Electronic Supplementary Information (ESI)

Synthesis of RGD-peptide conjugated near-infrared CdTe/ZnSe core-shell quantum dots for in vivo cancer targeting and imaging

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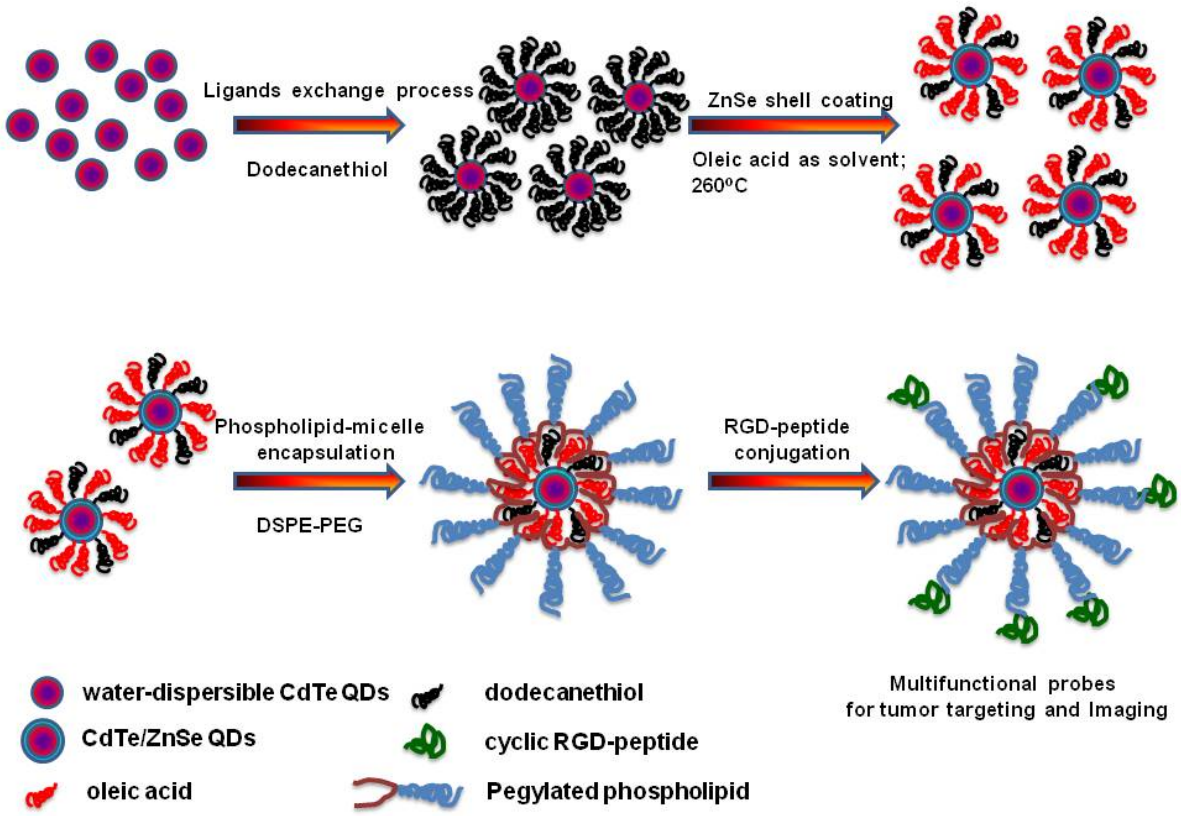
A. Synthesis of CdTe/ZnSe QDs

The hydrophilic moieties, mercaptopropionic acid, on the surface of CdTe QDs were replaced by dodecanethiol molecules to make them dispersible in chloroform. The particles were washed and cleaned with ethanol and centrifugation. The particles were then dried in vacuum as powder form for further use. Next, organically dispersible CdTe quantum dots solution was prepared by dissolving ~50 mg of CdTe QDs in ~4-5 mL of chloroform. Separately, 0.5 mmol of zinc acetate was dissolved in 10 mL of oleic acid. The reaction mixture was heated to 170^{o}C for ~30 minutes under an argon flow and then the CdTe QD solution was injected slowly under stirring into the hot reaction mixture. After ~10 minutes of heating, the reaction temperature was raised to 260^{o}C. Upon reaching the desire temperature, a 1 mL of TOP-Se was added drop wise into the reaction mixture. The reaction mixture was then held at ~260^{o}C for 2 hours. Next, an aliquot was removed via syringe and was injected into a large volume of toluene at room temperature, thereby quenching any further growth of the QDs. The QDs were separated from the surfactant solution by addition of ethanol and centrifugation.

