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Conference Paper

Cytotoxicity of Polyelectrolyte Microcapsules Encoded with Semiconductor Nanocrystals

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Abstract

Polyelectrolyte microcapsules are promising carriers of drugs and diagnostic agents for designing targeted and controlled delivery systems design. The use of quantum dots (QDs) as fluorescent labels in bioimaging is a promising approach to bioimaging tool development. The potential toxicity of QDs makes their applicability as fluorescent labels in vivo questionable. Therefore, the cytotoxicity of polyelectrolyte microcapsules encoded with semiconductor nanocrystals has been investigated.

Keywords: Polyelectrolyte microcapsules, semiconductor nanocrystals, cytotoxicity, theranostic agents.

1. Introduction

The use of polyelectrolyte microcapsules as a tool for targeted delivery and controlled release of pharmaceuticals, contrast agents, and fluorescent probes for in vitro and in vivo imaging is a promising approach to personalized diagnosis and treatment of various human diseases [1]. QDs are fluorescent semiconductor nanocrystals from 2 to 10 nm in diameter characterized by a wide absorption spectrum and a narrow emission spectrum, as shown in Fig. 1. A high photostability and a bright fluorescence signal make QDs advanced nanophotonic detection and imaging labels [2, 3]. However, the presence of heavy metals in the cores of the best QDs and the related potential toxicity make in vivo applications of QDs a questionable issue.

Preparation of polyelectrolyte microcapsules using layer-by-layer assembly of alternatively charged polymers allows drug and fluorescent dyes to be incorporated into the polymeric wall [4]. Encapsulation of QDs into the matrix structure of an interpolymeric complex of alternatively charged polymers, which limits direct interaction of QDs with...
living cells seems to be an advantageous approach to diminish the adverse effects of QDs \textit{in vivo}. Here, we evaluate the \textit{in vitro} cytotoxicity of diagnostic agents based on polyelectrolyte microcapsules optically encoded with semiconductor CdSe/ZnS core/shell QDs.

2. Materials and methods

2.1. Preparation of polyelectrolyte microcapsules encoded with semiconductor nanocrystals

Home-made calcium carbonate microbeads were prepared and used as the matrix cores [5]. The polycation poly(allylamine hydrochloride) (PAH, Sigma-Aldrich, USA) and polyanion (sodium 4-styrenesulfonate) (PSS, Sigma-Aldrich, USA) were used for polyelectrolyte layers formation. The formation of the multilayer polyelectrolyte shell on the surface of negatively charged calcium carbonate microparticles and their encoding with water-soluble CdSe/ZnS QDs were carried out by alternately applying oppositely charged layers of polymers and QDs as described earlier [6]. The polyelectrolyte microcapsules were subsequently obtained by removing the calcium carbonate core from the prepared encoded microparticles. The resultant encoded microcapsules were stored at 4°C in the dark.
2.2. Characterization of Polyelectrolyte microcapsules

The morphology, size distribution, and fluorescent properties of microcapsules were analyzed microscopically using Carl Zeiss Axio Scope A1 microscope (Carl Zeiss, Germany) and Texas Red optical filter (ex 596 nm/em 615 nm); image processing was performed using the ZEN software (Carl Zeiss, Germany).

2.3. Cytotoxicity

The toxicity of the polyelectrolyte microcapsules encoded with QDs was evaluated in vitro using the 3-(4,5-dimethylthiazol-2-yl)-2,5-difeniltetrazolium bromide (MTT) assay for cell viability analysis. SK-BR-3 human breast carcinoma cells were used as an in vitro cell model. The cells were cultured in RMPI medium at 37°C under a 5% CO₂ atmosphere. At the exponential growth phase, they were transferred to 96-well plates containing RMPI. The plates were incubated for 24 h under the conditions described above. Then, suspensions of QD-encoded microcapsules and placebo microcapsules at different concentrations were added into the wells, and the cells were incubated for another 24 h. All concentrations of the microcapsules were analyzed in triplicates. Cells incubated in the wells without addition of any agents either with addition of the solvent used for microcapsule resuspension were used as controls. MTT interaction with microcapsules was analyzed in the wells containing the medium with the highest microcapsule concentration.

After incubation, the cells were thoroughly washed with phosphate buffer (pH 7.4), and the MTT solution was added. The plates were incubated at 37°C and under 5% CO₂ atmosphere for 4 h. MTT assay is based on the transformation the water-soluble tetrazolium (MTT) salt (3-(4,5- dimethylthiazol-2-yl)-2,5-difeniltetrazolium bromide) into the insoluble purple formazan crystals accumulated in the cytoplasm. The amount of formazan depends on the activity of mitochondrial NAD(P)H-dependent cellular oxidoreductase and is proportional to the number of living cells, as explained in [7]. After the formation of purple formazan crystals, the supernatant was removed, and formazan crystals were dissolved in dimethyl sulfoxide. The optical density was measured by a Multiskan EX microplate reader (Thermo Fisher, USA). The cell viability (CV) was determined as follows: $CV = \frac{A_i}{A_0} \times 100\%$, where $A_i$ is the optical density of the wells containing microcapsule suspension and $A_0$ is the optical density of the control.
3. Results

The prepared calcium carbonate microbeads were characterized by a slightly negative surface charge (–5.4 ± 2.5 mV) and were used as templates for deposition of alternately charged polyelectrolyte layers. The resultant microcapsules consisted of ten polyelectrolyte layers and one layer of QDs deposited between the 5th and 6th layers of PAH.

Microscopy study has shown that the size of the resultant polyelectrolyte microcapsules ranges between 3 and 6 μm. The microcapsules are spherical hollow structures whose walls are intensely fluorescent due to incorporation of QDs and can be distinguished as individual objects, as shown in Fig. 2.

![Figure 2: A typical fluorescence microscopy image of the obtained polyelectrolyte microcapsules.](image)

The analysis of MTT assay data has shown that the placebo polyelectrolyte microcapsules have no toxic effects, with an average viability of SK-BR-3 cells being 100% at all the investigated concentrations, from 2 to $2 \times 10^4$ capsules per cell, while the cell incubated in the presence of the encoded polyelectrolyte microcapsules exhibit a viability of 75% within 24 h in the same range of microcapsule concentrations.

4. Discussion

Encoding of microbeads with QDs using the layer-by-layer method based on successive application of alternately charged polyelectrolyte layers of polycation PAH and polyanion PSS and solubilized QDs ensures effective immobilization of QDs in the polymer matrix, which provides optimal fluorescence properties of the microcapsules obtained.
The prepared polyelectrolyte microcapsules themselves do not exhibit toxicity, which indicates QDs as the cause of the cell viability decrease. QD encapsulation into the polymeric wall of the microcapsules prevents the contact and limits the time of the direct exposure of cells to them, which is beneficial in terms of their in vivo applications.

5. Conclusion

The data show that the developed polyelectrolyte microcapsules encoded with CdSe/ZnS QDs are characterized by bright fluorescence and low cytotoxicity, which offers the opportunity for development of new systems for delivery of diagnostic and theranostic agents and drugs on their basis.

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