In vivo Quantum-Dot Toxicity Assessment

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***Q**uantum dots have potential in biomedical applications, but concerns persist about their safety. Most toxicology data is derived from in vitro studies and may not reflect in vivo responses. Here, an initial systematic animal toxicity study of CdSe–ZnS core–shell quantum dots in healthy Sprague–Dawley rats is presented. Biodistribution, animal survival, animal mass, hematology, clinical biochemistry, and organ histology are characterized at different concentrations (2.5–15.0 nmol) over short-term (<7 days) and long-term (>80 days) periods. The results show that the quantum dot formulations do not cause appreciable toxicity even after their breakdown in vivo over time. To generalize the toxicity of quantum dots in vivo, further investigations are still required. Some of these investigations include the evaluation of quantum dot composition (e.g., PbS versus CdS), surface chemistry (e.g., functionalization with amines versus carboxylic acids), size (e.g., 2 versus 6 nm), and shape (e.g., spheres versus rods), as well as the effect of contaminants and their byproducts on biodistribution behavior and toxicity. Combining the results from all of these studies will eventually lead to a conclusion regarding the issue of quantum dot toxicity.*

Keywords:
- biodistribution
- nanostructures
- quantum dots
- toxicity

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1. Introduction

Advances in nanotechnology research are leading to a shift in the design of materials for medical, electronic, biological, and chemical applications. Biomedical nanotechnology has proposed many exciting applications,[1–5] but progress is currently impeded by a lack of understanding of how nanostructures interact with biological systems and the environment.[6,7] Many in vitro studies have suggested that nanomaterials induce toxic responses through mechanisms such as particle breakdown and the subsequent release of toxic metals[8–10] and the production of reactive oxygen species.[11] In addition, nanoparticle surface chemistry has been shown to influence toxicity in vitro.[12,13] These findings have made researchers, policy makers, industry, and the general public wary of nanotechnology developments. Despite these in vitro studies, there are few toxicological reports of nanomaterials using animal models, which is the preferred system for the toxicological evaluation of a novel agent and should be used to characterize the toxicity of nanomaterials.[14] In vitro cultures cannot replicate the complexity of an in vivo system or provide meaningful data about the response of a physiological system to an agent. For example, in the case of

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carbon nanotubes,[15] Manna et al. found toxicity in vitro, whereas Schipper et al. found no significant toxicity in vivo.[16] Similarly, Sayes et al. found that in vivo toxicology results for fullerenes were very different from in vitro data.[17]

Toxicity in vivo is determined by many parameters including dose, route of exposure, metabolism, excretion, and immune response. The toxicological profiles of nanomaterials might also be determined by nanomaterial chemical composition, size, shape, aggregation, and surface coating.

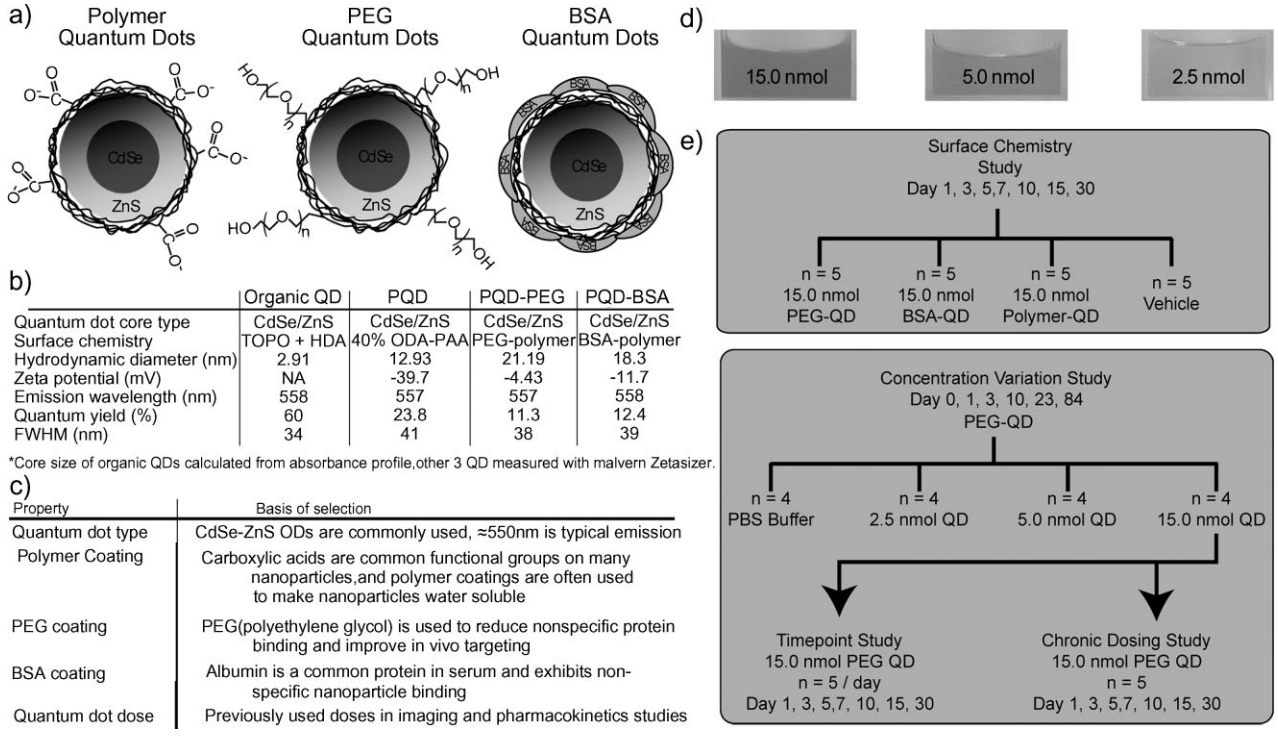
Quantum dots (QDs) represent a well-characterized family of engineered nanostructures and provide an excellent model system to test for toxicity of similarly sized materials. QDs can be prepared with tight size and shape distribution and various surface chemistries, they can be quantified in biological samples with conventional analytical techniques, and they can be imaged using electron and fluorescence microscopy. Furthermore, the observation and nature of QD toxicity in vitro is one of the most studied to date while there are few in vivo studies that have analyzed the physiological behavior of QDs and these studies did not focus on toxicity.[10,18,19]

