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One-pot synthesis of GSH-Capped CdTe quantum dots with excellent biocompatibility for direct cell imaging

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Abstract

In this work, we have developed one-pot aqueous synthesis of glutathione (GSH) binding CdTe quantum dots (QDs) for cell imaging. UV-Vis absorption spectrum, Fourier transform infrared spectrum, photoluminescence spectrum, and high-resolution transmission electron microscopy are applied to characterize the physical and chemical properties of the nanocomposites. The bioimaging efficiency of the GSH-capped CdTe QDs is further evaluated on Hela cells. The groups on the surface of QDs are able to bind to basic proteins, which are abundant in cell nuclei, enabling the application of QDs for direct cell imaging. Experimental results also indicate the GSH layer on the surface of QDs is able to reduce the cytotoxicity significantly. In conclusion, the as-prepared GSH-capped QDs are highly promising fluorescent probes for cell imaging in the near future.

Keywords: Bioengineering, Nanotechnology, Materials science

1. Introduction

Quantum dots (QDs) have been extensively investigated and attracted great interest to not only technical applications including light emitting diodes (LED) devices

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[1, 2] and solar cells [3, 4], but also fundamental biological studies such as fluorescent sensing approaches [5, 6, 7], biological imaging [8, 9], and drug delivery [10, 11]. Semiconductor QDs have numerous advantages over traditional fluorescent dyes [12, 13]. For example, the absorption is broad; the photoluminescence (PL) spectrum is always narrow and stokes shift is large; the photo-bleaching is low and the emission peak is tunable by controlling the size of QDs; multiple colors may be achieved with a single excitation. Moreover, these nanomaterials also feature versatile surface chemistry, which allows facile modification by different biomolecules [14]. Owing to these merits, semiconductor QDs have been widely applied in biological labeling and imaging [15, 16]. The most studied and applied semiconductor QDs include CdTe, CdS, CdSe, and PbSe [17, 18, 19]. For example, Adegoke and Park synthesized CdSe/In_2S_3 QDs with size-tunable optical properties. Six organic capping ligands were anchored onto the surface to minimize the interfacial surface defects [20]. Eley et al. prepared heterostructured CdS/ZnO, CdSe/ZnO, and CdTe/ZnO nanocrystals which exhibited more efficient photocatalytic decomposition of aqueous organic molecules under UV irradiation [21]. Witt et al. synthesized PbSe QDs and studied the photoinduced charge transfer between QDs and poly(3-hexylthiophene) [22]. These QDs are primarily prepared via a hot injection organometallic route. However, the synthesis in organic phase and the capped hydrophobic ligands not only decrease biocompatibility, but also makes it difficult for direct biological applications. Recently, different thiol-capped QDs have been prepared directly in aqueous solutions employing thiols as stabilizers [23]. Nevertheless, quantum yield (QY) is always too low [24]. Glutathione (GSH) is a thiol-containing tripeptide that can be easily found in most organisms, which is an important deoxidizer in biological system. It is able to bind heavy metal and form a phytochelatin shell [25]. Therefore, the structure can be mimicked, exchanging other thiols with GSH to stabilize QDs [26].

Based on the above consideration, in this report, we have synthesized GSH-capped CdTe QDs with high QY and excellent biocompatibility. UV-Vis absorption spectrum, Fourier transform infrared (FTIR) spectrum, PL spectrum, and high-resolution transmission electron microscopy (HR-TEM) have been used to characterize the physical and chemical properties of CdTe QDs. Multiple fluorescence emissions can be achieved by adjusting the size of QDs. In addition, it is proved in our experiment that the prepared QDs can be successfully applied as fluorescent probes to label and image Hela cells.

2. Experimental

2.1. Materials and chemicals

Sodium borohydride ($NaBH_4$), sodium hydroxide (NaOH), tellurium powder, cadmium chloride ($CdCl_2$), and GSH were purchased from Sigma (USA). Dulbecco's modified Eagle medium (DMEM) was purchased from Gibco (Gaithersburg,

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USA). Fetal bovine serum (FBS) was from Hangzhou Sijiqing Biological Engineering Material Co., Ltd. (Hangzhou, China). All other reagents were of analytical grade. Deionized water with a nominal resistivity higher than 18 MΩ cm is used throughout the experiments.