In our experiments, we noted the some challenges in coating the CdTe QDs with ZnSe shell by aqueous phase synthesis strategy. The failure of shell formation was resulted from these factors: (i) formation of quality ZnSe shell generally takes place at very high temperature; (ii) free ZnO particles will potentially formed at high pH environment; and (iii) degradation of ZnSe shell during processing of QDs due to the instability of shell. In addition, using aqueous phase for ZnSe shell coating, complicated and tedious steps are needed to remove the un-reacted Zn precursor from the QDs solution since Zn-complexes will precipitate together with QDs that makes them very difficult to be separated using centrifugation method. Thus, we have chosen to coat the CdTe core with ZnSe shell in the organic phase.



Scheme 1. Schematic illustration of solution phase synthesis of multifunctional CdTe/ZnSe QDs as biocompatible luminescent probe for targeted cancer imaging.

B. Characterization of NIR CdTe/ZnSe QDs

The micelle-encapsulated CdTe/ZnSe QDs were systematically characterized by transmission electron microscopy (TEM), powder X-ray diffraction (XRD), UV-Vis-NIR, and photoluminescence spectroscopy.

C. Preparation of phopholipid-micelle coated QDs

The QD stock chloroform solution (~2 mg/mL), DSPE-mPEG (1,2-Diacyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)]) chloroform solution (~10 mg/mL), and DSPE-PEG-Maleimide (1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Maleimide(Polyethylene Glycol)], (Ammonium or sodium Salt)) chloroform solution (~10 mg/mL) were mixed together at a weight ratio of 1:4:1. The PEGylated phospholipids were purchased from Avanti polar lipids, Inc. and Laysan Bio Inc. Each mixture was gently stirred for few minutes. A Labconco rotory evaporator with a water bath of 25^{o}C was used to evaporate the organic solvent. The lipidic film, deposited on the reaction vial, was hydrated with 3 to 5 mL of HPLC water and subjected to ultrasonication for 10 to 20 minutes using a bath sonicator. The resulting dispersion was filtered through a 0.45 μm membrane filter and kept at room temperature for further use. To remove the excess phospholipids from the QD dispersion, the micelle-encapsulated QDs were further purified using centrifugation at 10000 rpm for 15 minutes. The QD precipitate was re-dispersed in 1 to 2 mL of HPLC water (the water was

filtered with 0.2 μm membrane filter). The colloidal stability of the QDs was evaluated using dynamic light scattering. No precipitation was observed after two weeks of storage at 4°C. In terms of optical stability, no PL intensity loss was detected for more than weeks.

D. Conjugation of micelle-encapsulated QDs with thiolated RGD

1 mL of micelle-encapsulated QD stock solution was mixed with 0.5 mL of 1.2 mg/mL thiolated RGD-peptide solution (molar ratio of Maleimide to RGD-peptide is 1:2) and gently stirred for 60 minutes. Next, the resulting bioconjugate dispersion was further purified using centrifugation at 10000 rpm for 15 minutes. The QD precipitate was re-dispersed with 1 mL of HPLC water and kept at 4°C for further use.