Here we develop a systematic framework to study and characterize the effect of dosing and surface chemistry on the short- and long-term in vivo toxicity of semiconductor QDs in Sprague–Dawley rats. A continuation of this study would result in a complete toxicological assessment of QD composition (e.g., PbS, CdTe, CdS), size (2–6 nm), shape (e.g., semiconductor tetrapods or rods), and a wider range of surface functional groups and surface charges. This experimental model could be applied to other engineered nanostructures such as metallic nanoparticles, and the successful outcome of these studies would define how the chemical and physical nature of a nanomaterial relates to its toxicity and biodistribution.

2. Results and Discussion

Based on the findings of published reports,[10,18,19] we defined the parameters of the QDs used and the experimental conditions. We investigated the toxicity of CdSe–ZnS core–shell QDs of different surface chemistries using female Sprague–Dawley rats. The properties of the QDs and overall experimental scheme used in this study are described in Figure 1, including the rationale for selecting the QD type, surface chemistry, and dosing regimes. When studying the effect of surface chemistry, a vehicle control was used, consisting of an equal mixture of filtrate (solution) from the three types of QDs. Of note, we did not investigate the influence of each of the individual contaminants (e.g., unfiltered polymer, poly(ethylene glycol) (PEG), or bovine serum albumin (BSA)) on the biodistribution behavior. An excess of these contaminants or byproducts of these contaminants could potentially influence the biodistribution behavior and toxicity of the QDs since it might compete for cellular uptake and/or alter cellular response. The influence of contaminants from the formulation of the nanoparticle should be investigated in future studies.

Initially, we investigated basic physical signs of toxicity and the biodistribution of the QDs with inductively coupled plasma atomic emission spectroscopy (ICP-AES). ICP provides quantitative measurements of elemental metals in tissue, which cannot be determined with other common techniques such as fluorescence microscopy and transmission electron



	Organic QD	PQD	PQD-PEG	PQD-BSA
Quantum dot core type	CdSe/ZnS	CdSe/ZnS	CdSe/ZnS	CdSe/ZnS
Surface chemistry	TOPO + HDA	40% ODA-PAA	PEG-polymer	BSA-polymer
Hydrodynamic diameter (nm)	2.91	12.93	21.19	18.3
Zeta potential (mV)	NA	-39.7	-4.43	-11.7
Emission wavelength (nm)	558	557	557	558
Quantum yield (%)	60	23.8	11.3	12.4
FWHM (nm)	34	41	38	39

*Core size of organic QDs calculated from absorbance profile,other 3 QD measured with malvern Zetasizer.

Property	Basis of selection
Quantum dot type	CdSe-ZnS ODs are commonly used, ≈550nm is typical emission
Polymer Coating	Carboxylic acids are common functional groups on many nanoparticles,and polymer coatings are often used to make nanoparticles water soluble
PEG coating	PEG(polyethylene glycol) is used to reduce nonspecific protein binding and improve in vivo targeting
BSA coating	Albumin is a common protein in serum and exhibits non-specific nanoparticle binding
Quantum dot dose	Previously used doses in imaging and pharmacokinetics studies

Figure 1. QDs used and experimental framework of experiment. a–e) Surface chemistry of QDs used in this experiment (a), characterization of QDs (b), justification for the selection of various QD characteristics (c), concentrations of QDs injected (d), scheme of experiments (e). Organic QDs are coated with TOP and are soluble in organic solvents such as chloroform.

