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LaB_6 nanoparticles with carbon-doped silica coating for fluorescence imaging and near-IR photothermal therapy of cancer cells

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ABSTRACT

In this study, LaB_6 nanoparticles are used as a novel nanomaterial for near-infrared (NIR) photothermal therapy because they are cheaper than nanostructured gold, are easy to prepare and have an excellent NIR photothermal conversion property. Furthermore, the surface of LaB_6 nanoparticles is coated with a carbon-doped silica (C-SiO_2) shell to introduce a fluorescent property and improve stability and biocompatibility. The resulting LaB_6@C-SiO_2 nanoparticles retain the excellent NIR photothermal conversion property and exhibit a bright blue emission under UV irradiation or a green emission under visible irradiation. Using a HeLa cancer cell line, it is demonstrated that LaB_6@C-SiO_2 nanoparticles have no significant cytotoxicity, but their presence leads to remarkable cell death after NIR irradiation. In addition, from the observation of cellular uptake, the fluorescence labeling function of LaB_6@SiO_2 (LaB_6 core/SiO_2 shell) nanoparticles is also confirmed. These results suggest that LaB_6@C-SiO_2 nanoparticles may potentially serve as an efficient multifunctional nano-platform for simultaneous fluorescent imaging and NIR-triggered photothermal therapy of cancer cells.

1. Introduction

Photothermal therapy is an attractive therapy technique using photosensitizers to generate heat from light absorption and then kill cancer cells [1,2]. To avoid the non-specific heating of healthy cells, near-infrared (NIR) light is usually used, because the tissues are transparent in this window, and deep penetration can be achieved [3]. Furthermore, plasmonic nanomaterials are usually used as photosensitizers. They give this technique spatial selectivity.

Plasmonic nanomaterials with high optical absorption in the NIR region currently under investigation for photothermal therapy include gold nanorods [4–6], gold nanoshells [7,8], gold nanocages [9], single-walled [10–12] or multi-walled [13] carbon nanotubes, graphene or reduced graphene oxide [14] and germanium [15]. Among them, gold-based nanomaterials have received most attention. It is worth noting that lanthanum hexaboride (LaB_6) is also a metal-like plasmonic material. After grinding to nanoscale, it can exhibit strong NIR absorption via surface plasmon resonance [16–19]. Recently, it was demonstrated that LaB_6 nanoparticles processed an excellent NIR photothermal conversion property comparable with and even slightly superior to gold-based nanomaterials [20]. Because of the relatively low price, LaB_6 nanoparticles might be used as an alternative to gold-based nanomaterials for NIR photothermal therapy.

Recently, quantum dots have been used in biomedical imaging and diagnostics, owing to their high fluorescence quantum yields. Unfortunately, most of the highly luminescent quantum dots currently used for biomedical imaging are composed of toxic elements (Cd, Se, Te) [21–23]. Silica has been widely used for the surface coating of nanoparticles to improve their stability and biocompatibility. Also, its surface can be further modified with amines, thiols or carboxyl groups to bind the biological molecules covalently [24–27]. However, silica, which is usually synthesized by the condensation of tetraethyloxysilane (TEOS) via the Stöber process, is not luminescent [28]. So, in recent years, considerable effort has been made to dope it with fluorophores or coat it with luminescent layers to develop photoluminescent silica [29–33]. Note that photoluminescent silica also can be prepared by the addition of alkoxysilanes and/or carboxylic acids via the sol–gel route and heat treatment [34]. The photoluminescence from this type of silica was attributed to some defect-related mechanisms such as carbon impurities, photo-induced donor–acceptor pairs of nitrogen-related species and/or oxygen-related defects [35–38].

In this study, carbon-doped silica (C-SiO_2) was coated on the surface of LaB_6 nanoparticles via the modified Stöber method, followed by heat treatment using TEOS as the precursor and 3-aminopropyltriethoxysilane (APTES) as the carbon source. The coating of C-SiO_2 not only improved the dispersion and stability of LaB_6 nanoparticles in aqueous solution, but also introduced

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the photoluminescent property. Thus, the resulting LaB_6@C-SiO_2 nanoparticles could be used as a novel multifunctional nano-platform for simultaneous fluorescent imaging and NIR-triggered photothermal therapy of cancer cells, as illustrated in Fig. 1.

