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ORIGINAL PAPER

Liposome Encapsulation of Thiol-Capped CdTe Quantum Dots for Enhancing the Intracellular Delivery

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Abstract Although water soluble thiol-capped quantum dots (QDs) have been widely used as photoluminescence (PL) probes in various applications, the negative charges on thiol terminals limit the cell uptake hindering their applications in cell imaging. The commercial liposome complex (Sofast®) was used to encapsulate these QDs forming the liposome vesicles with the loading efficiency as high as about 95%. The cell uptakes of unencapsulated QDs and QD loaded liposome vesicles were comparatively studied by a laser scanning confocal microscope. We found that QD loaded liposome vesicles can effectively enhance the intracellular delivery of QDs in three cell lines (human osteosarcoma cell line (U2OS); human cervical carcinoma cell line (Hela); human embryonic kidney cell line (293 T)). The photobleaching of encapsulated QDs in cells was also reduced comparing with that of unencapsulated QDs, measured by the PL decay of cellular QDs with a continuous laser irradiation in the microscope. The flow cytometric measurements further showed that the enhancing ratios of encapsulated QDs on cell uptake are about 4–8 times in 293 T and Hela cells. These results suggest that the cationic liposome encapsulation is an effective modality to enhance the intracellular delivery of thiol-capped QDs.

Keywords Quantum dots · Living cells · Intracellular delivery · Cell imaging · Liposome encapsulation

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Introduction

Because of the excellent optical properties, such as the bright photoluminescence (PL), broad absorption region with the high extinction coefficients and the good photostability, quantum dots (QDs) have become promising fluorescence probes for biological staining and cell imaging [1–5]. For biological applications, the delivery of QDs into biological bodies such as the intracellular delivery for cell imaging, is thus a necessary requirement. However, most water-soluble QDs have the negative charges on their surface, which allows QDs to disperse in aqueous solution by the electrostatic repulsion [6–8] and also makes those QDs hardly bind on the surfaces of living cells because the most electric charges on cell surfaces are negative. Therefore, the efficiency of cell uptake for these QDs is low [9, 10]. The attempts to enhance the intracellular delivery of QDs have been made by surface modifications with various functional molecules, such as linking with cationic peptides and encapsulating with liposomes [1, 2, 11–16]. Liposomes have outstanding features to act as the biological delivery vehicle, because they are biocompatible,

biodegradable and less toxic. Lipsomes already became the most clinically established nanometer-scale systems for various drug deliverys [16–18]. Liposomes could form self-assembled vesicles, consisting of a lipid bilayer surrounding an aqueous cavity, so that both hydrophobic agents and hydrophilic compounds can be carried. The hydrophobic core/shell QDs were encapsulated by liposomes for the intracellular delivery as reported in several studies, but few works of liposome encapsulation on hydrophilic QDs could be found [12, 19–22]. As the delivery vehicle, the loading content of the hydrophilic compounds by liposome vesicles should be higher than that of hydrophobic ones, because the water phase compounds are contained in the central cavity whose volume is much bigger than that of the lipid bilayer where the organic phase compounds are embedded in. The high loading efficiency for QDs is the important index to fulfill the effective intracellular delivery, and thus the loading of hydrophilic QDs with liposome vesicles is worth investigating further.

Water-soluble QDs could be prepared by two ways. The organometallic synthesis way is the original one, in which the QD cores are constructed in organic solvents at a very high temperature such as 350 °C, then capped with a thin layer of a higher bandgap material such as ZnS to passivate their surfaces forming the core/shell structure, and finally these hydrophobic QDs should be reacted further with the ligand exchange to become water-soluble [23–25]. In contrast, a promising synthesis route using the water as a solvent and thiols as ligands, called hydrothermal route, has been developed recently and the water-soluble thiol-capped QDs could be prepared directly in one step synthesis [26]. These thiol-capped QDs such as CdTe QDs, having good PL properties also, have been used in biological labeling and cell imaging, but the negative charges on their thiol terminals may still limit the cell uptake [9, 10]. The enhancement of intracellular delivery would certainly improve the cell imaging and other staining works using these easily prepared QDs. In this work, we selected the cationic liposome complex as the loading vehicles to encapsulate these thiol-capped CdTe QDs for intracellular delivery. We found that the loading efficiency is extremely high as the 95% of QDs can be encapsulated in loposome vesicles and these liposome vesicles can remarkably enhance the intracellular delivery of QDs in three kinds of living cells.

