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铈铽共惨杂氟化镧纳米晶体的快速合成和荧光特性

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摘要:用一种简单的方法快速合成了水溶性铈铽共惨杂氟化镧纳米晶体。这种荧光纳米晶体直径只有 14 nm,具有很好的生物相容性,并且在活体干细胞中显示出很强的荧光。结果表明这种铈铽共惨杂氟化镧纳米晶体可作为一种有效的生物荧光标记材料。

关键词:生物标记;稀土元素;纳米晶体;荧光
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Rapid Synthesis of Biocompatible LaF_3:Ce,Tb Nanocrystals with Bright Fluorescence

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Abstract: Water-soluble fluorescent LaF_3:Ce,Tb nanocrystals were synthesized rapidly by using a simple route in aqueous solution at 75 ℃. The water-soluble, small nanocrystals with an average diameter of 14 nm were demonstrated to be biocompatible and exhibited bright fluorescence in living hepatocellular carcinoma cell line HCCLM6 cells. The results indicate that the LaF_3:Ce,Tb nanocrystals have the potential to be used as an alternative biological fluorescence probe.

Key words: biolabels; rare-earth; nanocrystals; fluorescence

Inorganic luminescent nanomaterials have been frequently discussed in terms of biological applications, including biolabelling, biochips, bioassays, contrast agents, and imaging techniques[1~6]. To realize suitable lumophores, particle size, degree of agglomeration, material biocompatibility, and optical properties under biologically relevant conditions, have to be considered [7]. Recent studies have focused on the semiconductor quantum dots (QDs) which offer a sharp spectral peak with a larger Stokes shift than organic dyes[8~11]. However, the semiconductors such as CdSe are eclipsed by high toxicity, and have raised serious concerns about health and environment[12,13].

Alternative biological nanocrystals(NCs) that exhibit unique optical properties are those based on rare-earth-doped fluoride phosphors [14~17]. LaF_3 has very low phonon energy and therefore minimizes the quenching of the excited-state rare-earth ions and thus has a high quantum yield [14]. Furthermore, it exhibits adequate thermal and environmental stability as well as large solubility for all rare-earth ions without deleterious effects[18], and therefore is considered as an ideal host

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material for luminescent rare-earth ions. Unlike QDs, the rare-earth-doped LaF_3 NCs have fluorescence originated from the transitions between *d-f* or *f-f* electron states which are dependent on their local symmetry and independent of their size[17,19]. Furthermore, the optical properties can be tuned by the doped rare-earth ions, and therefore fluorescent labels of different colors can be produced by varying the dopants. Owing to these properties, a few of biological applications of the rare-earth-doped LaF_3 NCs have been reported in recent years[20~22]. However, to the best of our knowledge, there has been no report on the fluorescence of rare-earth-doped LaF_3 NCs in living cells.

We report here a simple preparation method for LaF_3:Ce,Tb NCs. And their bright fluorescence in living hepatocellular carcinoma cell line HCCLM6 cells is also demonstrated. The highly water-soluble LaF_3:Ce,Tb NCs were synthesized directly in aqueous solution at low temperature (75 ℃). Photoluminescence examination showed that the prepared NCs emitted bright green fluorescence with a very sharp emission peak, a large Stokes shift and a broad excitation band. The cell biocompatibility of the NCs was evaluated by using a MTT assay with three kinds of cancer cells. By using a fluorescence microscope, the strong fluorescence of the NCs in the cytoplasm of the hepatoma cells was observed. Such spherical shaped, small (the average diameter is 14 nm), water-soluble, bright LaF_3:Ce,Tb NCs make our approach attractive for preparing alternative biolabels.

1 Materials and Methods

1.1 Materials

Rare-earth chlorides and ammonium fluoride were purchased from Sigma-Aldrich(St. Louis, MO, USA) and used as received. Human hepatocellular carcinoma cell line HCCLM6 cells[23] were provided by Liver Cancer Institute of Fudan University(China).

1.2 Methods

1.2.1 Preparation of LaF_3:Ce,Tb NCs

LaF_3:Ce,Tb NCs were synthesized via a very simple and rapid route. In brief, $LaCl_3$, $CeCl_3$ and $TbCl_3$ were dissolved in 40 mL of water with a total rare-earth ion concentration of 0.04 mol·L^{-1}. Then 10 mL of 1 mol·L^{-1} NH_4F solution was added, and the mixture was subsequently heated to 75 ℃ for 2 h under stirring. After being centrifuged and washed 3 times, the NCs were re-dispersed in water or dried in vacuum.

