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Toxicity Evaluation of Hydrophilic CdTe Quantum Dots and CdTe@SiO_2 Nanoparticles in Mice

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Quantum dots have drawn tremendous attention in the field of *in vitro* and small animal *in vivo* fluorescence imaging in the last decade. However, concerns over the cytotoxicity of their heavy metal constituents have limited their use in clinical applications. Here, we report our comparative studies on the toxicities of quantum dots (QDs) and silica coated CdTe nanoparticles (NPs) to mice after intravenous injection. The blood cells analysis showed significant increased level of white blood cells (WBCs) in groups treated with CdTe QDs as compared to the control while red blood cells (RBCs) and platelet counts were normal in treated as well as control groups. The concentration of biochemical markers of hepatic damage, alanine amino transferase (ALT) and aspartate aminotransferase (AST) were in the normal range in all the groups. However, renal function analyses of mice showed significantly increased in the concentration of blood urea nitrogen (BUN) and creatinine (CREA) in mice treated with CdTe QDs while remained within normal ranges in both the CdTe@SiO_2 NPs and control group. The results of histopathology showed that the CdTe QDs caused mild nephrotoxicity while other organs were normal and no abnormalities were detected in control and CdTe@SiO_2 treated group. These findings suggest that the nephrotoxicity could be minimized by silica coating which would be useful for many biomedical applications.

Keywords: Quantum Dots, CdTe@SiO_2, Cytotoxicity, *In Vivo*.

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1. INTRODUCTION

The unique optical properties of the Quantum dots (QDs) provide major advantages in fluorescence detection and imaging in molecular and cell biology such as luminescent probes and labels in biological applications, ranging from molecular histopathology, disease diagnosis, to biological imaging.[1–3] Compared to organic dyes they offer possibilities like multiplexed imaging and long-term investigations.[4, 5] The water soluble CdTe QDs have proved to be the choice of interest in biological labeling due to their biological compatibility.[6] Quantum dot surfaces have to be protected and functionalized to provide biocompatibility, biostability and suitable surface functions for biomedical applications.[7] The toxicity of QDs and nanoparticles continues to be investigated and debated,[8–10] a numerous of experiments have demonstrated that the modification of QDs' surface have a potent effects.[11, 12] Silica is preferred as an inert surface coating material mainly because it is non-toxic, stable, as well as biocompatible. Silanization of various semiconductor nanoparticles have shown outstanding superiority in protecting their surface characteristics.[13, 14] Silica coatings can preserve the encapsulated QDs from environmental factors which may affect the optical properties and stability.[15, 16] The common methods used for silica coating are Stöber and the microemulsion methods,[17] but there are still great challenges for preparing uniform and thin silica coating on individual nanoparticles.

Recently, several research groups have demonstrated the quantum dot and nanoparticles cytotoxicity *in vitro*.[18–26] The results of our previous studies showed that the CdTe QDs were much toxic to the HEK293 cells but the toxicity was much reduced after coating with silica. Herein, we evaluated the comparative toxicity of these nanoparticles in mice because a significant discrepancy exists between the conclusions drawn from *in vitro* and *in vivo*

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studies.[27–34] Therefore, it was imperative to study the cytotoxicity behavior of the CdTe@SiO_2 in mice and more importantly it provides the foundation for their practical application. Our results demonstrated that CdTe@SiO_2 nanoparticles were better biocompatible than CdTe QDs and expected to display great potential in applications such as *in vivo* molecular imaging and detection.

2. MATERIALS AND METHODS

2.1. Materials

Water for all reactions, solutions preparation was ultrapure water (18 $M\Omega \cdot cm^{-1}$) double distilled. All chemicals mentioned in the current investigations were used as received; cadmium perchlorate hydrate ($Cd(ClO_4)_2 \cdot 6H_2O$), Te powder (GuoYao chemistry agent corporation, 99.9%), Thioglycolic acid (TGA), tetraethyl orthosilicate (TEOS), *t*-octylphenoxypolyethoxyethanol (TritonX-100), glutaraldehyde, and 3-aminopropyltrimethoxysilane (APS) were obtained from Sigma–Aldrich.

