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Synthesis, characterization and biomedical application of multifunctional luminomagnetic core–shell nanoparticles

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ABSTRACT

It has been well-established that nanomaterials provide a robust framework into which two or more functional moieties can be integrated to offer multifunctional and synergetic applications. We report here the facile synthesis and systematical investigation of the luminomagnetic core–shell nanoparticles (NPs) with the magnetic Fe_3O_4 core coated with a silica shell incorporating fluorescent $[Ru(bpy)_3]^{2+}$. The luminomagnetic NPs were monodisperse and spherical in shape with a diameter of 60 ± 10 nm. The luminomagnetic NPs possessed not only the desirable optical signature of $Ru(bpy)_3^{2+}$ but also the distinctive magnetic profile of Fe_3O_4, where a strong red-orange emission and the super-paramagnetic characteristics with the saturation magnetization values ca. 10 emu/g were observed for the luminomagnetic NPs. As revealed by Alamar blue assay and flow cytometry analysis, the Fe_3O_4 NPs decrease the cell viability of HepG2 by ca. 10%, while an increase by ca. 10% on HepG2 cell proliferation was revealed after the silica shell was coated onto Fe_3O_4 NPs, suggesting that the silica shell serves as a protective layer to increase the biocompatibility of the luminomagnetic NPs. Confocal laser scanning microscopy, transition electron microscopy and magnetic resonance (MR) images confirmed that the luminomagnetic NPs can enter into the interiors of HepG2 cells without damage, highlighting their capabilities for simultaneous optical fluorescence imaging and T2 MR imaging. Taking advantage of versatility of silica shell towards different surface modification protocols, the luminomagnetic NPs were successfully functionalized with epidermal growth factor receptor (EGFR) antibody for HepG2 cell recognition. All the results illustrated that the luminomagnetic NPs should be a potential candidate for future cancer diagnosis and therapy.

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1. Introduction

Advances in nanotechnology have contributed to the development of multifunctional nanoparticles that enable the simultaneous imaging and therapy [1–14]. The ability to control the size, shape, and surface functionalities of gold nanoparticles (AuNPs) allows for both selective imaging and photothermal killing of cancer cells [6–8]. Through surface modification or combination with functional moieties, magnetic iron oxide NPs have shown a great promise as multimodality imaging probes for simultaneous target-specific diagnostics and therapeutics [9–14]. Biocompatible suspension of magnetic NPs in the presence of an alternating current (AC) magnetic field may be used to generate heat to kill cells by apoptosis. It has been well-established that magnetic hyperthermia can produce regional heating in the temperature range of 42–47 °C [15–17]. Considering the fact that tumor cells are hypoxic while normal cells are euphoric, tumor cells are, in general, more heat-sensitive when compared to normal cells in such temperature range [18]. To ensure stability and biocompatibility under physiological conditions, the surface coating of magnetic iron oxide nanomaterials should ideally be non-immunogenic and non-antigenic, prevent opsonization by plasma protein and exhibit high affinity towards the iron oxide core [13]. In addition to ligand exchange and cross-coupling reactions, silica shell coating strategy fulfills the above requirements and quickly becomes a popular choice of introducing a passivating layer on the surface of magnetic NPs due to the good biocompatibility of silica and its resistant to decomposition in vivo [19–25]. Another advantage of the silica surface is its versatility towards different surface modification protocols [26]. Organic fluorescent dyes [19–21], metal complexes [22] or quantum dots (QDs) [23,24] were incorporated into silica shell to form silica-coated core–shell NPs. However, organic dyes are susceptible to leaching and photobleaching problems, and QDs suffer

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from issues such as their potential toxicity, fluorescence intermittency and chemical instability [27,28]. Recently, $[Ru(bpy)_3]^{2+}$-encapsulated silica nanoparticles (SiNPs) were extensively applied in biodetection and biomedical diagnosis due to their high luminescence quantum yield and good stability [26,29–31]. In order to understand the interaction between nanomaterials and biological systems and to manipulate these interactions for the development of NPs with novel functions [32–36], we herein designed and synthesized the luminomagnetic core–shell NPs with the magnetic Fe_3O_4 core coated with a silica shell incorporating fluorescent $[Ru(bpy)_3]^{2+}$. The optical and magnetic properties of these multifunctional luminomagnetic NPs were systematically characterized, and their biocompatibility and their potential biomedical applications in cell imaging were also studied.

2. Material and methods

2.1. Synthesis of the luminomagnetic NPs

Firstly, the Fe_3O_4 nanoparticles were synthesized using a previously reported co-precipitation method with minor modification [37]. An aqueous solution of $FeCl_3$ (2 mL, 1 mol L^{-1}, Acros) and $FeCl_2$ (0.5 mL, 2 mol L^{-1}, Aldrich) in 2 mol L^{-1} HCl (Farco Chemical Supplies) was mixed. Then, the mixture was added into diluted NH_3 solution (25 mL, 0.7 mol L^{-1}, Ajax Chemical) under stirring for 30 min, followed by isolation of the black precipitate using magnetic decantation. The precipitate was stirred with diluted $HClO_4$ (25 mL, 2 mol L^{-1}, Beijing Chemical Works) and was then isolated by centrifugation. Then, the luminomagnetic SiNPs were prepared by modification of a water-in-oil reverse micelle method [31]. Typically, Fe_3O_4 nanoparticles were dissolved in D.I. water at the concentration of 2.0 mg mL^{-1}. Then, 7.7 mL cyclohexane, 2 mL Triton X-100, 1.6 mL hexanol, and 0.34 mL 2.0 mg mL^{-1} Fe_3O_4 nanoparticles were stirred for 30 min to generate a microemulsion system. Then, 80 μL 0.1 M $[Ru(bpy)_3]^{2+}$ and subsequent 40 μL tetraethoxy orthosilicate (TEOS) were added to the mixture, followed by the addition of 100 μL aqueous ammonia for the TEOS hydrolysis under stirring for 24 h. After that, ethanol was added to destabilize the micro-emulsion system. The luminomagnetic SiNPs were isolated via centrifugation and washed in sequence with ethanol and D.I. water to remove any surfactant and unreacted reactants.