1.2.2 Characterization

The transmission electron microscopy (TEM) and the high resolution TEM (HRTEM) images were observed with a JEOL 2010 HT and JEOL 2010 FET transmission electron microscope (operated at 200 kV), respectively. Powder X-ray diffraction (XRD) analyses were performed on a Bruker D8-advance X-ray diffractometer with Cu $K\alpha$ irradiation (λ=0.154 06 nm) at a scanning speed of 0.025°·s^{-1} over the 2θ range of 20°~80°. Fourier transform infrared(FTIR) spectra were recorded on an AVATAR-360 spectrometer. The fluorescence excitation and emission spectra were obtained with a Hitachi F-4500 fluorescence spectrophotometer.

1.2.3 MTT assay

To assess the biocompatibility of the LaF_3:Ce,Tb NCs, human hepatocellular carcinoma cell line HCCLM6 cells, human cervical cancer cell line HeLa cells and human breast cancer cell line MCF-7 cells were grown with the NCs, respectively. The cell viability was measured by a MTT assay. Cells were cultured in 96-well plates (approximately 1×10^4 cells per well) with a medium containing different concentrations of NCs for 48 h. Then, 20 μL of MTT solution (5 mg·mL^{-1} MTT in phosphate buffer solution, pH=7.4) was added to each well and incubated for 4 h at 37 ℃. After removing the medium, 150 μL of dimethylsulfoxide (DMSO) was added to each well to completely dissolve the crystals. The absorbance of the cell lysate at 570 nm was measured through enzyme photometer. Cell viability was expressed as a percentage of the control. All results are expressed as average±SD of ten samples.

1.2.4 Fluoresence microscopy

Human hepatocellular carcinoma cell line HCCLM6 cells were cultured in 6-well plates at 1×10^5 cells per well with a medium containing LaF_3:Ce,Tb

NCs for 48 h. The concentration of the NCs was 100 μg·mL. Then, the medium was taken out, and each well was rinsed thrice with 37 ℃ pre-heated Phosphate-Buffered Saline (PBS). The living cell fluorescence was observed by an inverted fluorescence microscope (Olympus IX 70), coupled with a digital camera (Nikon coolpix 5400) and Image-pro Plus 5.0 image analysis software.

2 Results and discussion

Fig.1a shows the TEM image of the LaF_3:Ce,Tb NCs. It can be seen that the NCs are of nearly spherical shape and have an average size of 14 nm. The lattice fringes are visible in the HRTEM image of a LaF_3:Ce,Tb nanocrystal and the corresponding fast Fourier transform (FFT) analysis clearly shows the hexagonal phase of LaF_3(shown in Fig.1b). Fig. 1c shows the XRD pattern of the LaF_3:Ce,Tb NCs, which is in good agreement with hexagonal phase structure known from the bulk LaF_3 crystal(PDF No.32-0483).

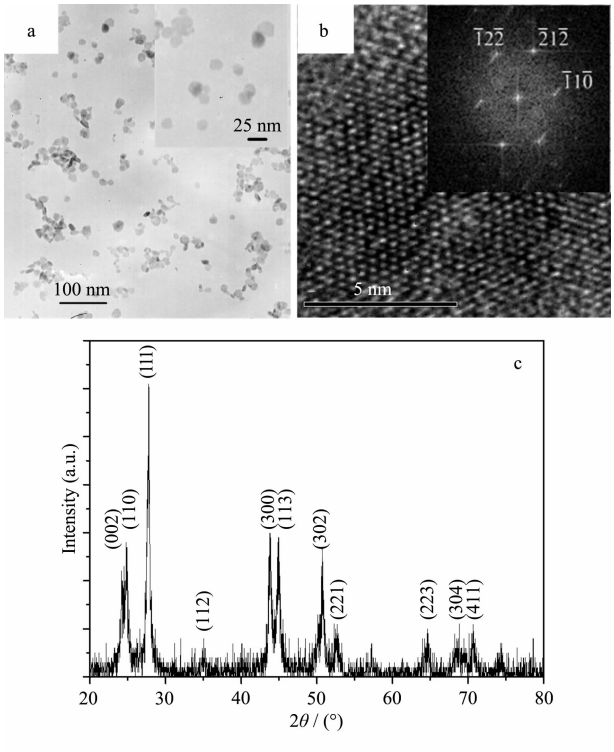

Fig.1　(a) TEM image of LaF_3:Ce,Tb NCs, the inset in (a) is the same particles at higher magnification; (b) HRTEM image and corresponding FFT image (inset) of a LaF_3:Ce,Tb nanocrystal. (c) XRD pattern of LaF_3:Ce,Tb NCs

Fig.2 gives the FTIR pattern of the LaF_3:Ce,Tb NCs. As the NCs are prepared in water, their surface may be covered by a large number of OH groups either chemically bonded or physically adsorbed to the surface. The absorption bands at around 3 430 and 1 630 cm^{-1} are attributed to O-H stretching and bending vibrations, respectively. The hydroxyl groups render the NCs well dispersed in water.

