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Phytotoxic effects of CdTe quantum dots on root meristems of *Allium cepa* L.

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Abstract

The effect of solutions of cadmium telluride quantum dots (CdTe QD) as powerful cytotoxic effectors was investigated using a standard *Allium cepa* L. test system. The diameters of synthesized CdTe QD derived from the optical data varied within 3 – 4 nm. Toxicity of experimental solutions of CdTe QD at the organism level were evaluated by measuring biomass growth of onion roots and cytotoxic influence was estimated based on proliferative activity of root meristem cells. All of CdTe QD experimental solutions in concentration 10 μM significantly inhibited the growth of *Allium cepa* L. roots, proliferative activity, and total dehydrogenase activity. Relation between the QDs size and their phytotoxicity was not found. However, the highest inhibiting impact was for QDs solution with a nanocrystals size of 3.5 nm. Their ability to penetrate into cells and interact with their intracellular components may cause inhibiting mitosis without fixed clastogenic and aneugenic effects. Solutions of CdTe QD at a given concentration can be considered as potent cytostatic agents for plant cells with antimitotic properties.

Introduction

The extraordinary properties of semiconductor quantum dots (QDs) have resulted in their widespread application in various fields, from light-emitting and photovoltaic to chemical sensing and biomedical applications (Kargozar *et al*. 2020; Shu *et al*. 2020; Wang *et al*. 2020). QDs of Cd-based II-VI compounds, such as CdS, CdSe, CdTe, are the most developed and understood in terms of both the synthesis and optical properties (Yadav *et al*. 2020). Their bright (with photoluminescence quantum yield up to 90 %) coloured emission can be tuned over the whole visible spectrum by varying the QD size or/and composition (i.e. forming allow QDs, e.g. $CdSe_xTe_{1-x}$). However, the toxicity of QDs remains a concern and delays their commercialization (Jang *et al*. 2018).

An efficient way to reduce the Cd toxicity and simultaneously boost their optical properties is covering QD with a shell of more environmentally

friendly material, e.g. ZnS, additionally stabilized by bio-compatible polymers (Raevskaya *et al*. 2007). The most recent trend in making fluorescent QDs more bio-friendly is synthesizing compounds not containing very toxic metals (Cd, Hg, or Pb), for instance Ag-In-S (Raevskaya *et al*. 2018), Cu-In-Se (Lox *et al*. 2018) or Cu-Zn-Sn-S (Stroyuk *et al*. 2018). The latter materials allow tuning of the light absorption and photoluminescence (PL) in the visible and near IR spectral ranges, while ZnO QDs are a good alternative for the UV range (Panasiuk *et al*. 2014). Unfortunately, an inherent drawback of all the above Cd-free QDs is the large spectral width of the PL band, 100 – 200 nm, which does not allow as fine color tunability as in the case of Cd-based QDs. Moreover, recent studies show toxicity also of QDs consisting of rather non-toxic elements (Kays *et al*. 2020). Therefore, intense research of II-VI QDs with advanced optical properties continues (Yeshchenko *et al*. 2020), currently focused mostly on developing various functionalization of the NC surface and reducing toxicity (Borovaya *et al*. 2016; Filali *et al*. 2019; Garmanchuk *et al*. 2019; Lima *et al*. 2019). Of special interest are water-soluble QDs synthesized directly in aqueous solutions using bio-compatible stabilizers (ligands) (Raievska *et al*. 2020).

Better understanding of the behaviour of QDs in various biological environments is needed, including their impact on human health, plants, etc. Since the possibility of accumulation, uptake, and trophic transfer is long been known for Cd ions (Bardáčová *et al*. 2017), allocation of Cd-based QDs has also been studied (Hu *et al*. 2010; Majumdar *et al*. 2019; Sun *et al*. 2021).

From the second half of the 20th century in ecotoxicological research, the *Allium*-test using various colorants is extensively used for toxicity investigation of various environmental pollutants due to its low cost and high sensitivity (Fiskesjö, 1997; Leme and Marin-Morales 2009). Studies on the effects of Cd ions using *Allium*-test have shown toxicity on macroscopic – growth responses and microscopic levels – decreasing of proliferative activity of root meristems with various clastogenic and aneugenic effects (Seth *et al*. 2008; Zou *et al*. 2012; Arya and Mukherjee 2014).

