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ORIGINAL PAPER

Preparation of liposomes loaded with quantum dots, fluorescence resonance energy transfer studies, and near-infrared in-vivo imaging of mouse tissue

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Abstract We report on a simple, fast and convenient method to engineer lipid vesicles loaded with quantum dots (QDs) by incorporating QDs into a vesicle-type of lipid bilayer using a phase transfer reagent. Hydrophilic CdTe QDs and near-infrared (NIR) QDs of type CdHgTe were incorporated into liposomes by transferring the QDs from an aqueous solution into chloroform by addition of a surfactant. The QD-loaded liposomes display bright fluorescence, and the incorporation of the QDs into the lipid bilayer leads to enhanced storage stability and reduced sensitivity to UV irradiation. The liposomes containing the QD were applied to label living cells and to image mouse tissue in-vivo using a confocal laser scanning microscope, while NIR images of mouse tissue were acquired with an NIR fluorescence imaging system. We also report on the fluorescence resonance energy transfer (FRET) that occurs between the CdTe QDs (the donor) and the CdHgTe QDs (the acceptor), both contained in liposomes. Based on these data, this NIR FRET system shows promise as a tool that may be used to study the release of drug-loaded liposomes and their in vivo distribution.

Keywords Hydrophilic QDs · Lipid-QDs · Fluorescence resonance energy transfer · NIR imaging in vivo

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Introduction

Semiconductor quantum dots (QDs) have been intensively studied in recent years due to their quantum size and surface effects. Compared with conventional organic fluorescent dyes, QDs have unique optical properties such as narrow emission, tunable spectra, and higher photostability. While these attributes suggest potential use as fluorescent probes [1–3], QDs present certain stability and biocompatibility issues in biological systems. Strategies that have been explored to overcome these limitations include surface coatings, functionalizing QDs with ligands or incorporating QDs into nanoparticles [4–7]. Surface modifications, however, often lead to decreases in QD fluorescence intensity and photostability, and some nanoparticles are not suitable for biological imaging in vivo due to the large size of microscales. In addition, some of these methods involve special equipment or complicated synthesis.

Liposomes are spherical vesicles consisting of a phospholipid bilayer surrounding an aqueous cavity. They are currently the most broadly used nanometer-scale system for drug delivery in clinical applications. Phospholipids are natural amphiphilic materials found in cell membranes and vesicles. Biomimetic lipid bilayers offer high biocompatibility and biodegradability with less toxicity. Lipid bilayer incorporation of hydrophobic QDs has been reported in several studies [8–10]. Lipid-QDs were engineered in order to retain lipid bilayer biocompatibility and surface versatility while preserving luminescent characteristics unique to QDs, thereby offering a novel means for

combining therapeutic and diagnostic applications in oncology. However, the procedure for synthesizing hydrophobic QDs is severe and most of the chemicals used are highly toxic and costly. So far, little work has been done in the area of liposome encapsulation of hydrophilic QDs.

In this work, hydrophilic CdTe QDs and near infrared (NIR) CdHgTe QDs were used to synthesize QD-loaded liposomes. We used a simple, fast and convenient method to engineer lipid-QDs by incorporating hydrophilic QDs into the vesicle lipid bilayer using an efficient phase transfer reagent. Encapsulation of QDs within liposomes membranes retained all QD fluorescent characteristics and made QDs more compatible with biological environments. Compared with bare hydrophilic QDs, the obtaining lipid-QDs vesicles exhibited remarkably improved fluorescence stability. Furthermore, lipid-CdTe was then successfully used to label living cells and lipid-CdHgTe was used for NIR in vivo imaging by means of NIR fluorescence. Liposomes loaded with QDs exhibited great potential both as NIR fluorescent nanoprobes and as traceable nanocarriers for real-time in vivo drug research.

In addition, a novel fluorescence resonance energy transfer (FRET) system between CdTe QDs (donor) and NIR CdHgTe QDs (acceptor) in the lipid bilayer was established in this work. Techniques for in vivo liposome distribution and drug release behavior are urgently needed. Prior research indicates that the drug release process for drug-loaded liposomes includes rupture and digestion of the phospholipid bilayer followed by fusion with the cell membrane. Therefore, the extent of rupture and the digestion rate of the lipid bilayer both play a key role in drug release, which in turn is closely related to changes in the structure of the lipid membrane [11, 12]. The FRET principle can be applied to study changes in the drug-loaded liposome membrane as a fluorescent nanoprobe [12]. While it has been demonstrated that changes in fluorescence signaling can reflect the extent of damage to the liposome membrane [13], to the best of our knowledge, the FRET between two types of QDs in lipid bilayer has not yet to be described. We established the FRET system between 556 nm-emitting CdTe and 655 nm-emitting CdHgTe QDs, given that embedding them in lipid bilayer can reduce the distance between them. We also calculated FRET efficiency and the distance between the donor and the acceptor. The stable fluorescent signals from lipid-QDs in living cells and NIR imaging in vivo suggested the potential value of using the NIR FRET system as a nanosensor to investigate the releasing behavior of drug-loaded liposomes and their distribution in vivo.

