang Y, Gu J, Qin G, Liu X, Ferrari M, Shen H (2014) Polycation-functionalized nanoporous silicon particles for gene silencing on breast cancer cells. Biomaterials 35:423–431
33. Zhang Y, Mi L, Xiong R, Wang P-N, Chen J-Y, Yang W, Wang C, Peng Q (2009) Subcellular localization of thiol-capped CdTe quantum dots in living cells. Nanoscale Res Lett 4:606–612
34. Ribeiro RT, Dias JMM, Pereira GA, Freitas DV, Monteiro M, Cabral Filho PE, Raele RA, Fontes A, Navarro M, Santos BS (2013) Electrochemical synthetic route for preparation of CdTe quantum-dots stabilized by positively or negatively charged ligands. Green Chem 15:1061–1066
35. Xiong R, Li Z, Mi L, Wang P-N, Chen J-Y, Wang L, Yang W-L (2010) Study on the intracellular fate of tat peptide-conjugated quantum dots by spectroscopic investigation. J Fluoresc 20:551–556
36. Niu L, Li Y, Li X, Gao X, Su X (2012) Study the cytotoxicity of different kinds of water-soluble nanoparticles in human osteoblast-like MG-63 cells. Mater Res Bull 47:3654–3659