STUDIES ON BIOLOGICAL ACTIVITY OF QDS IN VERSATILE MICROFLUIDIC SYSTEM

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ABSTRACT

QDs (quantum dots) offer many important benefits as fluorescent probes for biomedical imaging, but they may also pose risk to human health. Therefore, it is extremely important to carry out a systematic study of this relatively new and diverse group of materials. For evaluation of biological activity of QDs we propose a microfluidic system. With the usage of proposed device it is possible to observe morphological changes of cells, and simultaneously investigate their viability and proliferation as a result of cell culture incubation with nanomaterial solutions of various concentrations. Results of cytotoxicity studies of CdSeS/ZnS-MPA confirm usefulness of developed microsystem.

KEYWORDS: Quantum dots, microfluidic system, cytotoxicity, cell culture

INTRODUCTION

There is a wide range of nanomaterials (e.g. carbon nanotubes, fullerene derivatives), which are increasingly being used in many fields such as medicine, electronics, aerospace and energy production or storage [1, 2]. Each year, the prevalence of these materials will be enlarging which, besides to the obvious benefits, may also pose risks and negative effect to human health and the environment [3].

Quantum dots (QDs) are relatively new nanomaterials, which have gained enormous interest. Their unique optical properties in comparison to conventional organic fluorophores, makes them extremely valuable for many applications including biological imaging [4]. QDs constitute a very diverse group of nanostructures. Their synthesis is difficult and expensive, and usually limited amounts of high quality product are obtained. In our opinion advantages of microfluidics and utilization of microsystems can simplify and make research on QDs' bioactivity more accessible and faster.

EXPERIMENTAL

To evaluate quantum dots' toxicity we propose a novel, very simple and versatile microfluidic system. One of the biggest advantage of proposed system is a specially designed microchannel geometry, which allows to generate simultaneously four different concentrations of examined nanoparticles (Figure 1).

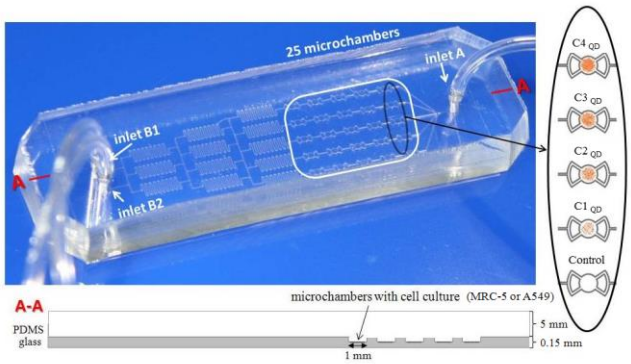

*Figure 1: Microsystem for quantum dots' biological activity studies. Cells' suspension 1*10^6 was introduced into the microsystem (inlet A). After 24h of cell culture QDs' solution (0.6 μM) and medium were infused into the microsystem through inlet B1 and B2 and treated on cells for 24 h under static condition.*

For fabrication of the system we used PDMS and a thin glass slab (24x60x0.13mm) as a bottom part of our system and this way we obtained system which is compatible with a confocal microscope. Such combination allows for precise analysis of morphological changes and identify cells' organelles, in which QDs were accumulated. Cell morphology, viability, proliferation, and QD uptake could be simultaneously investigated in our microsystem. Mentioned cellular events are very important for evaluation of QDs' biological activity. Our QD microscale cytotoxicity studies were conducted for the two different human cell lines: normal (MRC-5) and tumor (A549) lung fibroblast. At first we introduced cells' suspension $1*10^6$ into the microsystem. After cells had been cultured in microchambers for 24 hours, freshly prepared QDs' solution (0.6 µM) and medium were infused into the microsystem and treated on cells for 24 h under static condition. Intermediate solutions ($C3_{QD}$ = 0.45µM, $C2_{QD}$ = 0.3µM, $C1_{QD}$ = 0.15µM) were generated by concentration gradient generator (CGG). In the next stage cell viability test was performed. Fluorescent images of cells were acquired by an inverted fluorescent microscope and were analyzed. As a model nanostructure, which cytotoxicity was monitored we chose CdSeS/ZnS QD stabilized with mercaptopropionic acid (MPA) ligand (Figure 2). Mercaptopropionic acid-coated core-shell quantum dots were prepared via ligand exchange method according to modified Pong procedure [5].

