PREPARATION OF WATER-SOLUBLE SiO_2 COATING $Sr_2MgSi_2O_7:Eu^{2+}$, Dy^{3+} FOR CELLS IMAGE

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In the present study, afterglow materials of SiO_2 coating $Sr_2MgSi_2O_7$: Eu^{2+}, Dy^{3+} (SiO_2-PLNPs) of good water solubility were prepared by hydro-thermal co-precipitation method. Effects of SiO_2 coating on the luminescence property, afterglow performance and water solubility of SiO_2-PLNPs have been discussed by contrast with $Sr_2MgSi_2O_7$: Eu^{2+}, Dy^{3+} (PLNPs) phosphor. The phase structure examined by X-ray diffraction (XRD) and both them was tetragonal $Sr_2MgSi_2O_7$. From the excitation spectrum, they two can be excited by light with a wavelength of 275 nm and 355 nm. Subsequently emission peak was recorded at 470 nm. The afterglow decay curves of the PLNPs and SiO_2-PLNPs showed that optical signal can be detected with a persistent time of 2 h. Micro structure of PLNPs and SiO_2-PLNPs were characterized by scanning electron microscope (SEM) and transmission electron microscopy (TEM) which demonstrated the success for the surface coating. Suspension property test suggested SiO_2-PLNPs have a good water solubility compared with PLNPs. The death rate of cells was less than 10 % when SiO_2-PLNPs solution of different concentration treat mouse osteoblasts. Bright blue light spots were visible to the eye in the dark field when the above cells excited by a 470 nm excitation light.

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Keywords: $Sr_2MgSi_2O_7:Eu^{2+},Dy^{3+}$, SiO_2, Afterglow materials, Coating

1. Introduction

Long afterglow phosphors have been widely studied since the late 1990 s due to its good properties of persistent luminescence when removed the light source[1]. Silicate-based phosphors have good chemical stability and thermal stability[2-4]. The most influential silicate sustainable luminescent material was first put forward by Lin et al[5] in 2001. In recent years, a variety of long afterglow materials with silicate and their matrix have been developed concretely. Especially, $Sr_2MgSi_2O_7$: Eu^{2+}, Dy^{3+} possesses stable chemical properties, good water resistance and excellent optical properties. Various technology have been used to prepared $Sr_2MgSi_2O_7$: Eu^{2+}, Dy^{3+} Phosphor for luminescence property such as by sol-hydrothermal synthesis[6], solid state reaction[7],

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co-precipitation method[8] and so on. These synthetic methods are all subjected to high temperature processes, which would led to the big particles and agglomeration.

When the nanoparticles are targeted to cell, the light from afterglow nanoparticles can be used to biomarker[9]. So many techniques were used to synthesize water soluble afterglow nanoparticles. Particularly, surface modification played a increasing important role in this aspect. Zhang[10] et al. using a reverse microemulsion-mediated sol-gel method prepared Magnetic silica with core-shell structure, which was well dissolved and monodisperse in water. ActivationHoma Homayoni[11] et al. synthesized $Sr_2MgSi_2O_7$: Eu^{2+}, Dy^{3+} particles by coating with APTES, then conjugating with PpIX, and finally, coating withfolic acid to improve their water solubility. Leila HosseinRashidi[12] et al. used $Sr_3MgSi_2O_8$: Eu^{2+}, Dy^{3+} afterglow NPs to conjugate with variety of biomolecules, to qualitatively investigate cancer cells targeting.

In this study, we report the preparation of PLNPs and SiO_2-PLNPs by a new Hydro-thermal co-precipitation method. Structural characterizations were discussed on the basis of XRD, SEM and TEM analysis. luminescence properties of the obtained PLNPs and SiO_2-PLNPs have been investigated by fluorescence spectroscopy and afterglow attenuation curves. According to suspension property test, cytotoxicity studies and cell image, it was found that SiO_2-PLNPs has a significant promise for cells image.

