SYNTHESIS OF CdTe/ZnS CORE/SHELL QUANTUM DOTS AND THEIR *IN VITRO* TOXICITY ASSESSMENT

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Abstract

Quantum dots (QDs) are well known semiconductor nanocrystals that have been extensively investigated as visible drug carriers or imaging agents due to their unique optical properties. Nevertheless, potential toxicity is still one of the major issues that limit the advances of QDs into clinical studies. In this paper, water soluble CdTe core a CdTe/ZnS core/shell QDs stabilized with different ligands (3-mercaptoacetic acid, thioglycolic acid, glutathione and mercaptosuccinic acid) were prepared by a simple method using Na_2TeO_3 and $CdCl_2$. For comparison, ZnSe and ZnSe/ZnS QDs were also prepared. QDs cytotoxicity on Human Embryonic Kidney cells (HEK 293) was performed using MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) viability assay. Single core CdTe QDs were found to have severe toxic effects on cells due to the absence of a zinc sulfide shell, which currently decreases the toxicity of QDs.

Keywords:

Quantum dots, core/shell, toxicity, MTT

1. INTRODUCTION

Semiconductor nanocrystals, also known as quantum dots (QDs), are nano-scaled inorganic particles in the size range of 1–20 nm [1]. Due to their quantum confinement, QDs show unique and fascinating optical properties, such as sharp and symmetrical emission spectra, high quantum yield (QY), good chemical and photo-stability and size dependent emission [2]. Since QDs themselves are commonly capped by hydrophobic ligands for increased stability, ligand exchanging or attaching steps are essential for extending their application potential to the bio-imaging field. A variety of materials such as mercaptoacetic acid, peptides, lipids, streptavidin, and even dendrimers have been utilized to fabricate water-soluble QDs with high stability, as well as to reduce their toxicity to both animal cells and animals itself. QDs consist of groups II–Vi or III–V elements, but most of them consist of cadmium, selenium, or lead and they are frequently fabricated as core–shell structures [3]. Besides this semi-conductor core, QDs usually have their surface coated with different capping substances. The core and surface coating affect the QDs photochemical properties [4]. Unfortunately, these metals comprising QDs are well-known highly toxic materials that cause severe disease or adverse effects when exposed to humans or animals even at the low concentrations. Consequently, various studies have reported the cytotoxicity of QDs capped with various kinds of ligands on different cell lines [5].

The present work describes the synthesis and fluorescent behavior of water soluble core and core/shell QDs stabilized with various mercaptan ligands. The influence of core and core/shell QDs on HEK 293 cells was also evaluated by analyzing the cell viability (MTT essay) after 24 h incubation with the nanoparticles.

2. MATERIAL AND METHOD

2.1. Chemicals

All chemicals were purchased by Sigma Aldrich (Czech Republic) in ACS purity unless otherwise stated. Aqueous solutions were prepared using MilliQ water.

2.2. Synthesis of core QDs

The procedure for synthesis of glutathione (GSH)-capped CdTe QDs was adapted from the work of Duan et al. [6] with slight modifications. The synthesis of 3-mercaptopropionic acid (MPA)-capped CdTe QDs and thioglycolic acid (TGA)-capped CdTe QDs were adapted from the work of Wang et al. [7]. Sodium telluride was used as Te source. Due to the fact that sodium telluride is air stable, all of the operations were performed in the air avoiding the need for inert atmosphere. The synthesis of CdTe QDs and their subsequent coating were as follows: 114 mg of the $CdCl_2$.2.5 H_2O was diluted with 25 mL of water. During the constant stirring, 65 µL MPA (56 µl TGA or 150 mg GSH), 25 mg of sodium citrate, 2 mL of Na_2TeO_3 solution (c = 0.01 mol/L), and 10 mg of $NaBH_4$ were added into cadmium(II) aqueous solution. 1 M NaOH was then used to adjust the pH to 10 under vigorous stirring. The mixture was kept at 95 °C under the reflux cooling for 4 hours.

