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ORIGINAL PAPER

Fluorescence Imaging of Stem Cells, Cancer Cells and Semi-Thin Sections of Tissues using Silica-Coated CdSe Quantum Dots

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Abstract Trioctylphosphine oxide capped cadmium selenide quantum dots, synthesized in organic media were rendered water soluble by silica overcoating. Silanisation was done by a simple reverse microemulsion method using aminopropyl silane as the silica precursor. Further, the strong photoluminescence of the silica-coated CdSe quantum dots has been utilized to visualize rabbit adipose tissue-derived mesenchymal stem cells (RADMSCs) and Daltons lymphoma ascites (DLA) cancerous cells in vitro. Subsequently the in vivo fluorescence behaviours of QDs in the tissues were also demonstrated by intravenous administration of the QDs in Swiss albino mice. The fluorescence microscopic images in the stem cells, cancer cells and semi-thin sections of mice organs proved the strong luminescence property of silica-coated quantum dots under biological systems. These results establish silica-coated CdSe QDs as extremely useful tools for molecular imaging and cell tracking to study the cell division and metastasis of cancer and other diseases.

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Introduction

Semiconductor nanoparticles or Quantum Dots (QDs) posses superior optical properties compared to organic dyes, making them suitable for many biological applications, particularly in fluorescence cellular imaging [1, 2]. The conventional fluorescent tags mainly suffer from low photostability, poor quantum yield under biological conditions and interference from autofluorescence. The exceptional properties such as strong and long lived emission, broad excitation spectrum and size tunable emission, shown by QDs make them a potential substitute for organic dyes. Besides, QDs are better labelling agents in long-term imaging such as fluorescence marking of transport processes in cells and in tracking the path of single membrane-bound molecules [3, 4].

Although imaging of fixed cells is useful and sufficient for many applications, live cell microscopy is ideal for visualizing cellular processes, but is considerably more difficult. It has been shown that many cell types naturally engulf QDs through a nonspecific uptake mechanism. This mechanism was used to track the migration of breast tumour cells on a substrate coated with red emitting QDs; the fluorescence with in the cells were increased due to the uptake of QDs, leaving behind a dark path [2, 5, 6]. QDs have already been applied to visualize sentinel lymph node in gastrointestinal tract [7], pleural space [8], lung area [9], esophageal area [10], skin [11], axilla [12–15], and bladder area [16] in rodents and pigs. These studies confirm that QDs have opened up a new avenue for investigating biomolecular processes inside the cells of tissues. As far

as we know, most studies are focused on cellular and cancer imaging applications and there is almost no report on silica-coated CdSe QDs for stem cell imaging, cancer cell imaging and in vivo tissue imaging applications.

In current work, we report the in vitro cellular imaging studies, using rabbit adipose derived mesenchymal stem cells (RADMSCs) and in vitro cancer cell imaging studies with Daltons lymphoma ascites (DLA) cells. We have also performed the fluorescence imaging of semi-thin sections of tissues at the peak distribution time period, after the intravenous injection of silica-coated QDs into the Swiss albino mice. The obtained results provide useful indications for optimization of the diagnostic procedure for cancer and other diseases.

Materials and Methods

Preparation of Silica-Coated CdSe QDs

QDs were synthesized by following a reported procedure [17, 18] Cadmium oxide (0.067 g, 0.52 mmol), dodecylamine (3.8 g, 20.72 mmol), trioctylphosphine oxide (2.7 g, 6.9 mmol) and tetradecylphosphonic acid (0.40 g, 1.44 mmol) was heated to 300 °C under vacuum, until CdO dissolves completely to produce an optically clear solution. At this temperature, an injection mixture containing trioctylphosphine (5.2 mL) and Se (0.083 mmol) was introduced. After desired crystal growth, the reaction was arrested by reducing the reaction temperature down to ambient conditions. The QDs obtained were purified by reprecipitation with methanol and redispersed in dry chloroform for silica overcoating.

For overcoating CdSe QDs with silica, we modified reported procedure [19] as follows; A mixture of TOPO capped CdSe QDs in chloroform (400 mL, ~14 μM) and aminopropyl silane (0.075 g, 0.36 mM) was vortexed for 30 min, in an inert atmosphere. This mixture was added to Igepal CO-520 (1.3 mL) in cyclohexane (10 mL) and stirred for 30 min under dry conditions. Ammonia solution (150 μL, 33 wt %) was added drop wise and the stirring was continued for 1 day. The silica-coated QDs were purified by washing repeatedly with dry chloroform and redispersed in PBS buffer (pH 7.3). Dots were stored in dark at room temperature and the emission stability was investigated as a function of time, pH and ionic strength of the medium.