2.2. Synthesis of CdTe QDs and CdTe@SiO_2 NPs

CdTe QDs and CdTe@SiO_2 NPs were prepared as described previously.[35] Briefly, the CdTe QDs were synthesized by dissolving 1.262 g of $Cd(ClO_4)_2 \cdot 6H_2O$ (3.01 mmol) into 160 mL of water, and then adding 0.371 g of TGA (3.91 mmol) in continuous stirring. The H_2Te gas was added with stirring followed by drop wise addition of 13 mL of 0.5 M H_2SO_4 into an oxygen-free flask containing 0.257 g (0.589 mmol) of Al_2Te_3. After about 20 min, the resultant solution was heated to reflux under open-air conditions. The resulting QDs were stabilized by adding 0.371 g of TGA (3.91 mmol) in continuous stirring. TGA capped CdTe QDs were modified with silica shell according using reverse microemulsion method,[35] based on the hydrolysis of TEOS onto the CdTe QDs. Finally prepared CdTe@SiO_2 were washed with PBS (pH 7.4), isolated, and then saved for usage.

2.3. Photophysical Characterization

Prepared CdTe@SiO_2 NPs were characterized using JEOL JSM-5910LV scanning electron microscopy (SEM), high-resolution transmission electron microscopy (HR-TEM, JEM2010, at 200 kV), the photoluminescence (PL) spectra (Perkin Elmer LS 55 spectrofluorimeter).

2.4. Animal Studies

The study complied with standards for the care and use of laboratory animals (Laboratory Animal Center of Southeast University, Nanjing China). Thirty female BALB/c mice (20 ± 1 g, 6–7 weeks old) were obtained from the Animal Center of Southeast University and were divided into three test groups each containing ten. Each group of mice was separately housed in positive pressure (25–30 °C, 50–60% relative humidity, 12/12 hours light/dark cycle) and fed a commercial diet while water was available *ad libitum*. To study the toxicity of the NPs *in vivo*, the mice in group 1 and 2 were injected with CdTe QDs and CdTe@SiO_2 NPs solution respectively via tail vein injection at the dose rate of 5 nmol/mouse,[36] (100 μL in total) while the mice in group 3 were injected with 100 μL normal saline. The QDs, NPs and normal saline were sterilized before injection.