2. Experimental

2.1. Preparation of $Sr_2MgSi_2O_7$: Eu^{2+}, Dy^{3+}

The starting raw materials, $Sr(NO_3)_3$, $Mg(NO_3)_2$, $6H_2O$, EuO, Dy_2O_3, H_3BO_3, CTAB, EDTA and TEOS, all of analytical purity, were employed in this experiment. Firstly, the raw powders were weighed according to the nominal compositions of $Sr_2MgSi_2O_7$: Eu^{2+}(0.5 mol%), Dy^{3+}(1 mol%). $Sr(NO_3)_3$, $Mg(NO_3)_2$, EuO, Dy_2O_3 in nitric acid solution, H_3BO_3(5 mol%) in deionised water and TEOS（3.6 ml） in absolute ethanol were mixed in a beaker followed by addition of 0.004mol CTAB and EDTA. The molar ratio of the addition amount of EDTA to the metal ion in the mixed solution is 1:1. Then the solution's pH at 7 was prepared by slowly adding ammonia. After that the reagent solution was transferred into the Teflon-lined autoclaves, and was heated to 110℃ for 24 h. After cooling and filtration, the sample was dried at 65℃for 15 h to obtain the PLNPs precursor. Then heated to 550 ℃for 1h and continued heating to 900 ℃ for 2 h. PLNPs were obtained.

2.2. Preparation of SiO_2-$Sr_2MgSi_2O_7$: Eu^{2+}, Dy^{3+}

Above $Sr_2MgSi_2O_7$: Eu^{2+}, Dy^{3+} phosphors was stirred using mortar and pestle followed by addition of small amount of 5 mol/L NaOH solution. The powers in NaOH solution disperse evenly by ultrasonic cleaner. Then the solution was milled for 12 h. After separation, the powers in PVP solution disperse evenly by ultrasonic cleaner. The solution was centrifuged and then the precipitate scattered in mixed solution of 25 ml absolute ethanol, 5ml ultra-pure water and 2 ml ammonia. 200 uL Ethyl orthosilicate was added in the above solution at 0 ℃. This solution kept stirring for 12 h and then was centrifuged. At last the precipitation was washed using absolute ethanol for several times. SiO_2-PLNPs were obtained after drying.

2.3. Characterization of the samples of PLNPs and SiO_2-PLNPs

Powder X-ray diffraction (XRD) patterns were recorded with a scanning rate of 3◦min−1and 2ɵ value ranging from 10 to70◦ (D/MAX2500PC, Japan). The morphology and the crystal structure of the nano-composites were characterized by SEM (S-4800, Japan) and TEM (JEM-2010, Japan) with an accelerating voltage of 20Kv, 200 kV respectively. The Photoluminescence (PL) spectra of the products were measured using a Fluorescence spectrophotometer (F-700FL, Japan).

2.4. Cytotoxicity testing

Cells survival rate was test by MTT method which is a technique to detect living cells. Mouse osteoblasts were cultured in cell culture medium which containing 10% fetal bovine serum. Cells were cultured according to a density of 4×10^4 per pore (24 pore plate) in a 5 % CO_2 atmosphere at 37 °C for 24 h. Then SiO_2-PLNPs solution of different concentration (50 ~ 1000 μg mL-1) took place above cell culture fluid and then cultured mouse osteoblasts for another 24h. After that 10 μL of 3 mg • mL-1 MTT solution was added in cell culture medium and incubate for another 4 h under the same conditions. Carefully remove the liquid in the wells. Each well was added 150 μL dimethyl sulfoxide per pore. After shaking 10 minutes, wavelength absorbance was measured with a microplate reader at 570 nm.

2.5. Cell imaging

After culturing mouse osteoblasts for 24 h according to the above toxicity test conditions, the cell culture medium was removed and the medium containing SiO_2-PLNPs was added and cultured for 24h. Then these cells cleaned by 10 mM PBS (pH=7.4) for 3 times. Finally, the cells were imaged with an inverted fluorescence microscope equipped with a light source of Retiga 2000 cooled CCD and an X-cite series. The eyepiece magnification is WHN10, the objective is UPLFLN10 × / 0.30 and the excitation filter is BP330-385.

3. Results and discussion

The phase structure of PLNPs and SiO_2-PLNPs were analyzed by the powder X-ray diffraction (XRD) technique. The data were seen from the digital photographs in Fig. 1. It can be obtained that the diffraction peaks of PLNPs and SiO_2-PLNPs samples were in accordance with the JCPDS standard data file (PDF 75-1736). The phase structure of PLNPs and SiO_2-PLNPs was both $Sr_2MgSi_2O_7$. The diffraction peaks of SiO_2 coating as a organic compound weren't found. Because the process of surface modification didn't experience high temperature and the content of SiO_2 was too little. This implies that the phase structure of PLNPs haven't been changed after the surface modification with SiO_2 coating.