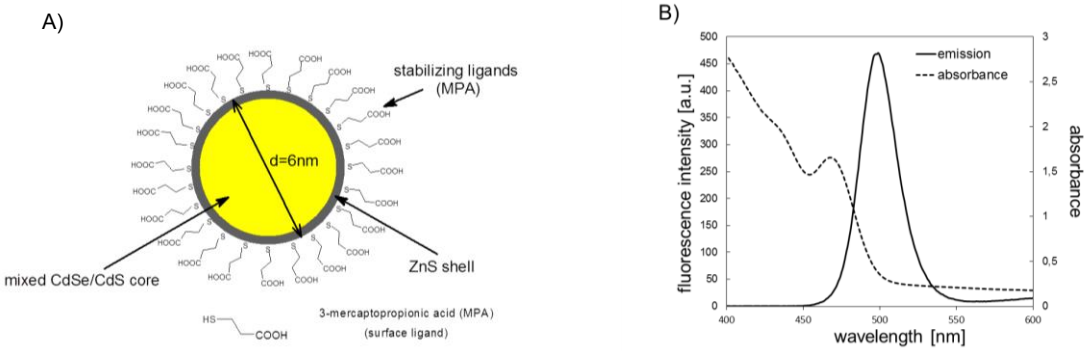

Figure 2: The schematic structure of quantum dots - CdSeS/ZnS-MPA: A) scheme and B) fluorescence and
absorbance spectrum.

RESULTS AND DISCUSSION

For both type of cells (normal and tumor) cultured in microchambers and 96-well microplate the same 20% decrease of their viability after 24h of incubation with the highest concentration (0.6 µM) of QDs was observed (Figure 3).

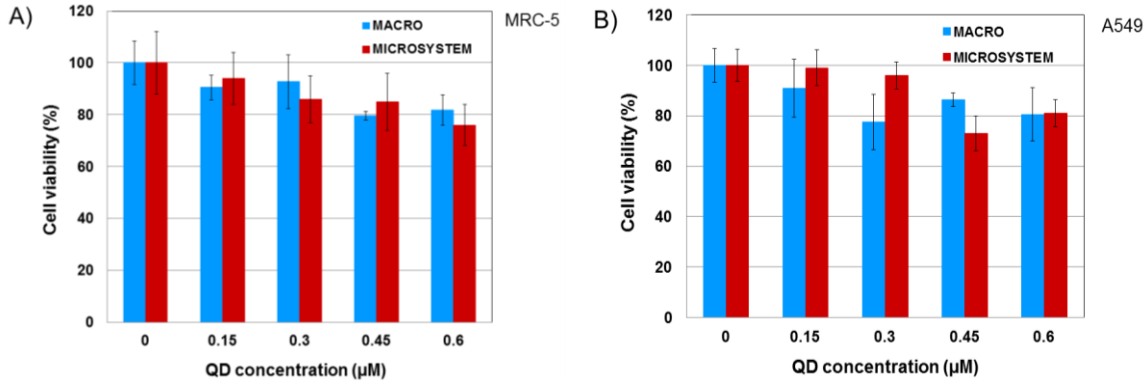

Figure 3: Comparison of cell viability in microsystem and 96-well plate studies: A) MRC-5, B) A549 cell culture.

The level of cells' morphology changes was qualitatively rated with the analysis of images captured by confocal microscope (FluoviewFV10i) (Figure 4). The shrinkage of cells was observed, proportionally to the concentration of QDs, for both cell cultures. However, greater reduction of cells size was observed for tumor cell line (A549).

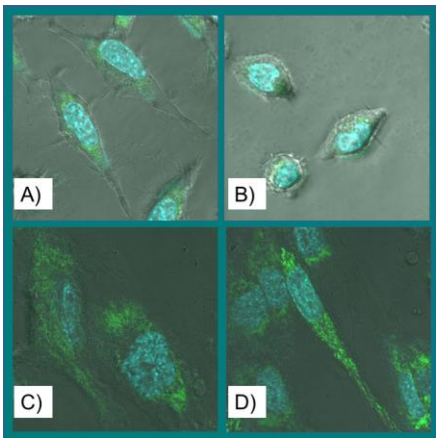

Figure 4: Comparison of cell morphology before and after cell incubation with QDs in microfluidic system: A)A549, B)A549 with QD, C)MRC-5, D) MRC-5 with QD. For visualization of the nuclear morphology, the nucleus was stained with the DNA-intercalating dye Hoechst 33342 and mitochondria with Rodamine 123.

CONCLUSION

In comparison to routine cytotoxicity tests, microfluidic system could provide an effective and high throughput analysis system and contribute to systematic evaluation of biological activity of various QDs' types and other nanoparticles.

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