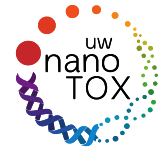




A NOVEL METHOD FOR THE DETECTION AND QUANTITATION OF INTACT QUANTUM DOTS IN TISSUE SLICES



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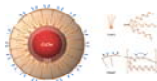


Introduction

Recent developments in the field of nanotechnology have produced a vast array of engineered nanoparticles. A group of these nanoparticles are semiconductor quantum dots (QDs). QDs are fluorescent nanocrystals composed of a semiconductor core containing heavy metals, such as CdSe or CdTe, surrounded by a stabilizing ZnS cap. QDs have applications in imaging due to their physico-chemical properties such as size-tunable emission, high photostability, and large Stokes shift resulting in broad absorption profiles. These characteristics allow QDs to be superior to conventional organic fluorophores in imaging applications. The surface of these QDs can also be modified with various ligands which renders them ideal for *in vivo* targeting applications. Because these particles possess novel structural, physical, and chemical properties, concerns have been raised about their safety and potential toxic effects in biological systems. *In vivo* toxicity studies have attempted to quantitate intact QD in tissues, but currently there is no rapid and cost effective method to do so. Quantitation of intact QD would allow better organ and tissue dose assessments rather than relying on Cd content determination which does not differentiate between degraded or intact QD. To address this, a new method for quantifying tissue QD content was developed. Furthermore, this method can be utilized to investigate differential QD degradation kinetics in mice with varying levels of glutathione, a cellular thiol critical for antioxidant defense.

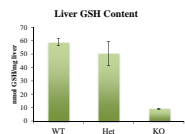
Quantum Dot Preparation

For these studies, we synthesized amphiphilic polymer-coated TOPO-PMAT CdSe/ZnS QDs. Ocean Nanosciences kindly supplied TOPO coated CdSe/ZnS core/shell QDs which were subsequently coated with PMAT. The TOPO-PMAT quantum dots (TOPO-PMAT QD) have a hydrodynamic diameter of 12.7 ± 0.5 nm as measured by dynamic light scattering and the core of these particles is 6.8 ± 0.5 nm in diameter. Prior to dosing, the QD solution was vortexed and diluted in sterile injection-grade 0.9% sodium chloride. This diluted solution was then again vortexed prior to administration.



Methods

- Gclm* transgenic mice were used for these studies. Previous data have shown that mice lacking the *Gclm* gene (KO; *Gclm*^{-/-}) have severely depleted liver glutathione content, while mice lacking one copy of the *Gclm* gene (Het; *Gclm*^{+/-}) have approximately normal levels (i.e. ~ 85% of WT; *Gclm*^{+/+}).



- Animal dosing: All animal procedures were approved by the University of Washington Institutional Animal Care and Use Committee. Specific pathogen free (SPF) *Gclm*^{+/+}, *+/+*, and *-/-* mice (all on a C57BL/6 background) were maintained under 12 hr light/dark cycle in modified SPF conditions. Mice were anesthetized with ketamine/xylazine and dosed with 6 µg/kg body weight Cd equivalent TOPO-PMAT QD solution via retro-orbital injection. After dosing, mice were allowed to recover from the anesthetic and returned to their cages. Mice were sacrificed at 1, 8, and 24 hours after dosing and their livers were removed for cadmium analysis and QD deposition.

- Cadmium content in liver tissue was analyzed by the UW Environmental Health Laboratories and Trace Organics Analysis Center using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) according to modified EPA method 6020A with an Agilent 7500ce ICP/MS.

- QD deposition in 7 µm thick frozen liver sections was analyzed by digital fluorescence microscopy utilizing multispectral unmixing and quantitated for total QD-specific fluorescence using Metamorph (Molecular Devices, Sunnyvale CA).

Results

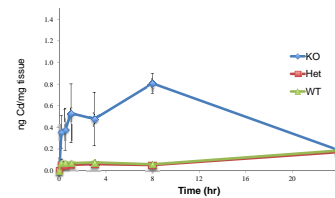


Figure 1. Cadmium Disposition Kinetics in the Liver.

Cd content in liver tissue at the times indicated was determined by ICP-MS. Data represent the mean ± sem. n=3-6/genotype/time point.

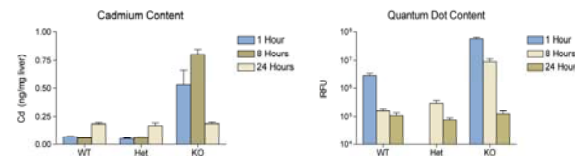


Figure 2. Cadmium Content in the Liver.

Cadmium content was determined by ICP-MS at different time points for the 3 *Gclm* genotypes. Data represent the mean ± sem. n=3-6/genotype/time point.

Figure 3. QD Content in the Liver.

QD deposition was determined by fluorescence microscopy at different time points for the 3 *Gclm* genotypes. Data represent the mean ± sem. n=3-4/genotype/time point.

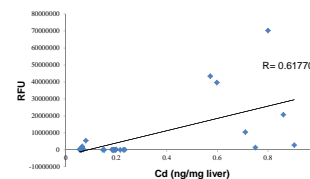


Figure 4. Quantum Dot/ Cadmium Correlation.

Regression analysis of cadmium content and QD content in liver revealed an R value of 0.617702, which suggests that not all Cd content data can be explained by QD fluorescence.

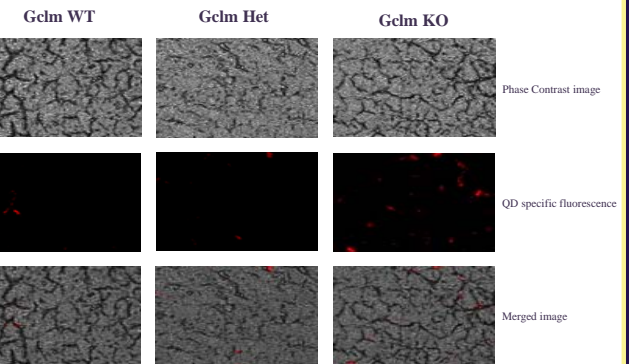


Figure 4. Microscopic Images of Liver Tissue for the *Gclm* Genotypes.

These images reveal the differences in QD content between *Gclm* genotypes and were obtained using phase contrast and fluorescence microscopy at 8 hours following QD administration. QDs are localized within the sinusoidal space, suggesting QD uptake by Kupffer cells.

Principal Findings

- Cadmium content was significantly higher in the *Gclm* KO mice, relative to either the Het or WT mice 8 hours after QD dosing.
- Liver fluorescence (a measure of intact QD) was likewise higher in the *Gclm* KO mice, relative to either the Het or WT mice at 1 and 8 hours after dosing.
- At 24 hours, both cadmium and QD content was similar across all 3 *Gclm* genotypes.
- The regression analysis of the two measures suggests that a significant portion of the measured Cd is due to intact QD.
- This method will be of great value for determining uptake and degradation rate of QD in a variety of tissues from QD-treated mice.

Acknowledgements

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