Previously in our investigation, *Allium*-test has also been used for the assessment of toxic impact of stabilized and non-stabilized nanoparticles of essential or biocide metals and their oxides, e.g. Cu, C_2O, Cu_2O, Fe, Fe_2O_3, Fe_3O_4, Zn, ZnO, Mn, Mn_3O_4, Mn_2O_7, Ag, Ag_2O (Konotop *et al*. 2014; 2019). The aim of this work was to investigate toxicity of experimental solutions of CdTe QD at levels of individual cells and entire organism using the standard *Allium cepa* L. test system.

Experimental

Nanomaterials

All chemicals used were of analytical grade or of the highest purity available. Milli-Q water was used as a solvent. CdI_2, NaOH, and thioglycolic acid (TGA ≥90 %) were purchased from Himlaborreactive (Ukraine). CdTe QDs were synthesized by means of colloidal chemistry, following the protocol described by us previously (Kapush *et al*. 2019). Briefly, the low-temperature colloidal synthesis was performed in the reactor with complete mixing and in the presence of TGA as a stabilizer. Deionized water was used as the dispersion medium. CdI_2 was dissolved in water and TGA was added under stirring, followed by adjusting the pH to 11 by dropwise addition of NaOH solution. H_2Te gas was passed through the solution using argon as a carrier gas. The CdTe precursors formed at this stage were subsequently converted into CdTe QDs after heating the solution at 100 °C. Within the given synthesis route (Kapush *et al*. 2019), the NCs of different size can be obtained by varying the magnitude of the electrical current flowing through the electrochemical cell in which the synthesis of the NCs took place. In particular, by changing the current from 0.1 to 0.5 A, a series of samples with the average NC size of 3 nm to 4 nm could be obtained, as estimated from the photoluminescence spectra in Fig. 1.

Photoluminescence

PL spectra were measured from colloidal NC solutions in a standard 10 × 10 mm quartz cuvette using Shimadzu RF-1501 fluorimeter.

Plant material and growth conditions

The phytotoxicity of water solutions of cadmium telluride quantum dots were studied using the standard test object *Allium cepa* L. (Fiskesjö 1985). For each option, 10 equal onion bulbs, cv. Goliant, were cultivated on experimental solutions of CdTe QD for 5 days at 25 °C. Control plants grew in distilled water. Experimental solutions were prepared by dilution with distilled water 100 times to achieve concentration of 10 µM of CdTe QD.

Analysis of growth response, genotoxic and cytotoxic effects

At the end of exposure time, fresh weight and length of roots per bulb were measured. Growth response was estimated according to the tolerance index (TI, %) calculated as the ratio of the fresh length or weight of roots of the experimental group to the same parameter of the control group (Wilkins 1978).

Onion roots were fixed in Clark solution (ethanol : acetic acid = 3 : 1) and after maceration in 1M HCl at the temperature 60 °C throughout 15 min were stained with 1 % (w/v) aqueous solution of toluidine blue. The temporary oppressed slides with root's tips were prepared and further analyzed under a light microscope (PrimoStar, Carl Zeiss) at 400-fold magnification.

Different phases of mitosis in cells of the root apical meristem were counted to calculate mitotic index, for evaluation of genotoxic effects of the experimental solutions. The mitotic index (MI, %) was presented as number of cells in the state of division to the total number of cells observed (3,000) (Eq. 1):

$$MI = (P+M+A+T)/(I+P+M+A+T) \times 100\ \% \quad (1)$$

where P, M, A, T, I are quantity of cells in pro-, meta-, ana-, telo-, and interphase of mitosis respectively (Konotop *et al*. 2019).