Experimental

Reagents

The chemicals were of A.R. grade or the best grade commercially available. $Cd(NO_3)_2{\cdot}4H_2O$, tellurium powder, $Hg(NO_3)_2{\cdot}1/2H_2O$, $NaBH_4$, chloroform, cholesterol, PEG-6000, mercaptoacetic acid (MPA) was acquired from Lingfeng Reagent Company (Shanghai, China, http://www.lingfengchem.com); Cetrimonium Bromide (CTAB) was obtained from Xiamen Pioneer Technology Inc. (Xiamen, China, http://www.xm-pioneer.com); L-α-phosphatidylcholine (PC, 98 %) was purchased from Aladdin Reagent Company (Shanghai, China, http://www.aladdin-e.com). All culture reagents were purchased from Gibco (USA, http://zh.invitrogen.com/site/cn/zh/home/brands/Gibco.html). Water for all reactions and solution preparation was double distilled.

Instrumentation

The absorption spectrum was measured using a UV2100 UV–vis spectrometer (Shimadzu, Japan, http://www.shimadzu.com). All fluorescence measurements were made with an RF-5301 spectrofluorophotometer (Shimadzu, Japan, http://www.shimadzu.com). In both experiments, a 1 cm path length quartz cell was used to measure the absorption or fluorescence spectrum. The transmission electron microscopy (TEM) images of the QDs and the lipid-QDs were acquired on a JEM-2100 transmission electron microscope (JEOL, Japan, http://www.jeol.com). The size distribution of the vesicles was measured using a Mastersizer 2000 laser particle size analyzer (Malvern Instruments, UK, http://www.malvern.com). Fluorescence images of lipid-QDs were visualized using an Olympus IX-51 fluorescent microscope and captured with a Retica digital camera. The pH values were measured with a pHS-25 pH meter (Shanghai INESA Scientific Instrument Co., Ltd. Shanghai, China, http://www.lei-ci.com). Confocal micrographs of cells were acquired on FV1000 confocal microscope (Olympus, Japan, www.olympus.com). The components of the NIR system were locally equipped as follows: for the fluorescence images, a NL-FC-2.0-763 laser (λ= 765.9 nm) light was coupled into NIR optical fiber bundles and defocused to provide a broad spot with even optical density shining on the surface of the mouse. A highly sensitive NIR CCD camera was positioned 10 cm above the subjects. An 800 nm long pass filter was put ahead of the CCD to block the excitation and ambient light, and thus to capture the emitted fluorescence from the tissue. All optical measurements

were performed at room temperature under ambient conditions.

Procedures

Preparation of liposomes loaded with QDs

Lipid vesicles with QDs were prepared using the classic rotation film method. CTAB was added as a surfactant to the aqueous solution of QDs in order to transfer negatively charged hydrophilic QDs to a chloroform solution via extraction. The long hydrocarbon chain in CTAB makes the resulting CTAB-coated QDs soluble in chloroform. Specifically, PC, cholesterol, CTAB and PEG-6000 were dissolved in 5 mL chloroform, and then transferred the solution to a 100 mL separating funnel. Different volumes of CdTe or CdHgTe QDs (at a QD concentration of 4.7×10^{-5} mol·L^{-1}) were added to the separating funnel, which was gently shaken and then left undisturbed for 15 min. The color of the upper QD solution faded gradually while the chloroform solution darkened, showing successful transfer of QDs from the aqueous to the organic phase.

The chloroform solution of lecithin-QDs was transferred to a 500 mL eggplant-shaped flask and dried via rotary evaporation to form a thin QD-containing lipid film. Two millilitres of PBS solution (pH=7.4) was then added to hydrate the dry lipid-QD film. Upon hydration of the film, multilamellar vesicles (MLV) self-assembled, incorporating the QDs into their lipid bilayer. Sonication using an ultrasonic bath led to the formation of small unilamellar vesicles (SUV) loaded with QDs (at a QD concentration of 50 μL·mL^{-1}). The fluorescence intensity and optical stability of bare QDs and lipid-QDs in vitro were measured during storage and exposure to UV irradiation. All the emission spectra were measured with an excitation wavelength of 370 nm.

Living cell labeling and NIR imaging in vivo

Human breast cancer cells (MCF7) were seeded at a density of 5×10^3 cells/well in 96-well plates and cultured in RPMI 1640 culture medium with 10 % fetal bovine serum in a humidified incubator with 5 % CO_2 at 37 °C for 24 h. The diluted lipid-CdTe and lipid-CdHgTe solution (at a QD concentration of 2 μmol·mL^{-1}) were added into the cells respectively. After incubation for 120 min, the cells were washed with PBS buffer and immediately observed under a confocal laser scanning microscope.

Kunming mice weighing approximately 20 g were denuded using a mixture of Na_2S (5 %) and starch, immediately followed by daubing camphor ice to avoid further skin erosion. After resting 24 h, the mice were anesthetized with an intraperitoneal injection of 150 μL ethyl carbamate (20 mg·mL^{-1}) and then immobilized in a Lucite jig. Afterwards, Lipid-CdHgTe was injected (10 μg·g^{-1}) through the tail vein. And then NIR imaging was performed in a dark room and the excitation light intensity of laser from the fiber bundle was adjusted to 26 mw. The images were collected at 10 s, 5 min, 10 min, and 20 min post-injection.