Materials and Methods

QDs

The thiol-capped CdTe QDs were made in our lab by hydrothermal route, which is believed to be a simple and efficient method [27, 28]. The details of the procedure have been described in our previous work [29]. Briefly, a typical procedure is as follows: with a molar ratio of 2:1, sodium borohydride was used to react with tellurium in water to prepare the sodium hydrogen telluride (NaHTe). Fresh solutions of NaHTe were then diluted by N_2-saturated deionic water to 0.0467 M for use in the following step. $CdCl_2$ (1 mmol) and thioglycolic acid (1.2 mmol) were dissolved in 50 mL of deionized water. Stepwise addition of NaOH solution adjusted the precursors solution to pH=9. Then, 0.096 mL of oxygen-free solution containing fresh NaHTe, cooled to 0 °C, was added into 10 mL of the above prepared precursor solution and stirred vigorously. Finally, the solution with faint yellow color was put into a Teflon-lined stainless steel autoclave with a volume of 15 mL. The autoclave was maintained at the reaction temperature (170 °C) for 40 min and then cooled to the room temperature by hydro-cooling process. The as-prepared CdTe QDs, dispersed in water, were precipitated from solution using excess ethanol. Then, these solutions were centrifuged to harvest QDs in the bottom of the centrifuging tube. The obtained QD powders were dried in vacuum and brought into a nitrogen-atmosphere box for subsequent use. A stock solution of QDs (1 mg/ml) was made before experiments. The PL quantum yield of obtained QDs is about 0.35 [29].

Cell Culture

U2OS (human osteosarcoma cell), Hela (human cervical carcinoma cell) and 293 T (human embryonic kidney cell) were cultured at 37 °C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Gibco BRL) in a humidified 5% CO_2 incubator. Cells were subcultured in tissue culture flasks to keep them in an exponential growth phase for use in experiments.

Liposome Encapsulation of QDs

Cationic liposome complex with the molecular weight of about 20kD (Sofast®) purchased from Sunma Biotechnology Co., Ltd was used in this study. This liposome complex has shown a high efficiency in gene transfection experiments to encapsulate genes for intracellular delivery. The liposome encapsulation of QDs was carried out by mixing the liposome complex with QDs in aqueous solution at various molar ratios from 0.45:1 to 0.1:1 for 10 min, respectively. The liposome vesicles were formed in these mixtures by agglomeration. Then these mixtures were centrifuged for 10 min at 15000 rpm to harvest the QD loaded liposome vesicles (QD-LVs) in supernatants. The hydrodynamic diameters of these obtained QD-LVs were

