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Terbium-Doped Gadolinium Oxide Nanoparticles Prepared by Laser Ablation in Liquid for Use as Fluorescence and Magnetic Resonance Imaging Dual-Modal Contrast Agents

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Abstract

Dual-modal lanthanide-doped gadolinium nanoparticles (NPs), which exhibit an excellent magnetic resonance imaging (MRI) spatial resolution and high fluorescence imaging (FI) sensitivity, have attracted intensive attention in biotechnology and nanomedicine applications. In this paper, terbium (Tb) ion doped gadolinium oxide (Gd_2O_3:Tb) NPs with varied Tb concentration were synthesized by a laser ablation in liquid (LAL) method. A characterization of the structure, morphology, and composition shows that these NPs are spherical with an excellent crystallinity. The effects of Tb ion concentration on the visible green fluorescence and longitudinal relaxivity were investigated, indicating that the fluorescence properties were significantly influenced by the Tb ion concentration, but all samples were still efficient T_1-weighted contrast agents. Furthermore, the optimum Tb doping concentration was determined to be 1%. The cell viability, cellular fluorescence imaging and *in vivo* MRI of this dual-modal nano-probe were studied, with the results

revealing that the Gd_2O_3:Tb NPs did not have a significant cytotoxic effect, making them good candidates for use as dual-modal contrast agents for MRI and fluorescence imaging.

Keywords: Gd_2O_3:Tb, Fluorescence, Magnetic Resonance Imaging, Laser Ablation in Liquid

Introduction

Dual-modal or multimodal contrast agents have attracted considerable attention in biomedical imaging.[1–8] It is known that magnetic resonance imaging (MRI) employing positive or negative contrast agents to enhance the image contrast is one of the most important and irreplaceable imaging techniques in cancer research, clinical trials, and medical practice.[9] This non-invasive technique provides high spatial resolution,[10] good soft-tissue contrast, excellent perception with tomographic capabilities, and outstanding anatomical detail and orientation.[4,11-12] However, the MRI's sensitivity is low, detecting approximately 10^{-9}–10^{-6} of label moles, compared to a higher (up to 10^{-12} label moles) detection level for an optical technique.[13] Moreover, a fluorescence composition can be further exploited through the use of contrast agents for targeted detection.[14] Therefore, dual-modal contrast agents that couple MRI's high resolution with optical imaging's high sensitivity allow for the implementation of potent contrast agents, which can improve the accuracy of fundamental diagnoses.

Rare earth-doped gadolinium (III) oxide (Gd_2O_3) nanoparticles (NPs) are suitable for use as dual-modal contrast agents with enhanced fluorescence properties due to their doping ions and higher longitudinal relaxivity (r_1) with MRI due to the material's matrix. The low cytotoxicity and attractive optical properties of lanthanide-based NPs, including high photostability, absence of blinking, extremely narrow emission lines, large Stokes shifts, and

long lifetimes, make them superior to other materials such as organic dyes, quantum dots, or semiconductor nanocrystals.[14] Moreover, the r_1 value, which denotes the performance of T_1-weighted MRI contrast agents, is proportional to the number of water molecules coordinated to Gd^{3+}'s unpaired electrons.[15] The Gd_2O_3 material offers a greater number of unpaired electrons for water hydration (seven maximum for the Gd^{3+} surface), and thus it has higher r_1 values than Gd- diethylenetriaminepentaacetate (DTPA) or other Gd-based chelates. Based on these merits, studies have emerged on different rare earth elements doped Gd_2O_3 with different fluorescence color for potential use as dual-modal contrast agents.[16–23]

Previous research on preparing doped Gd_2O_3 samples have focused on the polyol route developed by Bazzi *et al.*,[16] by a hydrothermal method,[2] or by other conventional wet chemical techniques, all of which contain reducing agents. Here, the full physical preparation approach, standard solid state combining with laser ablation in liquid (LAL) technique is used. This method is simple, free from reducing agent contamination, and is independent of the respective precursors. Additionally, this method allows for size control of the particles through a laser-induced size reduction,[24] choice of solution,[25] or adjustment of the laser parameters,[26–30] such as fluence, pulse duration, beam focus, and repetition rate. There is evidence that a smaller laser fluence,[28] reduced beam size,[29] and shorter pulses[27,30] facilitate the synthesis of smaller particles. As a result of these properties and merits, LAL has already been a general approach for synthesizing of variety of particles.