2.2. Synthesis of GSH-capped CdTe QDs

The quantum dots were synthesized according to a previously reported synthetic method with certain modifications [27]. Briefly, Cd^{2+}-GSH precursor solution was prepared by dissolving 3.6 mg of $CdCl_2$ and 15.4 mg of GSH in 49.6 mL of ultrapure water. After that, the pH was adjusted to be 8.5 with 1 M NaOH solution under vigorous stirring. Next, 2 mL of ice-cold $NaBH_4$ solution with the concentration of 25 g/L was prepared and 0.04 g of tellurium powder was added to the solution, which was then maintained at room temperature overnight in a syringe with a needle to release gas. The generated sodium hydrogen telluride (NaHTe) solution was then diluted for 12 times. Afterward, NaHTe solution was injected into the N_2-saturated Cd^{2+}-GSH precursor solution under vigourous stirring. The typical molar ratio of Cd^{2+}, HTe^{-}, and GSH was about 4:1:10. The mixture was heated to 100 °C and refluxed at different times to achieve different emission peaks of formed CdTe QDs.

2.3. Characterization of GSH-capped CdTe QDs

Fluorescence of CdTe QDs was measured by F-4600 fluorescence spectrophotometer (Hitachi, Japan) at room temperature. Excitation/emission spectra were obtained at an interval of 5 nm excitation wavelength. UV-vis absorption spectra were recorded using U-3900 UV-vis spectrophotometry (Hitachi, Japan) at room temperature. TEM images were taken by FEI Tecnai G20 transmission electron microscopy (FEI, USA) to observe the morphology of CdTe QDs. FTIR spectrum was acquired from an Agilent Cary 660 FTIR spectrometer (Agilent Technologies, USA).

2.4. Cell culture

Hela cell line was obtained from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. (Shanghai, China). The cells were seeded in DMEM supplemented with 10% FBS and antibiotics (100 μg/mL streptomycin and 100 IU/mL penicillin) at 37 °C in a 5% CO_2 humidified environment.

2.5. Cytotoxicity of GSH-capped CdTe QDs

The viability of cells was measured by cell counting kit-8 (CCK-8) method. Hela cells in the log phase were firstly seeded in a 96-well plate at a concentration of 1.0×10^4 cells/well, which were cultured overnight at 37 °C with 5% CO_2. Subsequently, the cells were treated with different amounts of GSH-CdTe QDs. After

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the cells were cultured for 2 h, 10 μL of CCK-8 solution was added to each well and the cells were further incubated for 2 h. The optical density (OD) of each well was recorded at the wavelength of 450 nm using Synergy HT multifunction microplate reader (BioTek Instruments, Inc., USA). After comparing the tested OD values with that of control cells, cell viability could be determined.

2.6. Direct cell imaging

The cells were treated with GSH-CdTe QDs for 1 h. Afterward, the QDs were removed and washed with PBS for three times. The fluorescence images of cells were then observed under Axio Observer A1 fluorescence microscope (ZEISS, Germany).

3. Results and discussion

The synthetic process of the water-soluble GSH-capped CdTe QDs is based on the reaction of Cd^{2+}-GSH and NaHTe, which is described in Fig. 1. The synthesis condition is not demanding. GSH is used to stabilize CdTe nanocrystals and acts as the capping ligand on the surface of the CdTe QDs. This biocompatible molecule is supposed to inhibit the release of harmful elements and contribute to the low cytotoxicity. The bond between SH and Cd may also passivate the surface trap states and thereby enhances the QY. Upon the addition of HTe^- to Cd^{2+}-GSH precursor, slightly yellowish solution forms immediately. After the mixture is heated to 100 °C, CdTe QDs begin to grow and the different heating duration is supposed to create CdTe QDs with different sizes, which influence the optical properties of the synthesized nanomaterials.

To demonstrate and understand the hypothesis in the synthetic process, the QDs are characterized by different instruments. TEM images shown in Fig. 2a confirm that the CdTe QDs possess a well-dispersed crystalline structure with an approximately spherical form. Average diameter of the formed nanomaterials is about 6 nm (Fig. 2b). To confirm the binding between the ligands and CdTe QDs, FTIR

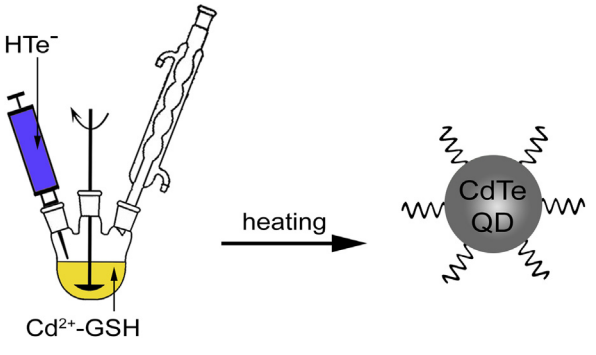

Fig. 1. Schematic illustration of the formation of GSH-capped CdTe QDs.

