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RESEARCH PAPER

Facile synthesis of near-infrared $CuInS_2$/ZnS quantum dots and glycol-chitosan coating for in vivo imaging

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Abstract This study describes the synthesis method of water-soluble, low-toxicity, photostable highly luminescent probes based on I–III–VI_2 type semiconductor quantum dots (QDs) and the possibility of tumor targeting in living animals. Cd-free high-quality $CuInS_2$/ZnS core/shell QDs were synthesized, and their surfaces were reacted with mercaptoundecanoic acid for aqueous phase transfer followed by reaction with glycol-chitosan; lastly, Arg-Gly-Asp (RGD) integrin-binding peptide was covalently attached for in vivo tumor targeting. Dowtherm A, a highly viscous heat-transfer organic fluid, was used to control semiconductor crystal growth at high temperature (>230 °C) during organic synthesis. The structural and optical properties of the resulting $CuInS_2$/ZnS QDs were investigated. The average diameters of $CuInS_2$ and $CuInS_2$/ZnS QDs were 3.0 and 3.7 nm, respectively. Cell toxicity and in vivo tumor targetability in RR1022 cancer cell-xenografted mice were further evaluated using cRGDyk-tagged glycol-chitosan-coated $CuInS_2$/ZnS QDs. Glycol-chitosan-coated MUA-QDs displayed a *Z*-average diameter of 203.8 ± 7.67 nm in water by dynamic light scattering.

Keywords $CuInS_2$/ZnS quantum dots · Glycol-chitosan · In vivo optical imaging · Nanoparticle · Nanobiomedicine

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Introduction

Semiconductor $CuInS_2$ nanocrystals have attracted great attention due to their low toxicity and eco-friendly properties when compared to heavy metals containing semiconductor nanomaterials such as cadmium (Cd) and lead (Pb) (Lee and Han 2015; Zang et al. 2017). $CuInS_2$ nanocrystals have been widely exploited in biological imaging studies; their inorganic nature overcomes drawbacks associated with conventional organic fluorescent dyes. The nanocrystals are very stable against photobleaching, have high photoluminescence brightness, are easily tunable in terms of color, and can provide multicolor photoluminescence (PL) ranging from visible to near-infrared (NIR) wavelengths (Li et al. 2009; Pons et al. 2008; Yong et al. 2010).

$CuInS_2$ is an I–III–VI_2 semiconductor with a direct band gap of 1.5 eV (which has been reported to lie between 1.2 and 1.5 eV), corresponding to emission wavelength of ~830 nm ($E = hc/\lambda$) (Lee et al. 2015; Panthani et al. 2008; Xie et al. 2009). Therefore, this

material might be a promising candidate for optical imaging in the NIR region. However, $CuInS_2$ synthetic methods have not been optimized so far because most of the $CuInS_2$ quantum dots (QDs) generated by published methods were relatively unstable and provided low luminescence efficiencies. In various methods, PL efficiency and photostability of $CuInS_2$ QDs have been enhanced by surface passivation that involves via growth a shell of a higher band gap material, typically ZnS, to form core/shell structured QDs (Speranskaya et al. 2013; Zhang et al. 2013).

However, $CuInS_2$/ZnS synthesis remains relatively complicated. Reported applications have been largely confined to solar energy conversion and light-emitting diodes (LED). Deng et al. (2012) reported a one-pot approach for in situ fabrication of the $CuInS_2$/ZnS core/shell structure. They tried the non-injection approach based on the use of dodecanethiol anion precursor. This precursor served as both ligand and sulfur source, minimized the number of reagents, and simplified the steps. For instance, after $CuInS_2$ core synthesis, $CuInS_2$-growth solution was used directly without an intermediate purification step for coating of ZnS shell. These QDs exhibited improved PL properties, with tunable emission peaks ranging from 550 to 800 nm and a maximum PL quantum yield of up to 80%. Recently, Tang et al. (2015) used ethylene glycol and highly viscous heat-transfer organic fluid, Dowtherm A, to overcome the difficulty of controlling semiconductor crystal growth, arising during moderate- or high-temperature organic synthesis. They developed a modular and simple approach to preparing stable water-soluble NIR Ag_2S QDs covering a wide range of spectra from 500 to 1200 nm using a viscosity modulated approach. In that study, the higher viscosity of Dowtherm A sufficiently slowed the reaction to enable the isolation of QDs with distinct emission wavelengths from 840 to 1200 nm.

Chitosan solubility depends on the ratio of free amino and *N*-acetyl groups (degree of deacetylation). Commonly, chitosan is water-soluble at an acidic pH and insoluble at natural pH because the majority of amines are deprotonated at the latter pH (Bajaj et al. 2012; Najafabadi et al. 2014a, b, 2015). To improve chitosan solubility over a broader pH range, the amine groups of chitosan were partially quaternized or conjugated with sugar moieties. Glycol-chitosan is a derivate of chitosan with 2-hydroxyethylether groups in the 6-O position to improve hydrophilicity. Glycol-chitosan is biocompatible and can be targeted at tumors, and so has been frequently used in various biomedical applications including drug delivery, small interfering RNA carrier, cancer imaging, and therapy (Na et al. 2011; Riva and Ragelle 2011; Yoon et al. 2014).

Herein, we report a novel non-injection-based and simple approach to generating monodisperse and highly luminescent $CuInS_2$/ZnS nanocrystals in Dowtherm A solvent, followed by transfer to an aqueous solution using 11-mercaptoundecanoic acid (MUA). The product was then chemically linked to glycol-chitosan ($–NH_2$ rich), and a target ligand cRGDyk motif was conjugated for tumor imaging.

Experimental section

Materials

Copper(I) iodide (CuI, 99.999%), indium(III) acetate ($In(Ac)_3$, 99.99%), zinc acetate ($Zn(OAc)_2$, 99.99%), 1-dodecanethiol (DDT, 98%), 1-octadecene (ODE, 90%), oleic acid (OA, 99%), oleylamine (OAm, 97%), Dowtherm® A (26.5% diphenyl +73.5% diphenyl oxide), 11-mercaptoundecanoic acid (MUA, 95%), and tetramethylammonium hydroxide solution (TMAH, 10 wt% in H_2O) were purchased from Sigma-Aldrich (St. Louis, MO). Glycol-chitosan ($[C_8H_{15}NO_5 = 205.21]_n$, n = 400, Mw ≈ 82,000 Da) was purchased from Wako Chemicals (Wako, Japan). The water used in all experiments had a resistivity higher than 18.2 MΩ·cm (Milli-Q, Millipore, Billerica, MA). All chemicals were used without further purification. Cyclo[R-G-D-y-K(mercaptoacetyl)] (= cRGDyk, 97%, Mw = 693.40 Da) was delivered by Peptron Inc. (Daejeon, Korea).

Synthesis of $CuInS_2$ QDs

$In(Ac)_3$ (0.2 mmol, 58.4 mg), CuI (0.2 mmol, 38 mg), 1-dodecanethiol (2 mL), oleic acid (0.3 mL), and Dowtherm® A (3 mL) were loaded into a 50-mL three-neck flask equipped with a reflux condenser connected to a nitrogen line. One neck was sealed with rubber septa (Sigma) and the other neck was inserted into a thermometer. The solution was degassed under N_2 purged at room temperature, and the nitrogen line was changed to a N_2 gas balloon. The temperature was then increased to 130~150 °C for 30 min using a heating mantle under a N_2 atmosphere. The color of the solution