Cytotoxicity assessment was providing by following the protocol of Majumdar *et al*. (2017) with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) which is based on potential of dehydrogenases of living cells to reduce MTT. For cell viability (CV) investigation, 10 mg of fresh root meristem was transferred to a microcentrifuge tube and 1 mL of 0.1 % (w/v) MTT aqueous solution was added into each tube and incubated 4 h in the dark. After incubation, the MTT solution was discarded and the root meristems were homogenized in 0.5 mL 2N KOH and 0.5 mL 99.99 % DMSO solution in microcentrifuge tubes. After the color change observed in the solution, the content was centrifuged at 5000×g at room temperature for 5 min. The clear supernatant was transferred to 1 mL spectrophotometry cuvette and the absorbance was determined at 570 nm. The optical density (O.D.) value was calculated for 1 mg of fresh tissue. CV was obtained by the formula (Eq. 2) and expressed as a percentage (%):

$$CV = (\text{O.D. of control set} - \text{O.D. of CdTe QDs set/O.D. of control set}) \times 100\ \% \quad (2)$$

Statistical analysis

Each experiment was performed at least triplicate. The data were subjected to analysis of variance (ANOVA) with subsequent Student's t-test or Duncan's multiple range test. Data are expressed as means of replicates ± standard deviation (bars) and were considered reliable at a significance level of $p<0.01$.

Results and Discussion

CdTe QD solutions analysis

The CdTe QDs used in this study were of several different sizes determined from the spectral position of the maximum of their PL emission, following the generally accepted calibration curves (Kamal *et al*. 2019). The PL spectra of the QDs solution are shown in Fig. 1a, while Fig. 1b shows normalized spectra, for a better visibility of the different spectral position of their maxima. The QD diameters derived from the optical data (3 – 4 nm) are attributed to the crystalline part of the QD as schematically shown in inset to the Fig. 1a.

An important parameter for biological applications and investigations using QDs is their hydrodynamic size, which includes the ligand shell and most often also the layer of solvent molecules. In our case,

when the thioglycolic acid was used as the used as the stabilizer and water as solvent, the hydrodyna-

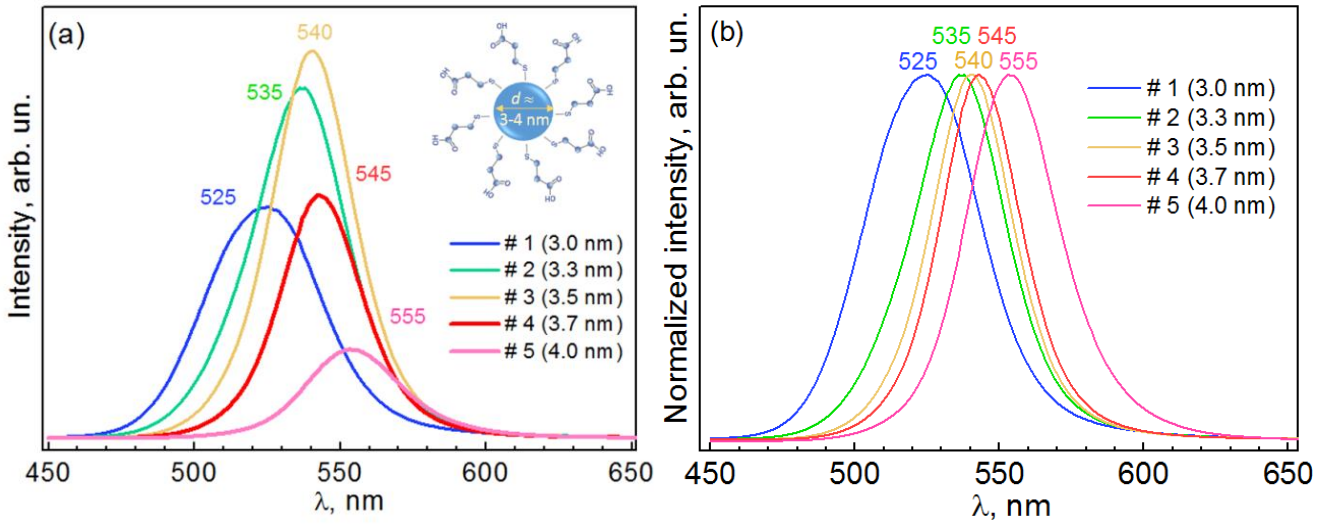

Fig. 1. Photoluminescence spectra of the CdTe QDs used in this work (measured in parental solutions). Absolute PL intensity is in (**a**) and (**b**) shows spectra with normalized intensity for better visibility of different peaks position for different samples (indicated on both figures). The inset to (**a**) schematically shows the QD stabilized by the ligand (TGA). Hereinafter: #1 – 5 are numbers of QD experimental solutions.

mic diameter of the QDs was expected to be larger by not more than 1 nm compared to the one determined from the PL spectra, i.e. 4 – 5 nm, for the series of the samples used in this work. The validity of the above estimation of the hydrodynamic QD size was confirmed by dynamic light scattering investigations of other samples prepared by the same synthesis approach.