2. Experimental and methods

2.1. Preparation of LaB_6@C-SiO_2 nanoparticles

LaB_6 nanoparticles of ~100 nm were prepared in an ethylene glycol solution containing dodecylbenzenesulfonic acid by a stirred bead milling process at an agitation speed of 2500 rpm (peripheral speed 10 m s^{-1}) and a grinding time of 6 h, according to previous work [20]. C-SiO_2 nanoparticles were synthesized via the modified Stöber method, with TEOS and APTES as the precursor and carbon source, respectively [28]. Typically, TEOS and APTES (total concentration 0.09 M; molar ratio of APTES/TEOS = 0%, 3% and 6%) were added to the mixture of 2-propanol (20 ml) and ammonia solution (29%, 0.5 ml). After stirring at 30 °C for 24 h, the resulting silica colloids were collected centrifugally, washed three times with ethanol, and then dried in vacuum. Finally, the white dried samples were annealed in air at 250, 300 or 350 °C for 2 h with a heating rate of 2 °C min^{-1} to yield the yellowish C-SiO_2 nanoparticles. LaB_6@C-SiO_2 nanoparticles were prepared according to the above procedures except that LaB_6 nanoparticles (5 mg) were dispersed in 2-propanol (20 ml), and the reaction was conducted by sonication for 3 h and stirring at 30 °C for another 21 h. Also, the molar ratio of APTES/TEOS and the annealing temperature were fixed at 6% and 350 °C, respectively. When the molar ratio of APTES/TEOS was 0, undoped SiO_2 and LaB_6@SiO_2 nanoparticles could be obtained for comparison.

2.2. Characterization

The transmission electron microscopy (TEM) analysis and energy dispersive X-ray (EDX) line scan of LaB_6@C-SiO_2 nanoparticles were carried out using a JEOL JEM-2100F at 200 kV. The mean hydrodynamic diameter and zeta potentials were measured on a Malvern Zetasizer Nano Z. The pH dependences of zeta potential for LaB_6@C-SiO_2 and LaB_6@SiO_2 nanoparticles (200 mg l^{-1}) were measured in a 0.1 M NaCl solution at pH 2–9, adjusted by NaOH and HCl. Fourier transform IR (FTIR) spectra were recorded on a Varian FTS-1000 FTIR spectrometer. The absorption and photoluminescence spectra were measured using a JASCO model V-570 UV–visible NIR spectrophotometer and a Hitachi F-4500 fluorescence spectrophotometer, respectively. The NIR photothermal conversion property of LaB_6@C-SiO_2 nanoparticles was investigated by placing 100 μl of phosphate buffered saline (PBS) containing LaB_6@C-SiO_2 nanoparticles (250 μg ml^{-1}) in a 96-well plate. The solution was irradiated by a CW 808 nm diode laser (Power Technology, USA) at a power density of 2.47 W cm^{-2} for 10 min. Its temperature was detected with a NIR thermal camera.

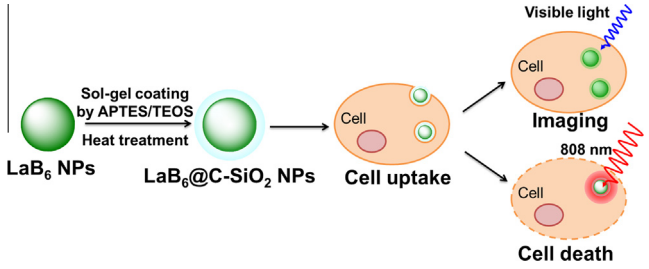

Fig. 1. Fabrication of LaB_6@C-SiO_2 nanoparticles for fluorescence imaging and NIR photothermal therapy of cancer cells.

