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Magnetic and optical properties of multifunctional core-shell radioluminescence nanoparticles

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Abstract

When X-rays irradiate radioluminescence nanoparticles, they generate visible and near infrared light that can penetrate through centimeters of tissue. X-ray luminescence tomography (XLT) maps the location of these radioluminescent contrast agents at high resolution by scanning a narrow X-ray beam through the tissue sample and collecting the luminescence at every position. Adding magnetic functionality to these radioluminescent particles would enable them to be guided, oriented, and heated using external magnetic fields, while their location and spectrum could be imaged with XLT and complementary magnetic resonance imaging. In this work, multifunctional monodispersed magnetic radioluminescent nanoparticles were developed as potential drug delivery carriers and radioluminescence imaging agents. The particles consisted of a spindle-shaped magnetic γ -Fe₂O₃ core and a radioluminescent europium-doped gadolinium oxide shell. Particles with solid iron oxide cores displayed saturation magnetizations consistent with their ~13% core volume, however, the iron oxide quenched their luminescence. In order to increase the luminescence, we partially etched the iron oxide core in oxalic acid while preserving the radioluminescent shell. The core size was controlled by the etching time which in turn affected the particles' luminescence and magnetic properties. Particles with intermediate core sizes displayed both strong magnetophoresis and luminescence properties. They also served as MRI contrast agents with relaxivities of up to 58 mM⁻¹s⁻¹ (r₂) and 120 mM⁻¹s⁻¹ (r₂*). These particles offer promising multimodal MRI/fluorescence/X-ray luminescence contrast agents. Our core-shell synthesis technique offers a flexible method to control particle size, shape, and composition for a wide range of biological applications of magnetic/luminescent nanoparticles.

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Electronic Supplementary Information (ESI): Supplementary information includes additional SEM images, zeta potential measurements, photographs of nanoparticles with encapsulated bromocresol green dye, ICP elemental analysis, and relaxivities in different concentration units. These materials are free of charge via the internet at <http://pubs.rsc.org>.

Introduction

Multifunctional magnetic and fluorescent nanomaterials have attracted broad interest because of their utility in biomedical applications such as bioimaging,^[1] drug delivery carriers,^[2] magnetic resonance imaging (MRI),^[3] bio-separation,^[4] fluorescent labeling,^[5–6] magnetic hyperthermia,^[7] and immunoassays.^[8] These magnetic particles can be magnetically guided, oriented, heated, and imaged using external magnetic fields. Meanwhile the particle fluorescence provides a sensitive label for imaging in cells and thin tissue sections.

Although biological tissues are essentially transparent to magnetic fields which facilitates *in vivo* magnetic control and imaging, fluorescence measurements are limited in thick tissue due to autofluorescence backgrounds and optical scattering. X-ray luminescence tomography (XLT) addresses these issues by using radioluminescent contrast agents for low background, high resolution imaging in tissue.^[9–10] Radioluminescence is well-suited for tissue imaging because both the X-ray excitation and near-infrared (NIR) emission penetrate deeply through tissue and because unlabeled tissues display minimal radioluminescence backgrounds. XLT images are generated by scanning a narrow X-ray beam relative to the sample and detecting the diffuse light escaping from the tissue using sensitive photodetectors. By restricting the X-ray excitation to a single narrow beam, the origin of the optical photons can be inferred regardless of how many times these photons scatter in tissue and where they emerge from the tissue. This enables tomography with a resolution limited by the X-ray beam width in the tissue. The principle is similar to scanning confocal and structured illumination optical microscopies, except that while multiphoton confocal microscopy is limited to tissue thicknesses of 1 mm or less,^[11] XLT can image through several centimeters of tissue because the X-rays are far less scattered than visible and NIR light. With a 1 mm X-ray beam and 1–100 cGy radiation dose, Pratz *et al.* achieved a spatial resolution of 1 mm through tissue-mimicking material based on the selective excitation of subpicomolar of 50 nm phosphor and optical detection of their luminescence.^[10] They also showed that XLT could image the cross sectional distribution of microsize phosphor particles in 4.5 cm of an agar tissue phantom.^[12]

Previously, we extended the XLT technique towards functional chemical imaging on implanted scintillator films using X-ray excited optical luminescence as a local light source for absorption spectroscopy of indicator dyes on the film surface. We developed a radioluminescent pH-sensing film based upon indicator dye absorption, and demonstrated 0.3 mm knife-edge resolution imaging through 1 cm of chicken.^[13] We also imaged patterns of silver deposition and dissolution on a scintillator film through 1 cm of pork tissue.^[14] Our technique is uniquely suited for studying chemical changes on the surface of implanted medical devices. The principle can also be extended to the third dimension for using particles with indicator dyes.