Characterisation of Silica-Coated CdSe QDs

The electronic absorption spectra were recorded on a Shimadzu Model UV-3101 scanning spectrophotometer; emission spectra were collected using SPEX-Fluorolog F112X spectrofluorimeter. For HRTEM studies, a drop of nanoparticle solution was placed on a carbon coated Cu grid and the solvent was allowed to evaporate. Specimens were imaged on a FEI Tecnai G^2 S-TWIN 300 kV high resolution transmission electron microscope.

Materials and Animals

For cellular imaging experiments, all chemicals and reagents used were purchased from Sigma-Aldrich, USA. The RADMSCs and DLA cells were maintained at 37 °C and 5% CO_2 in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% fetal bovine serum and antibiotics. The animal models used for the in vivo fluorescence study (Normal male Swiss albino mice of 6 weeks old, weighing 20-25 g) were from the animal house facility, Department of Biochemistry, University of Kerala. The animals were housed in an accredited, pathogen free, environmentally controlled facility (25±1 °C), and food and water were provided ad libitum. For maintaining the experimental animals, the institutional ethical guidelines were absolutely followed as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) rules, Government of India [Sanction No: IAEC-KU-9/06-07/BC-AA(8)(ii)]. The animals were weighed weekly and the food and water consumption were recorded.

In Vitro Cellular Imaging

RADMSCs were cultured on coverslips, and the medium was changed every 2 days, till 85% cell confluence was achieved. Then the cells were incubated at 37 °C with 5% CO_2 for 3 h after adding with silica-coated CdSe QD solution (100 nM) in PBS. After incubation, the coverslips were taken out, rinsed thrice with 37 °C pre-heated PBS, fixed with 3.7% paraformaldehyde and fluorescence cellular images were observed with a DM 6000 fluorescence microscope (Leica, Germany, 20x objective, equipped with DFC 300 FX digital camera.), with 488 nm argon laser to excite the QDs. Morphological examination of RADMSCs were carried out by phase contrast microscopy (Leica DM IL, Germany).

In Vitro Cancer Imaging

For cancer cellular imaging study, Daltons Lymphoma Ascites (DLA) cells were procured from Amala Cancer Research Centre, Thrissur, India and maintained in the peritoneal cavity of mice. Approximately 2 weeks were taken for the development of tumor in the peritoneal cavity and matured cells were aspirated from the peritoneum, washed with PBS and seeded on coverslips. Then, silica-coated CdSe QD solution (100 nM) was added to the cells.

Then cells were incubated at 37 °C with 5% CO_2 for 3 h. After incubation, the coverslips were taken out, rinsed thrice with 37 °C pre-heated PBS, fixed with 3.7% paraformaldehyde and fluorescence images were observed with a fluorescence microscope. Morphological examination of DLA cells were carried out using phase contrast microscope.

In Vivo Cellular Imaging

To study the fluorescence behaviours of the silica-coated CdSe QDs in vivo, the mice were injected with 100 μL of QDs in PBS buffer (10 nM/mouse) through the tail vein. The peak distribution time of the major organs (liver, kidney and spleen) were obtained utilizing an Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES, Optima 5300 DV instrument, Perkin Elmer, USA). At the peak distribution time intervals, the animals were euthanized by intraperitoneal injection of sodium pentothal. During autopsy, tested organ samples were stripped from the experimental mice for determining the fluorescence. For this, all tissues were fixed in 3% glutaraldehyde, and 0.1 M sodium phosphate buffer (pH 7.4) for overnight at 4 °C. Fixed tissues were washed with 0.1 M sodium phosphate buffer three times for 5 min each, and post-fixed with 1% OsO_4 for 1 h at room temperature. The fixed tissues were dehydrated by the following ethanol series: 30% for 10 min, 50% for 10 min, 70% for 20 min, 80% for 20 min, 90% for 20 min, 95% for 20 min, and 100% for 60 min, and then twice in acetone for 30 min each. Infiltration was carried out first with 1:1 resin (Poly bed 812): acetone for 1 h, 2:1 resin: acetone for 1 h, pure resin for 1 h, and finally with pure resin overnight. Tissues were placed for overnight in an oven at 65 °C, for embedding. The embedded blocks were then trimmed and sectioned by ultra microtome (LKB; Bromma 2088 Ultratome® V, Stockholm, Sweden), to obtain 0.5–1 μm semi-thin sections of organs were prepared. For in vivo cellular imaging, these tissue sections were directly examined with a fluorescence microscope.