Growth response

The obtained results testify that the experimental solutions of CdTe QDs vastly influence the growth of the roots of *Allium cepa* L. (Fig. 2).

Fig. 2. Growth response of *Allium cepa* L. on 5th day of cultivation on CdTe QD experimental solutions (#1 – 5 are numbers of QD experimental solutions).

Morphometric indices of onion roots under the cultivation on experimental solutions are presented in Fig. 3. All solutions of CdTe QDs inhibited the roots growth more than by 50 %, index of tolerance (TI) calculated by length (Fig. 3a) showed less obvious toxicity influence of experimental solutions than TI calculated by mass (Fig 3b). TI of plants grown on the first, fourth and fifth solutions (#1, #4, #5) of CdTe QDs did not differ significantly among themselves.

The CdTe QD aqueous solutions used in this study were chosen because these Cd-based semiconductor nanocrystals in contact with aqueous media have been shown to leach Cd ions,

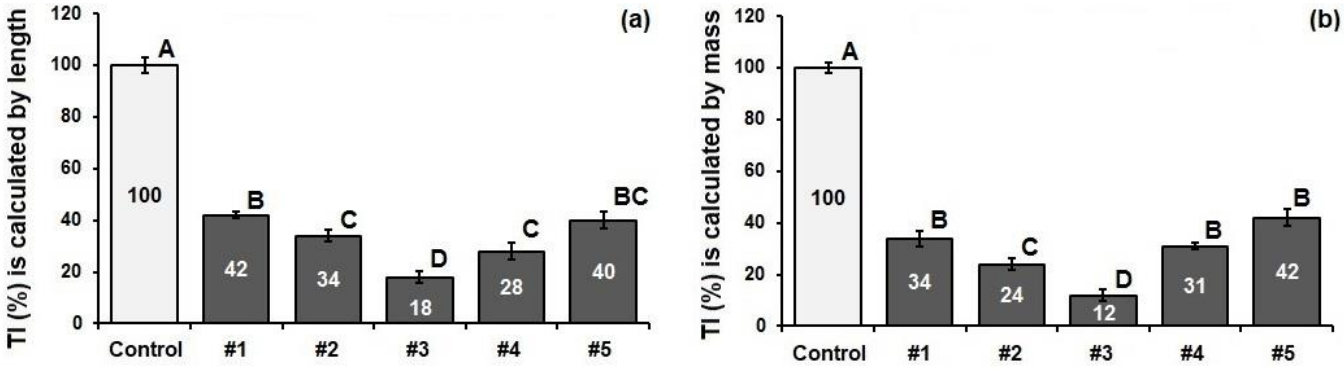

Fig. 3. Tolerance index (TI) of *Allium cepa* L. under cultivation on CdTe QD experimental solutions. TI (%) is calculated by length (**a**) and by mass (**b**) of roots of onion bulbs. Hereinafter: control comprises 100 %; means followed by the same letters were not significantly different at $p<0.01$ according to the Duncan's multiple range test (#1 – 5 are numbers of QD experimental solutions).

which are known for its high toxicity (El Rasafi *et al.* 2020). QDs are fluorescent unique semiconductor nanocomposites that are being widely developed for their strictly size-dependent physicochemical, optical and photophysical traits (Rocha *et al.* 2017). These traits have made QDs a suitable option for applications in various mainstream market products, including light-emitting devices such as displays of smartphones, computers and television (Hobson 2009). Furthermore, it has perspectives in biomedical research, mainly as more affective labels for multitarget simultaneous multicolor imaging of tissues and cells compared with other molecular biotechnologies and traditional fluorescent materials (Wang *et al.* 2020). Despite the fact that Cd-based QDs are causing agents of reactive oxygen species (ROS) in plant and animal cells and free radical damage cellular compartments, their mechanisms of phytotoxicity are still being studied (Michalet *et al.* 2005; Banerjee *et al.* 2021).