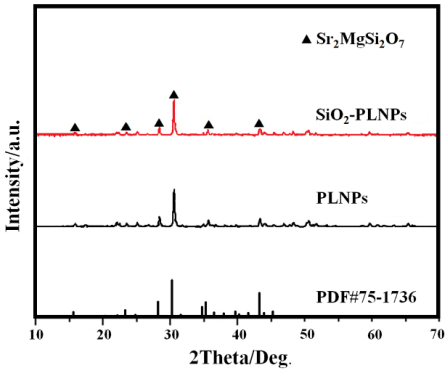

Fig. 1. The XRD patterns of PLNPs and SiO_2-PLNPs.

In order to confirm SiO_2 coated in the surface of PLNPs successfully, SEM and TEM patterns of PLNPs in Fig. 2(a, b) and SiO_2-PLNPs in Fig. 2(c, d) were displayed. The image (Fig.1a) showed the surface of PLNPs was rough and the existence of conglutination could be found at the edge of the grain.

From the image (Fig. 1b), it was easy to be observed the agglomeration of the powders. As seen from Fig. 2a, the particle of the SiO_2-PLNPs sample was ranging from 50nm to 200nm. SEM image (Fig. 2a) shows SiO_2-PLNPs sample with spherical shape. The smooth particles disperse evenly during the formation of SiO_2-PLNPs. As is clearly seen in Fig. 2d, SiO_2 coating was deposited on PLNPs surfaces, and SiO_2-PLNPs particles are produced. SiO_2 coating can improve the dispersivity of the samples and prevent the reunite of the particles in the process of growth.Due to the coating of SiO_2, PLNPs did not agglomerate during the growth process.This causes PLNPs to be slightly larger than SiO_2-PLNPs.

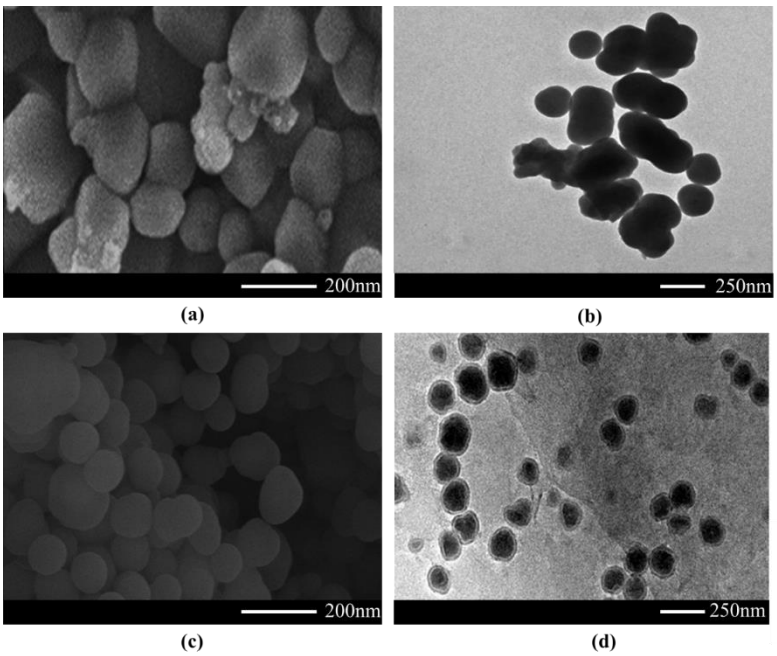

Fig. 2. (a) SEM images of PLNPs (b) TEM images of PLNPs (c) SEM images of SiO_2-PLNPs, (d) TEM images of SiO_2-PLNPs.

The excitation and emission spectra of PLNPs and SiO_2-PLNPs were used to examine the photoluminescent performance in Fig. 3. Both the spectra were found to be very similar in peak location as well as shape. The excitation spectra consist of broad bands from 250 nm to 450 nm, which peaked at 275 nm and 355 nm. The emission spectra were recorded in the range of 400 to 600 nm, which peaked at 470 nm.

