Semiconductor quantum dot toxicity in a mouse in vivo model

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Abstract. Quantum dots (QDs) are increasingly widely used in clinical medicine. Their most promising potential applications are cancer diagnosis, including in vivo tumour imaging and targeted drug delivery. In this connection, the main questions are whether or not QDs are toxic for humans and, if they are, what concentration is relatively harmless. We have carried out in vivo experiments with CdSe/ZnS fluorescent semiconductor core/shell QDs, which are currently the most widely used in research.

1. Introduction
QDs are highly fluorescent inorganic semiconductor crystals with a diameter from 2 to 10 nm [1]. They may consist of a semiconductor (CdSe, CdS, CdTe, InP, InAs, or PbSe) core alone or have a core/shell structure (usually, with a ZnS shell), the shell protecting the QD from oxygenation and enhancing the photoluminescence quantum yield [2]. Regarding optical properties, semiconductor QDs are characterized by an exceptionally bright photoluminescence and rock-solid photo- and chemostability. They have broad quasi-continuous absorption and narrow, sharp emission spectra with an approximately Gaussian shape and large (>100 nm) Stocks shifts [3]. The high brightness of fluorescent QDs is a result of high molar adsorption coefficients (several times higher than those of fluorescent dyes and proteins) combined with a high quantum yield [4]. Owing to the broad absorption spectra, different populations of QDs can be excited at the same wavelength, which can be very far from their respective emission bands, depending on the QD core size and composition. The size of QDs can be varied in the process of their synthesis, and the QD fluorescence colour directly depends on their diameters.

In recent years, QDs have been increasingly widely used in biology for cell targeting, imaging, and drug delivery due to their unique optical and physicochemical properties [5]. They are likely to replace the commonly used organic dyes because of their considerable advantages over the organic fluorophores [6]. The use of QDs in laboratory practice is also extending. Their most promising potential applications are in cancer diagnosis, including in vivo tumour imaging.

Cancer remains one of the leading causes of death in the world [7]. One of unsolved problems is differentiation of tumour-affected tissue from healthy tissue during surgery. In addition, noninvasive...
determination of metastases is highly demanded. These problems can be solved by in vivo tumour imaging. Many researchers address the issue of selecting the best method for in vivo tumour imaging using different substances, such as fluorescent proteins [8–13]. Advanced optical characteristics of QDs as compared to organic dyes call for detailed investigation of different aspects of QD use for in vivo optical imaging of tumours. In this connection, the behaviour of QDs in living organisms is of special interest. Certainly, the main questions are whether or not QDs are toxic for humans and, if they are, what concentration of QDs is relatively harmless.

2. Results and discussion

We have carried out in vivo experiments with fluorescent semiconductor CdSe/ZnS core/shell QDs, which are currently the most widely used in research. QDs coated with trioctylphosphine oxide (TOPO) were synthesized, solubilized, and modified with electrically neutral derivatives of polyethylene glycol (PEG) containing both thiol and carboxyl groups to make them soluble in water and aqueous buffer solutions and protect them from clustering [14].

Several stages of purification after coating QDs with PEG were performed so as QDs not to bear any toxic admixtures, such as unbound PEG or other substances. The stability of QDs was accurately measured during one month before the experiments in three different solutions, phosphate buffer, RPMI medium, and mouse blood serum, at different temperatures: room temperature and animal body temperature (37°C). Since QDs were stable under all these conditions, we could be sure that they would not aggregate or decompose shortly after they enter a living body. In addition, the physical characteristics of QDs, including their sizes and emission and absorption spectra (Figures 1, 2), were measured before the in vivo experiment.

![Figure 1. The absorption spectrum of CdSe/ZnS core/shell quantum dots.](image-url)
Figure 2. The emission spectrum of CdSe/ZnS core/shell quantum dots.

Seventy DMA/B6 hybrid mice were used to estimate the QD toxicity in vivo. A QD solution was injected intravenously. A pure solvent and PEG solution were used as negative controls. The mice were examined for about one month after QD injection. The survival rate was estimated throughout the study. Only in the group with the highest concentration of QDs did animals start to die during this period (survival rate, 75%).

During the experiment, we visually accessed the state of health of every animal and measured its weight (Figure 3). No significant weight loss was observed during four weeks.
Special attention was paid to the state of the animals’ hair and eyes. It was found that mice tolerated QDs well even at a concentration as high as 10 mg/kg. The mouse hair, teeth, and eyes looked like those of healthy animals (Figure 4).

![Figure 4. A mouse before the experiment (left) and a mouse three weeks after the injection of 10 mg/kg of QDs (right).](image)

One month after the injection, the internal organs were investigated. We used paraffin sectioning and haematoxylin–eosin staining to assess the state of the liver and kidneys every week during four weeks. Their morphology was the same as in healthy control mice (Figure 5).

![Control](image) ![20 mg/kg](image)

**Figure 5.** Liver and kidney haematoxylin–eosin staining at the fourth week of the experiment.

### 3. Conclusions

These results allow us to conclude that CdSe/ZnS QDs have no considerable toxic effect in the mouse *in vivo* model at concentrations up to 10 mg/kg, because there were no changes in organ morphology, physical parameters, and visually assessed general condition of mice and no weight variation during the four weeks of the experiment.
However, the QDs were found to be toxic at the highest concentration studied, 20 mg/kg body weight, with two-thirds of the animals dying shortly after the experiment was started. Further investigations will be performed to compare the single doses used in this experiment and the minimum therapeutic dose, so that we be able to use the mouse model for testing applications of QDs and their conjugates with biomolecules for tumours detection.

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