The root growth did not depend on the size of QD in the culture medium. Similarly to Modlitbová *et al.* (2018), bulbs of *Allium cepa* in control variant displayed significantly greater total length of the root system in comparison to the bulbs exposed to 10 μM of all CdTe QD experimental solutions. Toxicity of CdTe QD was connected with leaching of free Cd ions from CdTe QDs (Modlitbová *et al.* 2018). Furthermore, these authors claim that matching of the manifestation of toxicity between $CdCl_2$ and CdTe QDs showed no significant differences in growth response at both tested concentrations and both terms of exposure.

Cytotoxicity and genotoxicity

To understand the causes of growth responses of plant under the impact of CdTe QDs, microscopic investigation of *A. cepa* root meristem cells was conducted (Fig. 4).

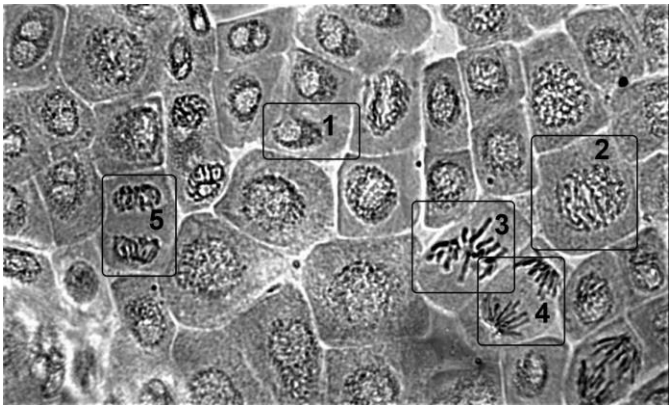

Fig. 4. Root meristem cells of control variant *Allium cepa* L. in the different phases of mitosis on the 5th day of cultivation (magnification 400-fold): 1 – interphase, 2 – prophase, 3 – metaphase, 4 – anaphase, 5 – telophase.

The effect of CdTe QDs solutions on the root cells division is shown in Fig. 5. The phytotoxic effect was noted for all experimental solutions – mitotic index of root meristem cells significantly differed from the control. All solutions of CdTe QDs inhibited proliferative activity of root meristems more than by 50 %. The most reduced proliferative

activity of cells of root apical meristem by 78 % was observed in plants grown on the third solution of CdTe QDs that accorded with TI of the same experimental option. First, second, fourth and fifth solutions (#1, #2, #4, #5) of CdTe QDs vastly inhibited the mitosis in root meristem cells by 51 %, 58 %, 59 % and 49 % respectively (Fig. 5).

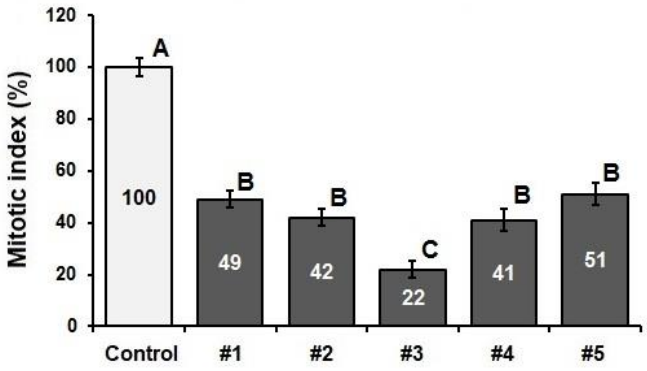

Fig. 5. Proliferative activities of cells of *Allium cepa* L. root meristems (vertical axis is MI, %) under cultivation on CdTe QD experimental solutions (#1 – 5 are numbers of QD experimental solutions).