Construction of FRET between CdTe QDs and CdHgTe QDs in lipid bilayer

Efficient FRET interactions require that two key criteria are satisfied: (1) donor and acceptor molecules must be in close proximity (typically 1–10 nm); and (2) the absorption spectrum of the acceptor must overlap with the fluorescence emission spectrum of the donor [14, 15]. In this study, the FRET system was established between CdTe and NIR CdHgTe QDs by adjusting the concentration, given that embedding them in lipid bilayer can reduce the distance between them in the nanometer scale. The FRET efficiency and the distance between the donor and the acceptor were then calculated.

Results and discussion

Characterization

The typical TEM images (Fig. 1a and b) showed that CdTe and CdHgTe QDs were uniform in size, averaging around 3–4 nm, and suitable for the lipid bilayer thickness. Figure 1c shows spherical morphology and homogeneous distribution of the lipid-CdTe. Liposomes are hollow lipid vesicles and their membranes are hard to observe via TEM unless the liposomes are negatively stained using a special reagent. Therefore, the dark spots suggested that the lipid-QDs were successfully synthesized. No free CdTe QDs dispersed in the field of vision. An image with higher magnification (Fig. 1d) clearly revealed that the 3-nm QDs (dark spots) were randomly distributed in the vesicle's lipid bilayer. Figure 1e shows that the lipid-QDs were well-proportioned with an average size of approximately 270 nm. The vesicles also had a narrow size distribution that was characteristic of solvent evaporation methods commonly used to prepare liposomes. Figure 2a, b depict fluorescence micrographs of lipid-QDs vesicles encapsulating CdHgTe and CdTe, respectively. Both types of lipid-QDs exhibited an intense fluorescent signal from the QDs present in the lipid bilayer. The results demonstrated that both the CdTe and NIR CdHgTe QDs in the lipid bilayer maintained good performance in terms of photoluminescence, which clearly revealed that the fluorophores were encapsulated in the liposomes.

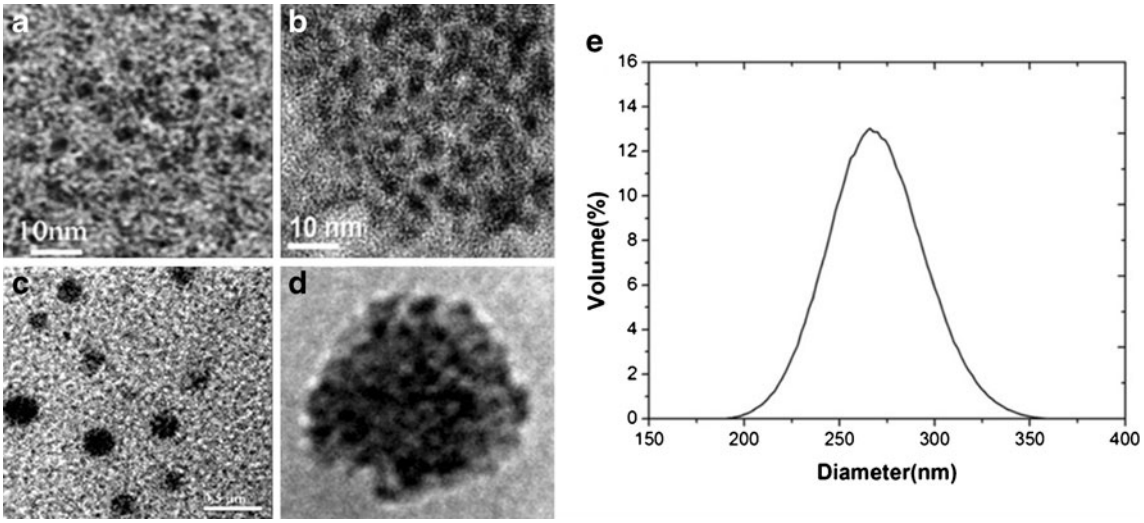

Fig. 1 TEM images of the prepared CdTe (**a**), CdHgTe (**b**) QDs and lipid-CdTe (**c**, **d**) and size distribution (**e**) of lipid-CdTe (at a CdTe concentration of 100 μL·mL^{-1})

Fluorescence properties of the QD-containing liposomes

Figure 3 shows the emission spectra of the original QDs and the liposome-coated QDs at the 370 nm excitation wavelength. The spectral characteristics of lipid-QDs were not dramatically different than those of bare QDs, except that the fluorescent intensity of lipid-QDs was lower than that of QDs alone (for the same total concentration of QDs) and the peak shifted slightly towards the red emission wavelengths. The peak emission spectrum shifted from 556 nm for original CdTe QDs to 562 nm, whereas that of the CdHgTe QDs was in the 655 nm to 660 nm range. These changes in QD optical properties upon incorporation with the liposomes were thought to be due to interaction between the entrapped QDs and the lipid bilayer.