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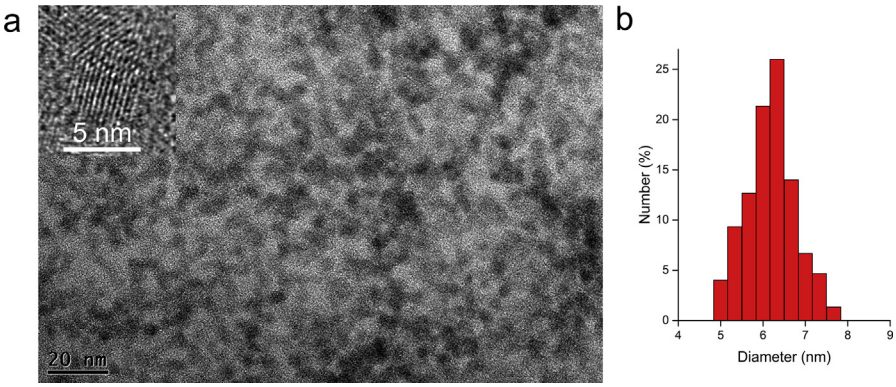

Fig. 2. Characterizations of GSH-capped CdTe QDs. (a) TEM image of GSH-capped CdTe QDs. Inset shows the high resolution TEM image. (b) Size-distribution histogram of the GSH-capped CdTe QDs.

spectrum is measured, which is depicted in Fig. 3. The peaks indicate existence of NH (3400 cm^{-1}), CH_2 (2930 cm^{-1}), COO-asymmetry (1590 cm^{-1}), COO-symmetry stretching (1400 cm^{-1}) and CN (1100 cm^{-1}) bands [28]. SH stretching vibration at 2557 cm^{-1} is not observed, which is due to the formation of a covalent bond between SH and Cd on the surface of the QDs [29]. Therefore, the FTIR spectrum has verified the capping of GSH on CdTe QDs. In addition, the surface of GSH-capped QDs shows negative charges, which is confirmed by the Zeta potential characterization (Fig. 4).

Fig. 5a presents the UV-vis absorption spectra and PL spectra of GSH-capped CdTe QDs with different growth time. The inset shows the photographs of the corresponding solutions illuminated under a UV lamp and their bright fluorescence of colors appears to be green, yellow and red. With prolonged reaction time, the PL emission peak is tuned from 520 nm to 620 nm (Fig. 5b). Green-emitting QDs is obtained with 2 h of reaction and yellow-emitting QDs is obtained with 3 h of reaction. To obtain

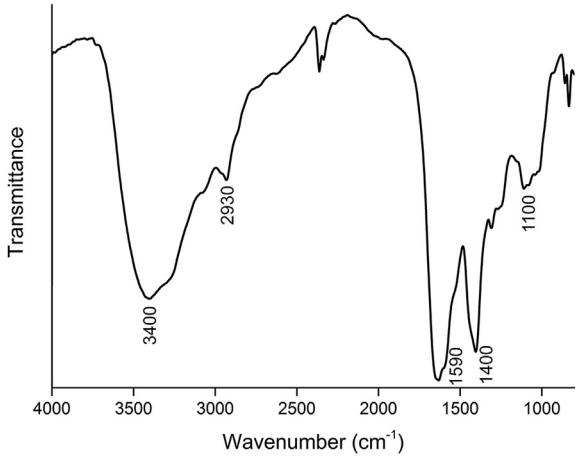

Fig. 3. FTIR spectrum of GSH-capped CdTe QDs. The peaks include 3400 cm^{-1}, 2930 cm^{-1}, 1590 cm^{-1}, 1400 cm^{-1} and 1100 cm^{-1}.