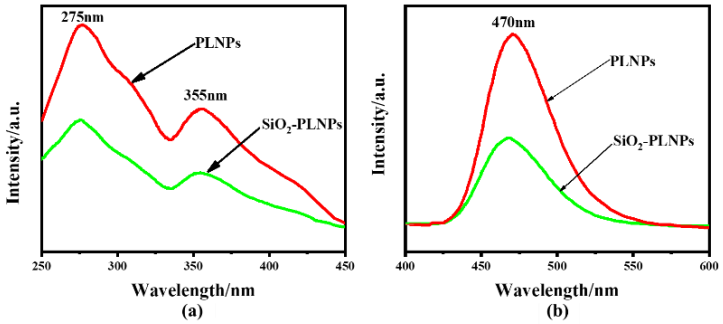

Fig. 3. The excitation spectra (a) and the emission spectra (b) of the PLNPs and SiO_2-PLNPs.

There was only one emission peak on the curve, and Dy^{3+} emission and excitation peak are not present in patterns[13], indicating that only a luminescence center formed by Eu^{2+} exists in $Sr_2MgSi_2O_7$ host lattice, and that Eu^{2+} ions occupy only one kind of site in the host lattice[14-15]. The peak intensity of PLNPs is better than SiO_2-PLNPs. Because some of the larger particles with good crystallinity were removed after centrifugation in possess of surface modification and the SiO_2 of the surface would lower the intensity of fluorescence spectrum.

The luminescence decay of SiO_2-PLNPs and PLNPs after 5 min exposured with UV irradiation was shown in Fig. 4. Both displayed a rapid decay and then a long-lasting phosphorescence. The decay curves can be well fitted by a double exponential function: $I=I_1\exp(-t/\tau_1) +I_2\exp(-t/\tau_2)$. SiO_2-PLNPs and PLNPs have different decay times. Curve (in Fig. 4) shows that the SiO_2-PLNPs, compared with PLNPs, has a poor afterglow property. This result is consistent with the data of the excitation spectra and emission spectra. However, we can still detect the optical signal after 2 h. It demonstrated that the luminescence properties of afterglow materials have been weaken after SiO_2 coating. But the luminescent properties of SiO_2-PLNPs was enough to meet the demand of cell image.

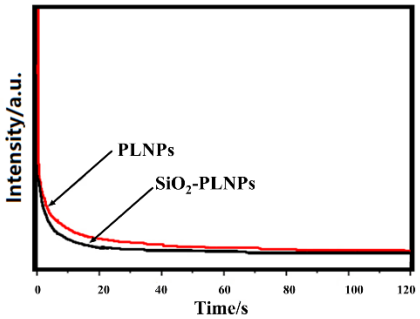

Fig. 4. Decay curves of the PLNPs and SiO_2-PLNPs.

Fig. 5a showed a comparison of the PLNPs solution and distilled water after ultrasound, and Fig. 5b showed a comparison of the above samples after standing for 10 minutes. As can be seen from the figure, after about 10min standing, PLNPs have been completely deposited in the bottom of the tube, the supernatant have no significantly difference with distilled water, which indicated that PLNPs materials have a poor water solubility. Fig.5c showed a comparison of the SiO_2-PLNPs solution and distilled water after ultrasound, and Fig. 5d showed a comparison of SiO_2-PLNPs solution and distilled water after standing for 80h. It can be seen that the suspension of SiO_2-PLNPs was stable even after standing for about 80 h. There was a big difference between SiO_2-PLNPs suspension and distilled water. This indicated that the coating of SiO_2 to PLNPs can improve its solubility. Because the coating of SiO_2 made the surface of PLNPs generated NH_2 groups which can prevent their aggregation.