Herein, we developed a novel and facile strategy to fabricate magnetic scintillators for MRI and XLT imaging. Magnetic fluorescent and upconversion nanoparticles have previously been reported with an iron oxide core and rare earth doped NaYF₄:Yb,Er shell.^[15–16] However, a challenge is that iron oxide quenches most of the fluorescence if it contacts the phosphor or is in proximity, even with a thin silica spacer layer.^[17–20] This demonstrates that absorption in the core modulates the luminescence signal, which would be a useful feature for generating chemical sensors with encapsulated indicators. However, the iron oxide core decreases the luminescence intensity and leaves no space for encapsulating drugs or dyes. In order to increase the luminescence intensity, we partially etched the iron oxide core in oxalic acid. The particles' optical and magnetic properties depend upon the core size,

which is controlled by varying the etching time. The etching process also produced a hollow space around the core which could be used for drug and dye encapsulation.

Experimental

Materials

Iron (III) chloride anhydrous and tetraethoxysilane (TEOS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gadolinium Nitrate, Europium nitrate, potassium dihydrogen phosphate, and sulfur powder (99.5%) were purchased from Alfa Aesar (Ward Hill, MA). Ethanol (anhydrous, denatured), urea, oxalic acid, ammonium hydroxide, and nitric acid were obtained from BDH Chemicals Ltd (Poole, Dorset, UK). Deionized (DI) water was purchased from EMD Chemicals Inc. (Gibbstown, NJ, USA). Polyvinylpyrrolidone (PVP K-30, MW 40,000) was purchased from Spectrum Chemicals (Gardena, CA). Agarose (melting point 88 ± 1 °C) was purchased from Shelton Scientific (Peosta, IA). All chemicals were used as received without further purification.

Characterization methods

Transmission and scanning electron microscopy (TEM) were performed on a H9500 operated at 300 kV and HD2000 microscope operated at 100 kV, respectively. An X-ray diffractometer (Rigaku; MiniFlex, Cu K α) was used to characterize the XRD pattern of the magnetic scintillators. For fluorescence spectra, 480 nm light was used to excite the scintillators.

To measure radioluminescence, X-ray was generated by a mini X-ray tube (Amptek Inc. MA, USA), the X-ray tube was operated with tube voltage of 40 kV and tube current of 99 μ A. The sample was mounted on a Leica Microscope (Leica DMI 5000M, Wetzlar, Germany) equipped with a DeltaNu DNS 300 spectrometer (Intevac-DeltaNu, Laramie, WY USA) with a 150 lines/mm grating blazed at 500 nm.

The Zeta-potential of the nanoparticles was determined using a Malvern Instruments Zetasizer Nano ZS with a 633 nm He-Ne laser. Prior to the experiment, the particles were diluted in distilled water (0.1 mg/ml). Magnetization measurements were performed at the designated temperature using vibrating sample magnetometer (VSM) option of physical property measurement system (PPMS, Quantum Design, USA), with the applied magnetic field sweeping between ± 3.0 Tesla at a rate of 50 Oe/sec. Determination of the gadolinium and iron content in a sample was performed by inductively coupled plasma (ICP)-(Optima 3100 RL; Perkin-Elmer).

In order to magnetically modulate the optical scattering from the magnetic luminescent particles, the particles were oriented and rotated in a magnetic field. A permanent magnet (NdFe, 1" in diameter, 3" in length, magnetized through its diameter) was attached to a stepper motor and controlled by motion control software (Si Programmer; Applied Motion Products, Watsonville, CV). Every 3s, the permanent magnet was rotated 90°, first clockwise and then anticlockwise.

All MRI experiments were performed on a Varian 4.7T horizontal bore imaging system (Agilent Inc, Santa Clara, CA). Samples, contained in 5 mm NMR tubes, were placed in a 63 mm inner diameter quadrature RF coil for imaging. MRI gradient echo scout images were collected in all three imaging planes (axial, coronal, and sagittal) for subsequent image planning, with repetition time (TR) = 100 ms, echo time (TE) = 4 ms, number of slices = 20, slice thickness = 2, matrix size 128 $\times$ 128, field of view (FOV) = 40 mm $\times$ 40 mm, number of acquisitions (NEX) = 2. Relaxivity measurements were then collected on a single 2 mm thick imaging slice, approximately perpendicular to the long axis of the NMR tubes. The single

slice, with FOV = 36 mm×36 mm, was imaged using a T_2 -weighted multi-spin echo imaging sequence with TR = 3000, NEX = 10, echo spacing = 4ms, number of echoes = 10, and 128×128 matrix. T_2^* -weighted images were collected using a gradient echo imaging sequence with TR = 500 ms, flip = 20°, 128×128 matrix, NEX = 10, and echo times = [1.5, 3, 4.5, 9, 15 ms]. Following data collection, images were analyzed using Matlab 2011a (The Mathworks, Inc., Natick, MA). Regions of interest (ROI's), encompassing approximately 70–80 voxels, were manually drawn in each sample, and the signals from those voxels averaged to obtain a mean signal for each sample. The same ROI was used to calculate the mean signal of the sample across all echo times.