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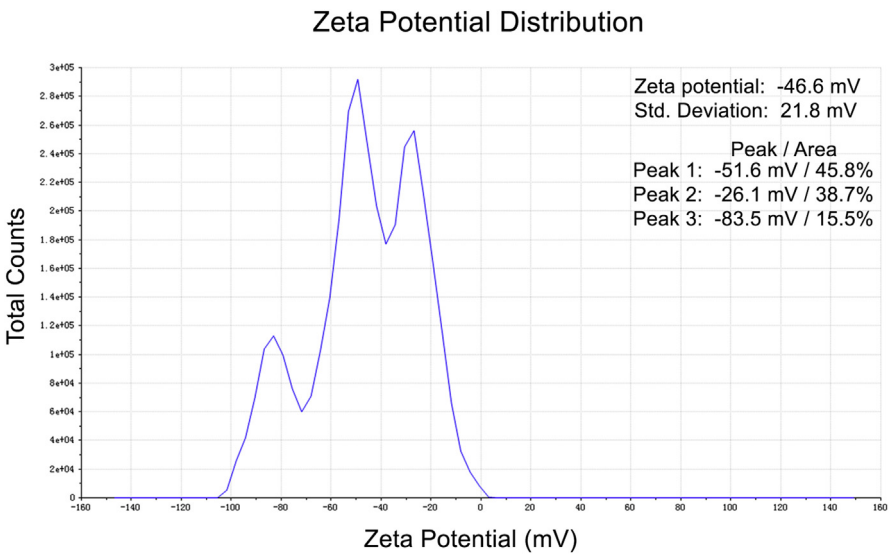

Fig. 4. Zeta potential distribution of GSH-capped CdTe quantum dots.

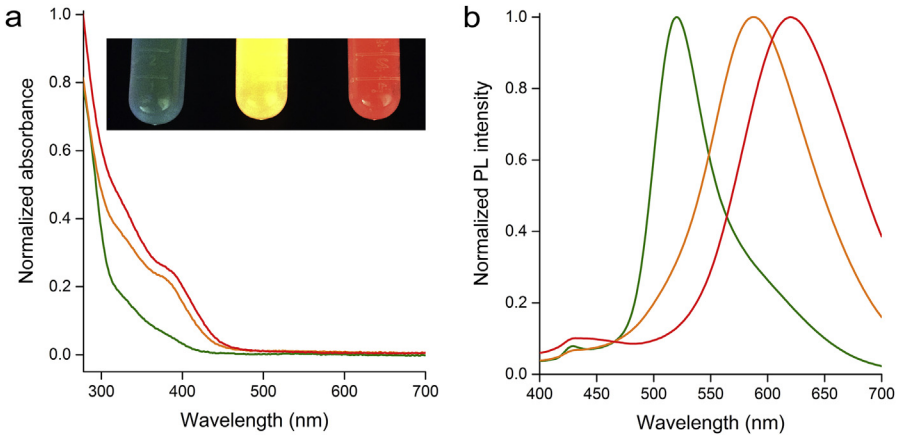

Fig. 5. (a) UV-vis absorption spectra and (b) fluorescence spectra (excited at 370 nm) of GSH-capped CdTe QDs with three different colors, which are shown in the inset. The CdTe QDs are prepared at different growth times, respectively.

red-emitting QDs, 5 h of reaction is needed. The best QY is 49%. In the following experiments, red-emitting QDs is applied for optimization and direct cell imaging. The influence of key synthesis parameters like pH value on the PL intensity of GSH-capped CdTe QDs is then assessed. As depicted in Fig. 6, the PL intensity reaches the maximal value when pH is adjusted to be 7. Thus, the pH value of 7 is used for further experiments.

The biocompatibility of the prepared QDs is a critical characteristic for future biological applications such as cell imaging. The cytotoxicity of GSH-capped CdTe QDs is evaluated by CCK-8 assays. Although CdTe QDs are supposed to be highly toxic for cells since Cd^{2+} are released during the incubation with the cells, GSH

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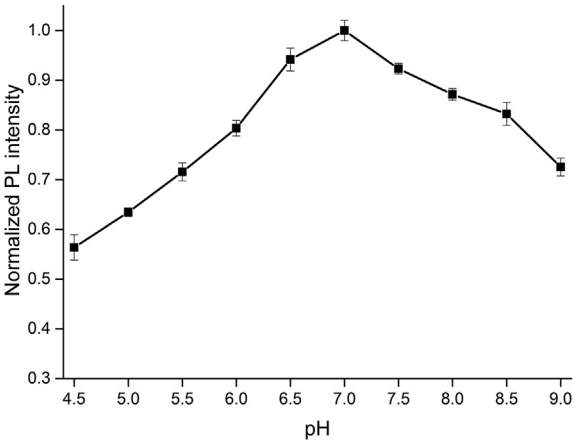

Fig. 6. PL intensity of GSH-capped CdTe QDs (red-emitting) at different pH values.

helps provide a CdS layer, which encapsulates the CdTe core and inhibits the release of heavy metal ions [30]. Therefore, the cytotoxicity is supposed to be reduced to a small extent. CCK-8 assay results indicate that cells maintain over 80% viability after the incubation of GSH-CdTe QDs with the concentration up to 500 μg/mL for 24 h (Fig. 7). The low cytotoxicity of the prepared nanomaterials are also significant after compared with the other CdTe QDs (Table 1).