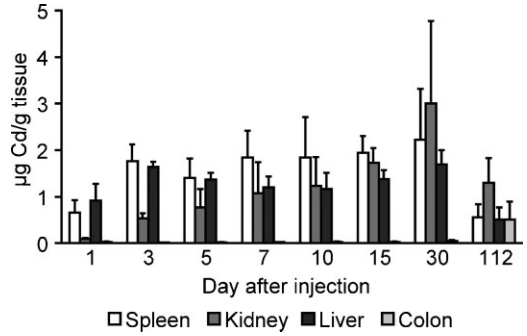


Figure 2. Biodistribution of QDs at varying time points after the injection of 15 nmol PEG QDs.

microscopy (TEM). Based on previous work, we are not using TEM since it is not quantitative[18] and fluorescence cannot be used since the QDs are green and tissue autofluorescence is in the same region of the spectrum.

2.1. Biodistribution Results

Throughout the study, rats did not exhibit unusual responses or behaviors (lethargy, weight-loss, etc.) compared to controls. Rat mass data (see Supporting Information) indicates no significant differences between the treatment and control groups. Representative biodistribution data for rats exposed to PEG–QD are shown in Figure 2. Figure 1 in the Supporting Information includes a buffer control, showing that endogenous Cd is negligible. The Cd levels detected in tissues from rats not treated with QDs were negligible, below 0.1 µg Cd g^{-1} wet tissue for both buffer and vehicle controls, which is in agreement with previously published QD studies by Fischer et al.[18] that showed between 0.1–0.3 µg Cd g^{-1} tissue for liver, spleen, and kidney. This is expected, as Cd has no known biological function, and laboratory rats purchased from a supplier have a highly regulated food supply and are not exposed to typical sources of Cd, such as occupational exposure, leafy vegetables, fish, cigarette smoke, and so on.[20] Even the vehicle controls, which consisted of suspension buffer with the QDs removed, yielded insignificant levels of Cd.

Additional data for rats exposed to varying concentrations of QDs and surface chemistries are available in the Supporting Information. The organ localization trends shown in these figures indicate that QDs initially target the liver and spleen and after several days to several weeks, QDs and/or their constituent metals are present in the kidneys as well. At one day after injection, the liver contains 0.92 µg Cd g^{-1}, and the kidneys contain 0.09 µg Cd g^{-1}, yet by 30 days after injection the liver contains 1.69 µg Cd g^{-1} and the kidneys contain 3.00 µg Cd g^{-1}.

The localization of QDs or their constituents in the kidney over several weeks is interesting and verifies previous work with PEG-coated CdTe–ZnS QDs by Yang et al.[21] This study also found that Cd concentration spiked immediately in the liver and spleen but required some time to accumulate in the kidneys. Early accumulation by the liver and spleen is expected[18] and is related to the clearance of nanoparticles from the blood by cells of the mononuclear phagocytic system. However, the results from the kidney are surprising since whole QDs cannot be renally cleared unless they are less than 5.5 nm in diameter[22] and the QDs used here are between 13 and 21 nm in diameter. Biodistribution data of three types of QDs with considerably different surface chemistries is shown in the Supporting Information and these three particular types of QDs show little difference in target organs after 30 days, suggesting that the QDs may be breaking down and behaving at least partially as constituent ions (Cd, Se, Zn, etc.). This result was unexpected since investigators previously found that QDs of different surface chemistries behave very differently in vitro.[9,13] However, further investigations are merited with extremely different surface groups, such as silica shells or various polymers of different surface charges.

A recent investigation by Mancini et al.[23] shows that digestive chemicals of phagocytic cells, hydrochlorous acid and hydrogen peroxide, can cause considerable QD degradation after 1 week of incubation. The authors in this study also observe that zinc leaches early in the phagocytic degradation process since it is located in the external shell of the QDs. Since Yang et al. found QD fluorescence several weeks after QD injection,[21] we expect that some QDs break down while others are left intact and that this breakdown is related to the integrity of the ZnS shell surrounding the QDs. The complexity of semiconductor nanoparticle breakdown, biodistribution, and interaction with endogenous proteins such as metallotheionein merits further investigation.

2.2. Histology Results

To continue the investigation of toxicity, histological assessment of tissues was conducted to determine whether or not the QDs themselves or their degradation products cause tissue damage, inflammation, or lesions from toxic exposure. Analysis was performed by a treatment-blinded veterinary pathologist (S.N.) and five representative organs were fixed, stained, and analyzed: liver, kidney, spleen, bone marrow, and lymph nodes. Bone marrow and lymph nodes were analyzed since a previous study showed a small accumulation of the QDs in those organs.[18] Overall, there were no apparent histopathological abnormalities or lesions related to treatment of these animals with the QDs. Representative histology results are shown in Figure 3.