Results and Discussion

Several research groups have described the use of QDs for sensitive bioassays and cellular imaging in vitro and in vivo [1, 12, 20–22]. But many aspects of this approach need to be further optimized particularly for in vivo applications. Our preliminary in vitro investigations proved the cytocompatibility and stability of silica-coated CdSe QDs under biological conditions [23].This was attributed to the little leakage of toxic core constituents, because of the good encapsulation offered by silica shell. Overcoating with silica shell prevents escape of the core constituents, thereby decreasing toxicity effects and also the silica surface renders the QDs aqueous friendly [24–26].The objective of this study was to demonstrate that silica-coated CdSe QDs can be used as diagnostic agents for long-life cellular imaging, cancer imaging using fluorescence microscopy. For this, we have successfully synthesized and characterized, silica-coated water soluble CdSe QDs and its in vitro and in vivo fluorescence behaviours were systematically carried out.

Characterization of Silica-Coated CdSe QDs

The photophysical characterisation of TOPO capped and silica-coated CdSe QDs are given in the Fig. 1. Silica overcoating resulted in a considerable blue shift, both in the absorption and emission peaks. This is attributed to the suppression of shallow traps and enhancement in band edge excitonic recombination as the amino groups in aminopropyl silane passivates the CdSe surface more efficiently than TOPO. The silica-coated CdSe QDs showed an excitonic absorption at ~545 nm and emission maximum ~555 nm. The QDs were also characterised by High

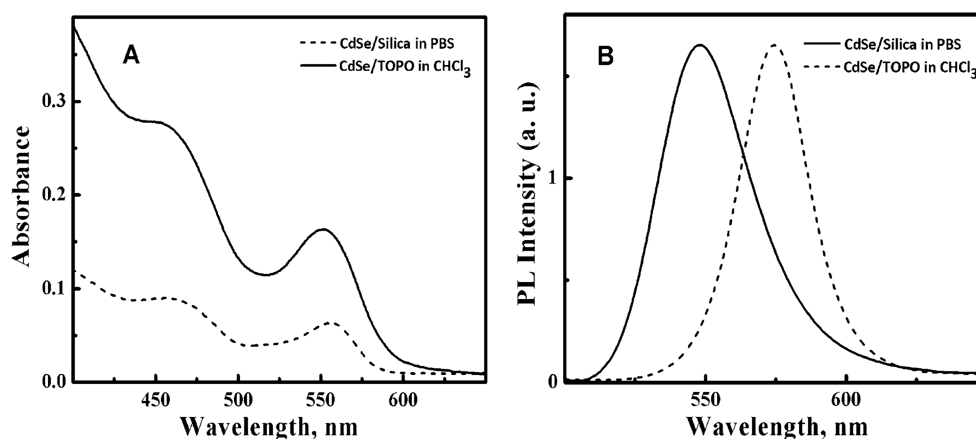

Fig. 1 Absorption (**a**) and emission (**b**) spectra of silica-coated CdSe QDs

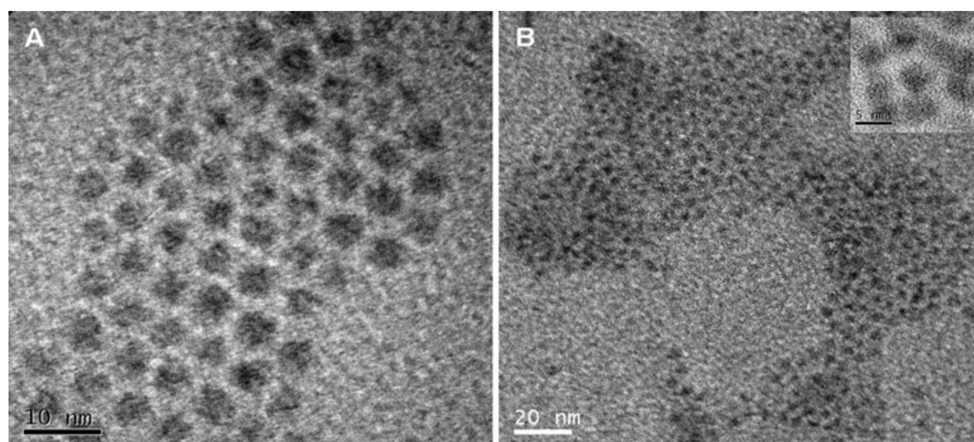

Fig. 2 HRTEM Images of (**a**) TOPO capped and (**b**) silica-coated CdSe QDs (Inset showing presence of very thin layer of silica shell)

resolution transmission electron microscope (HRTEM) and the results are shown in Fig. 2. TOPO capped QDs were found to be monodisperse with an average size of 4 nm. HRTEM images showed the presence of a thin silica shell (Inset Fig. 2b), and the overall size of QDs after silica coating was estimated as ~5.5 nm.

