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Synthesis and characterization of CdTe quantum dots embedded gelatin nanoparticles via a two-step desolvation method

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ABSTRACT

Novel CdTe quantum dots (QDs) embedded gelatin nanoparticles (CdTe/gelatin nanoparticles) were synthesized via a two-step desolvation method. The morphology and size distribution of the nanoparticles were characterized by transmission electron microscope (TEM) and laser particle size analyzer. They are presented spherically and relatively uniform with a diameter of 150 nm. The luminescent properties of the nanoparticles were investigated by using fluorescence spectrophotometry and fluorescence microscopy. The fluorescence stability of nanoparticles was tested in vitro. It was found that the nanoparticles were stable in water and phosphate-buffered saline (PBS) solution (pH 7.4) for at least 15 days. A possible formation mechanism of the CdTe/gelatin nanoparticles was also proposed. The inherent stability and biocompatibility indicate that the nanoparticles are expected to be promising candidates for in vivo biological imaging studies.

1. Introduction

In recent years, quantum dots (QDs) were emerging as an attractive alternative to traditional fluorescent organic dyes for biological labeling because of their unique, size-tunable spectral properties and excellent photostability [1,2]. As an important kind of visible light emitting QDs, CdTe was widely used for biological labeling, such as living cell imaging [3–5] and cancer marker targeting [6,7]. One challenge in the application of QDs is their stability and biocompatibility in biological system. As is well known, QDs synthesized in aqueous system were generally coated with small sulphydryl compounds such as mercaptoacetic acid and cysteamine. They were easily degraded by hydrolysis or oxidation, causing severe fluorescence quenching. Besides, Cd^{2+}, which was proved to be toxic in vivo [8], may dissolve from QDs and release to the biological system directly. To solve these problems, different techniques and materials have been used for incorporating QDs into polymer microbeads or nanoparticles [9–15]. For example, QDs incorporated poly (*N*-isopropylacrylamide) microspheres [9], bovine serum albumin (BSA) microspheres [10], polystyrene microspheres [11], polyelectrolyte microspheres [12] and chitosan nanoparticles [13,14] were synthesized recently. However, some of these polymer beads are large on microscale and not suitable for in vivo biological imaging, some of the synthesis methods need special equipment and reagents or complicated synthesis process.

Gelatin is a natural nontoxic, biocompatible biopolymer derived from collagens. Owing to its useful emulsifying, peptizing, stabilizing and binding properties, gelatin has been extensively used as a kind of biomaterial for preparation of nanoparticles which served as drug carriers and gene delivery systems [16–19]. More recently, gelatin was used as surface capping agent for in situ synthesis of QD–gelatin nanocomposites. Gelatin which added during QDs synthesis process efficiently caps free atoms protruding from the metalloid core and increases the rate of recombination by reducing the number of nonradiative centers [20]. However, no previous investigations were reported using gelatin as coating material in the synthesis of QDs embedded nanoparticles.

In this work, we prepared water soluble fluorescent gelatin nanoparticles embedded with CdTe QDs via a two-step desolvation method. The nanoparticles were characterized by size, optical properties as well as fluorescence stability. A possible formation mechanism was also proposed.

2. Experimental

2.1. Materials

CdTe QDs were locally prepared according to Ref. [21]. The refluxing time was 1.5 h and the QDs colloid was adjusted to 1.5 mM according to Te^{2-} concentration. Gelatin (type A, average molecular weight ~110 kDa) was purchased from Sinopharm Chemical Reagent Co., Ltd. Formaldehyde solution (40%) and acetone were obtained from Nanjing Chemical Reagent Co. Ltd.

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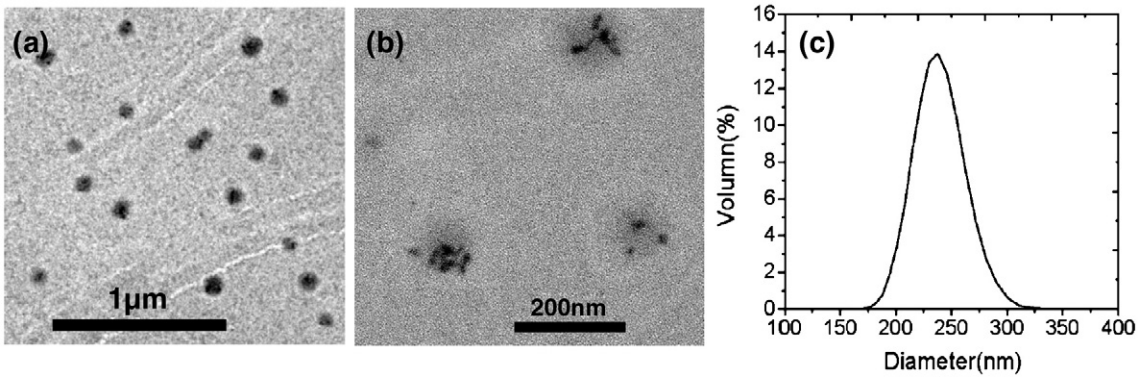

Fig. 1. TEM images ((a), (b)) and size distribution (c) of CdTe/gelatin nanoparticles.

