Fluorescent quantum dot as model imaging probe for studying plant uptake, systemic mobility, localization in plant tissue and potential fate of ultra-small nanoparticles

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ABSTRACT

Fluorescent quantum dots (Qdots) are widely used as biomarker in animal cell imaging and spectroscopy. In recent years, these Qdots have emerged in agricultural applications. Studies are primarily focused on nanotoxicity of ultra-small size water-soluble Qdots in plant systems. In this study, we have developed CdS:Mn/ZnS and ZnS:Mn/ZnS Qdots based model bio-imaging probes for studying plant uptake, distribution, localization and potential fate of ultra-small size (<10 nm) nanoparticles. These Qdots were synthesized using water-in-oil (W/O) microemulsion system. Qdots were surface-coated with biocompatible/ biodegradable materials such as N-acetyl cysteine (NAC). Stability of the coating and the core were evaluated using fluorescence confocal, fluorescence lifetime and Raman studies. Preliminary studies with N-acetyl cysteine (NAC) coated ~3-5 nm size ZnS:Mn/ZnS and CdS:Mn/ZnS Qdots demonstrated that these particles were readily uptaken by the snow pea (*Pisum sativum L.*, a model plant*)* vascular system. Fluorescence microscopy studies confirmed localization of NAC-Qdots in the intercellular regions. Germination and growth of the snow pea seeds were found to be strongly dependent on Qdot dosage and incubation time with Qdots. Cd containing Qdots exhibited heavy metal induced toxicity. Seed germination reached 100% within 48 hours of ZnS:Mn/ZnS NAC-Qdot exposure. Confocal, AFM and Raman imaging studies were conducted to localize Qdots in plant tissue. Based on our preliminary findings, it is suggested that NAC-Qdot could be potentially used as systemic bio-imaging probes for studying ultra-small size engineered nanoparticle uptake, mobility and fate in plant system.

Keywords: Quantum Dot, Nanoparticle, Plant uptake, Fate, Mobility, Bioimaging, Nanotoxicity

1 INTRODUCTION

Safe use of emerging engineered nanomaterials for crop protection is very essential as nanomaterials are receiveing increasing attention due to their great potential for sustainable agriculture and for improving the world's food supply (Handy et al., 2008, Jiang et al., 2011, Ray et al., 2009). Nanomaterials due to their small size and unique surface properties could become systemic once they are exposed to plant species. There are certain benefits of developing systemic nanomaterials (such as bactericides, micronutrient nanoparticles etc.) (Beyth et al., 2015). Once applied via soil drench or foliar spray methods, these nanomaterials by design would be uptaken by the plant system. Active species will then be released slowly from the nanomaterials over a period of time. Systemic bactericides, for example, can interact with phloem or xylem restricted bacterial pathogens, ultimately killing them (Huang et al., 2011). Nanomaterials should be ultra-small in size, preferably less tha n 10 nanometers (1 nm is a billionth of a meter). However, tracking these ultra-small size nanomaterials in plant system is extremely challenging. In this manuscript we report two ultra-small size fluorescently-active core-shell Qdots (CdS:Mn/ZnS and ZnS:Mn/ZnS Qdots) as model nanomaterial systems for studying plant uptake and localization of ultra-small nanomaterials. . These Qdots were coated with NAC which is a biocompatible antioxidant. Previous studies have also shown safe use of NAC in onions (Allium Cepa) (Souza et al., 2011).

It is expected that NAC-Qdots will be uptaken by the plant isse due to their small size and biocompatible surface coating. These NAC-Qdots were used to treat snow pea (Pisum *sativum*) seeds. Significant signs of boost in the growth was observed with minimal toxicity.

2 SYNTHESIS OF NAC COATED QDOTS AND CHARACTERIZATION

CdS:Mn/ZnS and ZnS:Mn/ZnS Qdots were synthesized at room temperature using a water-in-oil microemulsion method and further coated with N-acetyl cysteine (NAC) following our previously published protocols (Das et al., 2014, Das et al., 2015). Characterization of NAC-Qdot crystal size and crystallinity was performed using a High-Resolution Transmission Electron Microscopy (HRTEM, FEI Technai F30).