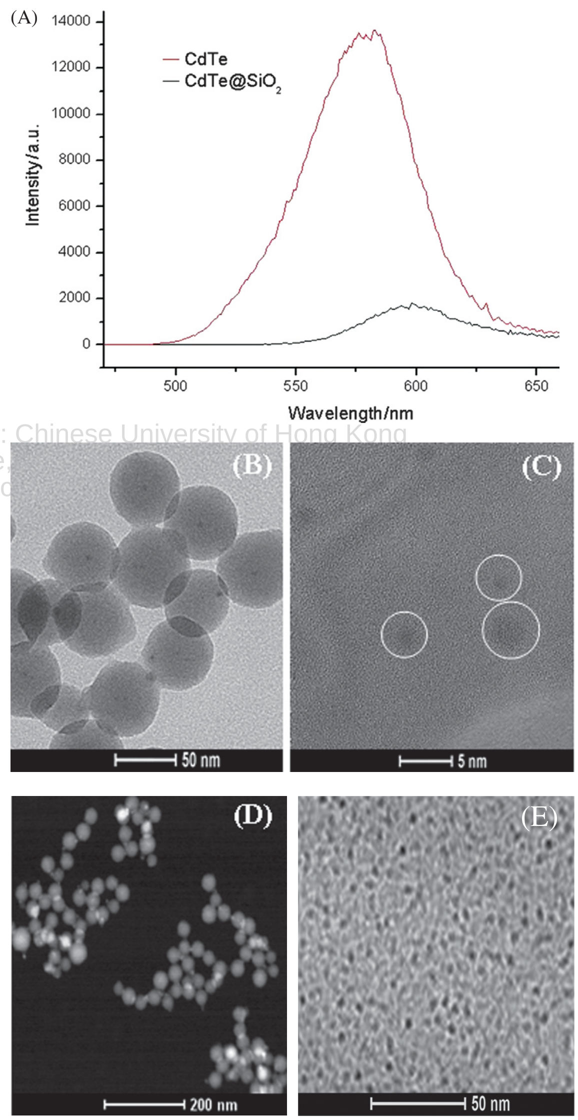

Fig. 1. (A) Photoluminescence spectra of CdTe Qds and CdTe@SiO_2 NPs. (B) TEM images of CdTe@SiO_2 NPs at 50 nm (B) magnification, HRTEM images of the CdTe QDs inside the silica shell (C). The white circle in each picture indicates a single CdTe QDs. The scanning transmission electron microscope (SEM) image of the silica coated CdTe QDs (D). TEM images of CdTe quantum dots (E).

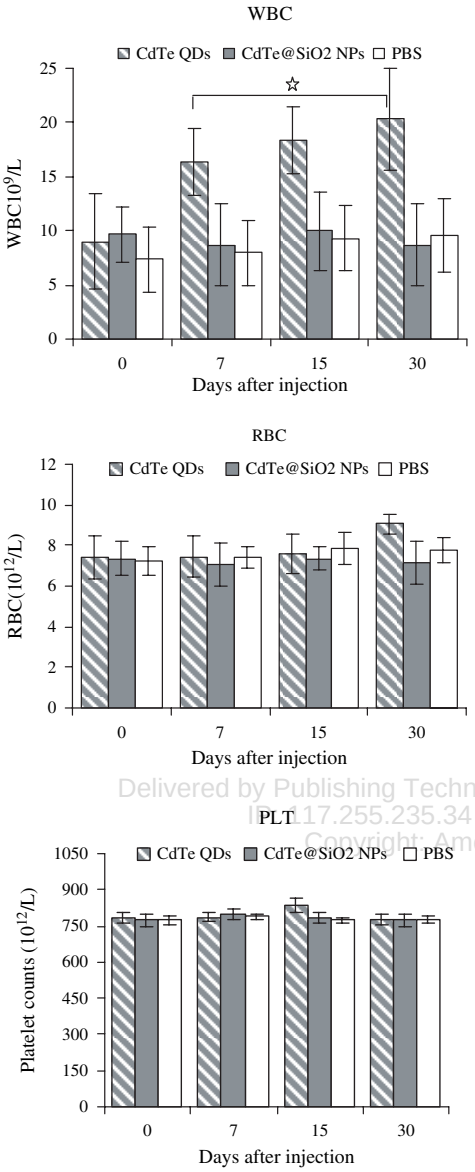

Fig. 2. Blood cell counts of mice. White blood cells (WBCs), and red blood cells (RBCs) Platelet counts (PLT) of experimental mice were measured at 0, 7, 15, 30 days after intravenous injection of CdTe QDs, CdTe@SiO_2 NPs and PBS. An asterisk indicates the WBCs were significantly higher than that in control group ($P < 0.05$). Data are shown as mean ± S.D. for three mice per group. While no significant difference was noted in PLT and RBCs counts of the treated and control groups.

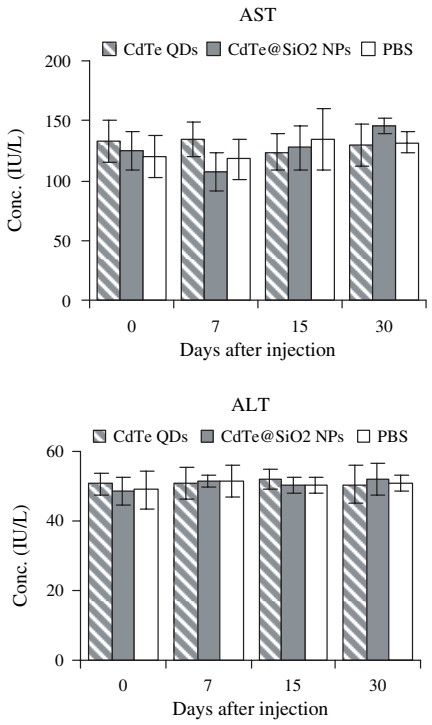

Fig. 3. The plasma levels of enzymes, AST, ALT (the marker of liver function) of mice measured at 0, 7, 15, 30 days after intravenous injection of CdTe QDs, CdTe@SiO_2 NPs and PBS. Data are shown as mean ± S.D. for three mice per group.