The synthesis of mercaptosuccinic acid (MSA)-capped CdTe QDs was adapted from the work of Zhu et al. [8]. $CdCl_2$.2.5 H_2O (c = 0.04 M, 4 mL) was diluted with 42 mL of water. 400 mg of sodium citrate, Na_2TeO_3 (c = 0.01 M, 4 mL), 160 mg of MSA and 50 mg of $NaBH_4$ were added under vigorous stirring. When the solution became green, the mixture was refluxed at 95 °C for 5 hours.

The synthesis of ZnSe QDs was adapted from the work of Huang et al. [9]. In a typical procedure, 148 mg of $Zn(NO_3)_2 \cdot 6H_2O$ and 276 mg of GSH were dissolved in 50 ml deionized water. Then 9 mg of Na_2SeO_3 and 0.2 g of $NaBH_4$ were added into the above mentioned solution under stirring. After several minutes, the pH value of the solution was adjusted to 10.5 by dropwise addition of 0.1 M NaOH. The mixture was then refluxed at 95 °C for 3 hours.

After cooled to room temperature, the QDs were precipitated by 2-propanol and dried under vacuum at 80 °C for 2 hours.

2.3. Synthesis of GSH-stabilized core/shell QDs

A typical synthesis of CdTe/ZnS or ZnSe/ZnS core/shell nanoparticles was adapted from the work of Liu [10]. The 40 mg of as-prepared core QDs sample was added to the 50 ml solution (pH = 8) containing 6.8 mg of $ZnCl_2$ and 61.4 mg of GSH. The solution was heated to 95 °C refluxed for 3 hours. After cooled to room temperature, the QDs were precipitated by 2-propanol and dried under vacuum at 80 °C for 2 hours.

2.4. Viability assays

The cytotoxicity induced by QDs on HEK 293 cell lines was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] assay [4]. The cells were seeded in 96-well plate in a volume of 200 µL and the density of $3x10^4$ cells/well. The cells were kept growing 24 hours prior to dosage. The medium was then replaced with a fresh medium containing core and core/shell QDs at various concentrations (0.05 - 1000 µg/mL). After 24 h of additional incubation at 37 °C, the medium was removed, the cells were gently washed with phosphate buffer solution (PBS, pH 7.4) and 100 µL of MTT solution (0.5 mg/mL in PBS) was added to each well. Samples were incubated for 3 h and formazan crystals were

dissolved in 100 μL of a sodium dodecylsulfate solution (10% of SDS in H_2O_2). After overnight incubation the absorbance was measured at 572 nm with Infinite M200 Pro, Tecan microplate reader.

3. RESULTS AND DISCUSSION

3.1. Synthesis of core/shell QDs

It has been reported that CdTe QDs can induce cell death due to the release of Cd^{2+}, whereas the CdSe/ZnS QDs present less cytotoxic effect due to the release of low Cd^{2+} concentration (<5 nM) which is confirmed by a Cd^{2+}-specific cellular assay [10]. Water-soluble CdTe/CdS QDs with high fluorescence synthesized recently are also cytotoxic due to the release of Cd^{2+} in biological environments. These QDs might not be suitable in long-term bio-application, especially *in vivo*. To overcome these shortcomings, we synthesized CdTe/ZnS QDs in aqueous solution. In the route, the CdTe QDs were firstly synthesized with TGA, MPA, MSA and GSH as the stabilizer in aqueous solution, then CdTe QDs were used as core template, highly fluorescent CdTe/ZnS QDs were obtained through *in situ* growth of ZnS shell on the surface of CdTe core in alkaline solution containing Zn^{2+} and GSH at 95 °C water-bath (see Section 2.3). For comparison, ZnSe and ZnSe/ZnS QDs were also prepared.