In Vitro Cellular Imaging

Stem cells offer an exciting new branch of therapy to treat a variety of conditions and diseases. It is therefore important to develop methods to monitor cell survival and location after transplantation. Owing to many advantages, we selected stem cells for cellular imaging experiments. Silica-coated CdSe QDs could effectively exhibit strong fluorescence in the stem cells (RADMSCs), which is known to in vitro florescence imaging, in the cytoplasm (Fig. 3b). Importantly, we did not observe any signs of morphological damage to the cells upon treatment with the silica-coated QDs (Fig. 3a). The observed an enhancement in fluorescence within cells and is attributed to the trapping of QDs in the endocytic intracellular vesicles [27]. Morphology of the cells in presence of QDs showed the good biocompatibility due to silica overcoating. Also, the strong luminescence proved that the electronic structure of QDs is not affected in the biological media. These results indicated that the silica-coated CdSe QDs might be a highly promising material for in vitro cellular imaging applications.

In Vitro Cancer Imaging

Figure 4b illustrates the fluorescence imaging of cancer cells (DLA) using silica-coated CdSe QDs. Uptake of QDs can be clearly observed from the robust fluorescence signal from the cells and was due to the internalization of QDs by endocytosis [2]. Phase contrast microscopic image shown the morphology of DLA cells, upon the treatment with the silica-coated QDs (Fig. 4a). Based on this result, it could be confirmed that the silica-coated CdSe QDs are very useful for in vivo cancer imaging using their relatively stable fluorescence emission.

In Vivo Cellular Imaging

On the basis of the promising in vitro cellular imaging results, the silica-coated QDs were further used for in vivo

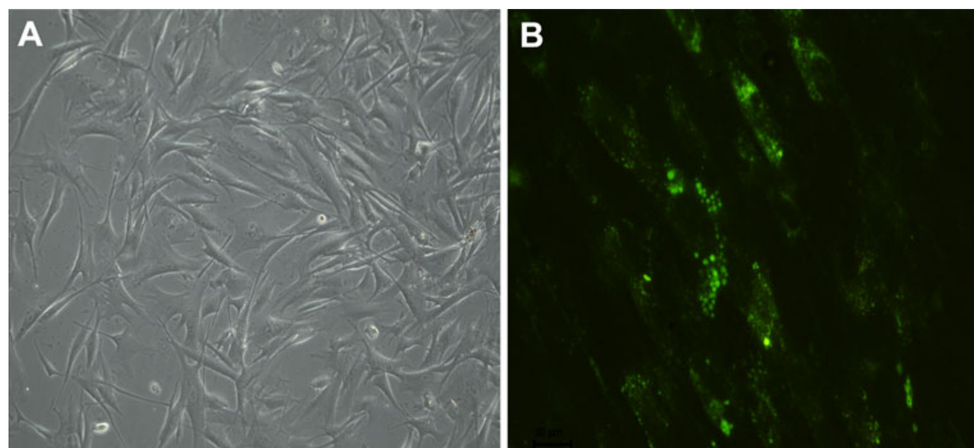

Fig. 3 In vitro fluorescence imaging of stem cells (RADMSCs) using silica-coated CdSe QDs. **a** Phase contrast microscopic image of RADMSC cells **b** Fluorescence microscopic image of RADMSCs

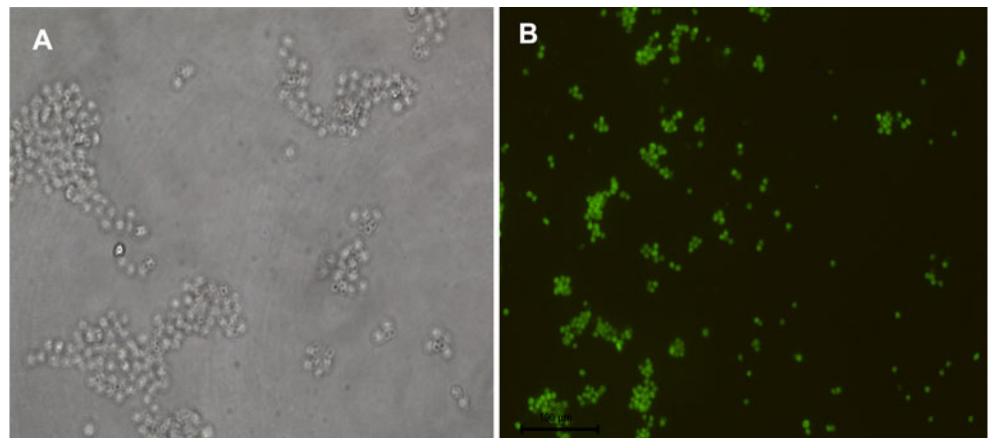

Fig. 4 In vitro fluorescence imaging of cancer cells using silica-coated CdSe QDs **a** Phase contrast image of DLA cells **b** Fluorescence microscopic image of DLA cells