In this paper, terbium-doped Gd_2O_3 NPs with the doping concentration varied from 0.5% to 20% are synthesized by a simple two-step approach: a standard solid-state technique and the LAL. The microstructure, morphology, composition, fluorescence, and magnetic resonance

(MR) properties of these NPs are fully characterized to investigate their potential as dual-modal contrast agents. The effects of Tb doping concentration on the NPs' fluorescence and MR properties were also evaluated, with the overall results indicating that the Gd_2O_3:Tb NPs at optimized Tb doping are a promising dual-modal contrast agent candidate for MR and fluorescence imaging.

Experimental procedures

Nanoparticle syntheses

Tb-doped Gd_2O_3 NPs with varied doping concentration have been prepared by a facile and general two-step up to top-down approach, which combines a standard solid-state technique with the LAL. The Gd_2O_3:Tb solid-state targets were prepared first by a standard solid-state technique (details are shown in the Electronic Supplementary Information (ESI)), with Gd/Tb element stoichiometric ratios of 99.5:0.5, 99:1, 95:5, 90:10, and 80:20. These solid targets were then ablated in liquid by a microsecond laser, with the Gd_2O_3:Tb target fixed on the bottom of a container filled with deionized water. A 1064 nm wavelength Nd:YAG microsecond laser with a 6 μs pulse duration, 100 Hz repetition, and 70 mJ/pulse power was focused onto the target's surface for approximately 30 min. The ablated colloids were aged for 24 h, and then the clear portion of the liquid was collected for use.

Structure, morphology, and composition characterization

The structure of the Gd_2O_3:Tb NPs with varied Tb concentration was characterized using an X-ray diffractometer (XRD, Rigaku, D/MAX-IIIA) with a Cu Kα radiation (λ = 1.54056 Å, 40 kV, 26 mA) at a 2°/s scanning rate. A transmission electron microscope (TEM, FEI,

Tecnai G2 Spirit; JEOL, JEM-2010 HR) and an X-ray photoelectron spectrometer (XPS, Thermo Scientific, ESCALab 250) were utilized to characterize the morphology and analyze the individual components, respectively.

Fluorescence characteristics

The fluorescence excitation and emission spectra, along with lifetime, were measured and analyzed by an Edinburgh Instruments spectrofluorophotometer (FLS 920) at room temperature, while visible fluorescence images of the Gd_2O_3:Tb NPs in deionized water (under laser excitation) were taken with a digital camera without filters. A nasopharyngeal carcinoma (NPC) S18 cell *in vitro* fluorescence image was taken with a fluorescence microscope (Olympus IX51), operating at an approximate 330–380 nm excitation wavelength. These cells were previously maintained at 37 °C under 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM), containing 10% fetal bovine serum (FBS), penicillin (100 units/mL) and streptomycin (100 mg/mL). After incubating with the Gd_2O_3:Tb NPs for 2 h, the cells were washed with a phosphate-buffered saline (PBS) to remove the dead cells and remaining Gd_2O_3:Tb NPs, and then prepared for the cell *in vitro* fluorescence imaging test.

MR Imaging

In vitro and *in vivo* MRI tests were performed by a 3.0 T TIM Trio clinical scanner (Siemens Medical Solutions, Erlangen, Germany). In the *in vitro* MRI tests, a concentration series (0–0.1 mM) of the 0.5%, 1%, 5%, 10%, and 20% Tb-doped Gd_2O_3 NP samples were prepared in 1.5 mL Eppendorf (EP) tubes. These then had a T_1-weighted phantom MRI applied, with an inversion recovery pulse of T_R (time recovery) = 3000 ms and T_I (time

inversion) = 500–2500 ms (providing 17 measurement points). The T_1 and R_1 (equals to 1/ T_1) of the Gd_2O_3:Tb NPs with varied Gd^{3+} concentration were determined by fitting the T_I to an exponential T_1 recovery model using a non-linear least square regression equation:[31–33] $S_{IR}(T_I) = S_0 [1 - 2\exp(-T_I/T_1) + \exp(-T_R/T_1)]$. The Gd^{3+} concentration was determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES, Thermo Fisher, iCAP 6500 Duo), followed by calculating the r_1 values: [34]

$r_1 = \Delta R_1/[Gd^{3+}$ concentration]

Animal experiments were performed in accordance with the National Institutes of Health guidelines on the rules of animal's research and our Institution's Animal Board. In the *in vivo* MRI test, four- to six-week-old BALB/c nude mice with NPC CNE-1 xenografted tumors (approximately 60 mm^3 in size) had anesthesia induced by a 0.1% mebumalnatrium (10 μL per g weight) intraperitoneal injection, followed by an injection of 15 μmol/kg Gd_2O_3:Tb in 100 μL of 0.4% sodium carboxymethylcellulose via the tail vein in the axial orientation. The mice were then subjected to a T_1 weighted MRI at different time frames after the intravenous administration, and compared to the Gd_2O_3:Tb-free control group. All data were analyzed by a picture archiving and communication system (PACS).