3.2. Optical properties of QDs

The reaction of synthesis of various types of QDs was monitored measuring the fluorescence spectra. We selected four kinds of mercaptan ligands such as MPA, TGA, GSH and MSA for the preparation of CdTe QDs. For ZnSe QDs, GSH was used as a stabilizing ligand. The emission spectra of typical CdTe QDs used in this study were measured at excitation wavelength of 390 nm. All peaks in characterized spectra showed a good symmetry and a narrow spectral width (see Fig. 1).The emission spectrum is displayed by one emission peak at 520 nm in the case of MSA-capped CdTe QDs, one peak at 510 nm in the case of GSH-capped CdTe QDs, one emission peak at 620 nm in the case TGA-capped CdTe and one peak at 516 nm in the case TGA-capped CdTe QDs. In the case of ZnSe QDs, one emission peak at 378 nm was observed. Heating of mixture solution of GSH, Zn^{2+} and core CdTe, ZnS NCs results in a red shift of the PL emission wavelength of the solution system, which implies that a ZnS shell is slowly growing *in situ* on the CdTe core. With the reflux proceeding, the excitonic absorption peak of nanoparticles shifts to the longer wavelengths (lower energies) as the NCs grow to larger size. This result agrees with the consequence of the quantum confinement effect. So, it could be concluded that the expected CdTe/ZnS QDs or ZnSe/ZnS QDs were successfully synthesized. Fig.1 also shows that with heating the mixture solution of GSH, Zn^{2+} and core CdTe NCs the PL efficiency of QDs system is improved. The reason might be that the heating causes the slow decomposition of partial GSH with the release of sulfur to form *in situ* a ZnS shell around the CdTe core, resulting in the enhancement of the PL efficiency. The formation of a ZnS shell also greatly inhibits further oxidation of CdTe QDs. The more important is that when GSH is used as the S precursor, the release rate of elemental S is slow enough to maintain the concentration below the critical level for nuclei formation. In contrast, directly injecting S^{2-} into the reaction system makes a rapid reaction take place between S^{2-} and Zn^{2+} ions, only leading to the formation of an inhomogeneous shell on CdTe surface and the single ZnS clusters, which limits PL enhancement [11].

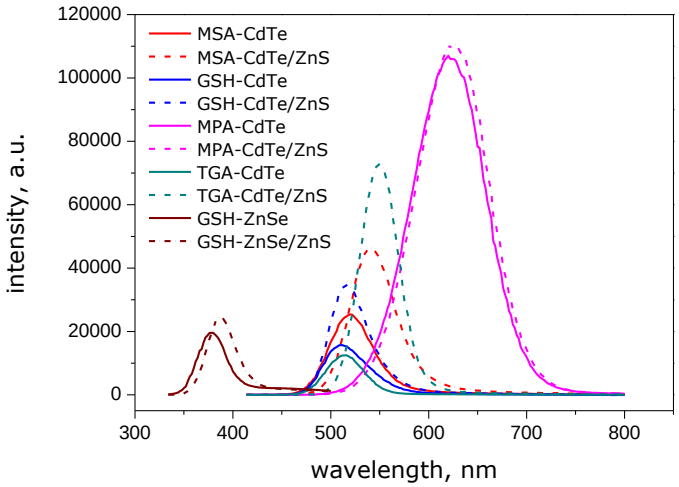

Fig. 1 Fluorescence spectra of prepared different types QDs

Table 1 Fluorescence intensity maximum and FWHM of prepared QDs

Type of QDs	λ [nm]	FWHM [nm]	Type of QDs	λ [nm]	FWHM [nm]
MSA-CdTe	520	48	MPA-CdTe/ZnS	630	88
MSA-CdTe/ZnS	542	50	TGA-CdTe	516	46
GSH-CdTe	510	56	TGA-CdTe/ZnS	550	48
GSH-CdTe/ZnS	516	46	GSH-ZnSe	378	38
MPA-CdTe	620	88	GSH-ZnSe/ZnS	388	38