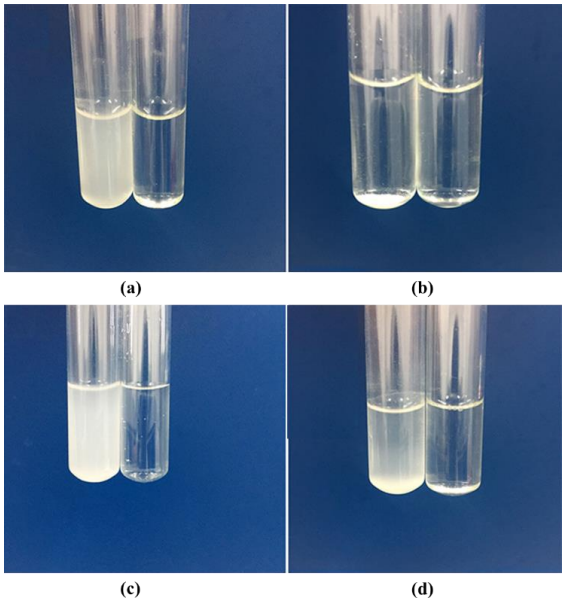

Fig. 5. Suspended photographs of the samples before and after surface modification with water:(a)-PLNPs Suspension photos,(b)-The photos of PLNPs suspension after standing, (c)-SiO_2-PLNPs Suspension photos, (d)-The photos of SiO_2-PLNPs suspension after standing.

MTT assay toxicology was carried out to demonstrate that SiO_2-PLNPs solution didn't harmful to cells. In Fig. 6, the cell viability is changing from 100%, 95%, 97%, 98%, 93%, 94%, 92% with the SiO_2-PLNPs concentration ranging from 0 mg/L, 50 mg/L, 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L, 500 mg/L. The cell viability is 96% which is the best condition at the concentration of 200 mg/L. In the worst situation, SiO_2-PLNPs just killed 8% of cells at a concentration of 300 mg/L. Overall, the cell viability reduced slightly with the increasing of SiO_2-PLNPs concentration. In fact, the MTT observations indicated that SiO_2-PLNPs have a little effect on cell viability. These results indicated that SiO_2-PLNPs are biocompatible.

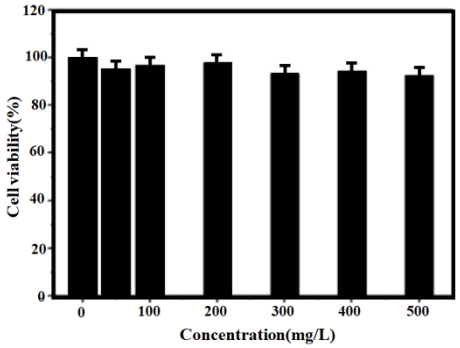

Fig. 6. Different concentrations of SiO_2-PLNPs solution for the treatment of mouse osteoblasts by MTT assay toxicology.

Images of cells in Fig. 7(a-d) were observed in different shading fields by fluorescence microscope. As is showed in Fig. 7a, mouse osteoblasts had a clear outline and grown very well in the bright field. The dark field image at the Fig. 7b shows that the cell itself does not emit light under the excitation of fluorescence microscope. As shown in Fig. 7c, SiO_2-PLNPs particles came into the cells and grown normally when SiO_2-PLNPs were added to the cell culture solution in the bright field. In the dark field(in Fig. 7d), bright blue light spots were visible clearly when the above cells(in Fig. 7c) excited by fluorescence microscope. The lighting time can persist for 2 h to support cell image.