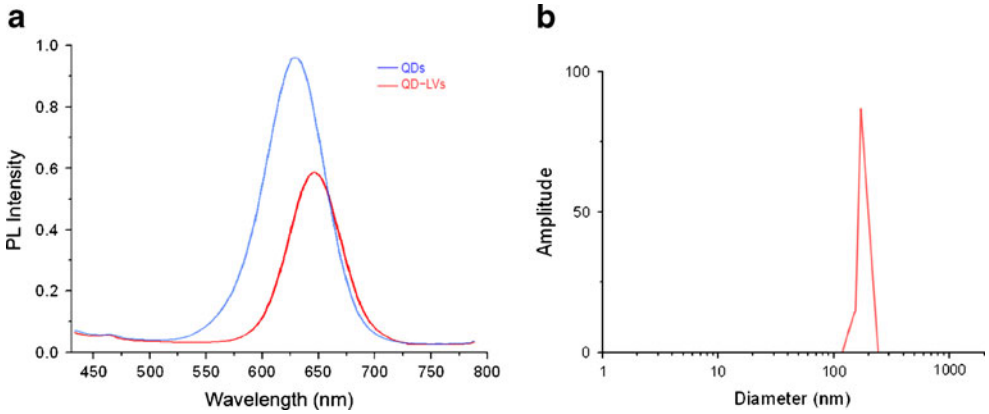

Fig. 1 **a** PL spectra of QDs (*blue*) and QD-LVs (*red*) excited at 400 nm. **b** Size distribution of QD-LVs measured by DLS

measured using the method of dynamic light scattering (DLS) (Malvern, Autoszer 4700).

The Loading Content of QDs in QD-LVs

The PL spectra and intensities of obtained QD-LVs in solutions were measured with a fluorescence spectrophotometer (Hitachi, F-2500), and compared with that of the QD solution at the concentration of 0.14 μM which was just the concentration used in liposome encapsulation. The PL in QD-LVs solution was totally come from the loaded QDs, so that the comparison of PL intensities between the QD-LVs solution and the unencapsulated QD solution could estimate the loading content of QDs in QD-LVs.

PL Imaging Measurements of QDs in Living Cells

Cells were incubated with unencapsulated QDs (0.14 μM) or QD-LVs for 0.5 to 4 h in different groups, and then washed with PBS to remove unassociated QDs or QD-LVs. These cell samples adhered on the cover slips were put in the stage of a confocal microscope (Olympus, FV-300, IX 71) in turn for PL imaging measurements. A 488 nm Ar^+ laser (Melles Griot, Argon ion) was used to carry out the excitation via a water immersion objective (60×), and a photomultiplier tube (PMT) with a 580–640 nm bandpass filter in a detection channel was used to record the PL signals of QDs and establish the PL images. With the z-scan mode, the three dimensional distribution of intracellular QDs and the relative PL intensities were measured. Differential interference contrast (DIC) images were recorded simultaneously in a transmission channel to exhibit the cell morphology.

Photobleaching Measurements of QDs in Living Cells

We selected some micro-regions in acquired PL images to measure the photobleaching of cellular QDs using the point-stay mode of the confocal microscope. Since the QDs have the high absorption coefficient at the short wavelength, a 405 nm laser (Coherent, Radius 405–25) was selected for the excitation. The laser beam with the power of 0.8 mW was focused on the selected spot of the cell by a 60× objective to do a continuous irradiation and the time-dependent PL decay was recorded by the PMT in the detection channel.

Measurements of Cellular Uptake by Flow Cytometric Analysis

To evaluate the difference of cellular uptakes for QD-LVs and QDs in a large number of cells quantificationally, the flow cytometric analysis was performed in a flow cytometer (FACS Calibur, Becton Dickinson). Cells (about 10^6 cells in 35-mm culture dishes) had been incubated with QDs or QD-LVs were washed with PBS for three times, and trypsinized with 0.25% trypsin-EDTA. Then the cell suspension was ready for the flow cytometric analysis. A 488 nm laser was used to excite cellular QDs in each flow cell, and the FL-2 channel (580–630 nm) was used to detect PL signals in turn in a train of flowing cells. At least 10^4 cells in each group were analyzed.

