FLUORESCENCE PROPERTIES OF QUANTUM DOTS

Šmerková K.[1], Blažková I.[1], Chudobová, D.[1], Vaculovičová M.[1, 2], Kopel P.[1, 2], Adam V.[1, 2],Kizek R.[1, 2]

[1]Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Zemedelska 1, 613 00 Brno, Czech Republic

[2]Central European Institute of Technology, Brno University of Technology, Technicka 3058/10, 616 00 Brno, Czech Republic

E-mail: xsmerkov@node.mendelu.cz

ABSTRACT

The aim of this study was the synthesis of CdTe quantum dots (QDs) and study of their fluorescence properties and their potential for the use in the bioimaging. QDs are small semiconductor nanoparticles (1 – 20 nm), which can be used in the imaging instead of the organic labels. The CdTe QDs were synthesised by microwave synthesis in an aqueous solution. As the source of telluride, Na_2TeO_3 was used, and as reduction agent, sodium borohydride was applied. Quantum dots were stabilized by mercaptosuccinic acid (MSA). According to reaction conditions (temperature: 50 – 130 °C), size of prepared quantum dots can be tuned. Synthesised QDs had got very good fluorescence properties and were used for the cell labelling. The QDs penetrated into the cells and stained plant cells, as well as human foreskin fibroblasts. But the changes in the cells shapes were observed, the reason could be the toxic effect of QDs, which should be more investigated.

For the usage of QDs in medicine, it is necessary to know their behaviour in the tissue. We investigated the behaviour of QDs in the chicken breast muscle tissue. After the direct injection of QDs into the muscle tissue, sufficient spreading of QDs in the tissue was observed and a significant linear increase of the fluorescence intensity of QDs with applied volume was determined. To detect the limiting depth for the signal detection, the tubes filled with different QDs was inserted into the different depths of the tissue. The intensity of the fluorescence of QDs depended on the size of QDs, therefore red QDs was possible to detect the most deeply (10 mm). Using the different emission filters, it is possible to distinguish between the different QDs. It enables the use QDs in the simultaneously labelling of different structures in the cells or the organisms.

Key words: quantum dots, fluorescence, microscopy, imaging, labels

Acknowledgments: Financial support by IGA TP1/2013 and PGS09_2012 is highly acknowledged.

INTRODUCTION

Quantum dots (QDs) are semiconductor nanoparticles. QDs are promising the next generation of fluorescent probes. They can be used to biosensing, biolabelling and delivery of therapeutic agents (Frasco and Chaniotakis 2010). QDs have got great fluorescence properties, they have high quantum yields, broad absorption spectra, narrow size tuneable emission spectrum and are photostabile (Drummen 2010). The studying of quantum dots is among the most emerging field of nanotechnology. Due to the properties of quantum dots, they can be used not only in *in vitro* but also in *in vivo* imaging. Quantum dots could be used in bioimaging instead of the traditional fluorescent materials (e.g., organic dyes, fluorescent proteins). The limitation in the biolabelling is the potential toxicity of QDs on cellular proliferation and homeostasis. The proper tuning of QD dose, surface ligand, and delivery modality can provide robust *in vitro* cell labelling reagents that exhibit minimal impact on cellular viability (Bradburne, Delehanty et al. 2013). Quantum dots can be applied in bioanalytical chemistry and biology as the specific labels of body tissues, antibodies, oligonucleotides, enzymes, etc. The conjugation of quantum dots with biomolecules opens the way for their use in the biological labelling. Quantum dots can be used in medicine, food safety control and environment monitoring (Medintz, Uyeda et al. 2005; Algar, Tavares et al. 2010). The limiting factor of the usage of QDs in the living organisms is the thickness of the tissue the light need to penetrate (Maestro, Ramirez-Hernandez et al. 2012). The next disadvantage of the *in vivo* imaging is the high autofluorescence of the tissue at low wavelengths, therefore it is preferable to study living organisms using near infrared radiation (NIR) (Frangioni 2003).