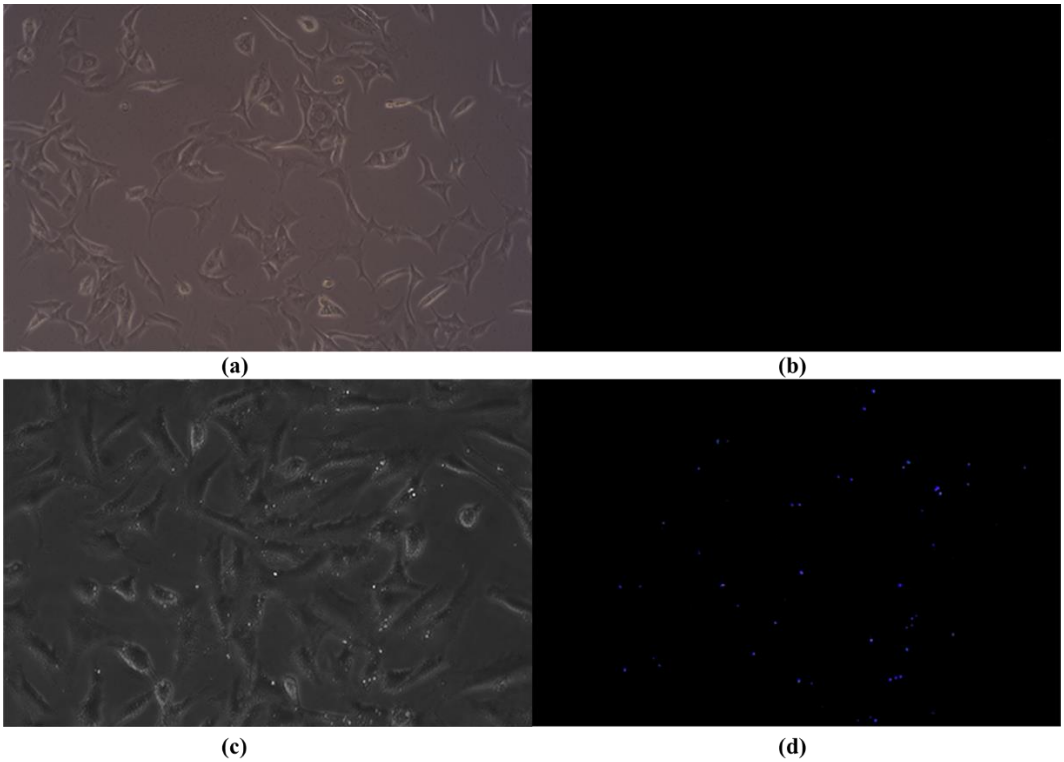

Fig. 7. Images of cells in different shading fields (a) Bright field cells (b) Dark field cells (c) Bright field co-cultured cells (d) Dark field co-cultured cells.

4. Conclusions

In this work, SiO_2-PLNPs particles were successfully achieved using a hydro-thermal co-precipitation method and compared with PLNPs phosphors on crystal structures and optical property and water solubility. The obtain samples can emit blue light with peak at 470 nm. The as-synthesized SiO_2-PLNPs composite displayed good water solubility by suspension property test. Totally, SiO_2-PLNPs have a little effect on cell activity. At the same, bright blue light spots could be visible clearly in cell image. Thus, SiO_2-PLNPs as a list of inorganic substance particles are a new promising material in the aspect of biological area.

Acknowledgements

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References

[1] I. P. Sahu, D. P. Bisen, N. Brahme, L. Wanjari, R. Tamrakar, Physics Procedia **76**, 80 (2015).
[2] X. U. Chao, L. U. Dianqing, Y. L. Xiong, Transactions of Materials & Heat Treatment **34**, 15 (2013).
[3] H. Jiang, B. Xu, Journal of the Chinese Ceramic Society **34**, 1154 (2006).
[4] Mahmood, H. M., Abdullah, M. A., Ramizy, A., Mohammed, A. S, Digest Journal of Nanomaterials and Biostructures. **15**, 2 (2001).
[5] Y. Lin, Z. Tang, Z. Zhang, X. Wang, J. Zhang, Journal of Materials Science Letters **20**, 1505 (2001).
[6] X. Wei,Y. Shen, G. Zuo, L. Hou, Y. Meng, F. Li, Ceramics International **41**, 13872 (2015).
[7] T. Aitasalo, J. Hassinen, J. Hölsä, T. Laamanen, M. Lastusaari, M. Malkanmki, J. Niittykoski, Journal of Rare Earths **27**, 529 (2009).
[8] W. Pan, G. Ning, X. Zhang, J. Wang, Y. Lin, J. Ye, Journal of Luminescence **128**, 1975 (2008).
[9] W. Chen, J. Biomed. Nanotechnol. **4**, 369 (2008).
[10] Y. Zhang, B. Zhen, H. Li, Y. Feng, Chinese Journal of Chemical Engineering, 2017.
[11] H. Homayoni, L. Ma, J. Zhang, S.K. Sahi, L.H. Rashidi, B. Bui, W. Chen, Photodiagnosis Photodyn Ther. **16**, 90 (2016).
[12] L. H. Rashidi, H. Homayoni, X. Zou, L. Liu, W. Chen, Photodiagnosis & Photodynamic Therapy **13**, 244 (2016).
[13] Y. Peng, X. Cao, C. Lei, L. Liu, Q. Li, S. Lian, Chin. J. Rare Earths Soc. **22**, 611 (2004).
[14] L. Jun, G. Qiang, Z. Kaiyan, G. Mingqiao, L. Jialin, Journal of Rare Earths **32**, 696 (2014).
[15] C. Shi, Y. Fu, B. Liu, G. Zhang, Y. Chen, Z. Qi, X. Luo, J. Lumin. **11**, 122 (2007).