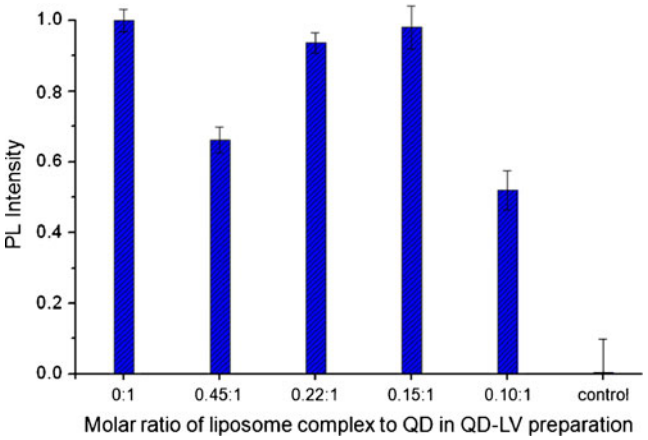

Fig. 2 QD loading percentages in QD-LVs groups prepared with different molar ratio of liposome complex to QDs

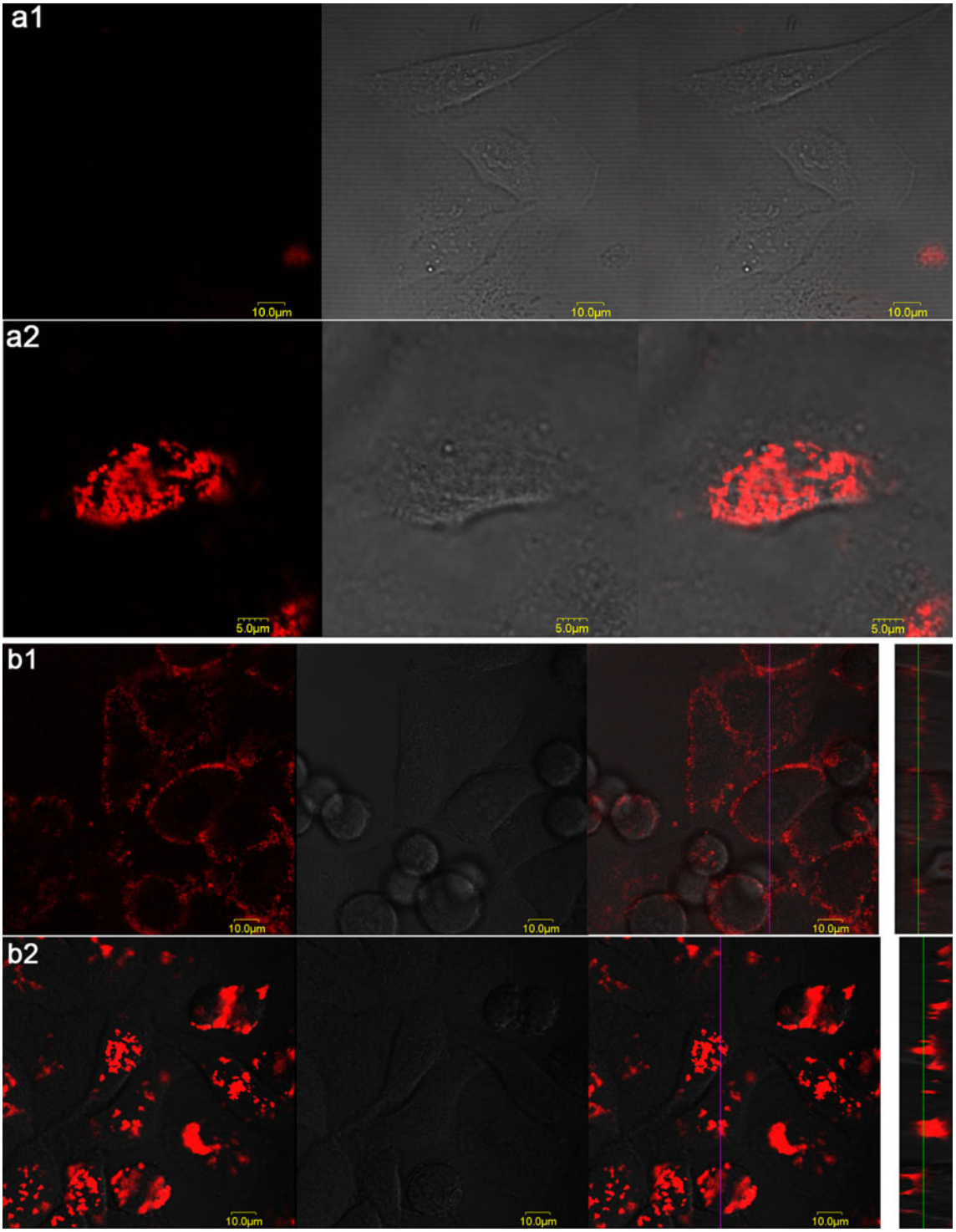

Fig. 3 Cellular uptakes of QDs and QD-LVs in different cell lines. Left: PL images; middle: DIC images; right: merged images. **a1** U2OS cells have been incubated with QDs for 4 h. **a2** U2OS cells have been incubated with QD-LVs for 1 h. **b1** Hela cells have been incubated with QDs for 1 h. The right profile shows the y-z distribution of cellular QDs obtained by the z-scan mode. **b2** Hela cells have been incubated with QD-LVs for 1 h. The right profile was also acquired by the z-scan mode. **c1** 293 T cells have been incubated with QDs for 3 h. **c2** 293 T cells have been incubated with QD-LVs for 0.5 h. **c3** QD-LVs loaded 293 T cells as in c2 have been kept in an incubator (37 °C) for another 4 h

