MONITORING OF QUANTUM DOT DISTRIBUTION IN PLANTS BY *IN VIVO* FLUORESCENCE IMAGING

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Abstract

Nanoparticle visualization has extensive potential for their utilization in targeted drug delivery tracking through wide range of biological environments. In this regard, quantum dot nanoparticles may represent a suitable option as they are characterized by very good fluorescent properties. This study is focused on application of quantum dot nanoparticles for fluorescence imaging of plants. A series of *in vivo* experiments was carried out on leaves of sunflower plant (*Helianthus annuus*). Leaves were immersed in water solution of CdTe and CdTe/ZnS quantum dots and were monitored (λ_{em} 535 nm and 600 nm respectively) for 8 hours at time intervals of 60 minutes using In Vivo Xtreme Imaging System (Bruker, Massachusets, USA). Captured images were further processed and the intensity of fluorescence analyzed by Bruker Molecular Imaging Software. Fluorescence imaging of plants encounters difficulties due to autofluorescence of biomolecules present in plants, including chlorophyll, carotene and xanthophyll. However, this can be suppressed by using adequate excitation and emission filters for fluorescence images acquisition. Moreover, visualization of nanoparticles in plants can be enhanced by multispectral imaging and spectral modeling for autofluorescence unmixing. In this study, evaluation regarding optimal characteristics and modifications of quantum dot nanoparticles for fluorescence imaging in plants is provided as well as recommendations on image acquisition setting and further image postprocessing.

Keywords: Quantum dot nanoparticles, *in vivo* imaging, fluorescence imaging, *Helianthus annuus* plant, nanoparticle transport visualization

1. INTRODUCTION

Nanomaterials gained immense popularity over the years in a wide range of biological applications, including the field of therapy, targeted drug delivery and diagnostics. Concerning this, semiconductor nanocrystals known as quantum dots (QDs) have been the centre of great recent interest [1]. They belong to a class of fluorophores that combine excellent fluorescence performance with a highly customizable surface for directing their bioactivity, producing a fluorescent probe that outperforms traditional organic dyes in many *in vivo* applications [2]. However, there is only a small body of literature dedicated to QDs use for transport tracking in plants.

In vivo imaging of plant leaves may represent a special challenge because of rich pigmentation, light-scattering starch granules and multiple layers of cell walls. In leaf tissues, biomolecules such as chlorophyll, xynthophyll and carotene as well as stressed or photodamaged cells, are the main source of autofluorescence [3]. While chlorophyll fluorescence of chloroplasts occupies the red/far-red region of the spectrum (650-750 nm), fluorescence of lignin present in cell walls is obtained in a wide range of visible spectrum (490-620 nm) [4]. Autofluorescence of cell walls can also be beneficial to locate different cell types and cell borders. On the contrary, strong chlorophyll fluorescence limits the use of red fluorescent markers in green leaves. Hence, the unique photophysical properties of quantum dots represent a promising platform for *in vivo* fluorescence monitoring in plants, as due to their tunable characteristics, the emission spectra can be shifted toward the minor autofluorescence regions.

Several studies have been proposed regarding nanoparticles visualization and examination in plants, including upconversion nanoparticles in *Phalaenopsis* and *Arabidopsis* plants [5]; QDs nanoparticles for plant chromosomes visualization [6]; or their potential toxicity on ryegrass, onion and chrysanthemum plants [7]. However, the uptake of nanoparticles by plants and how they affect their biochemistry is not well investigated. Generally, the first stage of the experimental design is the plant soaking of the nanoparticles from the solution or soil, followed by root or stem cutting and examination whether the particles can be detected in different parts and lengths. For this purpose, confocal laser scanning microscopy is usually used. To our knowledge, *in vivo* fluorescence imaging had not been widely used for monitoring of nanoparticle uptake by plants. However, we assume that whole leaf fluorescence imaging of nanoparticle represent a great potential thanks to its noninvasiveness and possible long term experiment follow-up.

In this study, we report on the uptake of CdTe quantum dot nanoparticles by *Helianthus annuus* L. plant. *In vivo* imaging by fluorescence scanner had been used for this experiment. Whole-leaf fluorescence images taken at periodic intervals confirm the uptake of the QDs nanoparticles to the petiole and main venation.

