Biomaterials 35 (2014) 1608–1617

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Magnetically engineered Cd-free quantum dots as dual-modality probes for fluorescence/magnetic resonance imaging of tumors

Ke Ding, Lihong Jing*, Chunyan Liu, Yi Hou, Mingyuan Gao*

Institute of Chemistry, Chinese Academy of Sciences, Bei Yi Jie 2, Zhong Guan Cun, Beijing 100190, China

ARTICLE INFO

Article history:
Received 12 September 2013
Accepted 27 October 2013
Available online 15 November 2013

Keywords:
Cd-free $CuInS_2$@ZnS:Mn QDs
Cytotoxicity
Fluorescence/MR dual-modality probe
Tumor imaging
In vivo

ABSTRACT

Magnetically engineered Cd-free $CuInS_2$@ZnS:Mn quantum dots (QDs) were designed, synthesized, and evaluated as potential dual-modality probes for fluorescence and magnetic resonance imaging (MRI) of tumors *in vivo*. The synthesis of Mn-doped core–shell structured $CuInS_2$@ZnS mainly comprised three steps, i.e., the preparation of fluorescent $CuInS_2$ seeds, the particle surface coating of ZnS, and the Mn-doping of the ZnS shells. Systematic spectroscopy studies were carried out to illustrate the impacts of ZnS coating and the following Mn-doping on the optical properties of the QDs. In combination with conventional fluorescence, fluorescence excitation, and time-resolved fluorescence measurements, the structure of $CuInS_2$@ZnS:Mn QDs prepared under optimized conditions presented a Zn gradient $CuInS_2$ core and a ZnS outer shell, while Mn ions were mainly located in the ZnS shell, which well balanced the optical and magnetic properties of the resultant QDs. For the following *in vivo* imaging experiments, the hydrophobic $CuInS_2$@ZnS:Mn QDs were transferred into water upon ligand exchange reactions by replacing the 1-dodecanethiol ligand with dihydrolipoic acid-poly(ethylene glycol) (DHLA-PEG) ligand. The MTT assays based on HeLa cells were carried out to evaluate the cytotoxicity of the current Cd-free $CuInS_2$@ZnS:Mn QDs for comparing with that of water soluble CdTe QDs. Further *in vivo* fluorescence and MR imaging experiments suggested that the PEGylated $CuInS_2$@ZnS:Mn QDs could well target both subcutaneous and intraperitoneal tumors *in vivo*.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Molecular imaging has stimulated intense interest as it will surely offer revolutionary tools not only for fundamental studies but also for clinical applications. The dilemma of imaging modality selection in the clinic is that each modality has its own unique advantages and intrinsic limitations, such as insufficient sensitivity or low spatial resolution, so it remains difficult to extract accurate and reliable biomedical information solely based on single imaging modalities [1,2]. Integrating the advantages of different imaging techniques is apparently an effective approach for improving the efficacy of clinical imaging diagnosis [3–5]. To date, the combinations of different imaging methods such as positron emission tomography (PET)/computed tomography (CT) [6] and PET/magnetic resonance imaging (MRI) [7] have already developed into commercial imaging instruments being adopted clinically. Although the optical imaging techniques have shown potentials in extracting detailed biomedical information with high imaging sensitivities and low cost in imaging facilities, the assist of anatomical information is essentially required. In this context, the combination of optical imaging techniques and MRI may represent another useful imaging modality pair for more accurate biomedical detections.

Nanoparticle provides an ideal platform for developing novel fluorescence/MR dual-modality probes especially for tumor imaging [3,4,8–10]. Different strategies have been developed towards this goal by integrating fluorescent quantum dots (QDs) with magnetic nanoparticles or individual magnetic ions for forming bifunctional nanoparticles, including epitaxial heterocrystalline growth, co-encapsulation of pre-made magnetic particle and QD, conjugation of magnetic chelates to QD, and doping QDs with transition metal ions, *etc*. The epitaxial heterocrystalline growth is commonly realized by directly coating superparamagnetic nanoparticles such as FePt, γ-Fe_2O_3, and Co nanocrystals with II–VI semiconducting materials for fusing them into either spherical core/shell particles or hetero-dimers [11–13]. However, the fluorescence quantum yield (QY) of the resultant bifunctional particles is generally low, typically below 5%, due to the quenching effect of the magnetic domains [11–13]. In contrast, bifunctional particles with QY higher than 10% can be obtained by encapsulating pre-made magnetic nanoparticles and QD into inert matrices such as silica or polymer [14–16]. Nonetheless, the resultant composite particles are typically larger than 50 nm and prone to higher uptake by the reticuloendothelial system (RES) in comparison with small

* Corresponding authors. Fax: +86 10 8261 3214.
E-mail addresses: jinglh@iccas.ac.cn (L.H. Jing), gaomy@iccas.ac.cn (M.Y. Gao).