***In vitro* cytotoxicity**

The *in vitro* cytotoxicity assay was obtained to evaluate the preliminary biocompatibility of the Tb-doped Gd_2O_3 as a contrast agent. The cytotoxicity of PC12, 293T, and CNE-1 cells incubated with different concentrations (10 μM, 1 μM, and 100 nM) of the Gd_2O_3:Tb NPs was evaluated using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assays for 24 h and 48 h, then compared with control

groups of either a culture medium (DMEM) as the negative control or lipopolysaccharide (LPS) as the positive. The cells were all cultured in DMEM, using 96-well plates cultured at 37 °C and 5% CO_2. After incubation with the Gd_2O_3:Tb NPs, 20 μL of MTS was added for an additional of 4 hours incubation. Finally, all of the culture media were replaced by 100 μL of dimethyl sulfoxide (DMSO) and the absorbance was measured by a microplate reader (Bio-Rad Laboratories, Inc., USA) at 490 nm.

Results and discussion

Prepared NPs' structure, morphology, and composition

Thorough XRD, TEM, and XPS analyses were performed to collect detailed information of the samples. Fig. 1 shows the XRD patterns of Tb-doped Gd_2O_3 NPs with concentrations of 0.5%, 1%, 5%, 10%, and 20%, which were compared to the monoclinic Gd_2O_3 bulk (PDF#42-1465) with lattice constants of a = 14.095 nm, b = 3.576 nm, and c = 8.796 nm. The results reveal that all of the NPs possess a monoclinic structure without any metallic terbium or terbium oxide phases. The structure of these Gd_2O_3:Tb NPs is identical to the bulk form of their Tb-doped Gd_2O_3 target (shown in ESI Fig. S1), indicating that the suitable Tb doping and laser ablation process did not alter the Gd_2O_3's structure and the LAL process only affected its morphology, not its crystal structure. The strong narrow peaks in the XRD patterns infer a good crystallinity and high purity of both the bulk Gd_2O_3:Tb targets and the prepared NPs. A TEM analysis of a typical 1% Tb-doped sample was utilized to examine the morphology, average size, and crystallinity of the prepared NPs. As shown in Fig. 2(a), a typical TEM image displayed spherical particles with an N = 644 particle size distribution

shown in the bar graph of Fig. 2(b). The average size, determined using a Gaussian fit, was approximately 8.4 ± 0.2 nm. A high-resolution (HR) TEM image and selected area electron diffraction (SAED) pattern are shown in Fig. 2(c) and Fig. 2(d), respectively. The HRTEM image's interplanar distance (d ≈ 0.310 nm) is in agreement with the d_{111} = 0.317 nm in the PDF#42-1465 database. Additional SAED patterns reveal a strong diffraction ring pattern of the monoclinic Gd_2O_3 phase. The Tb and Gd 3d XPS spectra were collected to determine the samples' elemental components and chemical state. As shown in Fig. 3, the typical Gd 3d spectra revealed that the Gd $3d_{3/2}$ and $3d_{5/2}$ peaks had binding energies of 1220.5 eV and 1188.7 eV, respectively, which are consistent with the published results of the Gd_2O_3 bulk.[35-36] The intensity of the Tb's 3d peaks increased with increasing Tb doping concentration, and the Tb $3d_{3/2}$ and $3d_{5/2}$ binding energies peak at 1277.5 eV and 1242.1 eV, respectively, showing that the Tb ions were effectively doped into the Gd_2O_3 matrix. However, the binding energy of Tb's $3d_{5/2}$ peak was slightly higher than the published results of terbium(III) oxide (Tb_2O_3), which may relate to the Tb atom's substitution in the Gd_2O_3 matrix as an extrinsic dopant, changing its crystal structure from that of pure Tb_2O_3.[37] The chemical state of Tb is still unclear, as there was little intensity difference in the Tb $3d_{5/2}$ peaks of Tb_2O_3 and TbO_2.[36-38]