E. Generation of in vivo tumor model

Pancreatic tumor animal models were first created in the athymic female mice by injecting Panc-1 cells at a concentration of 2-3 x 10^6 cells in a 100 μL suspension of Matrigel and medium mixture (1:1) maintained at 4°C. This mixture was then injected subcutaneously in one scapular region of the mice using a 1 mL Monoject tuberculin syringe with a 25g x 5/8" detachable needle. Tumor growth was monitored every 24 – 48 hours until a tumor size of approximately 5 mm^2 was obtained. Once the tumors reached to the appropriate size, the mice were injected with the cRGD-peptide conjugated QDs formulation as prepared above by tail vein injection with a volume of 150-200 μL per injection (~0.5 mg of bioconjugated QDs per 200 μL). After injection, mice were anesthetized with Aerrane: isoflurane, at an induction concentration of 5% isoflurane/1L O_2. Anesthesia maintenance concentrations were 2-3% isoflurane/1L O_2. Once proper plane of anesthesia was reached, the mice were imaged using the Maestro in-vivo optical imaging system at specific time points to monitor the distribution of functionalized QDs in the tumor-bearing mice.

F. In vitro cytotoxicity study

Human pancreatic carcinoma cell line (Panc-1) was used as the cell model and incubated in a 96-well plate with QDs concentration ranging from 0.1 to 1 mg/mL. After the treatment of 24 and 48 hours, the cytotoxicity assay was performed by recording the absorbance at 490 nm with a scanning multiwell reader (Opsys MR Autoreader, Dynex Technologies Inc., Chantlly, VA, USA) after adding 20 μL of the MTS solution and incubating for another 2 hours. Measurements were corrected from background (DMEM media with MTS reagent). The cell viability was calculated as the ratio of the absorbance of the sample well to that of the control well and expressed as percentage viability, assigning the viability of non-treated cells in control well as 100%.

The results of MTS assay shown in Figure S1 indicated that the PEGylated micelle-encapsulated NIR QDs did not cause significant cytotoxicity to the tested Panc-1 cells within the tested concentrations ranging from 0.1 to 1 mg/mL. The average cell viabilities were between 90-110% when compared with untreated cells. The low toxicity of the PEGylated micelle-encapsulated QDs is within our expectation because the PEGylated-phospholipids are generally known for their biocompatibility in vivo. More importantly, PEGylated phospholipids micelles are FDA approved biomaterial for human cancers chemotherapy treatment. Therefore, it is not surprising that the toxicity of phospholipid micelle-encapsulated QDs is low and can be regarded as a useful nanoplatform for traceable targeted imaging and delivery in vivo.

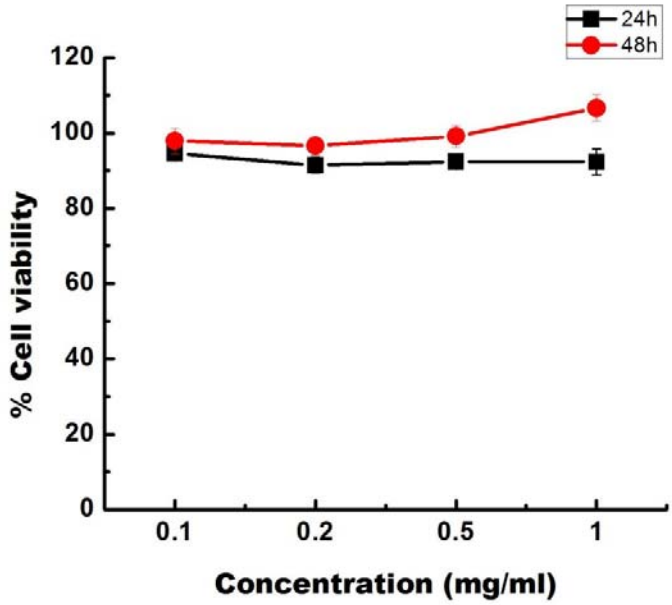


Figure S1. In vitro cell viability of Panc-1 cells treated with a series of concentrations of PEGylated micelle-encapsulated NIR QDs for 24 and 48 h, respectively. Percentage viability of the treated cells is calculated relative to that of untreated cells (with arbitrarily assigned 100% viability).

G. In vivo toxicity study

After treating the mice (n=5) with QDs formulation, the mice were dissected and major organs such as heart, liver, spleen, lung and kidney were harvested and rinsed with PBS and immobilized in paraffin for further hematoxylin & eosin (H&E) staining and pathological evaluation. No inflammation or damage was observed on the tissue sections.