Photostability investigation

In order to confirm that QD fluorescence was preserved after incorporation into the lipid bilayer and that the lipid-QD bilayers were fluorescent, the photostability of QDs was investigated. Interestingly, incorporation of QDs within the lipid bilayer to form lipid-QD vesicles led to enhanced photostability compared to that of bare QDs (at the same concentration). As shown in Fig. 4a and b, after 12 h of exposure to air at room temperature there was no significant photobleaching when the QDs were embedded within the lipid bilayer, compared to nearly 20 % loss of fluorescence intensity in the case of bare QDs. Furthermore, the effect of UV radiation was studied by exposure of QDs and lipid-QDs to a UV light source for 10 h after preparation. The bare QDs were photochemically unstable when exposed to UV light, as evidenced by a sharp reduction in fluorescent intensity (Fig. 4c and d). On the other hand, the lipid-QDs exhibited improved photostability after 10 h of UV exposure and only 20–30 % reduction of the initial fluorescence intensity was observed. These results indicate that the protection provided by the lipid bilayer could effectively prevent photo-oxidation of QDs while in storage and also protect against UV light exposure, which can be attributed to the tight packing of QDs within the lipid bilayer. The increased photostability of liposome-encapsulated QDs might be expected to improve their performance as fluorescence markers.

Biological fluorescence imaging for living cells and NIR imaging in vivo

To study the compatibility and interaction of novel lipid-QDs vesicles with the biological milieu, the lipid-CdTe and

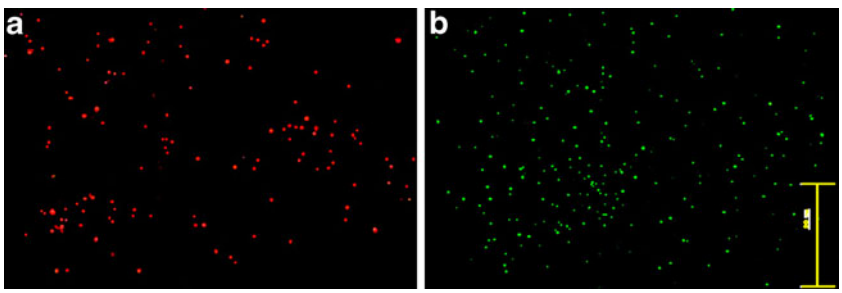

Fig. 2 Fluorescence micrographs of liposomes encapsulating CdHgTe (**a**) and CdTe (**b**) QDs (at a CdTe concentration of 100 μL·mL^{-1})

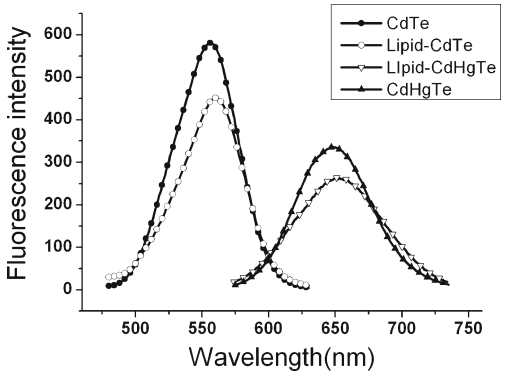

Fig. 3 Fluorescence spectra of the QDs before and after encapsulation in liposomes with the same concentration (4.7×10^{-6}mol·L^{-1})

lipid-CdHgTe were incubated at 37 °C with live human breast cancer cell (MCF7) cultures for 120 min. The cellular uptake was investigated using confocal laser scanning microscopy (CLSM). Figure 5a and b depict CLSM images of MCF7 cells with liposomes encapsulating CdTe and CdHgTe QDs, respectively. As shown, bright fluorescence was observed in the cells, which suggested uptake of fluorescent vesicles by the cells. Both types of QDs exhibited intense fluorescent signal, indicating that the living cells were successfully labeled and that the fluorescence of the probes was not quenched in the interior of cells. The control experiments using bare QDs were also performed. The negative charge on the thiol terminals of bare QDs limited cellular uptake, thereby hindering their potential application in cell imaging. Fluorescence signal can not be found in MCF7 cell membranes within 30 min after incubation with bare CdTe and CdHgTe QDs (image not shown). After 120 min, the fluorescence was observed in the cells but not bright (Supplementary Figure S1). Moreover, there were free CdTe QDs dispersed in the field of vision. This liposome system increased biocompatibility and stability of QDs, thus improving the imaging effects for cancer cell labeling. The lipid-QDs were efficiently internalized by MCF7 cells in a time-dependent manner. Fluorescent signal was found in MCF7 cell membranes within 30 min after incubation (Fig. 5c). Within 60 min, the vesicles were bound to the cell membrane and no fluorescence was observed in the cytosol (Fig. 5d). After 120 min incubation, as shown in Fig. 5e, lipid-CdTe vesicles were