cellular imaging experiments. For this systematic study, we used inductively coupled plasma optical emission spectroscopy (ICP-OES) to find the peak distribution time of silica coated CdSe QDs after intravenous injection in to Swiss albino mice. ICP-OES was used to measure the cadmium content of the organ samples collected at predetermined time intervals. The results showed that, peak concentration of QD accumulation occurred at 6 h in the major metabolic organs like liver, kidney and spleen (data not given). Liver is the organ of the reticuloendothelial system with the higher silica-coated CdSe QDs concentrations was observed after intravenous injection. QDs could be uptaken by the organs full of reticuloendothelial system, which was the reason that most of them were observed to be distributed in the liver and spleen. At the same time, the elements Cd were also found in the kidneys, which may be related to the elimination of QDs. Therefore, we have chosen this distributed time period for in vivo fluorescence study, we did not observe any fluorescence signal from control sections of liver, kidney and spleen at the preferred excitation wavelength. Interestingly, Fig. 5 illustrates the fluorescence microscopic images of semi-thin sections of liver, kidney and spleen showed stronger fluorescence intensity, and confirmed that this kind of silica-coated CdSe QDs might be a highly promising probe for cellular imaging and cell tracking application.

Conclusion

In summary, the TOPO capped CdSe QDs were made water soluble by over coating with silica, using aminopropyl silane as silica precursor. This water soluble fluorescent material showed essential properties required for both in vitro and in vivo cellular imaging applications. Silica-coated QDs were found to be robustly taken up by the stem cells (RADMSCs) and the cancer cells (DLA) in vitro, and exhibited strong luminescence in biological media, thereby highlighting their potential as luminescent probe for biomedical diagnostics. In addition, in vivo fluorescence study in semi-thin section of tissues also confirmed that these QDs are very useful for long-term imaging applica-

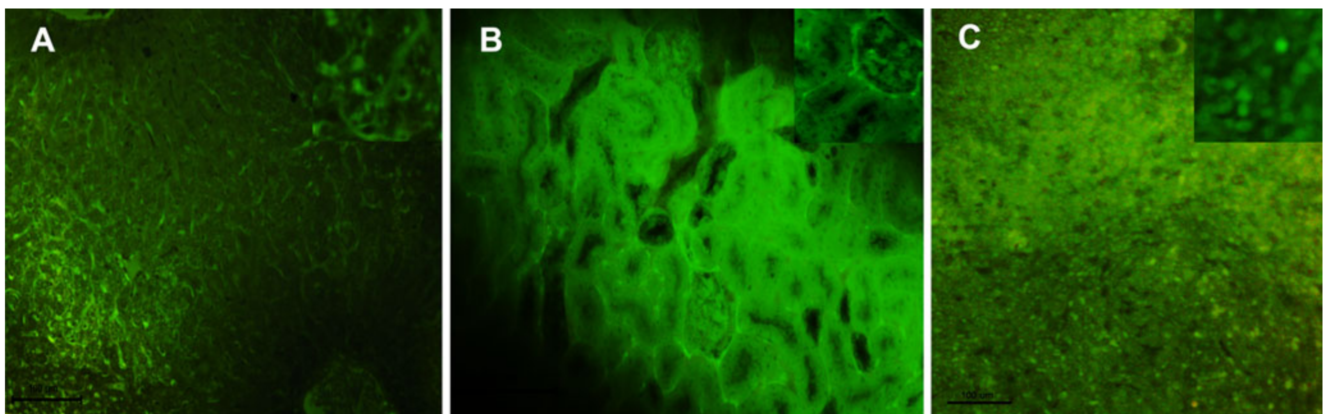

Fig. 5 In Vivo fluorescence imaging of tissues using silica-coated CdSe QDs **a** Liver **b** Kidney **c** Spleen (The images were obtained from semi-thin tissue sections on a fluorescence microscope). Insets (50x) image taken from same semi-thin section showing the strong single cellular level fluorescence

tions. Overall, this study implies that silica-coated CdSe QDs could be used as labelling agents for cancer cellular imaging and cell tracking applications for the study of cancer and other diseases.

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