2. MATERIALS & METHODS

2.1. Quantum Dots

CdTe QDs have been prepared according to published methods [8, 9]. Shortly, a solution of CdTe QDs was prepared by mixing of cadmium acetate dihydrate (53 mg in 76 mL of water) with mercaptosuccinic acid (MSA) (60 mg/mL) followed by addition of 1M NH_3 (1.8 mL). Then, a solution of sodium tellurite (6 mg/mL) was added and sodium borohydride (40 mg) was poured into the stirred solution. Volume was adjusted with water to 100 mL. Vials were heated at 60 °C in microwave oven Multiwave 300 (Anton Paar, Graz, Austria) (300 W, 10 min) to obtain green QDs.

Spare solution of ZnS was prepared by mixing solutions of zinc acetate (22 mg), MSA (30 mg), 1 M NH3 (0.9 mL) and 2.5 mL Na_2S (0.24 g/50 ml) [10]. Water was added to 50 mL.

Core shell CdTe/ZnS were obtained by mixing CdTe QDs with spare solution of ZnS in 1:1 ratio and heating in microwave oven at 60 °C.

CdTe-PVP QDs were obtained by adding 100 mg polyvinylpyrrolidone (PVP 40 kDa) to 50 mL of green CdTe QDs, shaking overnight and filtering of solution through a frit.

QDs were further characterized using fluorimeter Infinite M200 Microplate reader (Tecan, Switzerland) and dynamic light scattering (Zetasizer Nano ZS90, Malvern instruments, Malvern, UK).

In selected cases, quantum dots were precipitated by isopropanol (1:1 ratio) to remove the unreacted precursors of the synthesis following the procedure published in [11, 12].

2.2. Plant cultivation

As an experimental plant, sunflower (*Helianthus annuus* L.) Kongo hybrid was used. The achenes were sterilized (20 minutes in 20 % SAVO solution) and planted in perlite substrate. Then the achenes were germinated for seven days at 22 °C with photoperiod day/night 16/8 hours. After that, the grown seedling plants were transplanted into the hydroponic container containing Murashige-Skoog medium including vitamins (Duchefa Biochemie, Netherlands) and grown for 6 weeks under standard conditions at 22 °C, day/night 16/8 photoperiod and humidity of 55 %.

2.3. *In vivo* Fluorescence imaging of qds distribution

Monitoring of QDs distribution in sunflower leaf was performed using an In Vivo Xtreme Imaging System by Bruker (Massachusetts, USA). The parameters used for image acquisition were following: exposition time - 2 s, binning - 4x4 pixels, fStop - 1.1, field of view - 15x15 cm.

Monitoring of CdTe/ZnS quantum dots has been performed using excitation filter of 550 nm and emission filter 600 nm. Besides, CdTe-PVP QDs have been visualized by filters of wavelength λ_{ex} 480 nm and λ_{em} 535 nm.

For image postprocessing and analysis Bruker Molecular Imaging Software (Bruker, Massachusetts, USA) was used. This involved application of false color LUT to obtain pseudo-colored image for enhanced QDs visualization. Moreover, the image subtraction was used to register the QDs uptake over time. In Bruker Multispectral software (Bruker, Massachusetts, USA), the spectral modeling was performed in order to unmix QDs fluorescence and autofluorescence of the leaf.

3. RESULTS & DISCUSSION

In this study, the series of *in vivo* experiments has been done to evaluate the potential of CdTe-based quantum dots for monitoring of their distribution in sunflower plants.

Leaves of 7 weeks old sunflower plant were first cut. The petiole was immersed in a tube filled with quantum dot solution. The first image was captured at time 0 and subsequently, the distribution of QDs has been monitored at 60 minutes intervals for 8-hour period. Additional image was usually acquired after 24 hours from the start of the experiment.

When using crude CdTe QDs sample solution, a little uptake of the fluorophore through petiole and main venation was visible only for first 3 hours. After that, probably an obstruction in vascular bundle appeared and the soaking of QDs solution stopped.

Additionally, CdTe QDs solutions diluted 10 times with Milli-Q water have been examined. As can be seen from the **Figure 1**, the fluorescence of CdTe-PVP QDs is clearly visible after 2 hours from the beginning of the experiment and the QDs uptake to the main venation is increasing in time. In this case, the false coloring 1000-3000 counts was used. Moreover, the fluorescence image captured after 24 hours shows the QDs uptake also to the side venation of the leaf.