Since basic proteins are abundant in cell nuclei, negatively charged GSH-capped CdTe QDs are able to bind to positively charged proteins via electrostatic interaction at physiological pH. Therefore, the cellular accumulation of QDs may occur. The potential of GSH-capped CdTe QDs as fluorescent probes for biological imaging is then examined using Hela cells. As shown in Fig. 8, after incubated with GSH-capped CdTe QDs for 1 h, almost all cell nuclei are stained red, which indicate that the QDs have penetrated the cellular matrix swiftly and been enriched in cell nuclei. Meanwhile, no signs of morphological damage to the cells are detected

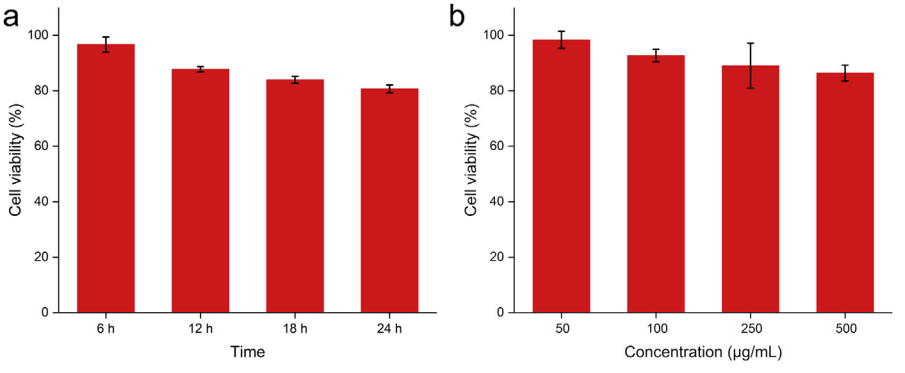

Fig. 7. (a) Relationship between the cell viability and incubation time of CdTe quantum dots. (b) Relationship between the cell viability and the concentration of CdTe quantum dots.

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Table 1. Assessment of the cell viability after treatment with different CdTe QDs.

CdTe QDs	Concentration (incubated for 24 h)	Cell viability (%)	Ref
Bare	60.12 μg/mL	50%	[31]
Amino coated	50 μg/mL	7%	[32]
Carboxyl coated	50 μg/mL	23%	[32]
Mercaptosuccinic acid capped	10.6 μg/mL	50%	[33]
GSH capped	500 μg/mL	>80%	This work

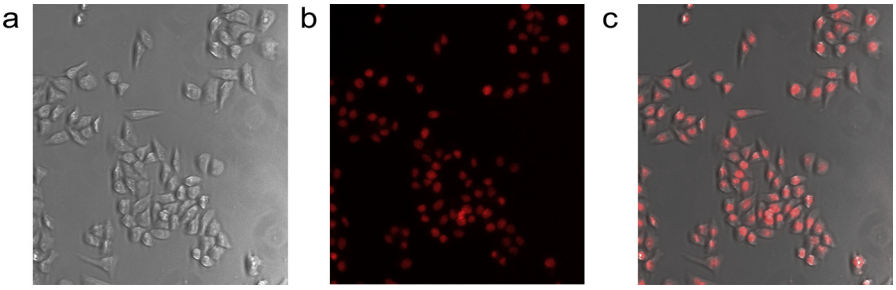

Fig. 8. Fluorescence images of Hela cells stained with GSH-capped CdTe QDs (red-emitting QDs). (a) Bright-field and (b) fluorescence images. (c) is the merged image.

upon the treatment of QDs, demonstrating the high biocompatibility directly. The prepared QDs are supposed to be excellent fluorescent probes for cell imaging.

4. Conclusions

In summary, GSH-capped CdTe QDs with bright fluorescence emission were successfully synthesized through one-pot process. The QDs have up to 49% QY and their size-dependent emission ranges from 520 to 620 nm. Compared to currently used QDs prepared in organic phase, GSH-capped CdTe QDs prepared in this work are water-soluble and can be easily linked with biomolecules. They also have many other merits like good stability, high biocompatibility, low cost and cytotoxicity. The QDs are further applied as effective fluorescent probes for direct cell imaging. Therefore, we believe the as-prepared QDs is promising to applications in certain biological and medical fields.

Declarations

Author contribution statement

P. Miao: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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X. Chen, Z. Guo: Performed the experiments; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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