Visible fluorescence spectra and lifetimes

The fluorescence spectra and lifetimes of 0.5%, 1%, 5%, 10%, and 20% Tb-doped Gd_2O_3 NPs were carefully examined, with the excitation and emission spectra depicted in Fig. 4(a). Under a 285 nm excitation (Fig. 4(a) inset), intense emission spectra were observed from the Gd_2O_3:Tb NPs with the varied Tb concentrations. The spectra displayed four emission bands

peaked at approximately 489 nm, 543.5 nm, 589 nm, and 621 nm in the 470–640 nm region, corresponding to Tb's energy level transitions from $^5D_4$ to $^7F_6$, $^7F_5$, $^7F_4$, and $^7F_3$, respectively. The intensity of these emission bands initially increase and then decrease with increasing Tb concentration. The dominant emission band at 543.5 nm is the NPs' visible green fluorescence. Fig 4(b) (left) shows a fluorescence image of the NPs taken by a digital camera without any filters. The NPs' energy transfer diagram is described in detail in Fig. 4(b) (right). A charge transfer occurs in the Gd_2O_3 under 285 nm excitation from the ground state $^8S_{7/2}$ to the excited state ^6I_J (J=7/2, 9/2, 11/2 …), and after a non-radiative decay from the ^6I_J to ^6P_J (3/2, 5/2, J = 7/2), there is an energy transfer between the Gd^{3+} and the Tb activator ion. The higher lying energy levels are unstable, leading to depopulation and non-radiative decay to the lower excited state $^5D_4$. Finally, visible emission occurs from the $^5D_4$ transition to the $^7F_3$, $^7F_4$, $^7F_5$, and $^7F_6$ energy levels. The intensity of the primary $^5D_4 \rightarrow {}^7F_5$ emission under the excitation of 285 nm for the samples with varies Tb concentration. The maximum intensity of this emission band peaks at 543.5 nm and is a function of the Tb doping concentration, first increasing with increasing Tb concentration, reaching maximum at 1% Tb doping, and then decreasing with the further increasing Tb concentration, as shown Fig. 5. The variation of maximum intensity with Tb concentration can be fitted linearly and exponentially in the low and high Tb doping concentration regions, respectively.

The fluorescence dynamics of the Tb dopant in the Gd_2O_3 host matrix for different doping concentrations were investigated using a 285 nm excitation of the $^5D_4 \rightarrow {}^7F_5$ transition. Fig. 6(a) shows the lifetime decay profiles of the Tb-doped Gd_2O_3 NPs with varied Tb concentration, which can be fitted with a mono-exponential curve to determine the delay

profile lifetime. Fig. 6(b) demonstrated that the lifetime decreased with increasing Tb doping concentration, and the typical lifetime value was 0.96 ms for the 0.5% and 1% Tb-doped Gd_2O_3 NPs. The possible explanations for the intensity and lifetime variation with the Tb doping concentration are provided in a later discussion.

***In vitro* MRI**

The *in vitro* MRI properties of the 0.5%, 1%, 5%, 10%, and 20% Tb-doped Gd_2O_3 NPs in deionized water as T_1-weighted MRI contrast agents were assessed by the 3.0 T Siemens Trio MRI scanner. Fig. 7(a) shows a series of *in vitro* MR images with a gradient Gd^{3+} concentration (from 0 to 0.1 mM) of each of the five varied Tb-doped Gd_2O_3 NP solutions compared with the commercial clinical MRI contrast agent Gd-DTPA. The results revealed that the MR images brightened with increasing Gd^{3+} concentration in each Gd_2O_3:Tb NP solution group. At an identical Gd^{3+} concentration, the MR image using the Gd_2O_3:Tb NPs as the contrast agent was considerably brighter than the Gd-DTPA. In addition, the MR image brightness of the Gd_2O_3:Tb NP solutions prepared with varied Tb doping concentrations remained nearly identical when the Gd^{3+} concentration was fixed, implying that the doped Tb ion had little effect on the magnetic resonance imaging. The r_1 was measured to understand its effectiveness from a quantitative perspective.[25] As shown in Fig. 7(b), the r_1, calculated from a linear fitting of the plotted relaxation rate ($1/T_1$) as a function of the Gd^{3+} concentration, was approximately constant (~15.5) and about four times larger than that of the Gd-DTPA (~4.0), except for the 20% Tb-doped sample's slightly lower value. Therefore, the qualitative and quantitative analyses suggest that these Gd_2O_3:Tb NPs can be used as highly efficient T_1-weighted MRI contrast agents.