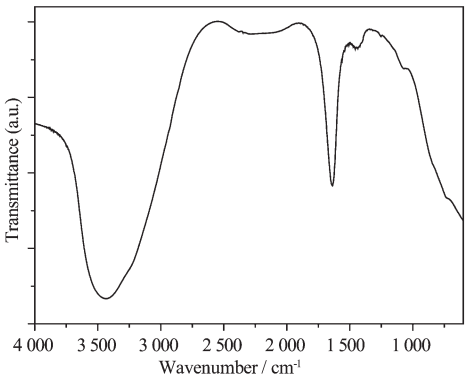

Fig.2　FTIR spectrum of the LaF_3:Ce,Tb NCs

Fig.3 shows the fluorescence excitation and emission spectra of LaF_3:45%Ce, 10%Tb(45% Ce^{3+} and 10% Tb^{3+} co-doped in LaF_3) and LaF_3:10%Tb (10% Tb^{3+} doped in LaF_3) NCs in aqueous solution, respectively. In general, rare-earth ions (such as Tb^{3+}) lack a broad excitation band and usually need to excite directly into the *f-f* transition (typically at 354 nm for Tb^{3+} shown in Fig. 3a). Due to the weak absorption and narrow line width, it is only useful for applications where a strong laser is used as an excitation source. To overcome this limitation, we co-doped Ce^{3+} and Tb^{3+} into the LaF_3 NCs, in which Ce^{3+} absorbs light, transfers energy to Tb^{3+} and makes Tb^{3+} emit green light. Due to the strong absorption in the wavelength range between 200 and 300 nm from the 4*f*-5*d* transition of Ce^{3+}[24], the LaF_3:Ce,Tb NCs can emit bright green fluorescence just under excitation of 260 nm (shown in Fig. 3a). The emission spectrum in Fig. 3b shows a typical emission band between 450 and 650 nm corresponding to the $^5D_4$ and ^7F_J(J=6~3) transitions of Tb^{3+}[25,26], and the emission peak with the highest intensity at 543 nm is attributed to the $^5D_4 \rightarrow {}^7F_5$ transitions. It is clear that the intensity at 543 nm of the LaF_3:45%Ce, 10%Tb NCs is 10.1 times that of the LaF_3: 10%Tb NCs. In addition to the typical emission lines of

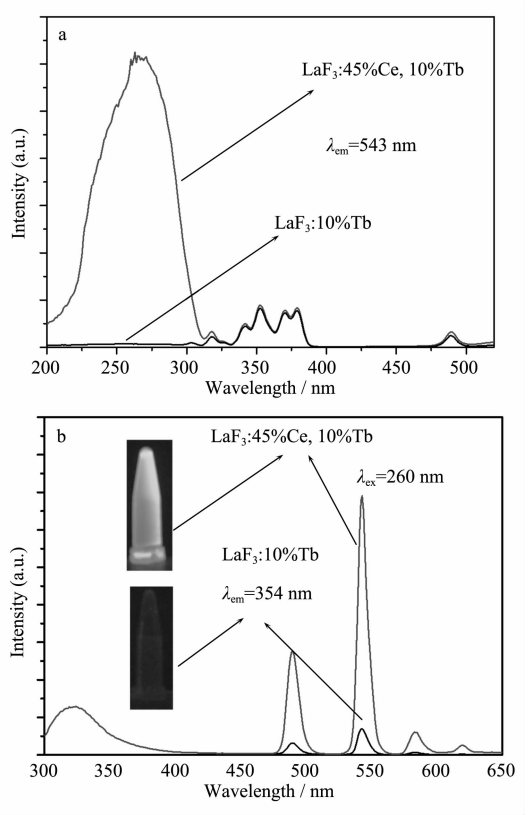

Fig.3 (a) Fluorescence excitation and (b) emission spectra of LaF_3:45%Ce, 10%Tb and LaF_3:10% Tb NCs in aqueous solution, respectively. The insets in (b) are the photographs which indicate the much stronger green fluorescence from the LaF_3:45%Ce, 10%Tb NCs exposed to UV light

Tb^{3+} ions, the spectrum of LaF_3:45%Ce, 10%Tb NCs also shows a broad emission band between 300 and 400 nm, which can be assigned to the 4*f*-5*d* transitions of Ce^{3+}, indicating that the fluorescence emission of Ce^{3+} is not completely quenched by Tb^{3+} ions. Furthermore, the luminescence quantum yield of the LaF_3:45%Ce, 10% Tb NCs was determined by using the method reported previously [15]. The quantum yield of the NCs in aqueous solution was found to be about 15%. As the LaF_3:Ce,Tb NCs are directly prepared in water, their surface may be covered by a large number of O-H groups, which usually cause non-radiative transition, resulting in the quenching of the fluorescence [15]. To improve the fluorescence intensity, passivating shells (such as LaF_3 and SiO_2) have usually been used to cover onto such fluoride NCs [17]. Consequently, the synthesis of the core/shell structures can protect the surface luminescent centers from water, resulting in higher luminescence quantum yield.