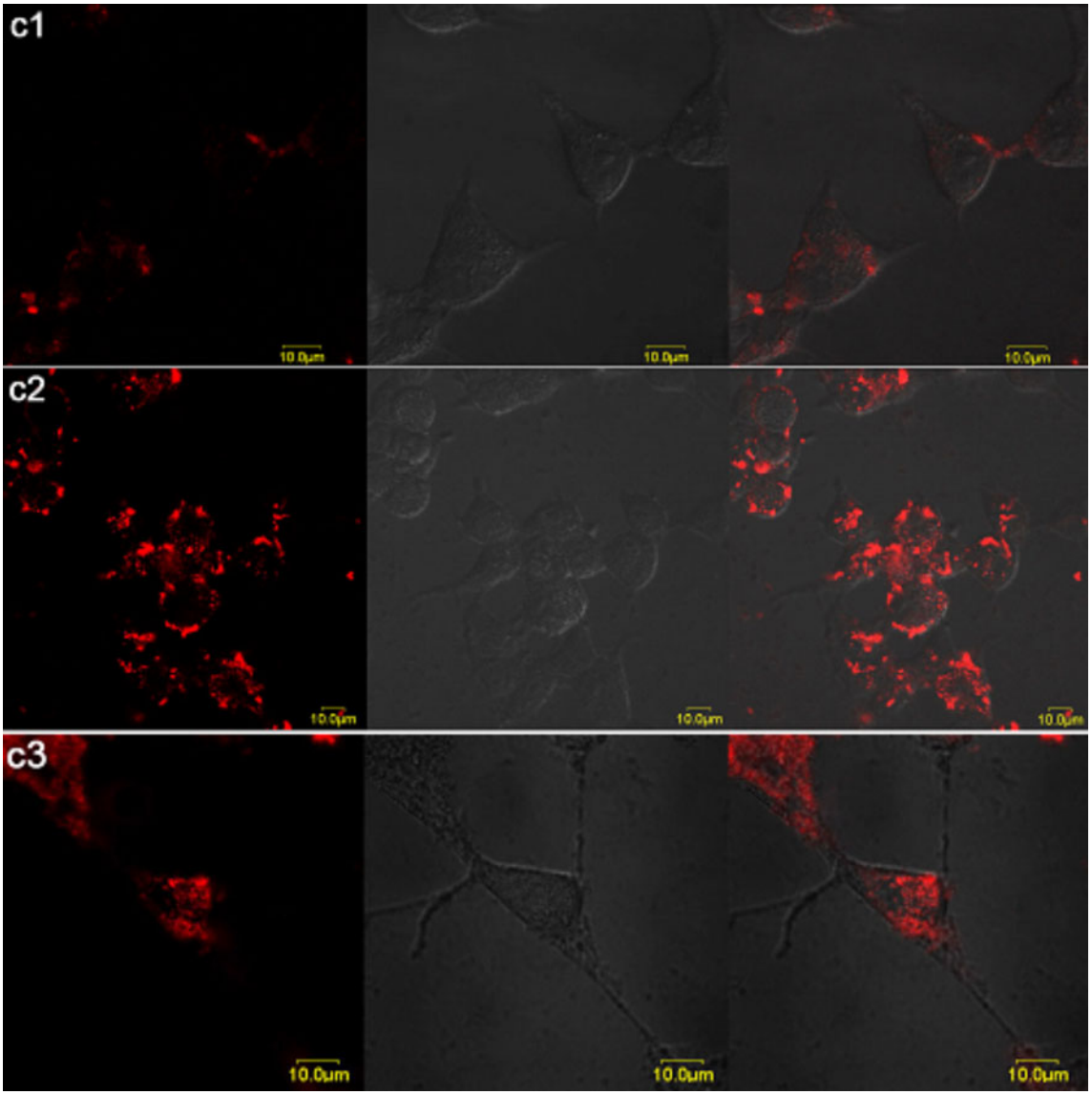

Fig. 3 (continued)

Results and Discussion

The Characterization of QD-LVs

The self-assembling ability of the used liposome complex was very strong, as the QD-LVs already formed 10 min post the mixing of the liposome complex with QDs. The strong PL from QD-LVs aqueous solution demonstrated that QD-LVs contained a large number of QDs (Fig. 1a). The PL peak of the QD-LVs solution was slightly red-shifted as compared to that of the uncapsulated QD solution, probably due to the interaction between the positive charges of the inner surface of the liposome vesicles and negative charges on the free terminals of thiols of the QD surface. The distribution of hydrodynamic sizes of QD-LVs in solution is showed in Fig. 1b measured by DLS, and their average size is about 150 nm. This size region of vesicles is suitable for cell uptake studies, as many previous works reported [19, 21, 30]. We have measured previously that the hydrodynamic sizes of unencapsulated QDs were about 20 nm [31]. There is no trace of particles around 20 nm in Fig. 1b, demonstrating that the harvested QD-LVs solution contained pure QD-LVs and did not include any unencapsulated QDs.

The Loading Content of QDs in QD-LVs

To enhance the intracellular delivery of QDs with liposome vesicles, the loading content is an important index to evaluate the efficiency. In QD-LVs preparations, different mixture groups of liposome complex and QDs with different molar ratios from 0.45:1 to 0.1:1, keeping the