Synthesis of spindle-shaped hematite particle

Monodispersed spindle-shaped hematite nanoparticles with controllable aspect ratios were fabricated according to the method described by Ozaki and coworkers^[21]. Typically, 100 ml of aqueous solution containing 2.0×10^{-2} M FeCl_3 and 4.0×10^{-4} M KH_2PO_4 were aged at 100 °C for 72 hours. The resulting precipitate was centrifuged and washed three times with water.

Synthesis of spindle-shaped hematite particle with silica shell

To obtain monodispersed hematite-silica core-shell nanoparticles, the PVP assisted coating method was used.^[22] The spindle-shaped hematite particles synthesized above were dispersed ultrasonically to a 80 ml solution containing PVP (0.6 g), water (6 ml), and ethanol (74 ml). The suspension was stirred using a magnetic stir bar at room temperature and a solution of TEOS (270 μl) in 20 ml ethanol was added, followed by 4 ml of ammonia hydroxide. After 3h, the reaction mixture was precipitated by centrifuging at 4000 rpm for 16 min. The particles were washed three times with ethanol and centrifuged to collect the product.

Synthesis of spindle-shaped magnetic scintillators

The products above were suspended in 180 ml distilled water with 1.8 g PVP and 11.34 g oxalic acid (0.5 M) incubated at 60 °C for certain time. The hematite partially dissolved particles were collected by centrifugation and rinsed with DI water twice. The obtained particles were resuspended with 3 ml $\text{Gd}(\text{NO}_3)_3$, 1.5 ml $\text{Eu}(\text{NO}_3)_3$ (80 mM), and 1.8 g PVP in pure water to form 300 ml of solution. 18 g of urea was added to the solution and the solution was maintained at 80 °C for 90 min. The precursor of magnetic scintillator was collected by centrifugation. The precursor of spindle-shaped precursor was calcined in a furnace at 600 °C for 60 min. The powder was then transferred to a tube furnace with a H_2/N_2 (5%/95%) flow at 450 °C for 3h. After that, the sample was calcined in the tube furnace at 350 °C for 24 h.

Preparing nanocomposites for MR Imaging

T_2 and T_2^* MR measurements were acquired for the spindle-shaped $\gamma\text{-Fe}_2\text{O}_3@ \text{SiO}_2@ \text{Gd}_2\text{O}_3:\text{Eu}$ particles at a series of concentrations (0.8 mg/ml, 0.4 mg/ml, 0.1 mg/ml, and 0.05 mg/ml). The particles were dispersed in 0.5% agarose gel at 80 °C and cooled to room temperature in NRM tubes to set the gel. The gel prevented settling and aggregation allowing MRI imaging several days after preparation.

Cell viability test

MCF-7 breast cancer cells were seeded at a density of 10,000 cells/well in a 96-well plate. Cells were stored at 37 °C at 5% CO_2 and attached to the plate overnight. Nanoparticles were suspended in media, sonicated for 10 minutes to disperse, and diluted to 250, 100, 50, and 10 $\mu\text{g/ml}$. Media was removed from wells and fresh media or nanoparticle in media was

added to each well. Five repeats were done for each concentration. Nanoparticles were incubated with cells overnight and the next day a Presto Blue assay (Life Technologies) was performed. Media was removed and 100 μ l of a 1:9 ratio Presto Blue in culture media was added to each well. Cells were incubated at 37 °C and 5% CO₂ for 45 minutes. Fluorescent intensity was taken with a plate reader with an excitation wavelength of 560 nm and an emission wavelength of 590 nm. Fluorescent intensity for each concentration of nanoparticle was normalized as a percentage of the fluorescent intensity of the control cells. Percent viability averages were plotted with error bars of one standard deviation.