2.5. Blood Analysis

Urine and blood samples were collected at the time intervals of 0, 7, 15, 30 days post inoculation (pi) and the body mass, temperature, exploratory behavior and appearance were recorded at the same time. Venous blood samples were collected in evacuated tubes containing EDTA, sodium citrate anticoagulants and non-anticoagulant agents using a standard sphenoid vein blood puncture from each group ($n = 3$) and were analyzed for standard hematology markers: red blood cell count (RBC), white blood cells count (WBCs), platelet counts (PLT). Liver function was evaluated with serum levels of alanine amino transferase (ALT), aspartate aminotransferase (AST). Nephrotoxicity was determined by blood urea nitrogen (BUN) and creatinine (CREA). These parameters were assayed using a Hitachi 7600 Automatic Biochemical Autoanalyzer.[27, 29]

2.6. Histopathology of Tissues

The histopathology of the tissues was performed as described previously,[37] with few modifications. The mice were killed at 30 days post treatment and their organs such as liver, spleen, kidneys, lungs (section 10 cm in length) and brain were collected, weighed and fixed with 10% buffered formalin. Haematoxylin and eosin-stained histological sections of the fixed organs were observed with light microscopy at ×400 magnification.

2.7. Statistical Analysis

The results were presented as mean ± SD. Statistical differences were evaluated using the t test and considered significance at $P < 0.05$.

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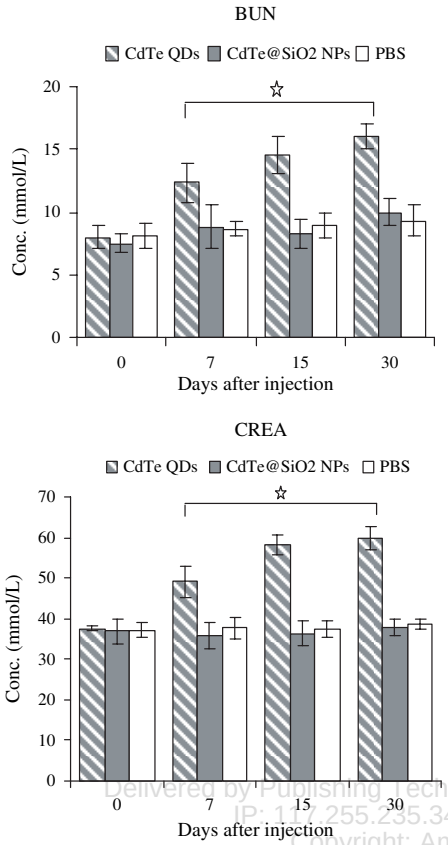

Fig. 4. The concentration of blood urea nitrogen and creatinine (BUN, CREA; the markers of kidney function) of experimental mice at 0, 7, 15, 30 days after intravenous injection of CdTe QDs, CdTe@SiO_2 NPs and PBS. An asterisk indicates the level of BUN and CREA were significantly higher than that of control group ($P < 0.05$). Data are shown as mean ± S.D. for three mice per group.

3. RESULTS AND DISCUSSION

3.1. Synthesis and Characterization of CdTe QDs and CdTe@SiO_2 NPs

The CdTe QDs and CdTe@SiO_2 NPs with the size of 3.8 nm and 65–70 nm respectively were synthesized. The size of CdTe in our preparation was about 3.8 nm calculated from the exciton peak position in absorption spectra according to the reference method.[38] Photoluminescence and absorption spectra of CdTe QDs and CdTe@SiO_2 NPs are shown in Figure 1(A). The PL spectra showed the characteristics of high-quality CdTe QDs with a well-pronounced band gap emission and no trap state emission. Some aliquots during incubation process exhibited a strong band edge and some shallow trap state emission and the aqueous dispersion of the resultant CdTe@SiO_2 particles exhibit reddish fluorescence. The TEM and HRTEM images of the silica coated CdTe and CdTe QDs are shown in Figures 1(B), (C) and (E) respectively which showed monodispersed CdTe@SiO_2 composite particles had a silica thickness of about 35 nm and have CdTe QDs core (single or multiple) inside the silica shell. It was noted that the quantum yield (QY) in CdTe@SiO_2 was quite lower than that of CdTe QDs which coincide with the previous reports.[35]