3 PLANT GERMINATION TESTS

A seed germination test was conducted on snow pea (*Pisum sativum seeds*) over a period of five days under

dark condition. Different concentrations of NAC-Qdots (0, 2, 5, 10, 20, 40, 60, 80, 100 μg/mL) were used for the treatment. Germination was considered successful when the coleoptiles were longer than 2 mm during our measurement at day 5. Three replicates were carried out for each treatment. Germination rate was calculated using following formula.

$$Germination\ \% = \frac{Number\ of\ Germinated\ Seeds}{Total\ Number\ of\ seeds\ in\ petri\ dish} \times 100$$

4 MICROSCOPY AND SPECTROSCOPY

Snow pea (Pisum *sativum*) plants treated with 40 ppm NAC coated CdS and ZnS Qdots were then used to determine the sensitivity of the microscopic and spectroscopic platforms for detection of Qdots inside plant samples.

4.1 Phase Contrast Microscopy

Samples were characterized under phase contrast (Olympus 1X71, Japan). Phase contrast images were acquired using excitation filter of 360/40 nm with a dichroic mirror of ZT365bcm and the emission filter used was 585/20nm.

4.2 Raman/AFM

Samples were also characterizaed by Raman spectroscopy (Witec alpha300 RA) under ambient conditions with excitation wavelength of 532 nm and using a Zeiss 20x objective. A Raman spectrum was collected across a region of interest of the sample. The settings of the spectrometer were set to 600g/mm grating and integration time of 0.05 s for our study. The maps representing the variations of intensity and/or peak positions maps of selected bands in the Raman fingerprint signture of the materials in the sample were reconstructed using the data analysis toolbox available in the Witec Project Plus software.

Atomic force microscopy (AFM) images were acquired in tapping (AC) mode (Witec alpha300 RA) under ambient conditions using a standard tapping-mode cantilever (Al coating, f_{res}=247 kHz).

5 RESULTS

The diameter of the particles used in this study was determined by HRTEM (**Figure 1**). We used cadmium and non-cadmium based NAC-Qdots as shown in the HRTEM images. The ultrasmall particles appeared to be sized in the range ~3-5nm. In addition, the fluorescence of the Qdots can be observed as the Qdots in a glass vial exhibited bright orange color for NAC-CdS:Mn/ZnS and a red-purple color for NAC-ZnS:Mn/ZnS Qdot. The insets the Figure 1 (A) and (B) show the particle size distribution of NAC coated CdS and ZnS Qdots with a maximum number of nanoparticles in the range of about 3.5 nm.

Figure 2 highlights the scheme used in our study. Snow pea (Pisum *sativum*) seeds were treated with the NAC-Qdot solution for 48 hours. After 48 hours, cross sections of the control and treated seeds were studied using phase contrast microscope. As can be seen in Figure 2, the fluorescence images obtained suggested the presence of the these Qdots in the intercellular regions in the plant seed tissues of the cross section (red regions in the images presented). Some of the treated seeds were allowed to grow further until their seedling phase. Treated seedlings displayed enhanced growth as compared to control seedlings.

The composition of the features observed in fluorescence images was confirmed by Raman confocal measurements. A large fluorescent background prevented us from resolving the signature of the plant. However a large shift of the fluorescence background is indicative of a change in the Qdots or an interaction between the Qdots and the plant tissue. The results are summarized in **Figure 3.**

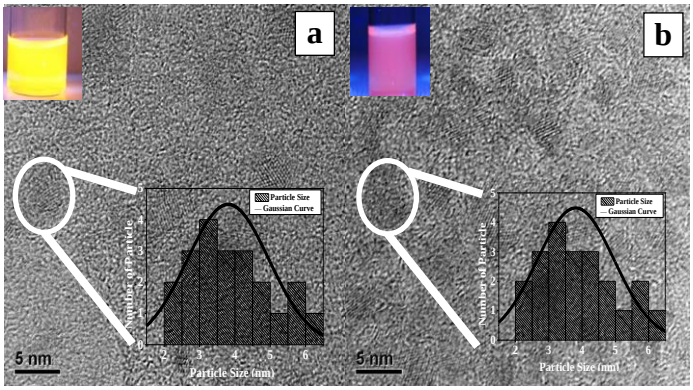

Figure 1. HRTEM images of (a) NAC coated CdS:Mn/ZnS and (b) NAC coated ZnS:Mn/ZnS Qdots.

