Conference Paper

In Vitro Cytotoxicity of CdSe/ZnS Quantum Dots and Their Interaction with Biological Systems

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Abstract

Semiconductor nanocrystals (quantum dots, QDs) have a wide range of potential application in multiplexed tissue and cell imaging, and for *in vivo* molecular diagnostics and therapy. Therefore studying of the toxicity of QDs and their influence on various cellular processes *in vitro* is necessary to understand their interaction with living systems.; The paper presents the results of studies on the evaluation of CdSe/ZnS QD cytotoxicity, as well as the results of studying their interaction with freshly prepared human monocytes *in vitro*.

Keywords: Quantum dots, semiconductor nanocrystals, cytotoxicity, *in vitro* models, monocytes.

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1. Introduction

Quantum dots (QDs) are luminescent inorganic semiconductor crystals [1]. In recent years quantum dots have been widely used for drug delivery [2] and cellular targeting and imaging due to their unique physicochemical and optical properties. QDs have broad excitation spectra and narrow, sharp emission spectra with approximately Gaussian shapes [3]. QDs are considered promising tools for imaging of cellular processes and immunodetection [4, 5] and have proved to be useful for developing more sensitive multiplexed cancer diagnostic systems [6]. Since QDs are becoming more popular in biomedical applications, the question of their toxicity has been raised. The physical and chemical properties of QDs have been well investigated, but little is known about the potentially harmful effects of QDs on the living body. Several commentaries have been made on this issue. QDs could be cytotoxic because they are oxidized while

entering the cell, and core ions (such as Cd^{2+}) are released into the cell environment [7]. Another opinion is that the toxic effect of QDs does not depend on the nanocrystal particle itself, but rather on the biochemical properties of the molecules on their surface [8].

Here, the interaction of CdSe/ZnS QDs with cell systems has been investigated in order to evaluate the QD toxicity and the permeability of cell membranes for QDs. In addition, the physico-chemical properties of QDs taken up by a primary cell culture were analyzed.

2. Materials and Methods

2.1. Quantum dot characterization

After the synthesis, the CdSe/ZnS QDs were transferred from an organic solution to the water phase through the ligand exchange reaction replacing hydrophobic surfactants on the QD surface by the three-functional polyethylene glycol (PEG) molecules with the thiol (SH-) group having a high affinity for the QD surface and the hydroxyl (OH-) group at the outer end of the SH-PEG-OH molecules in order to solubilize the QDs The absorption and fluorescence spectra of the QDs were recorded using a Tecan Infinite 200 Pro microplate reader (Tecan Trading AG, Switzerland). The particle size and zeta potential of QDs were measured by dynamic light scattering (DLS) using a Malvern Zetasizer Nano-ZS (Malvern Instruments Ltd., Worcestershire, UK).

2.2. MTT assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay was used to estimate cell viability. The toxicity of the QDs was evaluated *in vitro* using the SK-BR-3 human breast carcinoma cell line. SK-BR-3 cells were cultured in RPMI medium at 37°C in an atmosphere of 5% CO2. SK-BR-3 cells were transferred into 96-well plates (Costar, USA) containing a final volume of 180 µl/well of RPMI medium. After 24 hours of incubation at 37°C in an atmosphere of 5% CO2 the the QDs were added to the cells in different concentrations. Every concentration of QDs was analyzed in triplicate. After incubation of the cells with QDs at 37°C in an atmosphere of 5% CO2 cells were washed with phosphate buffer (pH 7.4). A solution of MTT was then added to the wells and cells were incubated for 4 hours at 37°C in an atmosphere of 5% CO2.

After formazan formation, the supernatant was removed and solubilization solution (dimethylsulfoxide) was added to each well to dissolve formazan crystals. The solubilisation solution was then thoroughly mixed to ensure complete solubilization. The absorbance of the solution was measured on a photometric analyzer Multiskan EX microplate reader (Thermo Fisher, USA) at the 570 wavelength.

2.3. INTERACTION OF QUANTUM DOTS WITH Freshly isolated human monocytes

Human monocytes were isolated using the ficoll reagent. Ficoll was added to the test tube, and fresh human whole blood was added to the top layer of it. The tube was centrifuged at 1500 rpm for 25 min. The layer of mononuclear cells was removed into a separate tube. The cells were washed with PBS containing heparin at a concentration of 5000 U/ml, and the tube was centrifuged for 15 min at 1500 rpm. The top layer was collected with a pipette. The precipitate was shaken and washed in 10 ml of RPMI-1640 medium at 1000 rpm for 8 min. The cells were counted, and 40–50 million cells per Petri dish were seeded in 1% RPMI-1640 medium with 2% of human serum. The Petri dishes were placed into an incubator for 1.5 h. Unattached cells were removed with a pipette, and the Petri dish with monocytes was gently washed with PBS. Afterwards, 20 ml of 1% RPMI-1640 medium (containing 2% of human serum) was placed into the Petri dish.

To analyze the interaction of water-soluble QDs-PEG-OH with monocytes, the QDs were added to the cell medium up to a final concentration of 1 mg/ml. After incubation at the 37°C for 24 h, the uptake of the QDs by human monocytes was studied using a Carl Zeiss Axio Scope A1 fluorescent microscope (Carl Zeiss, Germany) equipped with a Texas Red filter cube set (EXT540-580, DM595, BA600-660).

3. Results

CdSe/ZnS core/shell QDs were solubilized with three-functional polyethylene glycol (PEG) molecules with the thiol (SH-) group having a high affinity for the QD surface and the hydroxyl (OH-) group at the outer end of the SH-PEG-OH molecules as shown in Fig. 1.