2.2. Characterization of the luminomagnetic NPs

Morphology of the luminomagnetic NPs was characterized by a field emission scanning electron microscope (FE-SEM, Philips XL30 Esem-FEG) and a transmission electron microscope (TEM, Philips Tecnai 12 BioTWIN). Nickel grids were used for sample loading in the morphology characterization experiments by electron microscopes. Size distribution of the NPs was measured with a Malvern Zetasizer NanoZS instrument. UV–Vis and fluorescent spectra were obtained on an Agilent 8453 UV–Vis spectrometer and a Horiba Jobin Yvon FluoroMax-3 spectrometer, respectively. A vibrating sample magnetometer (VSM; Lake Shore Model 7300) was employed to characterize magnetic properties of the nanoparticles. The hysteresis of the magnetization was obtained by changing H between +10,000 and −10,000 Oe at 300 K. The heating rate of the luminomagnetic NPs was measured using the Hilgers' technique [17]. The frequency and amplitude of the magnetic field used were 63 kHz and 7 kA/m, respectively. The resultant temperature change of the 1.0 mL magnetic fluid that contained 50 mg luminomagnetic NPs dispersed in iodinated oil was monitored by an optical fiber thermometer probe.

2.3. Biomedical perspectives of the luminomagnetic NPs

HepG2 cells were cultured in an RMPI 1640 medium at 37 °C in a humidified 5% CO_2 atmosphere. The NP stock suspension (1.0 mg mL^{-1}) was prepared by dispersing NPs in sterile D.I. water under sonication for 30 min. The stock suspension was then diluted with an RMPI 1640 medium to prepare a series of NP suspensions with different concentrations for the cellular assays.

2.4. Alamar blue assay

Cell viability in the presence of NPs was evaluated using Alamar blue assay, as described in detail elsewhere [32]. HepG2 cells were seeded into a 96-well cell culture dish (6×10^4 cells per well) and incubated for 8 h at 37 °C. After that, cells were treated with NP suspensions (0.05, 0.10, 0.20 mg mL^{-1}) for different time intervals (24 h, 48 h and 72 h). Untreated cells were used as control and wells without cells were used as blank. After treatment, the medium containing NPs was removed, and phosphate buffered saline (PBS) was used to wash the cells once, followed by the addition of Alamar blue solution (2 mg mL^{-1} final concentration; 200 μL) and a subsequent 6 h further incubation. Each experiment was performed in triplicate. A microplate reader (excitation at 530 nm, emission at 584 nm) was used to record fluorescence intensity of each well. The relative cytotoxicity was expressed as a percentage of $[OD_{sample} - OD_{control}]/[OD_{control} - OD_{blank}]$.

2.5. SEM and TEM

The effects of the luminomagnetic NPs on cell morphology were observed by SEM, as described in detail elsewhere [35,36,51,52]. In the present study, HepG2 cells were treated with and without 0.05 mg mL^{-1} of the luminomagnetic NPs for 12 h, followed by being fixed in the primary fixative of 2% glutaraldehyde for 1 h and postfixed for 1 h at 4 °C with 1% osmium tetroxide. After dehydration, cells were sputter-coated with carbon, and subsequently imaged by a FEI Nova SEM system at accelerating voltage of 10 kV. And a Phillips Tecnai 12 instrument was employed to characterize the ultrastructural alterations and internalization of HepG2 cells, as described in detail elsewhere [35]. In the present study, HepG2 cells were treated with 0.05 mg mL^{-1} nanoparticles for 12 h, and the TEM system was operated at 80 kV.

2.6. Flow cytometer

HepG2 cells were incubated without and with 0.20 mg mL^{-1} the luminomagnetic NPs at 37 °C for 24 h, followed by being washed with ice-cold PBS (10 mM, pH 7.4). Then, the cells were re-suspended in PBS (200 mL) and incubated with DNA intercalating dye PI (20 mL, 1 mg mL^{-1}) in the presence of DNase-free RNase (20 mL, 10 mg mL^{-1}) at 37 °C for 1 h in the dark. DNA histogram was obtained with a Becton-Dickinson FACScan cytometer. The distribution of cells in the different cell-cycle phases was obtained through the analysis of the DNA histogram using Modfit software (Becton Dickinson Immunocytometry Systems, San Jose, CA).

2.7. Confocal laser scanning microscopy (CLSM)

Cellular uptake and internalization of the luminomagnetic NPs were observable by CLSM. HepG2 cells were incubated with the luminomagnetic NPs (0.05 mg mL^{-1}) for 12 h. Then, the cells were smeared on a clean glass slide, followed by being fixed with modified Karnovsky fixative (2% glutaraldehyde + 2% paraformaldehyde) in 0.1 M cacodylate buffer (pH 7.4) with 0.05% $CaCl_2$ solution at 4 °C for 2 h. The cells were then washed with PBS and examined by a Leica TCS SP5 confocal scanning system with an excitation wavelength at $\lambda = 488$ nm. The emission was detected by using a 610-nm long-pass filter.

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