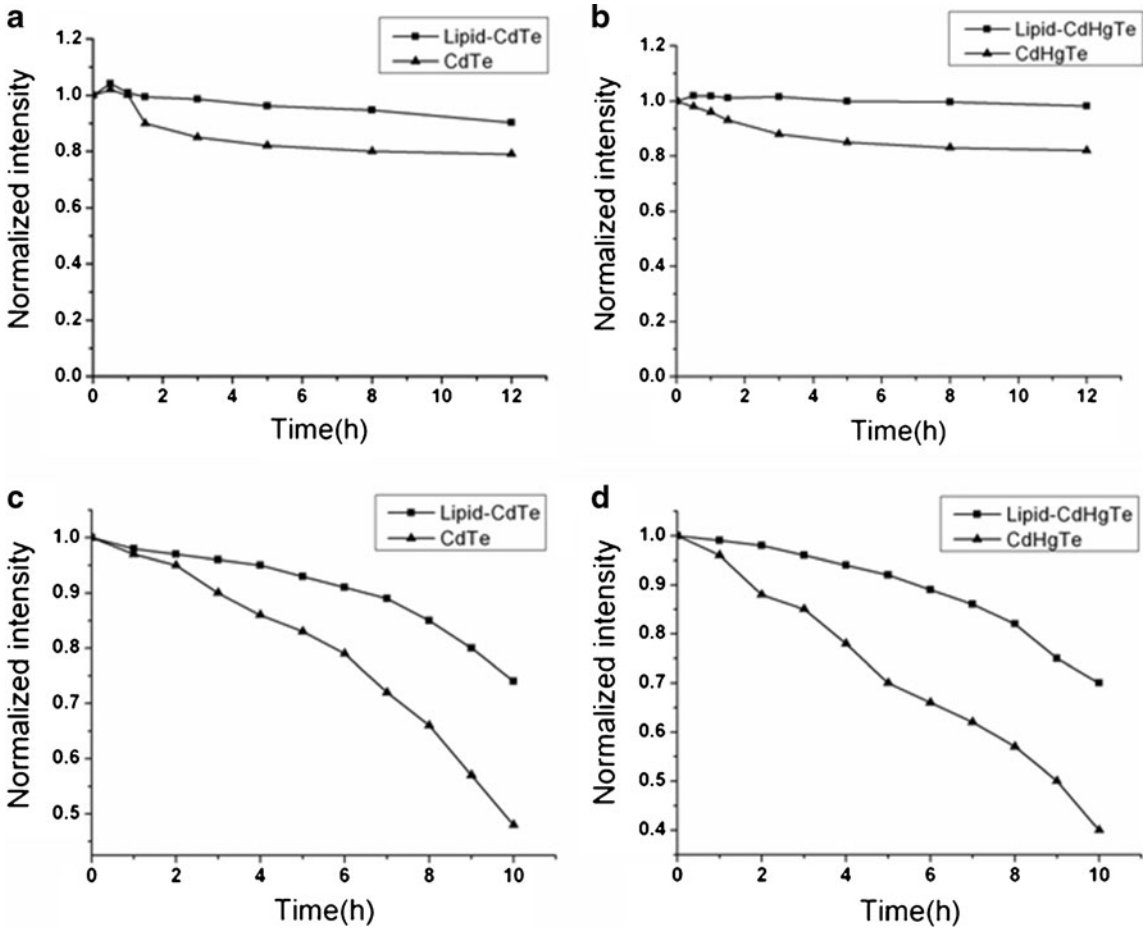

Fig. 4 Comparison of optical stability of QDs and lipid-QDs (at the same total QD concentration of 4.7×10^{-6}mol·L^{-1}) during storage (**a**, **b**) and exposure to UV irradiation with 365 nm (**c**, **d**)