2.3. Clinical Biochemistry and Hematology Results

Histology provides macroscopic and visual evidence of toxicity, but is not quantitative. To quantify the toxicity analysis of QDs, the next important step is an assessment of standard hematological and biochemical markers of the target organs (liver, kidney, and spleen) of QDs. When QDs are intravenously administered, the first physiological system they interact with is the blood and blood components. QDs and other nanoparticles are particulate materials within the size regime of viruses and large proteins, and consequently they may induce an inflammatory response and increase or decrease the activity of the immune system and alter related hematological factors such as white blood cell count.[6,24] Furthermore, the degradation of nanoparticle surface coatings or core material may occur

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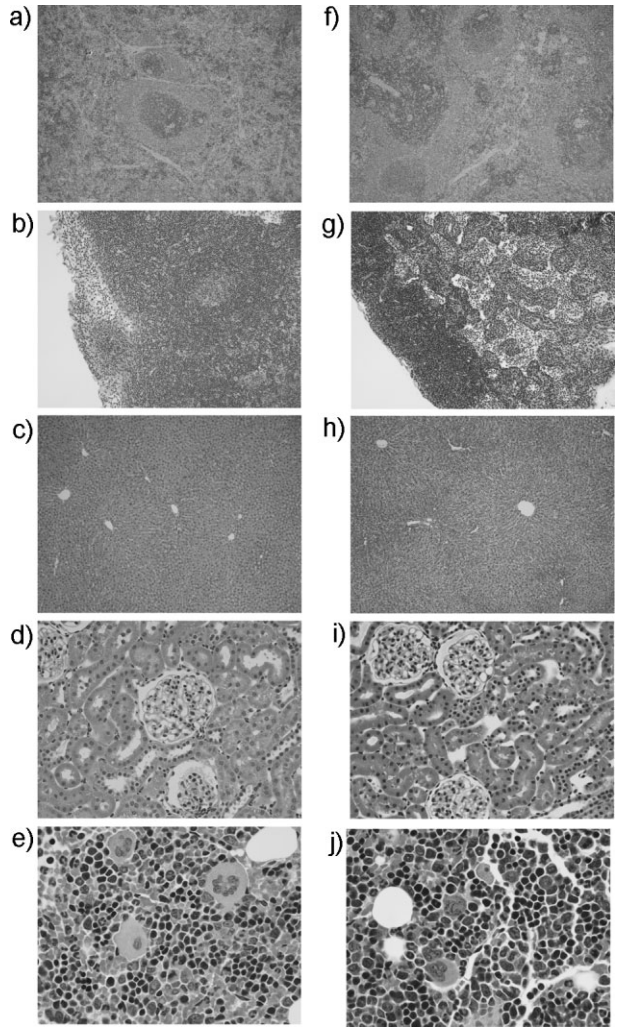

Figure 3. Representative organ histology for control and treated animals. For control animals (a–e) and chronically dosed animals (f–j) liver, kidney, spleen, bone marrow, and lymph node are shown. Our analysis shows that organs did not exhibit QD-derived signs of toxicity.

and could result in changes in hematology indicators such as red blood cells or hemoglobin due to Cd toxicity.[25] As a result, we selected the following standard hematology markers for analysis: red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, and white blood cell count. Representative hematology results are presented in Figure 4 and indicate that all measured factors were within normal ranges and did not indicate a trend associated with treatment. Furthermore, there were no significant differences in hematology measurements for QDs of different surface chemistries, also shown in Figure 4. Additional hematology data is available in the Supporting Information.

Once the nanoparticles leave the blood stream and reach the liver and kidneys, it is important to measure whether or not the particles themselves or their constituent materials can induce toxicity. The potential breakdown of QDs over a period of weeks and the resulting accumulation of Cd and Se ions in the liver and kidney make serum indicators of liver and kidney damage particularly useful. By measuring a variety of factors in the serum, it is possible to assess liver function, hepatocellular injury, and cholestasis (a reduction in the flow of bile).[26,27] In the rat, alanine aminotransferase (ALT) is specific for cellular damage in the liver.[26] Aspartate aminotransferase (AST) is also useful but not as specific. Reduction in liver function and cholestasis can be assessed by substances produced by the liver, including albumin, globulin, total protein, alkaline phosphatase (ALP), and total bilirubin. Indicators of kidney function include creatinine and urea, although these are not as specific or sensitive. Results did not indicate significant toxicity compared to control animals and reflected normal variation present in populations of animals.[26,28] Representative data is shown in Figure 5 and indicates that toxicity is not evident for all three QD surface coatings. For factors such as ALT and AST, fold-changes are indications of toxicity, as changes of 20% can result from exercise, a high body mass index, or hemolysis prior to separation of blood cell fraction.[27,29] Additional data is available in the Supporting Information.