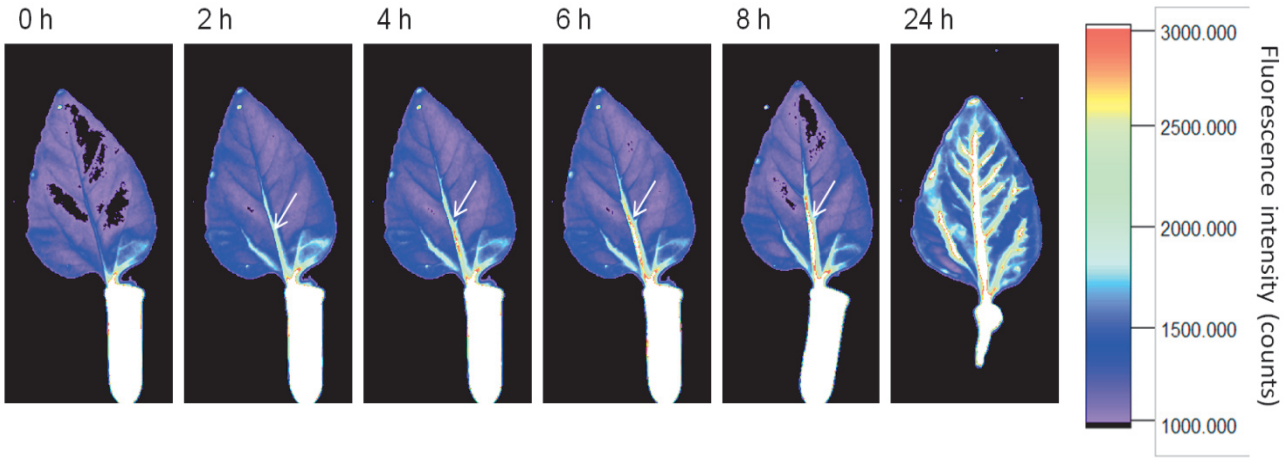

Figure 1 CdTe-PVP QDs (λ_{ex} 480 nm, λ_{em} 535 nm). Whole-leaf fluorescence images captured at time intervals as noted above pictures respectively

The images were further analyzed using Bruker Molecular Imaging Software. The fluorescence intensity was evaluated in three regions of the main venation (**Figure 2**, left). From the graph (**Figure 2**, right) we can confirm the QDs uptake by growing trend of the mean fluorescence intensity in regions *a* and *b*. In region *c*, the mean fluorescence intensity remains relatively consistent during first 8 hours, thus there is no evidence of QDs uptake to this region. However, QDs uptake can be confirmed from the fluorescence image captured after 24 hours, where the increased fluorescence intensity in region *c* is demonstrable.

