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Studies on influence of polymer modifiers for fluorescent nanocrystals' cytotoxicity

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ABSTRACT

The presented studies aimed at investigation of the effect of CdSeS/ZnS quantum dots (QDs) stabilized with hyperbranched polyglycidol and its carboxylated derivative on adenocarcinomic human alveolar basal epithelial cells (A549). The first stage of studies concerned the modification of quantum dots with both types of the tested polymers with the use of pyridine as an intermediate agent. Subsequently, cytotoxic effect of the prepared nanoparticles was examined after various incubation time using MTT test (cell metabolic activity assay). Our studies revealed that CdSeS/ZnS with a diameter of 6 nm, which were stabilized with hyperbranched polymers do not penetrate into cells, even after prolonged incubation time. Moreover, the cytotoxic effect of the tested QDs was observed over a range of tested concentrations (5–90 μM of Cd^{2+}). It was confirmed that tested nanoparticles had significant influence on cell culture viability. The examined cytotoxic effect of the tested quantum dots was dependent on the type of polymer applied and the experiments indicated, that the one bearing carboxylic moieties is more toxic to A549 cells.

1. Introduction

Quantum dots (QDs) have gained recently a considerable attention thanks to their interesting optical properties and wide range of potential applications including the use as noninvasive dyes for medical imaging or cells' labels in cellular tracing [1–3]. Their uniqueness lies in the combination of the semiconductor properties and small size, which give them specific quantum-confined properties – similar to single atoms, therefore they are often called 'artificial atoms' [4,5]. Typically, QD consist of a spherical core of a few nanometer diameter, which is a semiconductor crystal of single (eg. CdSe, CdTe, PbSe) or mixed salt (e.g. $CdSe_xS_{1-x}$) [6,7]. The core constituents often exhibit sensitivity to oxidation and hydrolysis, and therefore it is necessary to protect and separate the core surface from the environment by a material (also semiconductor), which is more resistant to aqueous environment and oxygen. Different semiconductors, which characterize themselves with a larger energy gap than the core (eg. ZnS) are commonly used as coating layers (so called shells). Their role is to passivate the core and prevent core-released ion leaching [8,9]. Moreover, the shells improve the QDs storage stability and allow to maintain high fluorescence of nanoparticles (high quantum yields). The outer part of the quantum dots has to be modified with hydrophilic ligands in order to allow formations of colloidal aqueous solutions of good stability. Therefore the number of methods, which allow for the transfer from the organic (where the QDs are typically stored to keep their properties and maintain stability) to aqueous phase has been described [10–13].

Due to forthcoming perspective of QDs application in bioimaging and therapeutics, it is necessary to examine their impact on living organisms very carefully. QDs cytotoxicity is one of the major issues, which can limit their clinical widespread application. Therefore, a number of studies, which are focused on the determination of the relationship between physicochemical properties of these nanocrystals and their biological activities are carried out [14–17]. The surface modifications of QDs e.g. attachment of suitable ligands or coating with appropriate polymers are often performed to obtain nanocrystals of the desired properties [18,19]. In this way nanoparticles can, among others, gain ability to penetrate into cells and their accumulation can be targeted, so specific cell organelles can be visualized. Moreover, QDs of certain properties (appropriately modified) can be able to entry only into particular cells e.g.

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tumor cells and in this way QDs can be used in angiogenesis studies [20].

Our interest has been drawn by range of features, which are offered by hyperbranched polyethers. Hyperbranched polyglycidol (HBPG) is a dendritic polymer obtained via one pot ring opening multibranching polymerization of glycidol [21]. HBPG exhibits high hydrophilicity and biocompatibility, similar to polyethylene glycol (PEG), thanks to the presence of polyether backbone as well as terminal hydroxyl groups with no charge in cellular environment [22]. Its low toxicity, facility of preparation and functionalization make HBPG an interesting molecule for drug delivery systems and cellular imaging, as well as for stabilization and modification of nanocrystals [23,24]. However, to the best of our knowledge, there are no reports regarding stabilization of QDs via ligand exchange using hyperbranched polyglycerol-based derivatives of low molecular weight.

Linear, hyperbranched and dendritic polymers pose an alternative to monodentate or bidentate thiol-based monolayers as QDs surface modifiers due to their superior stability in aqueous solutions and increased resistance to oxidation *in vivo* [23,25]. A large number of terminal, modifiable functional groups in case of dendritic or hypebranched structures offers great prospects for cellular imaging. Some of functional groups can stabilize QDs surface via multivalent interactions, while the other can be used for further (bio) functionalization of a nanostructure. Few strategies have been developed to prepare polymer-coated QDs: synthesis of QDs in aqueous medium containing polymer as stabilizer, intercalation of amphiphilic polymers into primary hydrophobic ligand shell or the ligand exchange [26]. For example, HBPGs modified with thioether moieties were utilized as modifiers for synthesis of CdSe and CdS nanocrystals in aqueous medium [23,27]. One can find also reports on utilization of hydrophilic polymers, which stabilize QDs through micelle-like structures [28,29]. The latter approach allows to retain good optical properties of QDs due to the lack of interferences in hydrophobic shell, however it leads to substantial increase of hydrodynamic radius. Ligand exchange strategy with the use of some polymers: hyperbranched polyethyleneimine (HPEI) [30], PEG-grafted HPEI [31,32], poly(maleic anhydride) [33] or polysaccharides [34], was applied to replace lipophilic surface modifiers (trioctylophosphine (TOPO) or oleylamine) directly. The indirect modification by means of pyridine use was carried out in a case of polyamidoamine-based dendrimers (PAMAM) [35], as well as linear polymers [36]. This strategy allows for obtaining QDs with a smaller diameter of ligand shell, which are more appropriate for *in vivo* imaging.