CdTe QDs capable of releasing free cadmium ions, leading to the disruption of DNA replication, chromosomal aberrations, gene mutation and the generation of ROS (El Rasafi *et al*. 2020). Su *et al*. (2010), on the model of human embryonic kidney cells, shown the higher level of cytotoxicity of CdTe QDs than that of Cd ions and that the cytotoxicity was not only because of free Cd ions releasing. Cytotoxicity was found to be closely depended on the size of quantum dots, with smaller (2 nm) diameter QDs demonstrating more toxic influence than larger (5 nm) QDs (Lovrić *et al*. 2005). Chen *et al*. (2014) demonstrated the potential toxic effects of QDs on inducing ROS generation, pro-oxidant scavengers' activity and DNA laddering in root and shoot tissues of wheat plants.

Investigation of root cells death or meristem cells viability based on method that shown (NAD(P)H) dependent dehydrogenase enzyme attendant in living cells is capable of reducing tetrazolium dye MTT. This enzyme activity takes place when the mitochondria are active therefore, the rate of reduction can directly relate to the number of viable cells. The metabolically inactive (dead) cells do not show this ability (Majumdar *et al*. (2017). Analysis of MTT-test results shown that first, second, fourth and fifth solutions of CdTe QDs (#1, #2, #4, #5 in Fig. 6) inhibited total dehydrogenase activity of root meristem cells approximately by 25 – 30 %.

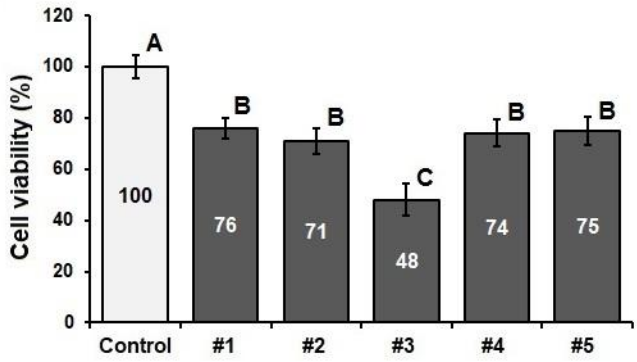

Fig. 6. The percentage of cell viability of root meristems of *Allium cepa* L. under cultivation on CdTe QD experimental solutions (#1 – 5 are numbers of QD experimental solutions).

The most reduced total dehydrogenase activity of cells of root apical meristem (52 %) was observed in plants grown on the third solution of CdTe QDs (#1) that accorded with both TI and MI traits of the same experimental option.

Since experimental solutions of CdTe QD affected growth parameters and proliferative features less than dehydrogenase activity of the root meristem, a relatively high cell viability rate could be explained by involving the CdTe QDs as cytostatic and no cytocidal effectors in the cell cycle of animal cells (Zalgevičienė *et al*. 2012; Garmanchuk *et al*. 2019). On the other hand, genotoxic influence of Cd ions was manifested in the form of various clastogenic and aneugenic breaches in anaphase and telophase steps of mitosis in root meristem cells of *Allium cepa* L. and *Glycine max* L. (Seth *et al*. 2008; Zou *et al*. 2012; Arya and Mukherjee 2014), disrupting the organization of cytoskeleton elements (Gzyl *et al*. 2015).

Conclusion

Recent review (Pagano *et al*. 2018) suggested that the extent to which quantum dots affect plant growth and development and their potential toxic effects may depend on QDs size, structure and stabilization characteristics. Nevertheless, even the shell presence around the QD core does not prevent the penetration and accumulation of QDs in plant

tissues (Modlitbová *et al*. 2018). Due to their size, the QDs used in our investigation could be absorbed by the onion root. However, links between the QDs size and their phytotoxicity were not found. All tested solutions with nanoparticles with a size of 3 – 4 nm inhibited root growth by more than 50 %, as evidenced by the decrease in TI by length, TI by mass and MI. The most unfavorable conditions for onion plants were in a solution containing QDs with a size of 3.5 nm with maximum absorption at 540 nm. The study did not detect genotoxic effects of QDs, although other studies have shown mitosis damages caused by Cd ions (Arya and Mukherjee 2014). The inhibition of plant growth could be explained by the cytostatic effect of QDs, but the mechanism remains unknown. We suggest future phytotoxicological investigation and prolonged ecotoxicology tests with QDs in environmentally relevant conditions to expand our knowledge about possible long-term effects on living organisms.

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Conflict of interest

Authors declare that they have no conflict of interest.

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