Results and discussion

Our objective was to synthesize radioluminescent particles with controllable size, shape, optical, and magnetic properties. We selected europium-doped gadolinium oxide as a matrix because of its photostability,^[23] its narrow and distinct spectral features under fluorescence and radioluminescence,^[24–25] its MRI contrast,^[26–30] and its relatively non-toxicity.^[26, 29–30] We selected a core-shell template synthesis route so that we could separately control the magnetic core and luminescent shell materials. Our initial studies employed spindle-shaped hematite cores and therefore generated spindle-shaped shells. However, the shape of the shell can be altered by using different core shapes by changing the hematite synthesis conditions.^[31–33] Although macrophage uptake of particles with diameters larger than 500 nm reduces circulation time and is an obstacle to particulate drug delivery,^[34] there are some reports that changes in particle shape strongly affect macrophage uptake and biodistribution. For example, Huang and Decuzzi *et al.* studied the clearance of a series of non-spherical silica/silicon nanoparticles with a length range of 300 nm~5 μ m and observed that the particle shape plays a crucial role in the circulation lifetime and biodistribution which are important for drug delivery applications.^[35–37] Champion and co-workers recently found that macrophages internalize ellipsoid particles 2~6 μ m long in a strongly orientation-dependent manner. They observed that it took only a few minutes to internalize particles with the high radius of curvature region contacting the macrophages, while particles contacting the macrophages on their low curvature sides were not internalized within 12 h.^[38] Our long term goal will be to study how shape and size affect the biodistribution using *in situ* XLT imaging.

Structure and morphology of magnetic luminescent nanoeyes (γ -Fe₂O₃@SiO₂@Gd₂O₃:Eu)

The template core-shell synthesis process is presented in Fig. 1. We first synthesized a monodispersed spindle-shaped hematite core according to methods described by Ozaki and co-workers.^[21] Our particles were spindle shaped with an average length of 400 nm and diameter of 80 nm, however, by varying the synthesis conditions, the nanoparticle size can be tuned from 120 to 550 nm, and the aspect ratio from spheres to prolate spheroids.^[31–32] Hematite is a chemically stable antiferromagnetic phase of iron oxide which can be later reduced to magnetite or iron and oxidized to ferrimagnetic maghemite (γ -Fe₂O₃). Hematite is used as a template because it is difficult to directly synthesize uniform spindle magnetite or maghemite particles, and these large particles often aggregate in magnetic fields especially at high particle concentration. The colloidal hematite particles had a positive zeta potential of +26 mV which kept them well dispersed in solution due to electrostatic repulsion. This positively charged hematite could not be directly used as template for growing a scintillator shell because the positively charged hematite would repulse the positively charged scintillator precursor (Gd(OH)CO₃:Eu, +11 mV), see Fig. S1, ESI. Instead, the hematite nanoparticles were first treated through a modified Stöber procedure^[39] to deposit a thin silica layer which reversed the surface charge (−36 mV), see Fig. S2, ESI. A scintillator shell was then successfully grown on these silica-modified hematite particles.

To increase the luminescence of the core-shell structure, and provide a hollow space for drug encapsulation, we etched the optically absorbing iron oxide core in oxalic acid solution at 60 °C for a controlled period of time. The obtained $\alpha\text{-Fe}_2\text{O}_3\text{@SiO}_2$ particles had a monodispersed spindle-shaped shell with a hollow space around the core and appeared like “nanoeyes” in TEM images. After 9.5 h of etching, the magnetic cores of these nanoeyes were cylinder-shaped with an average length of 150 nm and diameter of 60 nm. In addition, the length of the iron oxide core was tunable from 0 to 400 nm with different etching time (Fig. 2, 4).

To synthesize radioluminescent particles, the silica-coated core-shell particles were then coated with a layer of $\text{Gd}(\text{OH})\text{CO}_3\text{:Eu}$ through a homogeneous precipitation method from gadolinium, europium nitrate. In order to convert the $\alpha\text{-Fe}_2\text{O}_3$ to $\gamma\text{-Fe}_2\text{O}_3$, the product above was treated by H_2/N_2 (5%/95%) at 450 °C for 3h and calcined later in the air at 350 °C for 24 h. The resulting monodispersed nanoeyes ($\gamma\text{-Fe}_2\text{O}_3\text{@SiO}_2\text{@Gd}_2\text{O}_3\text{:Eu}$) had an average length of about 500 nm and diameter of 80 nm (Fig. 1B). They consisted of a ~10 nm silica intermediate shell and 40 nm scintillator outer shell which could be distinguished in TEM images such as Fig. 1C because of the different electron penetrability between the Gd_2O_3 , SiO_2 , and $\gamma\text{-Fe}_2\text{O}_3$ core.