The aim of this study was the synthesis of CdTe QDs and analysis of their fluorescence properties. The behaviour of QDs was observed in cell cultures and in the chicken breast muscle tissue.

MATERIAL AND METHODS

Preparation of QDs

All chemicals were purchased from Sigma-Aldrich and used without further purification. Cadmium (II) acetate $Cd(OAc)_2$ (10 mL; 5.32 g/L) was dissolved in ACS water (25 mL). Mercaptosuccinic acid (MSA) (1 mL; 60 mg/mL) was slowly added to stirred solution. Afterwards, 1.8 mL NH_3 (1 M) and 1.5 mL Na_2TeO_3 (4.432 g/L) was added. $NaBH_4$ (40 g) was poured into the solution under vigorous stirring. Subsequently the ACS water was added to the final volume of 100 mL, than the solution was pipetted (2 mL) into the vials, which were closed and put into the Microwave Reaction System (Multiwave 3000, Anton Paar, Graz, Austria). Microwave heating conditions: max. 300 W, temperature: 50 – 130 °C (QDs1 – 50 °C, QDs2 – 50 °C, QDs3 – 60 °C, QDs4 – 80 °C, QDs5 – 90 °C, QDs6 – 90 °C, QDs7 – 100 °C, QDs8 – 120 °C, QDs9 – 130 °C, QDs10 – 130 °C) 10 minutes rising of temperature, 10 minutes continuance and then cooling. Synthetized QDs were stored in dark at 4 ºC.

Fluorimetric analyses

Fluorescence spectrometer Tecan infinite M200 PRO (Grödig, Austria) was used for the fluorometric analyses. Samples of volume of 100 µL were placed in a Nunc microplate MaxiSorp (Thermo Fisher Scientific, Roskilde Denmark). The absorbance spectrum was measured (300 – 1000 nm). The highest absorbance was set as excitation and the fluorescence spectrum of QDs was measured (430 - 850 nm). The parameters were as follows: number of flashes: 5; emission wavelength step size: 5 nm; gain: 50.

Staining of the cells by QDs

The tobacco cells (200 µL) in the medium were incubated with 400 µL of QDs for 15 or 60 minutes (1400 rpm, 20 °C; Thermomixer® comfort, Eppendorf, Germany). After the incubation the cells were washed by PBS for three times and observed by fluorescence microscope. The human foreskin fibroblasts in microtitration plate with 200 µL medium were incubated with 50 µL of QDs. After the incubation the cells were washed by PBS for three times and observed by fluorescence microscope.

The inverted system microscope Olympus IX71S8F-3 (Olympus Corporation, Tokyo, Japan) was used for imaging of the cells. The images were captured by Camera Olympus DP73 and processed by Stream Basic 1.7 Software, the images resolution was 4800 × 3600 pixels. The parameters were follows: magnification: 100×, ISO 200.

Application of QDs into the muscle tissue

Quantum dots were applied directly into the chicken muscle tissue or into the tube (internal diameter of 2 mm) and it was inserted into the different depths of the tissue (0, 2, 5, 7 mm) and the fluorescence was detected. The fluorescence of QDs was detected by Carestream In-Vivo Xtreme Imaging System (Carestream Health, Inc., Rochester, USA) using specific filters for a given QDs. The images were analysed by Carestream molecular imaging software (Carestream Health, Inc., Rochester, USA) and processed by software PhotoFiltre Studio X.

RESULTS AND DISCUSSION

In this work, the CdTe QDs capped by mercaptosuccinic acid were synthesised by microwave synthesis. The different colour QDs were prepared by changing the temperature of the reaction (50 – 130 °C). The colour of QDs was detected (Fig. 1) in transilluminator (excitation: 312 nm). Using the low temperatures (50, 60 °C) blue light QDs were synthetized, in the high temperatures (130 °C) red colour QDs were prepared. From the blue colour to the red colour QDs grows the size of QDs (Cai, Hsu et al. 2007).