3.2. Cell viability of QDs

Quantum dots are gaining extensive attention due to the increase in biomedical applications such as imaging, cell tracking, and therapeutics. However, there is not enough knowledge on the toxicity of these rapidly emerging nanoparticles [12]. To address this issue, the present study investigated the effect of QDs on the viability of HEK 293 cells using MTT assay. Most commercially available QDs contain heavy metals in the core and shell which gets protected through a coating with different surface ligands having various size and chemical nature that are important determinants of their toxicity. Hence, in the present study, various QDs with four different coatings (GSH, MPA, TGA and MSA) at four different emission wavelengths were used to study the cytotoxicity on HEK 293 cells. In the MTT assay, HEK 293 cells were incubated for 24 hour in medium containing QDs at final concentration ranging from 0 to 1000 μg/mL. All MTT assay demonstrated that the cytotoxicity of QDs correlated with their concentration, i.e. cell viability decreased with increasing QDs concentration. It was found that ZnS shell induced better cell viability compared with core QDs. The CdTe/ZnS QDs stabilized with different ligands (MPA, GSH, TGA and MSA) did not show any cytotoxicity to the HEK 293 cells at the test concentrations up to 1 μg/mL. On the contrary ZnSe and ZnSe/ZnS QDs nanoparticles were nontoxic to the HEK 293 up to 100 μg/mL tested concentration. Among those tested, TGA CdTe/ZnS QDs were highly toxic to the HEK 293 cells with IC_{50} value of 3.97 ± 0.19 μg/mL. MPA CdTe/ZnS QDs were less toxic with IC_{50} value of 14.08 ± 0.91 μg/mL, GSH CdTe/ZnS QDs have IC_{50} value of 23.57 ± 1.21 μg/mL and MSA CdTe/ZnS QDs were least toxic with IC_{50} value of 104.79 ± 1.51 μg/mL (see Fig 2). The non-cadmium based ZnSe/ZnS QDs were showing IC_{50} value of 281.96 ± 0.21 μg/mL, which was much less toxic in comparison to cadmium based QDs (see Fig. 3).