0142-9612/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2013.10.078

counterparts. In contrast, coating QDs directly with paramagnetic metal chelates such as Gd chelates by self-assembly or covalent coupling is more favorable for achieving magnetic QDs without largely increasing the overall size of the particles [17–20]. Moreover, the presence of the paramagnetic metal chelates on the surface of QDs is in favor of T_1-weighted MR imaging. Nevertheless, the high binding affinity of the chelating ligand may heavily etch the QDs before it coordinates with the paramagnetic metal ions for forming the magnetic metal chelates on the QD surface [19,20].

Contrasting to the aforementioned methods, doping QDs with paramagnetic metal ions for achieving intrinsically paramagnetic QDs is superior because the overall size of the bifunctional particles can greatly be decreased down to <5 nm [21,22]. The small size of the particles is in favor of fast excretion of the intravenously administrated particle probes, largely reducing the possible side effects of the QDs within the body. Until now, doping II–VI QDs such as Cd(S, Se) [23–27], Zn(S, Se) [28–32] with Mn has been demonstrated to be a reliable approach for achieving fluorescence/magnetic bifunctional particles that can potentially be used for fluorescence/MR dual-modality imaging. The cytotoxicity of Cd^{2+} is an unavoidable problem for transferring the imaging probes to the clinic [33,34], although cadmium chalcogenide QDs are characterized by unique fluorescent properties [35–40] owing to their suitable exciton Bohr radii. In this context, zinc chalcogenide QDs are taken as suitable alternatives with respect to toxicities [28,32]. But their smaller exciton Bohr radii require the excitation photons to have much higher energy with wavelength typically below 400 nm. Apart from optical damage to tissue, the tissue penetration depth of the excitation light for exciting zinc chalcogenides nanoparticles is much limited [4].

Recent investigations suggest that I–III–VI QDs, such as $CuInS_2$ QDs, are very promising Cd-free candidates for *in vivo* applications [41–43], because they can be excited by incident light with wavelength up to 600 nm. Moreover, the photoluminescence (PL) emission covers a wide range from visible to near-infrared (NIR) with fluorescence QY up to 60% under optimized conditions if coated by ZnS shell [44–50]. Therefore, the magnetically engineered $CuInS_2$-based quantum dots may hold great potentials for producing fluorescence/MR dual-modality molecular imaging probes with greatly suppressed toxicity and suitable optical properties for *in vivo* imaging of tumors.

Following on from our previous studies on $CuInS_2$ nanocrystals [44] and *in vivo* tumor imaging based on versatile magnetic tumor probes [51–53], herein we report a new Cd-free dual-modality imaging probe constructed by doping ZnS coated $CuInS_2$ dots with Mn for achieving highly fluorescent and magnetic QDs. The ZnS coating has twofold functions in the designed particles, on the one hand, it was used to increase the fluorescence QY of the $CuInS_2$ core, on the other hand to reduce the fluorescence quenching effect caused by Mn-doping. Therefore, the impacts of the ZnS coating and the following Mn-doping on the optical properties of the $CuInS_2$ dots were systematically studied. A poly(ethylene glycol) (PEG) based hydrophilic ligand was designed and used for rendering resultant nanoparticles water soluble through ligand exchange. The optical and magnetic properties of the resultant bifunctional QDs were investigated and preliminary tumor imaging studies were carried out for showing their potential for detecting tumors *in vivo*.

2. Materials and methods

2.1. Chemicals

Indium(III) acetate ($In(OAc)_3$, 99.99%) was purchased from Alfa Aesar. 1-dodecanethiol (DDT, 97%) was purchased from Sigma-Aldrich. Copper(I) iodide (CuI, 99.995%), manganese(II) chloride tetrahydrate ($MnCl_2{\cdot}4H_2O$, 99%), stearic acid (SA, 98%), Zinc stearate ($ZnSt_2$) (90%) were purchased from Aladdin. DHLA-PEG2000 ligand was customized product provided by Beijing Oneder Hightech Co. Ltd. Acetone, cyclohexane, methanol, tetrahydrofuran, ether, dichloromethane, hydrochloric acid, and toluene were analytical reagent grade and purchased from Sinopharm Chemical Reagent Beijing, Co., Ltd. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, 98%), dimethyl sulfoxide (DMSO), Dulbecco's Modified Eagle Medium (DMEM, high glucose), and fetal bovine serum (FBS) were bought from Biodee Biotechnology Co., Ltd., Beijing, China. Other solvents and chemicals were used without further purification.