2.3. Cytotoxicity

For the study on the in vitro cytotoxicity of LaB_6@C-SiO_2 nanoparticles, WST-8 cell viability assay was performed by measuring the mitochondrial dehydrogenase activity, using a human cervix carcinoma HeLa cancer cell line. HeLa cells were cultured in Minimal Essential Medium (MEM) with 10% fetal bovine serum (FBS) under a 5% CO_2 atmosphere. First, 100 μl of cell suspension was seeded into each well of a 96-well culture plate at a density of 2×10^3 cells per well and incubated at 37 °C under a 5% CO_2 atmosphere for 24 h. Then, the medium in each culture well was replaced by 100 μl of the fresh medium containing LaB_6@C-SiO_2 nanoparticles at various concentrations (0–250 μg ml^{-1}), and the cells were further incubated at 37 °C under a 5% CO_2 atmosphere for an additional 72 h. Next, the medium was replaced with 100 μl of the fresh medium containing 10-times diluted WST-8, and the cells were incubated at 37 °C for another 2 h. Finally, cell viability was analyzed by measuring the absorbance of the colored medium (450/690 nm) using an ELISA plate reader (Infinite 200 series, TECAN).

2.4. Photothermal therapy

For the quantitative evaluation of NIR photothermal therapy capability, HeLa cells in the MEM medium with 10% FBS were seeded in a 96-well culture plate at a density of 5×10^3 cells per well and were incubated at 37 °C under 5% CO_2 atmosphere for 24 h. Then, the medium in each culture well was replaced by 100 μl of the fresh medium containing LaB_6@C-SiO_2 nanoparticles (250 μg ml^{-1}). After incubation at 37 °C under a 5% CO_2 atmosphere for another 24 h, NIR irradiation (808 nm, 2.47 W cm^{-2}) was applied to each well for 0–12 min. Next, the cells were washed in PBS to remove the nanoparticles non-specifically adsorbed or remaining in the medium. Subsequently, the medium was replaced with 100 μl of the fresh medium containing 10-times diluted WST-8, and the cells were further incubated at 37 °C under a 5% CO_2 atmosphere for 1.5 h. Finally, cell viability was analyzed by measuring the absorbance of the colored medium (450/690 nm) using an ELISA plate reader. For the qualitative study, HeLa cells were incubated with LaB_6@C-SiO_2 nanoparticles (250 μg ml^{-1}) according to the above procedures, except that a 24-well culture plate was used, and the cell density was 5×10^4 cells per well. After being washed by PBS to remove nanoparticles, cell viability was characterized by a live/dead kit containing calcein AM and Ethidium homodimer-1 (EthD-1), where green fluorescence from calcein AM indicated live cells and red fluorescence from EthD-1 indicated dead cells. Briefly, 0.1 ml of PBS containing 2 μM calcein AM and 4 μM EthD-1 was added to each well and was incubated at 37 °C under 5% CO_2 atmosphere for 30 min. Live and dead cells were visualized by using an inverted fluorescence microscope (Olympus, IX71).

2.5. Cell uptake and fluorescence imaging

For the observation of in vitro cellular uptake of LaB_6@C-SiO_2 nanoparticles, the HeLa cells in the MEM medium with 10% FBS were plated onto rounded glasses, which were placed in the wells of a 24-well-microtitration plate at 37 °C under a 5% CO_2 atmosphere for 24 h (1×10^5 cells per well). Then, the medium in each culture well was replaced by 100 μl of the fresh medium containing LaB_6@C-SiO_2 nanoparticles (250 μg ml^{-1}), and the cells were incubated at 37 °C under a 5% CO_2 atmosphere for another 24 h. Finally, the cells were washed in PBS and then fixed with 4% formaldehyde in PBS, permeabilized with 0.5% Triton X-100 in PBS at 37 °C for 30 min, and blocked with 2% bovine serum albumin (BSA) in PBST (0.1% Tween-20 in PBS). For cytoplasm staining,

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