Tb doping concentration effects on fluorescence and MR imaging

Fluorescence and r_1 were studied as a function of the Tb doping concentration. The $^5D_4 \rightarrow {}^7F_5$ emission intensity under the excitation of 285 nm changed rapidly with the Tb ion concentration (shown in Fig. 3). The fluorescence density of samples below 1% Tb increased linearly with the Tb concentration, which can be ascribed to the linearly increasing number of D energy levels. Above 1% Tb doping, a significant exponential decrease in the emission intensity was observed. Since the XRD results did not reveal the presence of a second phase, this decrease related to the decreasing average distance between Tb ions, which allow efficient resonant energy transfer among the reactive Tb activators. Energy is therefore delivered to quantum centers on the surface of the NPs, leading to fluorescence quenching.[39] A more detailed mechanism requires further research. The Tb doping concentration effect on the lifetime decay spectra is shown in Fig. 6(b), with little difference found in the lifetime values for the 0.5% and 1% Tb-doped Gd_2O_3 samples. However, when the Tb concentration increased further, the lifetime value decreased due to the quenching effect of excessive Tb ions. Both the fluorescence intensity and lifetime analyses indicated that the 1% Tb doping concentration was the most efficient. It can also be noted that the Tb doping concentration didn't cause a significant difference in the r_1 values, with only a slight decrease for the 20% Tb doping sample. All samples possessed high r_1 values resulting from the large number of water molecules coordinated to unpaired Gd^{3+} electrons, coupled with the large average surface/volume ratio of ~8.4. Balancing the fluorescence and r_1, the optimal stoichiometric Gd/Tb atomic ratio is 99:1.[15, 40]

Cell viability, Cellular fluorescence, and *in vivo* MR imaging

To investigate the toxicity of the Gd_2O_3:Tb NPs used as dual-modal contrast agents, PC12, 293T, and CNE-1 cells were employed to evaluate the *in vitro* toxicity. Fig. 8(a) shows the cell viability of PC12, 293T, and CNE-1 cells incubated with 1% Tb-doped Gd_2O_3 NPs for 24 h compared to Gd-DTPA and negative or positive controls. The results indicated no significant difference between the Gd-DTPA and the Gd_2O_3:Tb NPs, with the latter not significantly affecting viability for these three cell types. Additionally, the CNE-1 cells were co-incubated with the Gd_2O_3:Tb NPs at the same concentration series for 24 h and 48 h. Fig. 8(b) demonstrated that over 85% of the CNE-1 cells possessed viability, which is an insignificant difference compared to that of the Gd-DTPA

The cellular fluorescence, *in vivo* MR imaging, and cell viability of the prepared Gd_2O_3:Tb NPs with an optimized 1% Tb concentration were systematically investigated for use as dual-modal contrast agents due to their excellent fluorescence and *in vitro* MR imaging characteristics. Cellular fluorescence imaging was performed with a fluorescence microscope to examine the Gd_2O_3:Tb NPs' capacity for optical labeling in cellular studies. Fig. 9(a) shows the bright-field image of S18 cells after 2 h incubation with Gd_2O_3:Tb NPs, with no significant damage observed after the cells took up the NPs. Since the cellular swallowing process is size dependent with an optimum NP size of 50 nm,[41-42] the Gd_2O_3:Tb NPs with a 8.4 nm mean size can be taken up, facilitating the optical labeling. As shown in Fig. 9(b) and 9(c), the fluorescence image and merged image, respectively, clearly indicate that the green fluorescence was observed from these intracellular NPs swallowed by the S18 cells, making the Gd_2O_3:Tb NPs suitable as fluorescence imaging contrast agents.