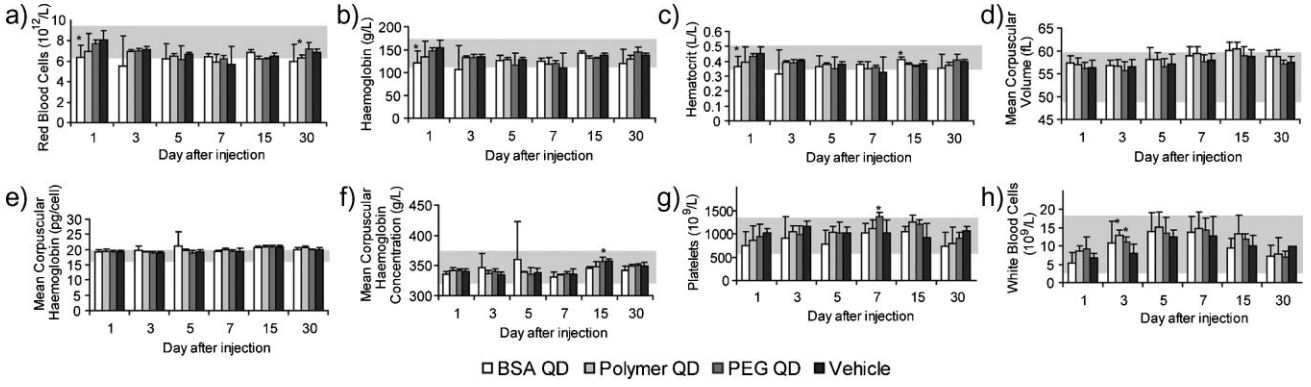

Figure 4. Hematology results from animals treated with QDs of varying surface chemistry or a vehicle control. a–h) These results show mean and standard deviation of red blood cells (a), hemoglobin (b), hematocrit (c), mean corpuscular volume (d), mean corpuscular hemoglobin (e), mean corpuscular hemoglobin concentration (f), platelets (g), white blood cells (h). Grey bars indicate the range of values obtained from healthy Sprague–Dawley rats, as reported by Petterino.[28] Error bars represent standard deviation. Statistical analysis was performed with a two-sample t-test, unknown and unequal variances, comparing each sample group to the related control group. * denotes statistically significant results at $\alpha = 0.05$. These findings were incidental and did not indicate a trend in toxicity or fall outside of the expected range for Sprague–Dawley rats.

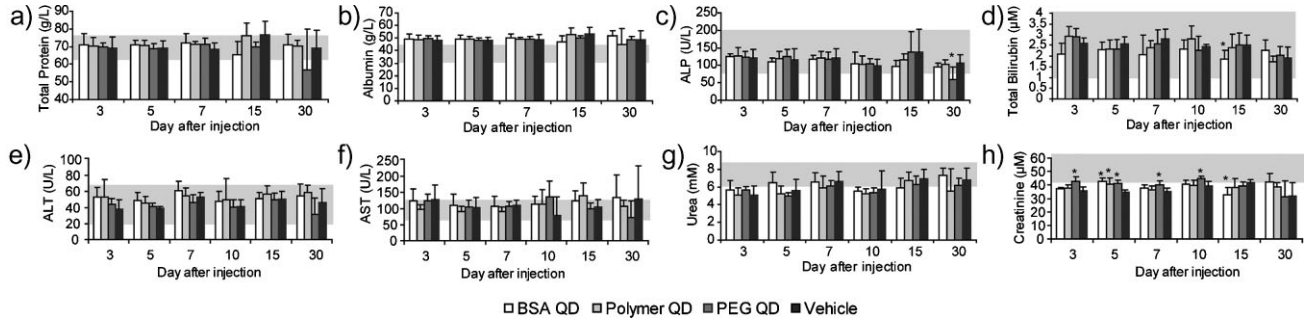

Figure 5. Biochemistry results from animals treated with QDs of varying surface chemistry or a vehicle control. a–h) Results illustrate mean and standard deviation of total protein (a), albumin (b), ALP (c), total bilirubin (d), ALT (e), AST (f), urea (g), creatinine (h). Grey bars indicate one standard deviation around the mean as reported by Loeb and Quimby.[26] Error bars represent standard deviation. Statistical analysis was performed with a two-sample t-test, unknown and unequal variances, comparing each sample group to the related control group. * denotes statistically $\alpha = 0.05$. These findings were incidental and did not indicate a trend in toxicity or fall outside of the expected range for Sprague–Dawley rats.