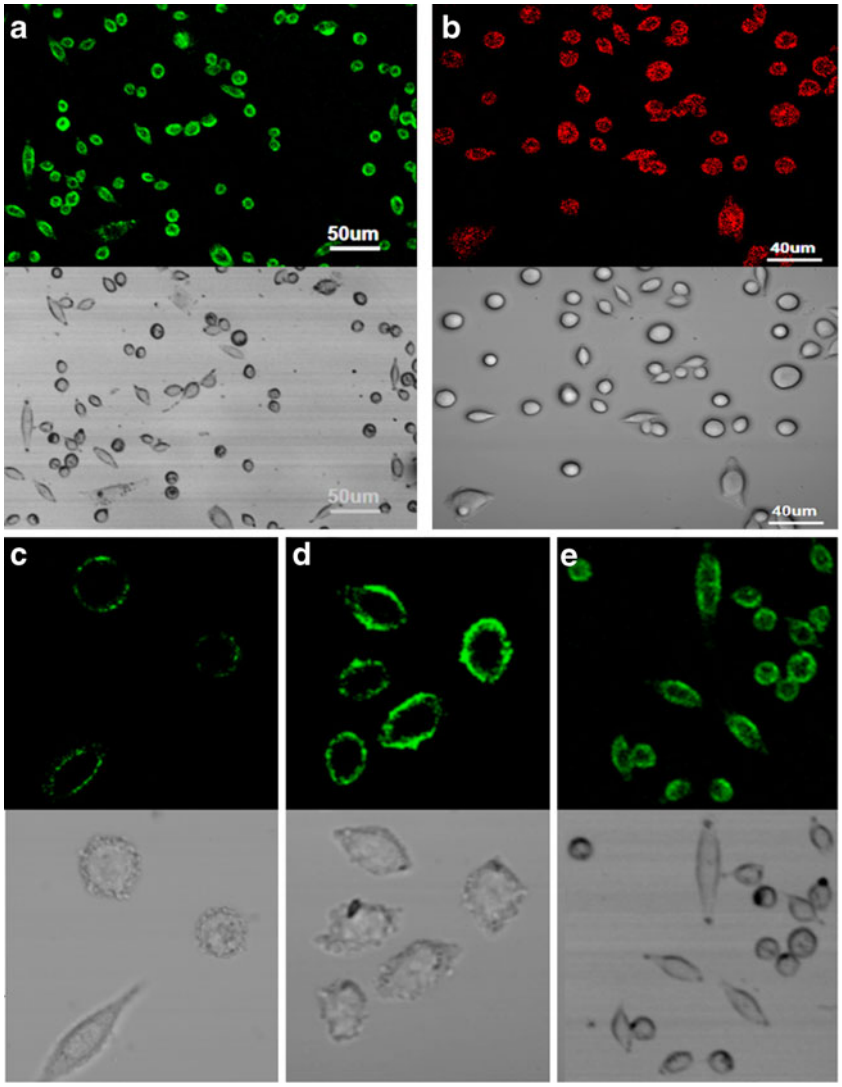

Fig. 5 CLSM bright field and fluorescence images of MCF7 incubation with lipid-QD after different time in vitro (at the QD concentration of 2 μmol·mL^{-1}, (**a**): lipid-CdTe 120 min, (**b**): lipid-CdHgTe 120 min, (**c**): lipid-CdTe 30 min, (**d**): lipid-CdTe 60 min, (**e**): lipid-CdTe 120 min)

capable of intracellular trafficking and could be imaged throughout the entire cell.

A major factor limiting in vivo use of QDs is the putative toxicity. Heavy metal ions such as Cd^{2+} released from unmodified QDs are potentially toxic to living cells. Surface coating of QDs, however, seems to prevent direct contact between QDs and the cells. In our work, the lipid-QD vesicles did not show any cytotoxic effects after 120 min of incubation with living cells and no morphological change of the cells was observed during the incubation period. All these findings suggest that the lipid-QD vesicles are biocompatible with living cells, and capable of cellular uptake as well as intracellular trafficking.

To demonstrate the capability of in vivo imaging, the NIR CdHgTe QDs were used for imaging in living animals. The NIR light can offer great advantages for deep tissue imaging applications because autofluorescence and absorbance from tissue-intrinsic chromophores reach their minima in this range. In addition, the NIR light possesses the capability of penetrating living tissues several centimeters in depth. As a result, biosensing in the NIR region is attracting increasing attention [16, 17]. Figure 6 represents a series of in vivo images taken by the NIR imaging system at different time intervals after lipid-CdHgTe vesicle injection. When we used bare CdHgTe, the fluorescence signal was very weak (Supplementary Figure S2). After 10 μg·g^{-1} of lipid-CdHgTe was injected to the mouse through the tail vein, fluorescence images were acquired at 10 s, 5 min, 10 min and 20 min. In the imaging process, fluorescence signal was observed immediately after lipid-QD injection (10 s post injection), as shown in Fig. 6a. Figure 6b reveals that the liposome distributed all over the vessels via the circulation, which resulted in strong fluorescence signal throughout the entire body. Liver tissue exhibited particularly strong fluorescence. Fluorescence signal was observed to accumulate in liver and spleen within 10 min, as can be seen in Fig. 6c and d, at which point the signal decreased sharply from the rest of the body. The images showed that within 20 min of