3.2. *In Vivo* Experiments

3.2.1. Physical Parameters

The 2 mice of CdTe QDs treated group died on 25th day pi. Before death, these mice appeared lethargic, less active, and body-weight losses. Postmortem of these dead mice showed no obvious changes in all the tissues except kidney which showed nephritis and nephropathies with enlargement in size. All other mice had normal eating, drinking, urination or neurological status. Body weight and temperature were observed every day and the fluctuations were no greater after treatment than before treatment. Overall, these parameters suggested minimal systemic effects of both the CdTe QDs and CdTe@SiO_2 NPs.

3.2.2. Blood and Biochemical Markers

The QDs and NPs being foreign particles may affect the immune system or induce an inflammatory response, which would be indicated by changes in hematological factors, therefore, standard hematological and biochemical markers were monitored. Hematological results indicated the mice in CdTe@SiO_2 and PBS injected groups showed normal ranges of hematological and biochemical markers which were not significantly different between groups in samplings of 0, 7, 15 and 30 days post inoculation (Figs. 2–4). However, in mice injected with CdTe QDs indicated significantly increased WBC count in group at 7, 15 and 30th day with maximum value at 30 days after injection (Fig. 2). This might be due to the accumulation and probable inflammation caused by the QDs in kidney tissues. Ruan et al. (2011),[29] have also observed elevated level of WBCs at high dose (100 μg) of magnetic nanoparticles. Similarly, serum biochemistry assay were measured weekly and showed no sign of liver injury as the both the AST and ALT were in normal range (Fig. 3). Indicators of kidney function—blood urea nitrogen (BUN) and creatinine (CRE) were significantly higher in CdTe QDs injected group (Fig. 4). Overall, the results of the hematology and blood chemistry indicated that the CdTe QDs cause mild to moderate renal damage.

3.2.3. Histological Analysis

Histological analysis of tissues from treated groups of mice and a control revealed no obvious pathological changes in the liver, spleen, lungs and brain (Fig. 5). Hepatocytes in the liver samples were observed to be normal, and there were no signs of inflammatory response.