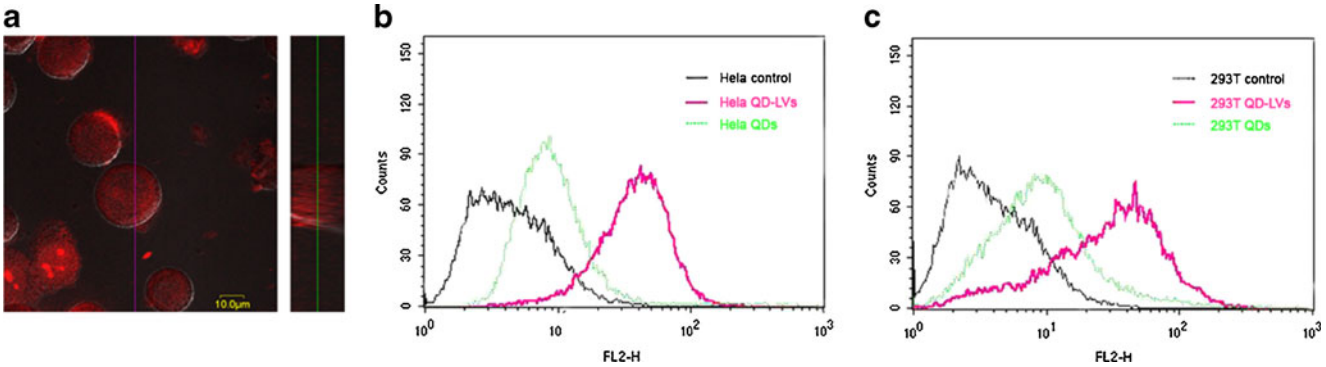

Fig. 4 Flow cytometric analysis of cellular uptakes of QDs and QD-LVs. X-axis represents the relative PL intensity of the labeled cell and the y-axis represents the number of cells. **a** Hela cells have been incubated with QD-LVs for 3 h, trypsinized then viewed by the confocal microscope. **b** Hela QDs: Hela cells have been incubated with QDs for 3 h. Hela QD-LVs: Hela cell have been incubated with QD-LVs for 3 h. Hela control: without any treatment. **c** 293 T QDs: 293 T cells have been incubated with QDs for 3 h. 293 T QD-LVs: 293 T cell have been incubated with QD-LVs for 3 h. 293 T control: without any treatment

QD concentration at 0.14 μM, were tested. The PL intensities of these prepared QD-LVs solution groups were measured and compared with that of the unencapsulated QDs solution with the original concentration of 0.14 μM. Then the QD loading percentages in these different QD-LVs groups can be estimated. As shown in Fig. 2, the QD loading content can reach about 95% at the QD-LVs group with the liposome complex/QDs molar ratio of 0.15:1. Therefore, this liposome complex (Sofast®) provided a simple and feasible way to effectively encapsulate QDs.

The Enhancement of Intracellular Delivery by QD-LVs

The QD-LVs prepared with the best molar ratio of liposome complex/QDs was used to check the enhancement of intracellular delivery further, as compared to unencapsulated QDs. When U2OS cells were incubated with 0.14 μM QDs for 4 h, almost no cellular QDs can be seen in the PL image (Fig. 3a1). While the strong PL image in U2OS cells that have been incubated with QD-LVs for 1 h demonstrates a remarkable accumulation of QDs in the cells (Fig. 3a2). In the case of Hela cells, after 1 h incubation the unencapsulated QDs only slightly bound on the cell surfaces, but QD-LVs extensively existed in cells post 1 h incubation (Fig. 3b1-2). The 293 T cells also resisted the unencapsulated QDs as only a small amount of QDs were internalized into cells after 3 h incubation (Fig. 3c1). In contrast, the cell uptake for QD-LVs was very fast as the PL image already became bright when 293 T cells had been incubated with QD-LVs for 30 min (Fig. 3c2). Concluding the results in these three cell lines, QD-LVs can fast and effectively penetrate into the living cells facilitating studies of cell imaging. Moreover, the cellular QD-LVs remained their vesicle form. When these QD-LVs treated cells were incubated in a incubator at 37 ^{0}C for another 4 h, the cellular liposome vesicles degraded as leaked QDs diffusely distributed in the cytoplasm (Fig. 3c3), indicating that liposome vesicles functioned as the carriers to deliver QDs into cytosol.