2.2. Synthesis of CdTe/gelatin nanoparticles

CdTe/gelatin nanoparticles were prepared by a two-step desolvation method developed by Coester et al. with modification [16]. Briefly, 10 ml of 5% gelatin solution was prepared at 50 °C under stirring. After the solution cooled down to room temperature, gelatin was desolvated by slowly adding an equal volume of acetone and kept for sedimentation. The supernatant was discarded and the sediment was dissolved in 10 ml as-prepared CdTe QDs colloid with pH near 11. Then 7 ml acetone was added to the gelatin–CdTe QDs mixture dropwise until the solution became turbid. CdTe/gelatin nanoparticles were cross-linked with 0.5 ml formaldehyde solution over night at room temperature. Purification was done by a five-fold centrifugation at 14,000 rpm for 5 min to remove desolvating agent and the excess free gelatin and QDs.

2.3. Characterization

TEM images were acquired on a JEM-2100 transmission electron microscope (JEOL, Japan). The size distribution of the nanoparticles was measured using a Mastersizer 2000 laser particle size analyzer (Malvern Instruments, UK). The UV–vis absorption spectra were acquired on a UV2100 UV–VIS spectrometer (Shimadzu, Japan). The fluorescence measurements were made with a RF-5301 spectrofluophotometer (Shimadzu, Japan). Fourier transform infrared spectroscopy (FTIR) spectra were recorded on a FTIR-8400S Spectrophotometer (Shimadzu, Japan). CdTe QDs for FTIR measurement were precipitated by adding isopropyl alcohol to the colloidal solution and then separated by centrifugation and dried. All samples were thoroughly milled with KBr. Fluorescence image was visualized using an Olympus IX-51 fluorescent microscope and captured with a Retica digital camera.

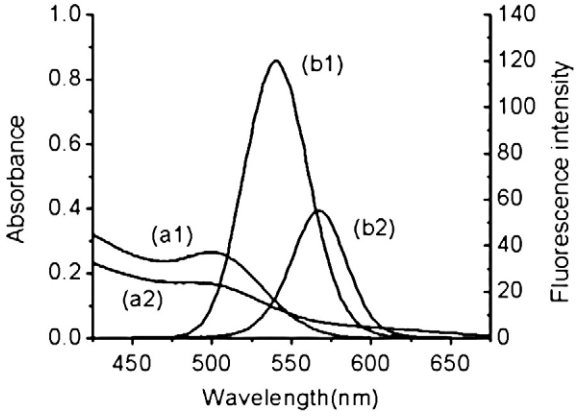

Fig. 2. UV–vis absorption and fluorescence spectra of CdTe QDS ((a1), ((b1)) and CdTe/gelatin nanoparticles ((a2), ((b2)).

2.4. Fluorescence stability in vitro

A fixed amount of CdTe/gelatin nanoparticles was stored in pure water and phosphate-buffered saline (PBS) solution (pH 7.4) over a period of 15 days. The nanoparticles were centrifuged down and redispersed in equal volume of water or PBS before measuring the fluorescence intensity on days 0, 2, 4, 5, 10 and 15.

3. Results and discussion

TEM image (Fig. 1(a)) showed that the size of CdTe/gelatin nanoparticles was uniform with an average size around 150 nm. No free CdTe QDs dispersed in the field of vision. An image with higher magnification (Fig. 1(b)) clearly revealed that CdTe QDs were encapsulated in the interior of gelatin nanoparticles. Some agglomerates could be observed as the distance between QDs shortened to a large extent due to their interaction with the reactive amino groups of gelatin molecules through electrostatic force. The size distribution of the nanoparticle measured by using laser light scattering (LLS) technique was shown in Fig. 1(c). It was found that the nanoparticles had a narrow size distribution (Polydispersity Index = 0.01) with the volume average hydrodynamic diameter of 237 nm. Since gelatin nanoparticles absorb water and undergo a swelling process in aqueous medium [22], the hydrous radius determined by LLS is a little larger than the particle size acquired from TEM.

The UV–vis absorption and fluorescence spectra of the CdTe/gelatin nanoparticles were investigated compared with CdTe QDs. As shown in Fig. 2, the maximum UV–vis absorption wavelength of the CdTe/gelatin nanoparticles did not change remarkably compared with free QDs. However, the fluorescence emission wavelength of the encapsulated CdTe QDs was red-shifted from 542 to 570 nm. This might occur when immobilized in gelatin nanoparticles because the close proximity of the QDs resulted in the increase of the dipole interaction between them [23,24]. When the aqueous solution of CdTe/gelatin nanoparticles was excited by a UV lamp at 365 nm, it emitted bright green fluorescence. Under a fluorescence microscope, monodispersed nanoparticles with bright green color can be observed (Fig. 3).