3. Conclusions

Even at high doses (15 nmol), QDs do not cause significant toxicity in Sprague–Dawley rats over the course of this long-term study. Chronically dosed rats, which received a total of 60 nmol of QDs over 4 weeks, did not experience QD toxicity. This result is reasonable if one considers that 200–300 μg Cd g^{-1} concentrations are required to cause significant renal damage.[20,30] Notably, one study[20] gives the mean level of kidney Cd as 20–35 μg Cd g^{-1} in humans, indicating that the levels present in QD-treated rats (maximum 3.00 μg Cd g^{-1} in the kidney) are insufficient to cause toxicity.

In summary, this investigation provides evidence that QDs were not toxic to the animals studied, which differs from previous in vitro results. Studies of this nature should be expanded to a wide range of nanoparticles and surface chemistries to characterize nanoparticle toxicity in vivo using standard clinical measurements.

4. Experimental Section

QD synthesis and Hydrophilic Coating: Standard organometallic protocols were used to synthesize ZnS-capped CdSe QDs.[31–34] Briefly, 20.0 g of trioctylphosphine oxide (TOPO) were heated to 340 °C under argon gas and 2.0 mL of CdSe stock solution (Se powder (0.162 mol) and dimethyl cadmium (0.232 mol) in trioctylphosphine (TOP)) were injected. The temperature was dropped to approximately 290 °C after 1 min. Four aliquots (4.0 mL) of ZnS capping solution (diethylzinc (0.232 mol) and hexamethyldisilithiane (0.232 mol) in TOP) were added to cap the CdSe QDs. After the last aliquot, the temperature was lowered to 100 °C and the solution stir overnight in argon gas. To prepare these QDs for surface modification, we removed unreacted reagents via a precipitation procedure. Excess methanol was added to the reaction vessel and a precipitate was observed instantaneously. The solution was centrifuged (3 700 rpm) for 5 min. The supernatant was removed and chloroform was added, followed by additional methanol to reprecipitate the QDs. This was repeated a total of four times. After synthesis, QD concentrations were determined using Beer's law and spectrophotometry absorbance measurements using standard procedures.[35]

Next, we rendered the surface of these QDs biocompatible and water-soluble using a previously established procedure.[36] Specifically, we prepared a 40% octadecylamine-modified poly (acrylic acid) via carbodiimide-linking chemistry in 1-methyl-2-pyrrolidinone. The monomer mole ratio of octylamine to poly (acrylic acid) was 40%, which led to a polymer containing 40% octadecylamine grafted on poly(acrylic acid) since the efficiency of this reaction is near 100%. The characterization and purification procedures of synthesizing these polymers are described in our previous publication.[36] To coat the surface of the QDs with this polymer, we mixed 0.0015 mol of the QDs with 0.45 mmol of the polymer in chloroform. This reaction was stirred for 1 h at room temperature, followed by air evaporation in a fume hood. Next, distilled water was added and the pH was modified to 10 in the reaction flask. Afterward, we sonicated the solution for 30 min and heated to 75 °C for 10 min, followed by further sonication. Finally, we filtered the solution through a 0.22-μm Millipore syringe filter. We further purified using size exclusion chromatography (Sephadex G25). Finally the optical properties were measured using UV–Vis and fluorescence spectroscopy (Shimadzu UV-1601PC; Jobin–Yvon Fluoromax-3, Edison, NJ). For assessment of the size of the QDs with polymer coating (PQDs), we used dynamic light scattering (Malvern Zetasizer Nano-ZS, Worcestershire, UK) as described previously.[31,37] ICP-AES (Perkin Elmer Optima 3000 ICP-AES, Waltham, MA) was used to determine Cd concentration, which could be correlated to the PQD concentration.

One year following these experiments, QDs were filtered with an Amicon MW 10 000 size exclusion filter and the Cd and Zn content of the filtrate was compared to that of the stock solution. Cd and Zn in the supernatant were present in amounts of 0.34% and 0.29%, respectively, as a percentage of the Cd and Zn in the stock solution.

QD conjugation to PEG and BSA: PEGlyated PQDs (PQD–PEG, MW 3 000) and BSA-conjugated PQDs (PQD–BSA) were prepared using an *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide (EDC) reaction. A final molar ratio of 1:500:4000 PQD/amino-PEG/EDC or 1:20:4000 PQD/BSA/EDC in phosphate buffer (10 mmol, pH 7.4) were mixed in a reaction vial and allowed to react at room

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temperature for 30 min and followed by purification through a Sephadex G25 column to remove excess polymer. In this experiment, we collected the void volume and used the PQDs in the void volume for subsequent studies. Of note, the G25 column may not remove all of the uncoated PEG. Although the Sephadex G25 separates proteins at a molecular weight of 1 to 5 kDa and the PEG is in this size range, the hydrodynamic radius of PEG is generally larger than a protein, which governs the separation of molecules.[38] The PQD–PEG and PQD–BSA conjugates were compared to unconjugated PQD and nonspecifically bound BSA and PEG QDs using gel electrophoresis (a successful conjugation shows a slower migration rate than unconjugated PQD and a different migration position than that for nonspecific binding). See Supporting Information for QD characterization data, including absorbance (Supporting Information, Figure 9a) and fluorescence (Supporting Information, Figure 9b) measurements, zeta potential (Supporting Information, Figure 10) and gel confirmation of conjugation (Supporting Information, Figure 11).