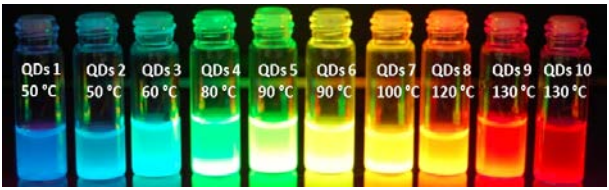

Fig. 1 Colour of QDs was observed in the transilluminator (excitation: 312 nm) and whole visible light spectrum was observed.

The absorbance and fluorescence spectrum of QDs was measured by fluorescence spectrometer Tecan. The detected absorbance spectrum showed the absorbance of all QDs in the low wavelengths. After the QDs excitation by the irradiation of 400 nm, the growing fluorescence intensity of the QDs with their size was detected. The red QDs (QDs 10) had the best fluorescence properties and seem to be good for the *in vivo* imaging. The potentiality of the QDs usage in the bioimaging was tested on the plant and human cells. The tobacco cells were incubated with QDs (QDs 10) it was found, that 15 minutes incubation with QDs is sufficient for the tobacco cells staining (Fig. 2). In the case of human cells (Human foreskin fibroblasts), was necessary to incubate the cells a longer time. After 4 hours of the incubation the QDs in the human cells were detected. But the changes in the cells shapes were observed, the reason could be the toxic effect of QDs.

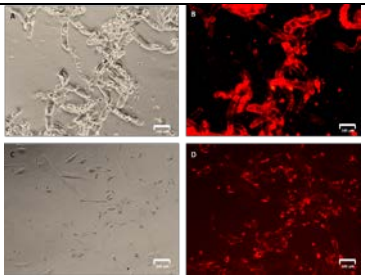

Fig. 2: The staining of the cells by QDs. The cells were observed by fluorescence microscope with the magnification: 100×: A) Tobacco cells in the ambient light after the 15 minutes incubation with the red QDs (QDs10, excitation: 640 nm, emission: 695 nm); B) Fluorescence of the tobacco cells after the 15 minutes incubation with QDs10 detected with the excitation filter: 545-580 nm and emission filter: 610IF; C) Human foreskin fibroblasts in the ambient light after the 240 minutes incubation with QDs10; D) Fluorescence of the QDs10 in the Human foreskin fibroblasts after the 240 minutes incubation with QDs10.

Next step was a study of the QDs behaviour in the muscle tissue. For the analyses the chicken breast muscle tissue was used and the fluorescence was detected by Carestream In-Vivo Xtreme Imaging System. Quantum dots were injected into the muscle tissue and the fluorescence was detected depending on the applied amount and depth of the injection. For the applications of the different QDs volumes into the muscle tissue, the syringe was firmly attached to the stand to ensure the application of the QDs into the same place. Fig. 3 shows the distribution map of the QDs fluorescence applied into the muscle tissue into the 3 mm depth in an amount of 100, 200, 300, 400 and 500 μL. Sufficient spreading of QDs in the tissue was observed. The fluorescence intensity as well as the spatial distribution of the quantum dots in the tissue with the applied amount increase. Furthermore, it was found that after the QDs application into the muscle tissue, the spatial distribution occurs almost immediately and a further area expansion of the fluorescence signal have not occurred even after a longer time (3 hours). For the QDs usage in the diagnosis, it is important to know the maximal depth, in which is possible to detect the fluorescence signal of studied QDs. The direct application of QDs into the tissue was not suitable for the limiting depth detection, because of the spreading in the tissue. For this reason, the non-fluorescence rubber tube was used. The tube was filled with QDs and inserted into the different depths into the tissue. The tube avoided the QDs spreading in the tissue and the exact depth could be determined. Green (QDs 5) and yellow (QDs 7) QDs was possible to detect in the depth of 7 mm. Red QDs was possible to detect up to the depth of 10 mm. It is due to the best fluorescence properties of red QDs.