QDs thanks to their unique properties can find application in medicinal diagnostics and pharmaceutical studies and therefore so much attention is focused on studying their cytotoxicity. Because QDs are wide and varied group of nanocrystals a very extensive research have to be carried out in order to fully explore the properties of these nanoparticles [37–39]. Such studies should cover the influence of many parameters, such as: size, shape, surface charge and surface modifications [40]. Moreover, the detailed investigation should allow for selection of QDs group which can be safely used *in vivo*.

This work focuses on the biological activity studies of CdSeS/ZnS QDs and its correlation with the properties of applied QDs and surface ligands used. The evaluation included: QDs size, stability of fluorescence, cytotoxicity and ability of passive penetration into cells via endocytosis at different concentrations and incubation times. Studies were carried out on human pulmonary cells – A549 cell line, which is often used as a cell model to investigate alveolar cell function.

2. Experimental section

2.1. Materials

Core-shell $CdSe_xS_{1-x}$/ZnS alloyed quantum dots (CdSeS/ZnS) stabilized with hexadecylamine were purchased from Sigma-Aldrich (concentration of 1 mg mL^{-1} in toluene, 6 nm, emission at 490 nm). Pyridine, cadmium nitrate, mercury(II) nitrate, sodium hydroxide and sodium dihydrogen phosphate were all purchased from Sigma-Aldrich. Anhydrous ethanol, hexane, sodium chloride,

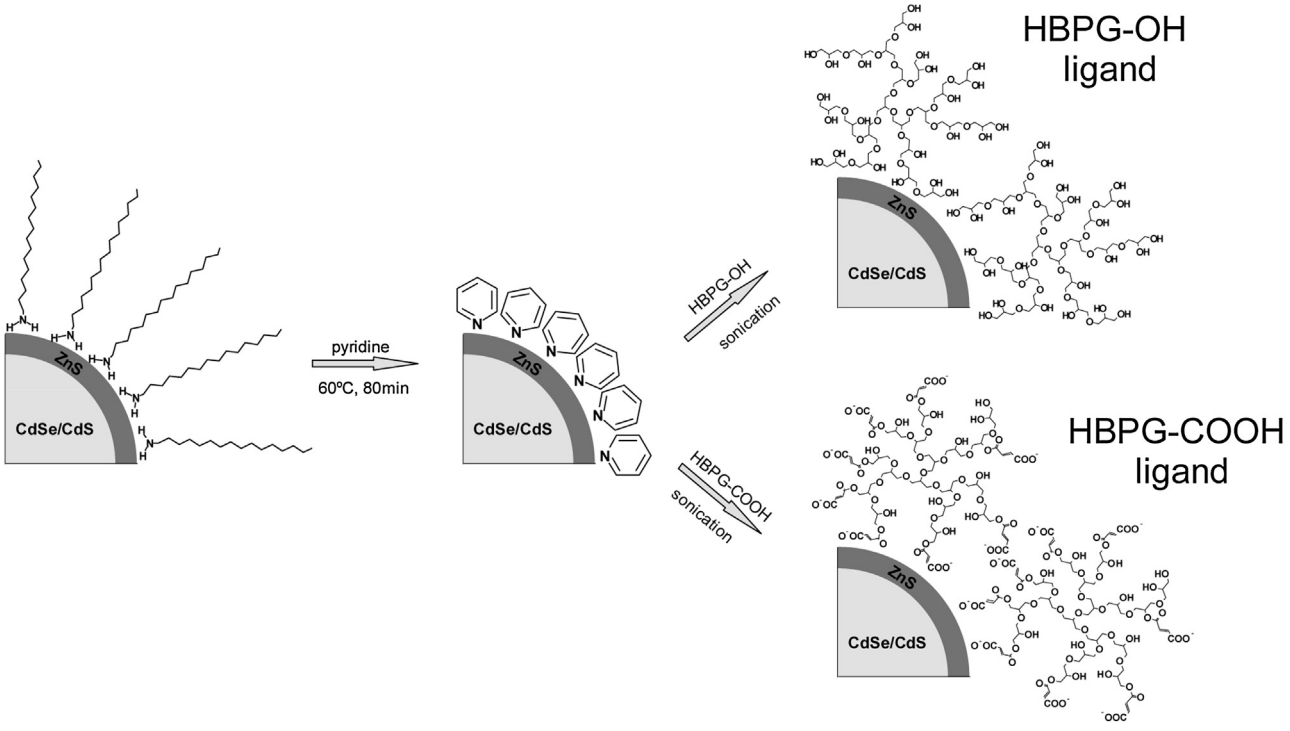

Fig. 1. Scheme of indirect ligand exchange via pyridine intermediate layer.