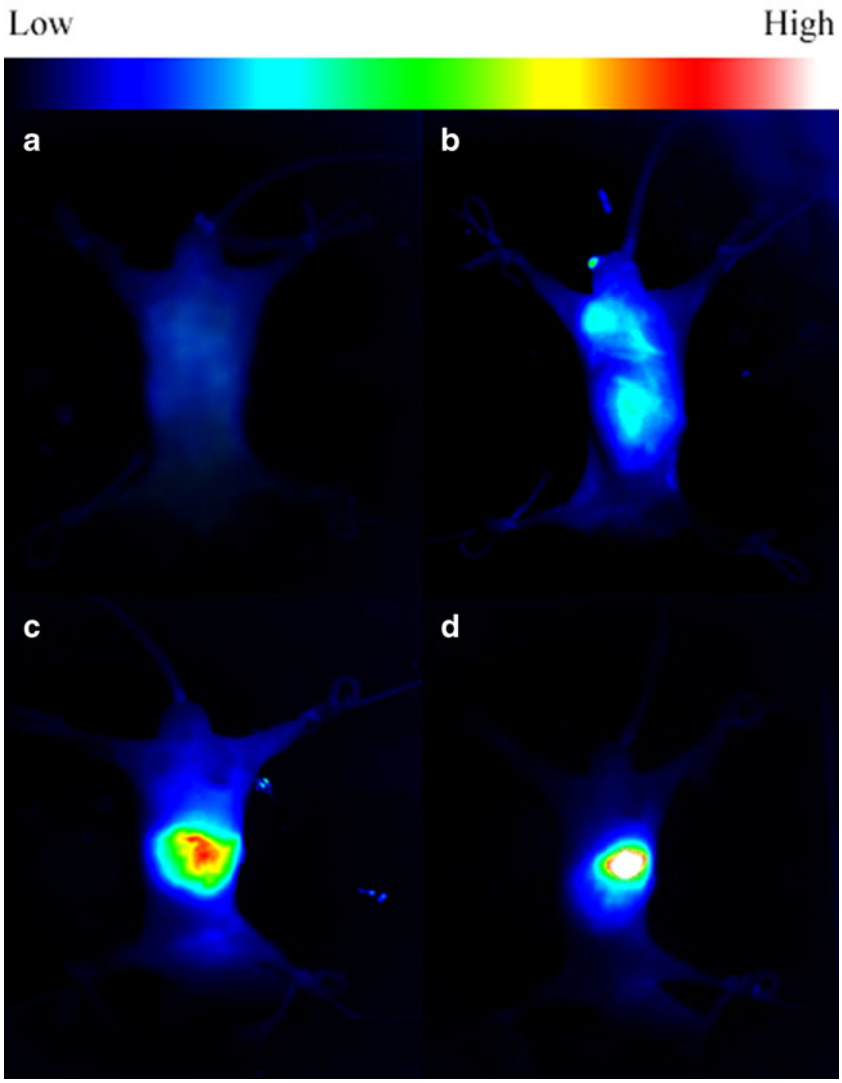

Fig. 6 NIR images of the denuded mouse after lipid-CdHgTe was injected (10 μg·g^{-1}) via tail vein. (**a**) to (**d**) represent fluorescence image of the mouse after injection for 10 s, 5, 10 and 20 min, respectively

the injection, QD-loaded liposomes were taken up mainly in the liver and spleen by macrophages of the reticulo-endothelial system. Additionally, the injection of 10 μg·g^{-1} lipid-CdTeHg did not appear to induce significant toxicity. No abnormal behavior was observed in any of the in vivo mouse imaging experiments. This suggests that lipid-QDs maintain an acceptable fluorescence signal in vivo and might serve as ultrasensitive probes for in vivo multiplex imaging.

FRET between two types of QDs in lipid bilayer

In this study, the FRET system was established between CdTe and NIR CdHgTe QDs, given that embedding them in lipid bilayer can reduce the distance between them. Figure 7 illustrates the absorption and emission spectra obtained from bare CdTe (line a and b) and CdHgTe (line c and d) QDs. The maximal absorption and emission peaks of the CdTe QDs were at 528 nm and 556 nm, while those of CdHgTe QDs were at 594 nm and 655 nm. There was appreciable overlap between the emission spectrum of the CdTe QDs (donor) and the absorption spectrum of the NIR CdHgTe QDs (acceptor), indicating that the energy transfer from donor to acceptor occurred with high probability. To minimize the contribution of direct excitation to acceptor

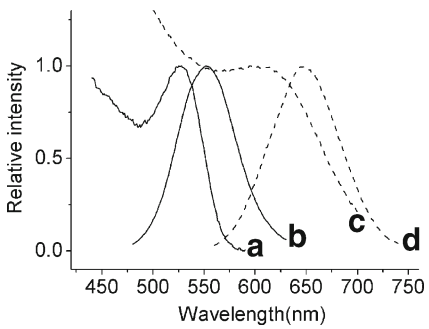

Fig. 7 Normalized absorption and emission spectra of CdTe QDs (line **a** and **b**) and CdHgTe QDs (line **c** and **d**)

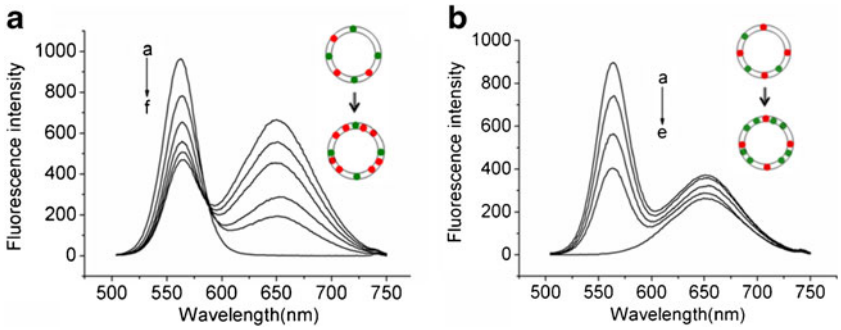

Fig. 8 **a** Fluorescence quenching of CdTe QDs: the volume of CdTe QDs was 200 μL and CdHgTe QDs were 0, 100, 150, 200, 250, 300 μL (from **a** to **f**); **b** Fluorescence enhancement of CdHgTe QDs: the volume of CdHgTe QDs was 150 μL and CdTe QDs were 200, 150, 100, 50, 0 μL (from **a** to **e**)

fluorescent intensity, the FRET measurements were performed by exciting the donors at 370 nm.