Preparation of QDs for Injection: Depending on the experiment (refer to Figure 1) 0–15 nmol PQD–PEG or vehicle or buffer control solutions were transferred to an Amicon Ultra centrifugal filter tube with a regenerated cellulose 10 000 MWCO filter insert (Millipore, Cork, Ireland) and centrifuged for 15 min at 3 000 rpm. The concentrated PQD–PEG solution was diluted to 200 μL with 10 mM phosphate buffer solution (PBS) and passed through a 0.22-μm Millipore syringe filter (Fisher Scientific, Ottawa, ON) for sterilization. 15 nmol of QD represents 201.2 μg Cd (PQD), 212.8 μg Cd (PEG–QD) and 163.3 μg Cd (BSA) depending on the type of QD used. Light scattering measurements showed that empty micelles were not present after purification.[39]

Animal Injections and Sample Collection: Animals were purchased, maintained, and handled with protocols approved by the Division of Comparative Medicine, University of Toronto. Female Sprague–Dawley rats were obtained from Charles River laboratories at 11 weeks of age and were housed 2 per cage in a 12 h/12 h light/dark cycle, and were given food and water ad libitum. Animals were injected with 200 μL of buffer, vehicle control, or buffered QD solution as per the schematic in Figure 1C. At increasing time points after injection (1, 3, 5, 7, 10, and 30 days) animals were weighed and assessed for behavioral changes. Using a standard saphenous vein blood collection technique, blood was drawn for hematology analysis (potassium EDTA collection tube) and serum biochemistry analysis (lithium-heparin collection tube). Analysis of standard hematological and biochemical markers was performed by the Samuel Lunenfeld Research Institute's CMHD Mouse Physiology Facility. For blood analysis, 300 μL of blood was collected from rats and separated by centrifugation into cellular and plasma fractions. Upon the completion of the last time point, rats were sacrificed by isoflurane anesthetic and angiocatheter exsanguination with PBS. From each group of four or five treated rats, one was fixed with 10% buffered formalin following PBS exsanguination. During necropsy, the following organs were collected and weighed: liver, kidneys, spleen, heart, lungs, brain, colon (section 10 mm in length), muscle (5 mm in diameter), thyroid, lymph nodes, and bone marrow (from the femur). From the fixed rats, the liver, kidneys, spleen, bone marrow, and lymph nodes were sectioned for histology.

Pharmacokinetic Analysis: Organ samples were homogenized with a Pro250 homogenizer. A representative homogenous sample of each organ was digested in 70% nitric acid and Cd content was quantified with ICP-AES to determine the total Cd in the original organ.

Histology: Organ samples were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Liver, kidney, spleen, lymph node, and bone marrow slides were examined by light microscopy by a blinded veterinary pathologist (S.N.).

Statistics: Statistical analysis was performed with a two-sample t-test, unknown and unequal variances, comparing each sample group to the related control group at a significance level or 0.05.[40]

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[1] M. Ferrari, *Nat. Rev. Cancer* **2005**, *5*, 161.
[2] W. Cai, D.-W. Shin, K. Chen, O. Gheysens, Q. Cao, S. Wang, S. Gambhir, X. Chen, *Nano Lett.* **2006**, *6*, 669.
[3] V. Bagalkot, L. Zhang, E. Levy-Nissenbaum, S. Jon, P. Kantoff, R. Langer, O. Farokhzad, *Nano Lett.* **2007**, *7*, 3065.
[4] D. R. Larson, W. R. Zipfel, R. M. Williams, S. W. Clark, M. P. Bruchez, F. W. Wise, W. W. Webb, *Science* **2003**, *300*, 1434.
[5] S. D. Perrault, C. Walkey, T. Jennings, H. C. Fischer, W. C. W. Chan, *Nano Lett.* **2009**, *9*, 1909.
[6] A. Nel, T. Xia, L. Mädler, N. Li, *Science* **2006**, *311*, 622.
[7] J. Kuzma, *J. Nanopart. Res.* **2007**, *9*, 165.
[8] A. Derfus, W. C. W. Chan, S. Bhatia, *Nano Lett.* **2004**, *4*, 11.
[9] C. Kirchner, T. Liedl, S. Kudera, T. Pellegrino, A. Javier, H. Gaub, S. Stölzle, N. Fertig, W. Parak, *Nano Lett.* **2005**, *8*, 331.
[10] R. Hardman, *Environ. Health Perspect.* **2006**, *114*, 165.
[11] J. Lovrić, S. J. Cho, F. M. Winnik, D. Maysinger, *Chem. Biol.* **2005**, *12*, 1227.
[12] J. Ryman-Rasmussen, J. Riviere, N. Monteiro-Riviere, *J. Invest. Dermatol.* **2006**, *127*, 143.
[13] J. Lovrić, H. S. Bazzi, Y. Cuie, G. R. A. Fortin, F. M. Winnik, D. Maysinger, *J. Mol. Med.* **2004**, *83*, 377.
[14] H. Fischer, W. C. W. Chan, *Curr. Opin. Biotechnol.* **2007**, *18*, 565.
[15] S. Manna, S. Sarkar, J. Barr, K. Wise, E. Barrera, O. Jejelowo, A. Rice-Ficht, G. Ramesh, *Nano Lett.* **2005**, *5*, 1676.
[16] M. Schipper, N. Nakayama-Ratchford, C. Davis, N. Kam, P. Chu, Z. Liu, X. Sun, H. Dai, S. Gambhir, *Nat. Nanotechnol.* **2008**, *3*, 216.
[17] C. Sayes, A. Marchione, K. Reed, D. Warheit, *Nano Lett.* **2007**, *7*, 2399.
[18] H. Fischer, L. Liu, K. Pang, W. C. W. Chan, *Adv. Funct. Mater.* **2006**, *16*, 1299.
[19] B. Ballou, B. Lagerholm, L. Ernst, M. Bruchez, A. Waggoner, *Bioconjugate Chem.* **2004**, *15*, 79.
[20] J. A. Ryan, H. R. Pahren, J. B. Lucas, *Environ. Res.* **1982**, *28*, 251.