An *in vivo* MRI experiment was also performed with several groups of mice. Fig. 10 shows two

typical groups, (a) and (b), of T_1-weighted MR pcolor (pseudo color) images of NPC CNE-1 xenografted tumors in BALB/c nude mice with an administrated 15 μmol/kg Gd^{3+} concentration, considerably smaller than that of the standard Gd-DTPA (0.1–0.2 mmol/kg).[43] The control groups, (a_0) and (b_0), are the uninjected mice. The T_1-weighted images in the axial orientation of the two groups were obtained at 20, 30, 40, and 55 min and 30, 40, 45, and 55 min after the Gd_2O_3:Tb NP intravenous administration. Group (a) showed an increasing contrast enhancement of the NPC CNE-1 xenografted tumor (white arrow) with time, from 20 to 55 min after injecting the Gd_2O_3:Tb NPs. Where as in group (b), the contrast enhancement reached a maximum at approximately 30 min after the injection and then decreased with time. Results indicate that the Gd_2O_3:Tb NPs initially accumulate in the tumor and other parts of the body before being gradually metabolized. Although they cannot target the tumor, these NPs still can provide a clear contrast enhancement for the tumor within a suitable time. The gray scale MR images and dynamic enhancement curve of xenografted tumors are shown in ESI Fig. S2.

Conclusions

Tb-doped Gd_2O_3 NPs with doping concentrations of 0.5%, 1%, 5%, 10%, and 20% were synthesized by a full physical method of solid state technique plus LAL method. The structure, morphology, and composition of these NPs were fully characterized, indicating a good crystallinity and spherical shape. The fluorescence and longitudinal relaxivity were further examined with emphasis on the Tb doping concentration. The results demonstrated that these NPs possess an efficient fluorescence due to the extrinsic dopant (Tb ion) and the high r_1 value resulting from the Gd_2O_3 matrix host. For the optimized 1% Tb ion doped samples, cellular fluorescence, *in vivo* MR imaging, and cell viability were all investigated.

The Gd_2O_3:Tb ion is capable of optical labeling, efficient for MR imaging, and does not cause significant cytotoxic effects. Our research suggests that the Tb ion doped Gd_2O_3 is a promising dual-modal contrast agent candidate for fluorescence and MR imaging.

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Supporting Information Available:

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Figure captions

Fig. 1. XRD patterns of 0.5%, 1%, 5%, 10%, and 20% Tb-doped Gd_2O_3 NPs compared to the monoclinic bulk phase Gd_2O_3 (PDF#42-1465).

Fig. 2. (a) Typical TEM image of the Gd_2O_3:Tb NPs. (b) A size distribution histogram of the NPs and their mean size calculated from Gaussian fitting. (c) HRTEM image of Gd_2O_3:Tb NPs. (d) Corresponding SAED pattern of the sample.

Fig. 3. Gd 3d and Tb 3d XPS spectra of the Gd_2O_3:Tb NPs with 0.5%, 1%, 5%, 10%, and 20% Tb doping concentrations.

Fig. 4. (a) Emission spectra of 0.5%, 1%, 5%, 10%, and 20% Tb-doped Gd_2O_3 NPs, and (inset) the excitation spectra. (b) (left) Green fluorescence image of the colloidal Gd_2O_3:Tb NPs in deionized water, and (right) illustration of the energy transfer between the Gd^{3+} and activator Tb^{3+}.

Fig. 5. Fluorescence intensity, peaked at 543.5 nm, as a function of the Tb doping concentration, and the linear-exponential fit with respect to the Tb doping concentration.

Fig. 6. Lifetime properties of (a) Lifetime decay of the 0.5%, 1%, 5%, 10%, and 20% Tb-doped Gd_2O_3 NPs, and (b) lifetime values as a function of Tb doping concentration.

Fig. 7. (a) *In vitro* T_1-weighted MR images of commercial Gd-DTPA compared to the Gd_2O_3:Tb NPs with varied Tb doping concentrations, as indicated. (b) Plots of the relaxation rate ($1/T_1$) as a linear function of Gd^{3+} concentration, the slope is longitudinal relaxivity (r_1) of contrast agents.

Fig. 8. Toxicity assays of (a) cell viability of PC12, 293T, and CNE-1 cells incubated with 1% Tb-doped Gd_2O_3 NPs for 24 h compared to Gd-DTPA, and (b) the viability of CNE-1 cells incubated with 1% Tb-doped Gd_2O_3 NPs for 24 h and 48 h compared to Gd-DTPA.

Fig. 9. *In vitro* cell fluorescence images including: (a) bright field, (b) fluorescence, and (c) merged images of the two, for S18 cells incubated with 1% Tb-doped Gd_2O_3 NPs taken with a fluorescence microscope.

Fig. 10. *In vivo* pcolor MR images of two BALB/c nude mice groups (a), (b) with NPC CNE-1 xenografted tumors (a, b are different mice) before and after injection of the 1% Tb-doped Gd_2O_3 NPs (15 μmol/kg) measured at varied times.