The fluorescent intensity of the FRET system could be adjusted by changing either the concentration of the donor or the acceptor. In our work, the change of fluorescence spectra of lipid-CdTe/CdHgTe with gradually increasing quantities of CdHgTe QDs and a fixed amount of CdTe QDs was studied. As expected, a significant enhancement of acceptor fluorescence intensity and the corresponding quenching of the green donor emission were observed when increasing the volume of acceptor from 0 to 300 μL as shown in Fig. 8a, which confirmed the process of FRET.

To further investigate the FRET process, the change of fluorescence spectra was studied by gradually increasing the quantity of CdTe QDs while keeping the amount of the CdHgTe QDs fixed. As shown in Fig. 8b, changing the volume of donor from 0 to 200 μL, a gradual enhancement of acceptor fluorescence intensity was observed, which indicates that a FRET process occurs between CdTe QDs and CdHgTe QDs in lipid bilayer.

The FRET efficiency and the distance between the donor and the acceptor were also calculated. According to Ref [18, 19], the FRET efficiency could reach 51 % (at a CdTe QDs concentration of 4.7×10^{-6} mol·L^{-1} and CdHgTe QDs 7.1×10^{-6} mol·L^{-1}). And the distance of the donor–acceptor pair was found to be about 8 nm [19, 20], which was smaller than 10 nm, a criterion value for energy transfer phenomenon to occur, indicating that the energy transfer from CdTe QDs to NIR CdHgTe QDs occurred with high probability. According to Forster's theory, effective FRET interactions require that donor and acceptor molecules must be in close proximity [14]. As a powerful technique for probing short distance-dependent interactions between the donor and the acceptor, the strong distance dependence of FRET efficiency has been widely exploited in studying the structure of proteins and nucleic acids, antigen-antibody interactions, detection and visualization of intermolecular associations, and the development of intermolecular binding assays [21–23]. The unique characteristics of QDs could enhance FRET as an effective technique for providing a fast, sensitive and non-destructive measurement. Moreover, the characteristic absorption bands in the visible to NIR region make 655 nm-emitting hydrophilic CdHgTe QDs a suitable acceptor for the FRET system, especially in NIR region. Although many QD-based FRET systems have been reported and widely used, most of them were in visible region. The main challenge of moving a QD-based FRET system from the visible to NIR window involved finding a suitable donor–acceptor pair. Therefore, we formed a FRET system between 556 nm-emitting CdTe and 655 nm-emitting CdHgTe QDs because embedding them in lipid bilayer via phase transfer reagent reduced the distance between them. The stable fluorescent signals from lipid-QDs in living cells and NIR imaging in vivo indicate that the NIR FRET system might be expected to be a promising candidate as FRET-based nanosensor for investigation of drug-loaded liposome releasing behavior and distribution detection in vivo.

Conclusion

In the present work, novel and highly luminescent QD-loaded liposomes were synthesized via a fast and convenient method. The synthesis of hydrophilic QDs was simple, low cost and less polluting. Therefore, the critical factor for QD-loaded liposomes synthesis involves the transfer of hydrophilic QDs. The long hydrocarbon chain in CTAB makes the resulting CTAB-coated QDs soluble in chloroform. The protection provided by the lipid bilayer could effectively prevent QDs from photo-oxidation during storage and when exposed to UV light. Showing bright fluorescence under the fluorescence microscope, the lipid-QDs were homogeneously distributed. QDs encapsulated by liposome complexes were stable, biocompatible and maintained fluorescent capacity in vitro and in vivo. We studied the ability of the fluorescent lipid vesicles to label living tumor cells in vitro and NIR imaging in vivo. The self-organized lipid-QDs with small sizes showed improved biocompatibility and were qualified for imaging living cells and mice, illustrating the great potential of NIR for bio-imaging in vivo.

In addition, we established a novel NIR FRET system between two types of QDs encapsulated in the lipid bilayer. The whole procedure for synthesizing lipid-QD-based FRET was simple, rapid and specific, and the energy transfer efficiency approached 51 % (at a CdTe concentration of 4.7×

10^{-6} mol·L^{-1} and CdHgTe 7.1×10^{-6} mol·L^{-1}). The NIR FRET system using two QDs could lead to novel and advantageous uses for FRET-based nanoprobes of lipid bilayer rupture, which lays the foundation for future liposome drug release studies. Moreover, with the aid of NIR CdHgTe QDs in liposomes, the probes are expected to be promising candidates for the investigation of drug-loaded liposome releasing behavior in vivo. Clearly, the potential use of QD-based FRET as a nanosensor for drug release has just begun to be realized and its application to pharmaceutical analysis is still in its infancy. Further work, such as the quantitative analysis of fluorescence intensity in NIR imaging in vivo, is ongoing in our laboratory.

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