The solubilized CdSe/ZnS QDs were characterized by excellent homogeneity, with the sizes varying from 10 to 12 nm (Fig. 2A) and the fluorescence maximum at 590 nm (Fig. 2B).

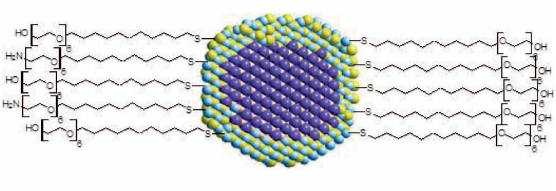

Figure 1: Schematic representation of the water-solubilized CdSe/ZnS QD structure.

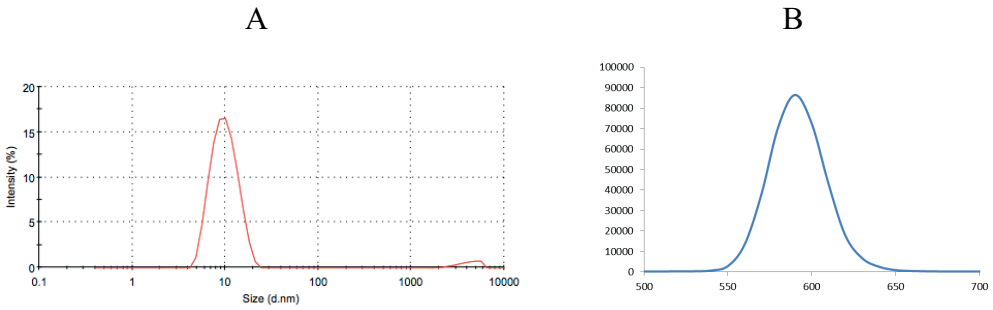

Figure 2: CdSe/ZnS core/shell QD size distribution (A) and fluorescence spectrum (B).

The data show that, at concentrations from 1.2 to 3.7 μg/ml, the QDs exhibit low cytotoxicity, with the cell survival rate between 80 and 100%. At concentrations above 3.7 μg/ml, the QDs become very cytotoxic, with the cell survival rate of 20% or less.

It has also been found that the monocytes take up the QDs within 48 h of incubation in primary culture, and the cell vital activity in the presence of QDs was entirely preserved. It is worth mentioning that the QD fluorescent properties in the human monocyte primary culture were unchanged, as shown in Fig. 3.

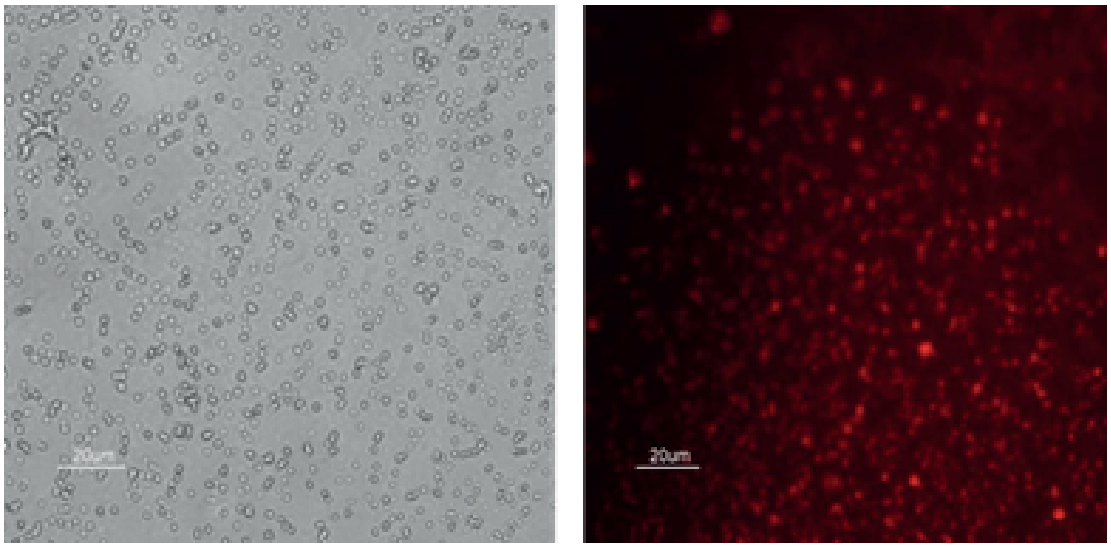

Figure 3: A fluorescence microscopy image of CdSe/ZnS core/shell QDs taken up by monocytes.

4. Discussion

Thus, the obtained data permit us to conclude that nontoxic concentrations of CdSe/ZnS core/shell QDs vary between 1.2 and 3.7 μg/ml. This has permitted us to identify the limit of CdSe/ZnS QD concentration at which they manifest themselves as reasonably safe, nontoxic agents for cell culture applications. The knowledge of the toxicity limits, when applied to cell cultures, allow us to preliminarily estimate the potential effect of CdSe/ZnS core/shell QDs on *in vivo* models.

Knowing that QDs are engulfed by a monocyte culture without losing their fluorescent properties allow us to consider CdSe/ZnS core/shell QDs to be a useful tool for *in vivo* imaging of cellular processes, cancer cells detection, and development of drug delivery systems. Using QDs in imaging tools could bring cancer diagnosis to another level due to the unique physical and chemical properties of QDs.

5. Conclusions

It has been shown that the obtained QDs are characterized by a narrow fluorescent spectrum and size distribution. The nontoxic concentrations of QDs have been determined and fluorescent properties of QDs have been characterized after the uptake of QDs by monocytes. In brief, our data pave the way to the development of safe QD-based tools for *in vivo* and *in vitro* diagnostic and therapeutic applications.

Acknowledgments

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