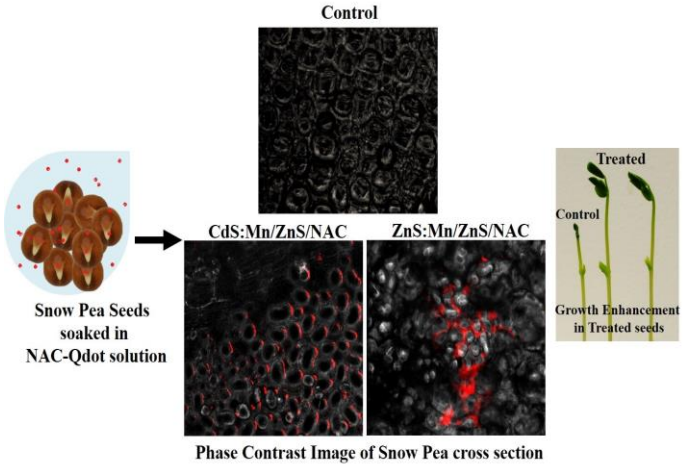

Figure 2. Scheme displaying NAC-Qdot uptake by snow pea (*Pisum sativum*) seeds. The direct comparison of

sections obtained on control and treated sections shows the presence of Qdots, appearing in red. The Qdots are located in the intercellular regions for the tissues. These presence of the NAC-Qdots was showns to enhance the growth of the plants.

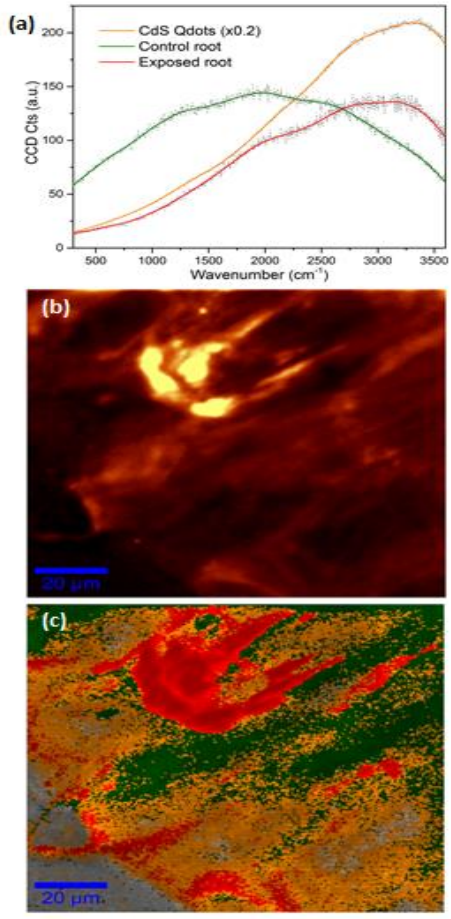

Figure 3. Overview of the Raman study of the NAC-Qdot uptake by snow pea (*Pisum sativum*) seeds. (a) Average Raman spectra of the NAC-coated Qdots (yellow), signature of plant tissue loaded in Qdots (red) and Qdot-free plant tissue (green). (b) In the epidermis, the Raman response at each point was integrated over the full spectrum to reconstruct the chemical map. (c) k-mean cluster analysis of the Raman signatures over the same region revealing the Qdots diffusion over the phloem (Red: high quantum dot concentration, orange: moderate quantum dot concentration, green: low quantum dot concentration).

6 CONCLUSION

Our result shows that the NAC coated ultra small sized fluorescent ZnS:Mn/ZnS and CdS:Mn/ZnS Qdots accelerated the growth of snow pea (Pisum *sativum*) plants and confirmed the growth is dose dependent. Spectroscopic and imaging techniques were used in translocation of the Qdot inside plant cells. These Qdots could potentially serve as surrogate fluorescent markers for studying uptake, mobility and potential fate of ultra-small size bactericide nanoparticles such as zinc oxide based bactericides/micronutrient.

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