The Evaluation for the Intracellular Delivery Enhancement by Flow Cytometric Analysis

The intracellular delivery enhancement of QD-LVs was further measured by flow cytometric analysis in a large number of cells. Having been incubated with QDs or QD-LVs and then washed by PBS for three times, cells were trypsinized with 0.25% trypsin-EDTA. After trypsinization, these cells became suspended in solution with the sphere-like form (Fig. 4a). These cell samples and the control sample (without any treatment) were measured comparatively in the flow cytometer, and results for Hela and 293 T cells are shown in Fig. 4b and c. From these data, the average PL values of P_c (control cells), P_{QD} (QD treated

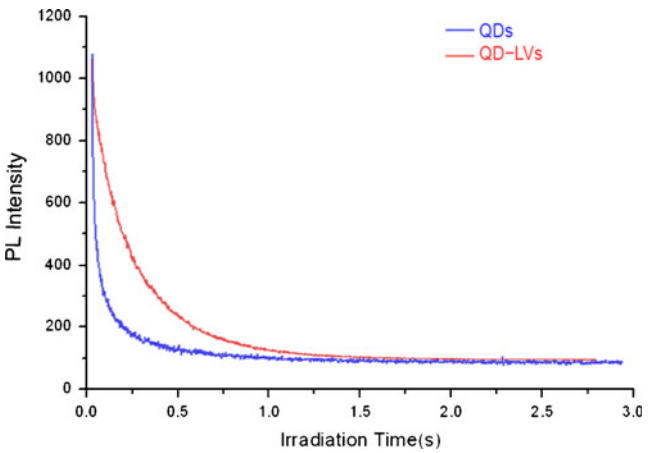

Fig. 5 Photobleaching of QDs (*blue*) and QD-LVs (*red*) in 293 T cells as a function of irradiation time. A 0.8 mW 405 nm laser was used to carry out a continuous irradiation on a selected micro-region of the cell

cells) and $P_{QD\text{-}LV}$ (QD-LV treated cells) were obtained for each cell line. Then the enhancing ratio (β) of cell uptake for QD-LVs/QDs can be assigned in the following formula.

$$\beta = \left(P_{QD-LV} - P_c\right)/\left(P_{QD} - P_c\right)$$

The enhancements of QD-LVs on cell uptake of 293 T and Hela cells are about 4–8 times, estimated by this formula. These results confirm the positive rule of liposome encapsulation on QD intracellular delivery.

The Photostability of QD-LVs in Living Cells

We have found in the previous work that CdTe QDs in living cells still can be photobleached, though they are more stable than organic dyes such as FITC [32]. It was also reported recently that the liposome encapsulated CdSe/ZnS QDs in toluene have increased stability under UV irradiation as compared to unencapsulated ones, demonstrating a potential advantage for biological applications. Herein, we further compared the photostabilities of QD-LVs and QDs in 293 T cells. Some small regions in obtained PL images as Fig. 3c were selected to measure the photobleaching. As shown in Fig. 5, although both cellular QDs and QD-LVs can be photobleached under a continuous laser irradiation, the bleaching rate of cellular QDs is obviously faster than that of cellular QD-LVs. Further inspection revealed that the photobleaching curves in Fig. 5 contain two components of the fast decay and slow decay, which can be fitted with the biexponential form of "A1exp (t/τ1)+A2exp(t/τ2)", where the τ1 is the time constant of the fast decay and the τ_2 is that of the slow decay. For these bleaching curves, the fast component plays a dominant role, so that the τ_1 is the dominant parameter to judge the bleaching rate. The τ_1 of cellular QDs is about 0.02 s and that of cellular QD-LVs is around 0.30 s, indicating that about 15 times improvement on photostability could be achieved by QD-LVs.

Conclusion

With a commercial liposome complex (Sofast®), water-soluble CdTe QDs can be easily encapsulated to form QD-LVs with the hydrodynamic size of about 150 nm. The QD loading content in QD-LVs reaches 95%, demonstrating a very high loading efficiency. The QD-LVs effectively enhance the intracellular delivery in U2OS, Hela and 293 T cells. The enhancing ratios of QD-LVs on cell uptake, as compared to unencapsulated QDs, are about 4–8 times for 293 T and Hela cells. In addition, the cellular QD-LVs are much photostable than cellular QDs. These results suggest that cationic liposome encapsulation is a promising way to enhance the intracellular delivery of water-soluble QDs.

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