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RESEARCH PAPER

Fabrication and Cell Imaging of Room Temperature Phosphorescent Silica Nanomaterials

ZHANG Man-Man[1], WANG Li-Qiang[1], XIAO Dan[2], ZHUANG Zhen-Jing[1,*]

[1] Institute of Molecular Medicine, Huaqiao University, Fujian 362021, China
[2] College of Chemistry, Sichuan University, Chengdu 610065, China

Abstract: **A** room temperature phosphorescent (RTP) silica nanomaterial was prepared using sol-gel method and characterized by TEM, XRD and photoluminescence spectra. The results demonstrate that the obtained sample was silica with a diameter of about 50 nm. The maximum fluorescence excitation and emission wavelengths of the silica nanomaterical were 280 and 335 nm, and the maximum phosphorescence excitation and emission wavelengths of the silica nanomatericals were 280 and 440 nm. The sample possessed the room temperature phosphorescence that was stable against environmental changes. The sample was stored in air at ambient conditions and its phosphorescence remained unchanged after 3 month demonstrated its long-term stability. Furthermore, the obtained RTP silica nanoparticle is noncytotoxic and can serve as bioprobes for cellular imaging.

Key Words: Silica; Sol-gel; Room temperature phosphorescence; Cytotoxicity; Cell imaging

1 Introduction

Silica has drawn much attention in preparing luminescent material as the doped substrate due to its low toxicity, good chemical stability, thermal stability and insulating properties[1–3]. Dye-doped silica nanoparticles or silica coated quantum dots, for instance, were widely used in cell imaging[4–8]. However, most of these methods were time-consuming, expensive, and commonly associated with issues like potential toxicity and dye-leaking. Green *et al*. reported highly emissive broad phosphors which were synthesized from a tetraalkoxysilane sol-gel precursor and a variety of organic carboxylic acids[9]. Recently, Zhao *et al* also reported the preparation of a room temperature phosphorescent mesoporous silica nanomaterial and attribute its phosphorescence to the carbon-based impurities in the silica structure[10]. In this study, a room temperature phosphorescent (RTP) silica nanoparticle was prepared by sol-gel method under the optimized conditions. The photoluminescent property and cytotoxicity of the obtained samples were also investigated. The results demonstrated that the obtained nanoparticles were biocompatibility and stable against environmental changes. Furthermore, the as-prepared silica nanoparticles can be tracked in vitro, suggesting the great potential of this technology in biological imaging.

2 Experimental

2.1 Instruments and reagents

Tetraethyl orthosilicate and sodium citrate were purchased from Aladdin Industrial Corporation (China). Isopropanol and dimethyl sulfoxide (DMSO) were purchased from Sinopharm Chemical Reagent Co., Ltd (China). Thiazolyl blue tetrazolium bromide (MTT, M5655) was purchased from Sigma-Aldrich Inc (USA). Roswell Park Memorial Institute 1640 medium and Fetal Bovine Serum (FBS) were purchased from Life Technology (USA). NCI-H446 cells were obtained from American Type Culture Collection (ATCC, USA). LysoTracker Red was purchased from Invitrogen (Karlsruhe,

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* Corresponding author. Email: zhuangzhenjing@hqu.edu.cn
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Germany). All other reagents were purchased from Aladdin Industrial Corporation (China). All chemicals were used without additional purification. Redistilled water was used for the fabrication of silica nanoparticles.

Optical experiments were performed at room temperature under ambient air conditions. Photoluminescence measurements were performed using a fluorescence spectrophotometer (Hitachi F-7000, Japan). The TEM and EDX were recorded using electron microscope (JEOL JEL-2010F, Japan) at 200 kV. The powder X-ray diffraction (XRD) spectra were recorded on an X-ray diffractometer (PANalytical X' Pert Pro). The formazan concentration was finally quantified using a spectrophotometer (Infinite M200, Tecan, Switzerland). Laser-scanning confocal fluorescent (Leica, TCS-SP5, Germany) was used for fluorescent cellular imaging.