FTIR spectra of CdTe QDs, native gelatin and CdTe/gelatin nanoparticles were given in Fig. 4. In the spectrum of CdTe QDs (curve (a)), peaks at 1633 cm^{-1} and 1461 cm^{-1} represented carbonyl stretching vibration and methylene scissoring vibration of mercaptoacetic acid coated on the surface of QDs. Peaks in the spectrum of gelatin (curve (b)) at 1654 cm^{-1} and 1544 cm^{-1} corresponded to amide (carbonyl stretching vibration) and amide II (N–H bending vibration) bands. As expected, in the case of the

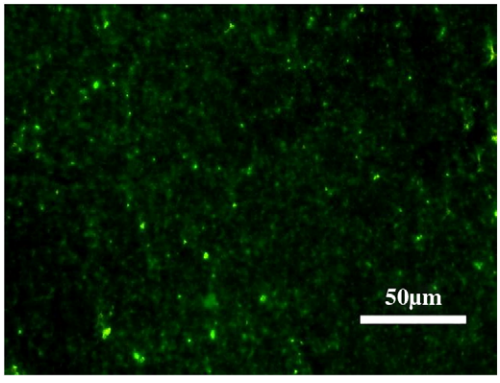

Fig. 3. Fluorescence microscope image of CdTe/gelatin nanoparticles. Objective magnification: 20×.

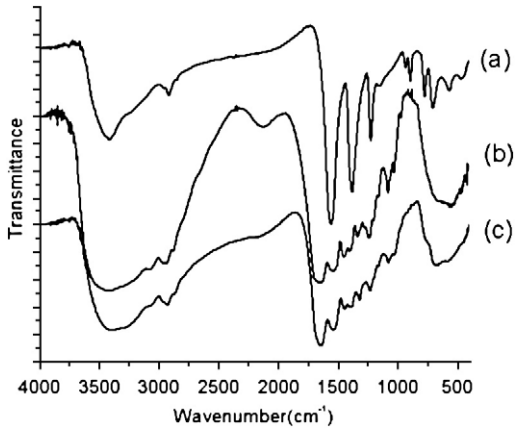

Fig. 4. FTIR spectra of CdTe QDs (a), gelatin (b) and CdTe/gelatin nanoparticles (c) recorded in KBr pellets.

CdTe/gelatin nanoparticles (curve (c)), the characteristic peaks of CdTe QDs disappeared, and the shape of the spectra was similar to curve (b). However, in the spectrum of nanoparticles, the peak of amide went through a blue-shift to 1641 cm^{-1}. It was noted that Cd^{2+} on the surface of QDs can strongly coordinate with oxygen atoms of carbonyl groups [25,26]. This complexation effect could cause the electron cloud between carbon and oxygen atoms moved to the direction of oxygen, which led to the decrease of vibration frequency of carbonyl groups [27].

The fluorescence stability results of CdTe/gelatin nanoparticles in pure water and PBS solution revealed that the maximum emission wavelength of the fluorescence spectra and the fluorescence intensity stayed essentially the same in both media after storage for 15 days. This suggested that the protection provided by the gelatin coated surface was sufficient enough to prevent the photo-oxidation damage and salt effect to the encapsulated QDs.

We considered that the CdTe/gelatin nanoparticles were formed through the process as follows: CdTe–gelatin nanocomposites were obtained firstly after the QDs and gelatin solution mixed. Because of the large surface to volume ratio and large surface energy, CdTe QDs are inclined to adsorb on gelatin. Moreover, it was found that the reactive amino groups in gelatin molecules were prone to bind to carboxylic group coated QDs through electrostatic force. When acetone was added into the CdTe–gelatin nanocomposites, gelatin nanoparticles formed and CdTe QDs were incorporated and immobilized into the gelatin matrix. After the introduction of formaldehyde solution (served as cross-linking agent), a large number of free amino groups on the side chains of gelatin combined together and CdTe/gelatin nanoparticles with more compact and stable structure were obtained finally.

4. Conclusions

CdTe/gelatin nanoparticles were synthesized via a two-step desolvation approach. The method is simple, low cost and highly efficient. The fluorescent nanoparticles prepared were water soluble and stable for at least 15 days. The inherent stability and biocompatibility indicate that the nanoparticles are expected to be promising candidates for in vivo biological imaging studies. Moreover, with the aid of these "visible" QDs embedded, investigations of in vitro and in vivo behaviors of drug and gene loaded gelatin carriers should be carried out by various optical and imaging techniques.

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