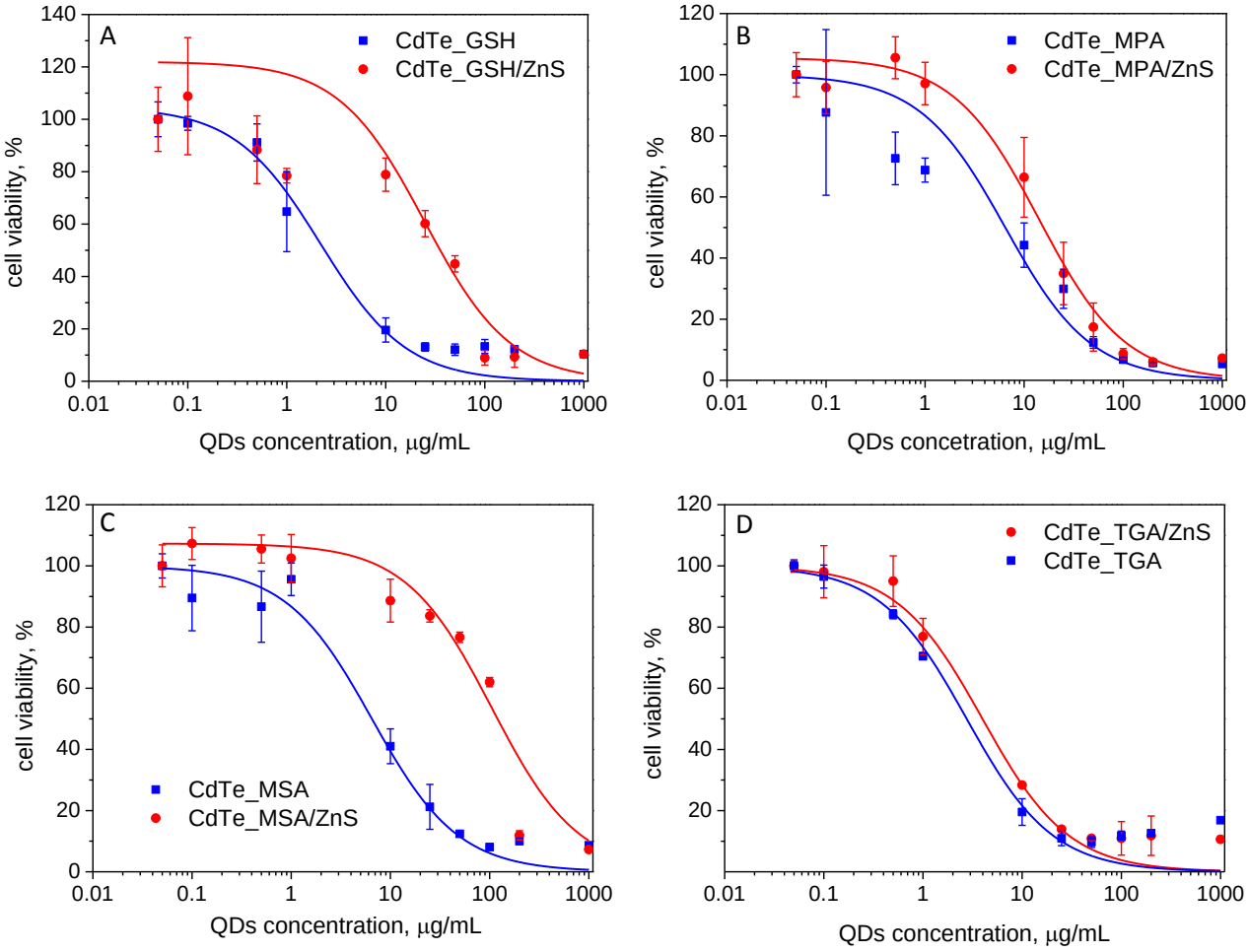

Fig. 2 Cytotoxic activity of CdTe and CdTe/ZnS QDs stabilized with GSH (A), MPA (B), MSA (C) and TGA (D) to HEK 293 cells using MTT assay after 24 hour of incubation.

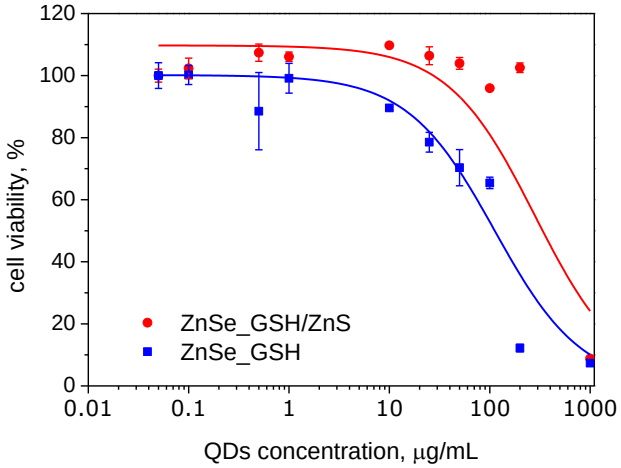

Fig. 3 Cytotoxic activity of ZnSe and ZnSe/ZnS QDs stabilized with GSH to HEK 293 cells using MTT assay after 24 hour of incubation.

4. CONCLUSION

Water soluble CdTe QDs stabilized with different ligands (MPA, TGA, MSA and GSH) and ZnSe QDs modified with GSH were prepared by a simple one step method using Na_2TeO_3 and $CdCl_2$. All prepared types of QDs were subsequently covered with ZnS shell to enhance the PL efficiency. The toxicity of prepared QDs were tested on HEK 293 cells using MTT assay. It was found that ZnS shell not only improved the PL efficiency but also reduced the toxicity. MSA CdTe/ZnS QDs were the least toxic of all prepared types CdTe QDs. The results also demonstrate that the non-cadmium based ZnSe/ZnS QDs were much less toxic in comparison to cadmium based QDs.

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