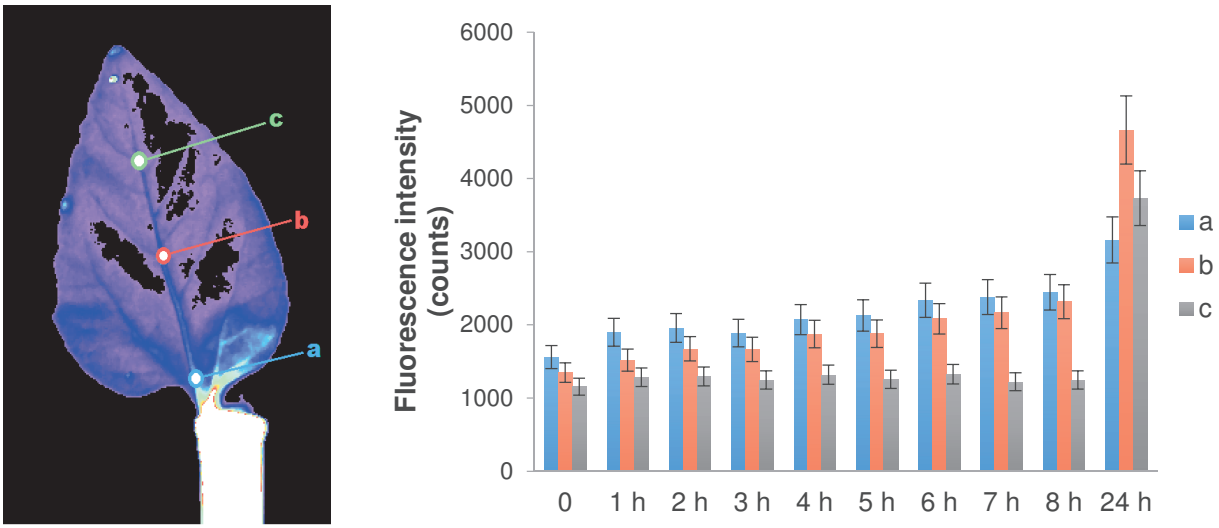

Figure 2 CdTe-PVP QDs (λ_{ex} 480 nm, λ_{em} 535 nm). Fluoresence intensity was analyzed in 3 leaf regions (*a-c*) of the main venation as illustrated in the picture (left). Graph (right) shows the mean fluorescence intensity in these regions as a time-dependent variable

Similarly, when using crude CdTe/ZnS QDs sample solution, the uptake was visible only for approximately 3 hours from the start of the experiment. Nevertheless, the Milli-Q water diluted CdTe/ZnS QDs solution provided better outcomes, as QDs accumulated in the main venation when examined after 24 hours from the start of the experiment (**Figure 3**, left).

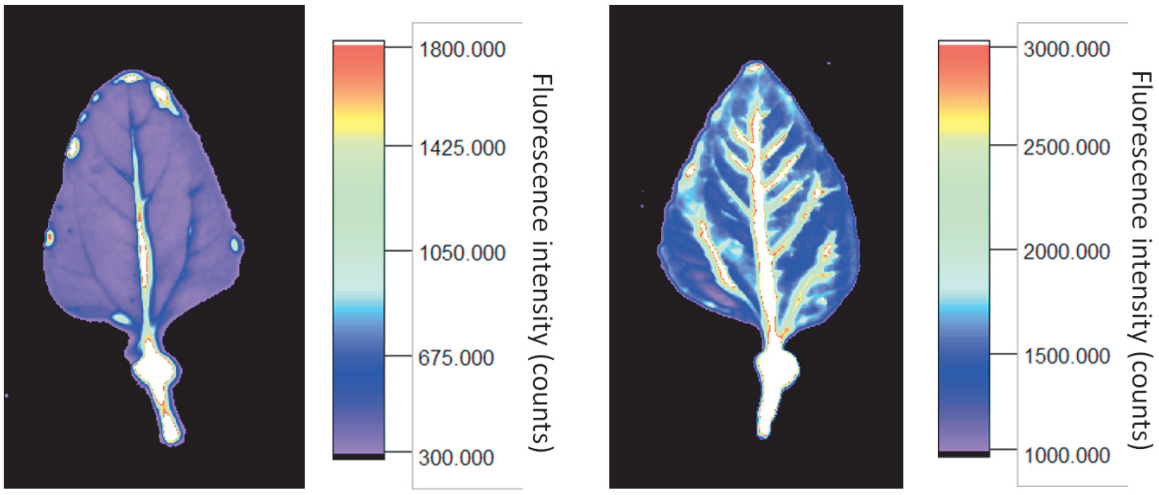

Figure 3 Comparison of CdTe/ZnS (λ_{ex} 550 nm, λ_{em} 600 nm) (left) and CdTe-PVP QDs (λ_{ex} 480 nm, λ_{em} 535 nm) (right). Fluorescence images captured at 24 hours from the start of the experiment

Furthermore, precipitation of quantum dots by isopropanol has been done and Milli-Q water solution of precipitated QDs has been examined in this set of experiments. Nonetheless, this procedure has not led to any visible plant uptake.

4. CONCLUSION

The fundamental aspect of this research was to evaluate the potential of CdTe-based QDs for monitoring of their distribution in plants by *in vivo* fluorescence imaging. For this purpose, core shell CdTe/ZnS and

CdTe-PVP quantum dots have been examined in leaves of sunflower plant. The most promising results were obtained by monitoring of CdTe-PVP QDs diluted with Milli-Q water as after 24 hours from the beginning of the experiment QDs were visible in the petiole, main venation and side venation of the leaf. These particular quantum dot nanoparticles have several unique properties including average size of 5.6 nm and their emission spectra in the green region of visible spectrum, so the chlorophyll autofluorescence of the leaf can be appropriately suppressed.

Results of our preliminary experiments on young whole plants of sunflower encourage us for further examination of these particular quantum dot nanoparticles and *in vivo* QDs distribution monitoring. Quantum dots may represent a stable platform for targeted drug delivery and visualization to be used in agriculture.

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