[21] R. S. H. Yang, L. W. Chang, J.-P. Wu, M.-H. Tsai, H.-J. Wang, Y.-C. Kuo, T.-K. Yeh, C. S. Yang, P. Lin, *Environ. Health Perspect.* **2007**, *115*, 1339.
[22] H. Soo Choi, W. Liu, P. Misra, E. Tanaka, J. Zimmer, B. Itty Ipe, M. Bawendi, J. Frangioni, *Nat. Biotechnol.* **2007**, *25*, 1165.
[23] M. C. Mancini, B. A. Kairdolf, A. M. Smith, S. Nie, *J. Am. Chem. Soc.* **2008**, *130*, 10836.
[24] M. Dobrovolskaia, S. McNeil, *Nat. Nanotechnol.* **2007**, *2*, 469.
[25] R. Karmakar, R. Bhattacharya, M. Chatterjee, *BioMetals* **2000**, *13*, 231.
[26] *The Clinical Chemistry of Laboratory Animals*, (Eds.: W. F. Loeb, F. W. Quimby), Taylor & Francis, Philadelphia **1999**.
[27] D. R. Dufour, J. A. Lott, F. S. Nolte, D. R. Gretch, R. S. Koff, L. B. Seeff, *Clin. Chem.* **2000**, *46*, 2027.
[28] C. Petterino, A. Argentino-Storino, *Exp. Toxicol. Pathol.* **2006**, *57*, 213.
[29] N. McIntyre, in *The Liver in Biology and Disease*, (Ed.: E. E. Bittar), Elsevier, Amsterdam **2004**, pp. 291–315.
[30] F. N. Kotsonis, C. D. Klaassen, *Toxicol. Appl. Pharmacol.* **1977**, *41*, 667.
[31] T. Pons, H. Uyeda, I. Medintz, H. Mattoussi, *J. Phys. Chem. B* **2006**, *110*, 20308.
[32] B. Dabbousi, J. Rodriguez-Viejo, F. Mikulec, J. Heine, H. Mattoussi, R. Ober, K. Jensen, M. Bawendi, *J. Phys. Chem. B* **1997**, *101*, 9463.
[33] Z. Peng, X. Peng, *J. Am. Chem. Soc.* **2001**, *123*, 183.
[34] J. Klostranec, Q. Xiang, G. Farcas, J. Lee, A. Rhee, E. Lafferty, S. Perrault, K. Kain, W. C. W. Chan, *Nano Lett.* **2007**, *7*, 2812.
[35] W. Yu, L. Qu, W. Guo, X. Peng, *Chem. Mater.* **2003**, *15*, 2854.
[36] R. E. Anderson, W. C. W. Chan, *ACS Nano* **2008**, *2*, 1341.
[37] W. Jiang, S. Mardyani, H. Fischer, C. Chan, *Chem. Mater.* **2006**, *18*, 872.
[38] C. J. Fee, J. M. Van Alstine, *Chem. Eng. Sci.* **2006**, *61*, 924.
[39] R. E. Anderson, W. C. M. Chan, unpublished results.
[40] D. C. Montgomery, G. C. Runger, in *Applied Statistics and Probability for Engineers*, John Wiley & Sons, Inc, Weinheim, Germany **2003**, Ch. 10.

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