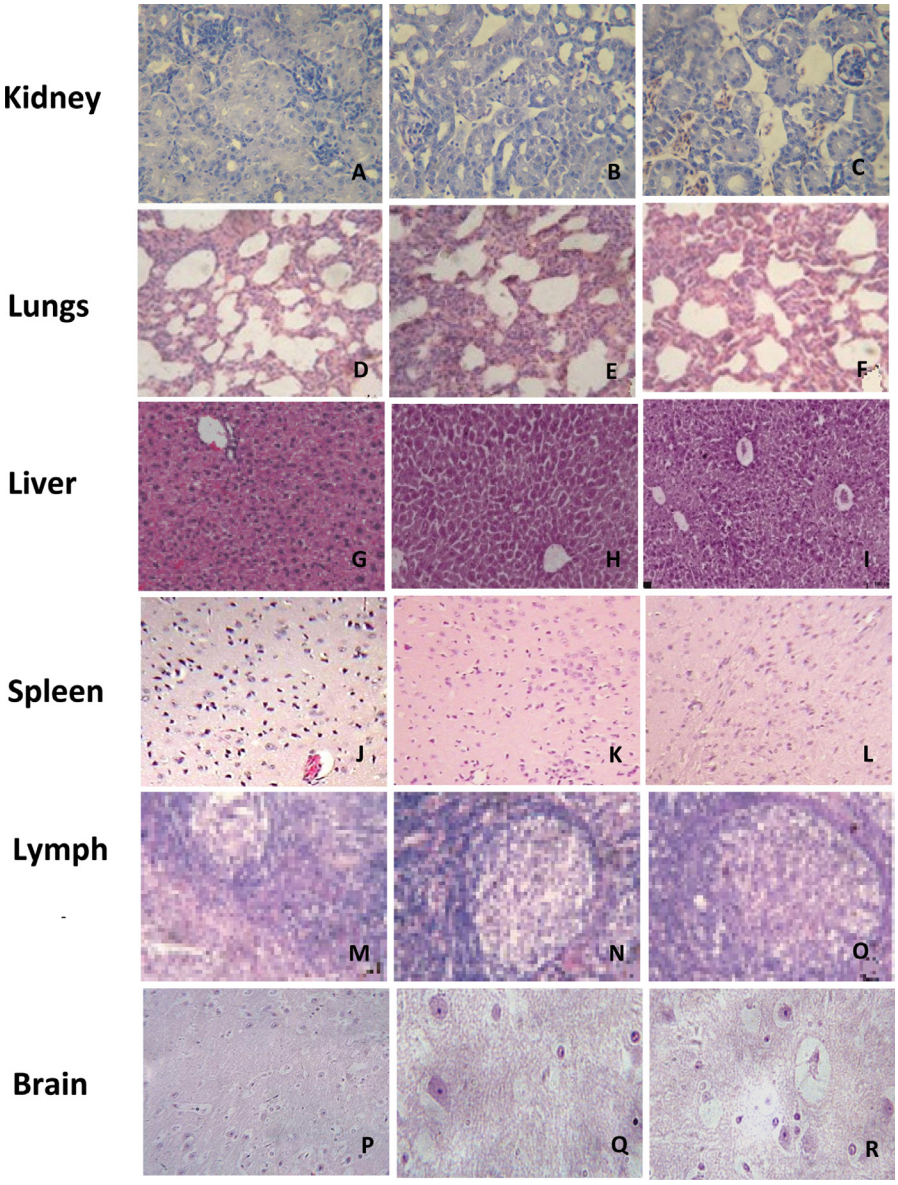

Fig. 5. Histological images from the major organs of the mice 30 days after intravenous injection of the CdTe QDs, CdTe@SiO_2 NPs and PBS (left to right respectively). No obvious pathological changes in the major organs of treated and non treated mice were observed except kidney tissue showed mild epithelial degeneration and focal necrosis in CdTe QDs treated group (A). Images were taken at ×400 magnification with standard haematoxylin and eosin staining.

No pulmonary fibrosis, inflammation or granulomas was observed in the lung samples which is different from the finding of Ho et al. (2011)[39] However, kidney section of the mice treated with CdTe QDs showed mild epithelial degeneration and focal necrosis as shown in Figure 5, which might be due to the releasing free cadmium ions that accumulate in the kidney after quantum dot formulation slowly degrade in the liver and spleen because the natural accumulation sites of Cd^{2+} ions are the liver and kidneys.

Choi et al. (2009),[40] have demonstrated the cytotoxic effects of using quantum dots with cadmium-based cores (for example, CdTe and CdSe) were associated with leaching of heavy metal ions, which may interfere tissue function by causing cell death. Previous studies on quantum dot toxicity have shown that coating the cadmium-based core with another material,[41] such as polymer coating can reduce or eliminate toxicity both *in vitro* and *in vivo*.[20] Recent reports on tissues distribution of the bioconjugated nanoparticles in mice showed that these were distributed

in lymph nodes, kidneys, liver, lung, and spleen and brain (Schipper et al. 2009; Al-Jamal et al. 2009).[42–44]

4. CONCLUSIONS

In conclusion, mice intravenously injected with ~125 mg kg^{-1} of silica coated CdTe nanoparticles formulation survived without any evidence of toxicity. All measured biochemical markers were in the normal range. Histopathological analyses showed that the CdTe QDs has very low nephrotoxicity which could be overcome by coated with silica. These results suggest a potential role of the CdTe@SiO_2 in biomedical applications such as bio-labeling, *in vivo* tumors imaging and pathogenic detections.

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