Journal of Luminescence 135 (2013) 281–287

Contents lists available at SciVerse ScienceDirect

Journal of Luminescence

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

Synthesis and luminescence of Eu^{3+} doped hydroxyapatite nanocrystallines: Effects of calcinations and Eu^{3+} content

Yingchao Han [a],*, Xinyu Wang [a], Honglian Dai [a], Shipu Li [a,b]

[a] Biomedical Materials and Engineering Center, Wuhan University of Technology, 122 Luoshi Road, Hongshan, Wuhan 430070, Hubei, PR China
[b] State Key Laboratory of Advanced Technology for Materials Synthesis and Processing, Wuhan University of Technology, 122 Luoshi Road, Hongshan, Wuhan 430070, Hubei, PR China

ARTICLE INFO

Article history:
Received 19 May 2012
Received in revised form
11 September 2012
Accepted 21 September 2012
Available online 29 September 2012

Keywords:
Hydroxyapatite
Nanocrystalline
Europium
Luminescence
Cell label

ABSTRACT

Eu^{3+} doped hydroxyapatite (Eu-HAP) luminescent nanocrystallines were prepared by ultrasound assisted precipitation method. Aiming to seek a compromise proposal for achieving high luminescence and nanocrystallines, the effects of calcinations temperature and Eu^{3+} doping content on luminescence property as well as phase composition, crystal size and crystallinity of Eu-HAP crystals were investigated. The results showed that the luminescence of Eu-HAP was enhanced by the thermal treatment and the increasing Eu doping content. However, the higher temperature and the excess Eu resulted in the size increase and phase composition change of HAP respectively. The 2% Eu doping content and calcinations temperature of 600 °C were preferred for preparing Eu-HAP nanocrystallines with strong luminescence. The Eu-HAP nanocrystallines were about 20–40 nm in diameter (TEM) and could be well dispersed in water to form aqueous suspension of about 140 nm (DLS intensity-averaged diameter). Eu-HAP nanocrystalline could be used as a fluorescent probe for cell labeling.

1. Introduction

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAP) is the inorganic component of human bone and tooth, exhibiting good biocompatibility and bioactivity. Consequently, the HAP correlative artificial bone substitutes such as bulk ceramic, powder, coating and composite materials have been extensively studied and used in biomedical applications [1]. Recently, nanoscale HAP has displayed some especial behaviors differing from bulk HAP, for example enhancing densification and mechanical strength, improving osseointegrative properties [2,3]. Especially, the apatite nanoparticles show a high solubility in acidic pH environment of cellular endolysosomes and the adsorbed molecules (gene or protein) can be escaped from the dissolving apatite [4,5]. Therefore, HAP nanoparticles are considered as biocompatible and biodegradable carriers of gene, drug and protein. As a delivery vehicle, it is required to reveal the cellular endocytosis and localization in cells, distribution and degradation in vivo for HAP nanoparticles. Nowadays, fluorescent labeling is an effective and commonly used tool to observe the targets in vitro and in vivo. Moreover, fluorescent labeling can realize the continuous and nondestructive observations, which is helpful for monitoring the delivering progression of carriers. The alternative is to replace the calcium ions in HAP crystal lattice using rare earth ions as luminescent centers. The emitted luminescent properties of rare earth ions show narrow emission bandwidths, high photochemical stability and long fluorescence lifetime (up to several milliseconds) [6,7]. The hexagonal apatite permits the substitutions of many rare earth ions without the change of crystal structure [8–11] and the doping rare earth ions are stably located in the crystal lattice.

The luminescent properties of rare earth ions in natural and synthesized apatite have been studied for a long time [12–15]. Recently, the rare earth ions doped apatite nanoparticles have attracted more attention on the application as cell labeling material due to their strong luminescence under visible-light excitation [16–21]. The previous results showed that the luminescent properties of rare earth doped apatite nanoparticles were much related with the thermal diffusion of rare earth ions to the Ca^{2+} sites in crystal lattice. The rising temperature could enhance the crystallinity of apatite and accelerate the diffusion of rare earth ions to the Ca^{2+} sites, resulting in the enhancement of luminescence. Also, this would lead to the increasing of particle size up to micron-size, which was not favorable for the internalization of particles while labeling cells. That is to say, there exists an incompatible relationship between luminescence and particle size of rare earth doped apatite. Therefore, it is required to seek a compromise proposal for preparing high luminescent and nanosize rare earth doped apatite particles.

Herein, the Eu^{3+} ions doped HAP (Eu-HAP) nanocrystallines were synthesized by ultrasound assisted precipitation method. The effects of calcinations temperature and Eu doping content on phase composition, crystal size, crystallinity and luminescent

---

* Corresponding author. Tel.: +86 27 87651852; fax: +86 27 87880734.
E-mail address: hanyingchaowhut@yahoo.cn (Y. Han).

0022-2313/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jlumin.2012.09.029

properties of Eu-HAP were investigated. The potential application of Eu-HAP nanocrystallines as cell labels was evaluated for Bel-7402 human liver cancer cells.

2. Materials and methods

2.1. Preparation of Eu-HAP nanocrystallines

The Eu-HAP nanocrystallines were prepared by ultrasound assisted precipitation method as the following process. Firstly, $Ca(NO_3)_2 \cdot 4H_2O$, $Eu(NO_3)_3 \cdot 6H_2O$ and $Ca(H_2PO_4)_2 \cdot 2H_2O$ were dissolved in distilled water respectively. According to the molar ratios of (Eu)/(Eu+Ca)=0.25–4% and (Eu+Ca)/P=1.67, the mixture of $Ca(NO_3)_2$ aqueous solution and $Eu(NO_3)_3$ aqueous solution was poured into the $Ca(H_2PO_4)_2$ aqueous solution under vigorous stirring. After the pH value was adjusted to about 10 by $NH_3 \cdot H_2O$, the suspension was treated by ultrasound for 0.5 h and then kept for 24 h. Next, the precipitation was washed by distilled water at least three times and then dried by freeze–drying method. Finally, the dried precipitation was calcined at 300–600 °C for 1 h to obtain samples.