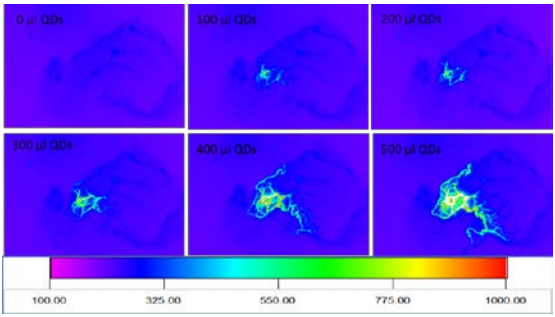

Fig. 3 Quantum dots (QDs 1) injected into a muscle tissue in a volume of 0-500 µL. Excitation wavelength: 410 nm, emission wavelength: 535 nm, exposure time: 1 s, binning: 1×1 pixels, field of view: 7.2 × 7.2 cm.

The Carestream In-vivo Xtreme Imaging System allows the intensity detection of the fluorescence. As well as the fluorimeter it detects only intensity of the radiation, no colour of QDs. But if we use different emission filters, the Carestream In-vivo Imaging System allows distinguishing between the different QDs in the tissue.

In the Fig. 4 is chicken muscle tissue with inserted tubes (3 mm deeply) filled with QDs. Each QDs were detected using the same excitation filter and different emission filters to distinguished the different colour QDs (green, yellow and red QDs). This enables the use of QDs in the simultaneously labelling of different structures in the cells or the organisms.

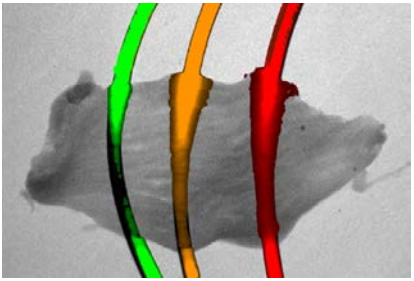

Fig. 4 Fluorescence of QDs in tubes in the muscle tissue (3 mm deeply, 300 µL). Overlay of four images: X-ray, fluorescence of green, yellow and red QDs, excitation: 480 nm, emission: 535 nm (green QDs), 600 nm (yellow QDs), and 700 nm (red QDs).

CONCLUSIONS

QDs have god great fluorescence properties and can be used for cells staining. The limitation of the QDs usage in bioimaging is their toxicity and inability to penetrate a thick layer of the tissue. The synthesised CdTe QDs was possible to detect up to the depth of 10 mm. By using the different emission filters, it is possible to distinguish between the different QDs in the tissue. It enables the use them in the simultaneously labelling of different structures in the cells or in the organisms.

REFERENCES

Algar, W. R., A. J. Tavares, et al. (2010). "Beyond labels: A review of the application of quantum dots as integrated components of assays, bioprobes, and biosensors utilizing optical transduction." Analytica Chimica Acta **673**(1): 1-25.

Bradburne, C. E., J. B. Delehanty, et al. (2013). "Cytotoxicity of Quantum Dots Used for In Vitro Cellular Labeling: Role of QD Surface Ligand, Delivery Modality, Cell Type, and Direct Comparison to Organic Fluorophores." Bioconjugate chemistry **24**(9): 1570-1583.

Cai, W., A. R. Hsu, et al. (2007). "Are quantum dots ready for in vivo imaging in human subjects?" Nanoscale Research Letters **2**(6): 265-281.

Drummen, G. P. C. (2010). "Quantum Dots-From Synthesis to Applications in Biomedicine and Life Sciences." International Journal of Molecular Sciences **11**(1): 154-163.

Frangioni, J. V. (2003). "In vivo near-infrared fluorescence imaging." Current Opinion in Chemical Biology **7**(5): 626-634.

Frasco, M. F. and N. Chaniotakis (2010). "Bioconjugated quantum dots as fluorescent probes for bioanalytical applications." Analytical and Bioanalytical Chemistry **396**(1): 229-240.

Maestro, L. M., J. E. Ramirez-Hernandez, et al. (2012). "Deep tissue bio-imaging using two-photon excited CdTe fluorescent quantum dots working within the biological window." Nanoscale **4**(1): 298-302.

Medintz, I. L., H. T. Uyeda, et al. (2005). "Quantum dot bioconjugates for imaging, labelling and sensing." Nature Materials **4**(6): 435-446.