Fig.4 shows the cell biocompatibility of the LaF_3:Ce,Tb NCs. Three kinds of cell lines, including human hepatocellular carcinoma cell line HCCLM6, human cervical cancer cell line HeLa and human breast cancer cell line MCF-7, were used to evaluate the cell biocompatibility of the NCs. These cell lines were from different tissue sources. All the cells were grown for 48 h with the medium containing LaF_3:Ce,Tb NCs with concentration ranging from 20 to 100 $\mu g \cdot mL^{-1}$. The cell viability was measured using a MTT assay. The results show that the viability of the cells grown with the LaF_3:Ce,Tb NCs are similar to that of the control within the concentration range, suggesting that the NCs are biocompatible to these cells.

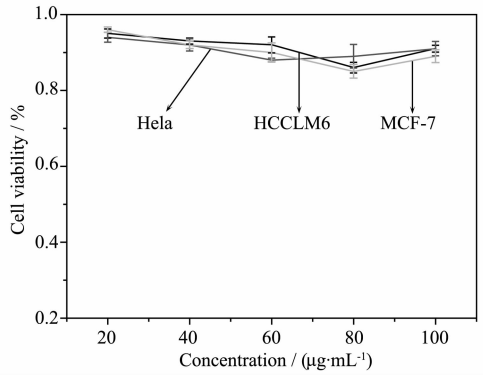

Fig.4 Viability of cells(including human hepatocellular carcinoma cell line HCCLM6, human cervical cancer cell line HeLa and human breast cancer cell line MCF-7) after being grown with different concentrations of the LaF_3:Ce,Tb NCs for 48 h

Fig.5 shows the bright fluorescence of the LaF_3:Ce, Tb NCs in the cytoplasm of the hepatoma cells. No conspicuous cell death is observed. These results further indicate that the NCs are not cytotoxic to the cells. The fluorescence of CdSe/ZnS QDs in hepatoma cells has been reported by our group[27,28]. Here the LaF_3:Ce,Tb NCs inside the living hepatoma cells through endocytosis also show bright fluorescence. The results indicate that the NCs can be alternative materials for some biomedical applications, such as biolabelling, contrast agents and tissue imaging. It should be noted that only little uptake of the NCs to the hepatoma cells is observed. The poor passive targeting ability of the

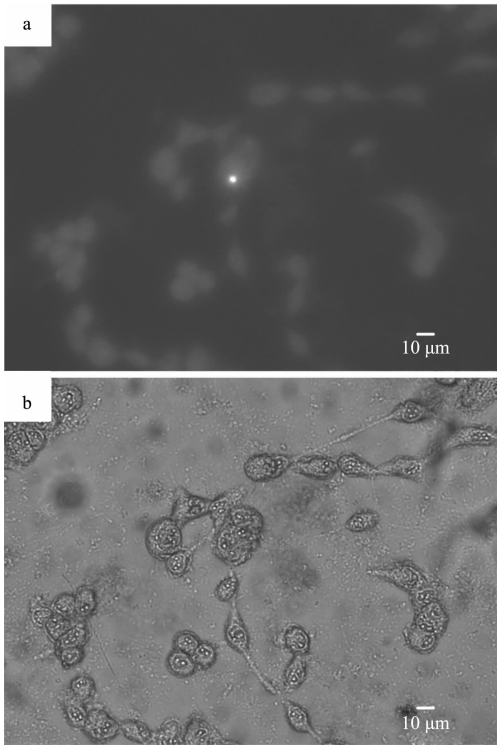

Fig.5 (a) Fluorescent image of LaF_3:Ce,Tb NCs in the cytoplasm of the living hepatoma cells with excitation wavelength of 250 nm; (b) The same samples were observed by using the bright-field mode of the fluorescence microscope.

NCs to the hepatoma cells shows the possibility of further active hepatic tumor targeting measurements.

3 Conclusions

In conclusion, a simple method was developed to prepare LaF_3:Ce,Tb NCs. The NCs have an average size of 14 nm and could be well dispersed in water. The NCs were demonstrated to be biocompatible to three kinds of cancer cells. The strong fluorescence at 543 nm of the NCs was observed both in aqueous solution and in living hepatoma cells.

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