2.2 Synthesis of non-metal-doped RTP silica nanoparticles

Approximately 5 mL of sodium citrate solution of certain concentration was dissolved in 25 mL of isopropanol, and the mixed solution was stirred continuously for 1 h. Then 25 mL of TEOS was added to the solution under magnetic stirring. After stirring for 2 h, 1.0 mL of ammonia was added drop by drop. The solution was stirred continuously for another 12 h, and then the product was allowed to age for 12 h at room temperature. Finally, calcinations was performed in a muffle furnace (SX2-4-13, Shanghai Yuejin Medical Equipment Co., Ltd., China), and the RTP silica nanoparticles were obtained.

The obtained samples were ground into powder before use.

2.3 Cell viability

The cytotoxicity of the RTP SiO_2 nanoparticles was evaluated by the MTT assay. NCI-H446 cells were dispersed in 96-well plates (90 μL in each well containing 1×10^4 cells per well). Approximately 10 μL of the obtained silica sample dispersed in DMSO with different concentrations was added to 96-well plates containing NCI-H446 cells (final concentrations of the samples were 100, 50, 10, 1, 0.1 μg mL^{-1} respectively, six replicates per concentration). The incubation was carried out for 24 and 48 h in a humidified atmosphere at 37 °C with 5% CO_2. Subsequently, 20 μL of MTT (5 mg mL^{-1}) was added into each well and the cells were incubated for a further 4 h. Then the medium in each well was removed carefully and replaced with 150 μL DMSO. The 96-well plates were shaken for 10 min to dissolve the formazan crystals completely. The formazan concentration was finally quantified using a spectrophotometer by measuring the absorbance at 490 nm with background correction at 630 nm. The cell viability was defined by Cell viability (CV). CV is the percentage of the average absorbance of experimental group and the average absorbance of blank control group. The experiments were repeated three times.

2.4 Cells imaging analysis

NCI-H446 cells (1×10^5) were cultured (37 °C, 5% CO_2) in glass bottom cell culture dishes from NEST (diameter: 20 mm) for 14 h. Then the above silica sample dispersed in DMSO was added (the final concentrations of the samples were10 μg mL^{-1}) and the incubation continued for another 24 h. NCI-H446 cells were then cultured with 2 mL fresh cell culture medium and 1 μL LysoTracker Red (final concentration is 50 nM) at 37 °C for another 30 min. After that, the staining working solution was removed, and 2 mL of fresh DMEM culture medium was added and finally observed under confocal laser scanning microscopy. The maximum excitation wavelength and emission wavelength of LysoTracker Red were 577 and 590 nm, respectively.

3 Results and discussion

3.1 Synthesis of RTP silica nanoparticles

Green *et al*[9] synthesized highly emissive broad phosphors from a tetraalkoxysilane sol-gel precursor and a variety of organic carboxylic acids and discussed the effect of these organic acids and silica precursors on the luminescent properties of the obtained phosphors. Here, RTP silica nanoparticles were fabricated via a sol-gel method. The influence of the trisodium citrate concentration and calcination temperatures on the obtained RTP silica nanoparticles was investigated. As shown in Fig.1A, it was found that as the concentration of sodium citrate increased, the luminescence intensity of the RTP silica nanoparticles increased first and then decreased, and reached its maximum as the concentration of sodium citrate was 0.035 M. Figure 1B demonstrates that the sample displays the strongest luminescence when the calcination is performed at 650 °C for 1 h. Therefore, the sodium citrate concentration of 0.035 M and the calcination performed at 650 °C for 1 h were selected for the preparation of RTP silica nanoparticles.

3.2 Structure and composition characterization of RTP silica nanoparticles

The morphological and composition of the obtained materials were carried out by TEM, EDX, and XRD. The TEM image (Fig.2A) showed that the RTP silica nanoparticles had an average dimension of about 50 nm. EDX analysis illustrates that the samples were composed of Si, C and O elements (Fig.2B). The XRD of the phosphors (Fig.2C) showed only one diffraction peak of non-crystalline silica was observed, further demonstrating that the RTP silica nanoparticles were silica.