2.2. Synthesis of Mn stearate ($MnSt_2$)

In a typical synthesis of Mn precursor [28], 2.84 g (10 mmol) of SA was dissolved in 15 mL of methanol. The resultant mixture was heated to 50–60 °C to form a homogeneous solution. After the reaction system was cooled down to the room temperature, 20 mL of methanol solution containing 0.91 g (10 mmol) of TMAH was introduced, and then the mixture was kept under stirring for 15 min. Subsequently, 10 mL of methanol solution containing 0.99 g (5 mmol) of $MnCl_2{\cdot}4H_2O$ was dropwise introduced into the above mixture under vigorous stirring, which generated white $MnSt_2$ precipitates that were collected by centrifugation, washed by methanol for several times and then dried under vacuum. The final product was stored under N_2 protection before further use.

2.3. Synthesis of $CuInS_2$ seeds QDs

The $CuInS_2$ seeds were prepared according to a slightly modified method previously reported [44,47]. In brief, 0.0584 g (0.2 mmol) of $In(OAc)_3$ and 0.038 g (0.2 mmol) of CuI were mixed with 12 mL of 1-dodecanethiol. The reaction mixture was firstly degassed under vacuum for 30 min, and then nitrogen gas was introduced to purge the reaction solution. After 30 min, the resultant mixture was heated to 200 °C, and the reaction was allowed for 120 min under nitrogen protection. During this process, the reaction mixture changed from turbid white to transparent yellow when the reaction temperature reached 160 °C and then remained transparent throughout the reaction. In the meantime, the color of the reaction solution progressively changed from light yellow to yellow, red, and finally dark brown. The $CuInS_2$ QDs were precipitated by acetone and isolated by centrifugation, redispersion in cyclohexane, and subsequent precipitation by using acetone for three cycles, the nanocrystals finally obtained were redispersed in cyclohexane for further experiments.

2.4. Synthesis of $CuInS_2$@ZnS QDs

Following the aforementioned procedures for synthesizing $CuInS_2$ seeds, a parallel reaction mixture was prepared by 120 min reaction at 200 °C. Without applying the purification procedures, 0.632 g (1.0 mmol) of $ZnSt_2$ was introduced at room temperature. The resultant reaction mixture was then heated up to 230 °C to initiate the ZnS coating process. A series of aliquots were extracted at 230 °C for monitoring the particle growth. The purification procedures for the collected particles were the same as those described above.

In parallel, one more sample was prepared by introducing 0.253 g (0.4 mmol) instead of 0.632 g (1.0 mmol) $ZnSt_2$ into a parallel reaction system for producing $CuInS_2$ QDs with thinner ZnS coating layer following the procedures mentioned above.

2.5. Synthesis of $CuInS_2$@ZnS:Mn QDs

Following the aforementioned procedures for synthesizing $CuInS_2$@ZnS QDs, a parallel reaction mixture was prepared by 120 min reaction at 230 °C. Without applying the purification procedures, 2 mL of DDT solution containing 0.124 g (0.2 mmol) of $MnSt_2$ was introduced when the reaction mixture was cooled down to 180 °C to initiate the Mn-doping process. A series of aliquots were extracted at 180 °C for monitoring the doping process. The purification procedures for the collected particles were the same as those described above. The sample obtained by 180 min of reaction, i.e., sample A, was used in the following experiments.

In parallel, two more sample was prepared, i.e., sample B and sample C. Sample B was prepared by using 0.311 g (0.5 mmol) instead of 0.124 g (0.2 mmol) $MnSt_2$. By the same procedures for sample A, sample C was prepared based on $CuInS_2$@ZnS QDs with thinner ZnS shell.

2.6. PEGylated $CuInS_2$@ZnS:Mn QDs

100 mg of hydrophobic $CuInS_2$@ZnS:Mn QDs were mixed with 1 g of DHLA-PEG in 50 mL of toluene. The mixture was kept under stirring under nitrogen at 60 °C for 2 h. The resultant PEGylated QDs were precipitated by 150 mL of ether, and then collected by centrifugation. After decanting the supernatant, 20 mL of Milli-Q water was introduced to redisperse the particles. The particle solution was subsequently purified by ultrafiltration at 5000 g using a 30 KD centrifugal filtration device (Millipore). The condensed solution was then ready for further experiments.

2.7. Cytotoxicity assay of PEGylated $CuInS_2$@ZnS:Mn QDs

The colorimetric MTT assay was performed to assess the cytotoxicity of the PEGylated $CuInS_2$@ZnS:Mn QDs for comparing with that of CdTe QDs. Specifically,

Download English Version:

https://daneshyari.com/en/article/10227456

Download Persian Version:

https://daneshyari.com/article/10227456

Daneshyari.com