2.2. X-ray Diffraction (XRD)

Powder sample was placed in the specimen holder and then determined by Powder X-ray Diffraction (XRD, D/Max-IIIA, Rigaku Co., Tokyo, Japan), using Ni-filtered CuKα radiation (λ=0.154184 nm) in the step scanning mode, with tube voltage of 35 kV and tube current of 30 mA. The XRD pattern was recorded in the 2θ range of 10°–70°, with a step size of 0.02° and step duration of 0.5 s.

The crystallinity degree and crystallite size of sample were calculated. The crystallinity degree (X_c) of Eu-HAP powders can be evaluated by the following Eq. (1):

$$X_c \approx 1 - V_{112/300}/I_{300} \tag{1}$$

where I_{300} is the intensity of (3 0 0) diffraction peak and $V_{112/300}$ is the intensity of the hollow between (1 1 2) and (3 0 0) diffraction peaks of HAP [22].

The crystallite size of Eu-HAP powders can be calculated by Scherrer's formula as Eq. (2):

$$X_{hkl} = k\lambda/\beta_{1/2}\cos\theta \tag{2}$$

where X_{hkl} is the crystallite size (nm); λ the wavelength of monochromatic X-ray beam (nm) (λ=0.15418 nm for CuKα radiation); $\beta_{1/2}$ the full width at half maximum for the diffraction peak under consideration (rad); θ the diffraction angle (°); and k is a constant varying with crystal habit and chosen to be 0.9 [23]. The (0 0 2) diffraction peak was used to calculate the crystallite size by the Scherrer's formula due to its most distinct reflection in the XRD pattern.

2.3. Fourier Transform Infrared (FT-IR) Spectroscopy

Fourier Transform Infrared Spectroscopy (FT-IR, Nexus, Thermo Nicolet, USA) was utilized to analyze the FT-IR spectra of Eu-HAP powders. The spectra were recorded at room temperature between 400 cm^{-1} and 4000 cm^{-1} at 4 cm^{-1} spectral resolution.

The peaks assigned to the OH vibrations of HAP indicate the crystallinity degree of HAP. The ratio (R_{631}) of the area of OH band at 631 cm^{-1} to the total area of the bands (631 cm^{-1}, 603 cm^{-1}, 565 cm^{-1}) between 670 and 480 cm^{-1} was calculated to show the change of crystallinity degree of HAP [17].

2.4. Thermogravimetric Analysis–Differential Scanning Calorimeter (TG–DSC)

The freeze–dried precipitation was determined by Thermogravimetric Analysis–Differential Scanning Calorimeter (TG–DSC, STA 449C, NETZSCH, Germany) for the analysis of the changes of sample during the calcinations procedure.

2.5. Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES)

The contents (wt%) of Ca, Eu and P in samples were determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES, Optima 4300DV, PE Co. Ltd., USA).

2.6. Morphology observation

The sample was observed by Scanning Electron Microscopy (SEM, JSM-5610LV, JEOL, Japan) and High Resolution Transmission Electron Microscope (HRTEM, JEM-2100 F, JEOL, Japan).

2.7. Dynamic Light Scattering (DLS)

Eu-HAP powders (10 mg) were dispersed in distilled water (10 mL) with addition of sodium heparin (4 mg) and treated using ultrasonic cleaner (40 kHz and 250 W) for 0.5 h to obtain transparent suspension. The averaged particle size (ZAve) and the size distribution of Eu-HAP suspension were determined by Zetasizer 3000HS (Malvern, UK) based on the intensity of scattered light.

2.8. Photoluminescence spectrum

The photoluminescence spectra of the samples were recorded using a 970CRT Fluorescence Spectrophotometer (Shanghai Sanco, China). The excitation spectrum between 345 nm and 435 nm was recorded with an emission wavelength at 618 nm. The emission spectrum between 580 nm and 735 nm was recorded with an excitation wavelength at 394 nm.

2.9. Cell labeling using Eu-HAP nanocrystallines

The internalization of Eu-HAP was observed by Confocal Laser Scanning Microscopy (CLSM, Leica TCS SP2 AOBS, Germany). Bel-7402 human liver cancer cells, obtained from the China Center for Type Culture Collection of Wuhan University (CCTCC, Wuhan, PR China), were seeded at a density of 40,000 cells per well for 24 h at 37 °C in 5% CO_2, and then incubated with the dispersed Eu-HAP nanocrystallines for 5 h. Before CLSM observation, cells were washed using PBS.

3. Results

The photoluminescence properties of Eu-HAP (Fig. 1) showed dependences on the calcinations temperature and Eu doping content. With a controlled Eu doping content of 2%, the luminescence spectra of Eu-HAP were gradually enhanced along with the rising of calcinations temperature to 600 °C. At low temperatures of 300 °C and 400 °C, only one weak excitation peak at 393.8 nm and one weak emission peak at 614.1 nm were detected. After calcinations at 500 °C, three excitation peaks were observed at 393.8 nm, 381.2 nm and 360.9 nm respectively accompanied with the appearance of two emission peaks at 614.1 nm and 696.9 nm. After the further calcinations at 600 °C, the excitation and emission spectra were becoming sharp and strong. The strongest and two other smaller excitations were located at 393.8 nm and 360.9,