X-ray diffraction (XRD) was performed on the nanocomposite samples in order to investigate their structure and composition. Fig. 3A shows the XRD pattern of the precursor of nanoeyes ($\alpha\text{-Fe}_2\text{O}_3\text{@SiO}_2\text{@Gd}(\text{OH})\text{CO}_3\text{:Eu}$). The X-ray diffraction pattern of $\alpha\text{-Fe}_2\text{O}_3$ is clearly distinguished from the broad peak at about 30° from the porous $\text{Gd}(\text{OH})\text{CO}_3\text{:Eu}$. The XRD pattern of nanoeyes (Fig. 3b) exhibits the characteristic diffraction peaks of cubic structure of Gd_2O_3 . The $\gamma\text{-Fe}_2\text{O}_3$ core peaks in Fig. 3 b are indiscernibly weak because the cores have a small volume percentage and cross-section compared to the Gd_2O_3 . However, the XRD pattern of $\gamma\text{-Fe}_2\text{O}_3$ can be readily distinguished under a thin shell (~10 nm) of $\text{Gd}_2\text{O}_3\text{:Eu}$ (Fig. 3c).

Magnetic and optical and properties of magnetic luminescent nanoparticles

In order to elucidate the magnetic properties of the nanoeyes, we synthesized similar shell structures with solid iron oxide cores and hollow particles with dissolved cores by incubation of the $\alpha\text{-Fe}_2\text{O}_3\text{@SiO}_2$ in oxalic acid for 0 h and 17 h, respectively (Fig. 4). The solid-core nanorice ($\gamma\text{-Fe}_2\text{O}_3\text{@SiO}_2\text{@Gd}_2\text{O}_3\text{:Eu}$) and hollow nanorice ($\text{SiO}_2\text{@Gd}_2\text{O}_3\text{:Eu}$) both are monodispersed with a radioluminescent shell shape almost identical to the nanoeyes (Fig. S3, ESI).

The room temperature magnetic hysteresis loops of magnetic nanorice, nanoeyes, and hollow nanorice are shown in Fig. 5. The hollow nanorice ($\text{SiO}_2\text{@Gd}_2\text{O}_3\text{:Eu}$) are paramagnetic, with minimal hysteresis and a magnetic susceptibility of $1.23 \times 10^{-4} \text{ emu g}^{-1} \text{ Oe}^{-1}$ and showed no sign of saturation up to applied fields of 30 kOe. From the TEM images (Fig. 4) the core is approximately 13% of the volume and a similar percentage of the mass. The iron oxide was converted to maghemite ($\gamma\text{-Fe}_2\text{O}_3$) via H_2 reduction followed by oxidization in air (350 °C 24 h), and the particle colour changed from red with the hematite core to brownish with the maghemite core. The nanorice magnetization curve (Fig. 5A) had two components, a hysteric ferrimagnetic (or ferromagnetic) component with a coercivity of 308 Oe and a saturation magnetization of ~7.6 emu/g, and a paramagnetic component (linear slope at large applied fields). The saturation magnetization of the ferrimagnetic component is approximately 10% of the saturation magnetization of bulk $\gamma\text{-Fe}_2\text{O}_3$ (74 emu/g).^[40] These results are consistent with a core ~10% maghemite by weight and a shell ~90% by weight which is also consistent with the TEM images and ICP elemental analysis data. The hysteresis curve for nanoeye (Fig. 5B) had a smaller ferrimagnetic component with a saturation magnetization of 2.3 emu/g, which is consistent with a 3-fold reduction in weight

per cent of maghemite. The coercivity decreased to 165 Oe likely due to the decreases in the aspect ratio of the iron oxide core. Although the saturation magnetization of the partially dissolved nanoeyes is smaller than the nanorice, the magnetization is still strong enough for rapid magnetophoretic separation (inset figure of Fig. 5).

In addition to magnetophoretic separation in magnetic field gradients, the particles are expected to align with external magnetic fields due to their magnetic shape anisotropy. This alignment is useful in developing sensitive and rapid immunoassays, viscosity sensor, and improved intracellular sensors with orientation and shape-dependent properties.^[41–43] For example, orientation of non-spherical particles has been shown to affect phagocytosis rates for adherent macrophages.^[38, 44] In addition, the ability for particles to rotate is useful for determining if particles are bound, while the rotation rate in solution is useful for measuring viscosity and viscous drag.^[42] We demonstrate that the magnetic nanoeyes rotate in response to a rotating external magnetic field by measuring the scattering signal intensity as the particles rotate in response to a changing magnetic field. Experimentally, we placed a solution of nanoeyes in water/glycerol ($v_{\text{water}}:v_{\text{glycerol}}=1:9$) on a glass slide and observed the scattering intensity with a dark field microscope. The nanoeyes were orientated by an external magnetic field which rotated by 90 ° in every 3 s. As depicted in Fig. 6, the scattering intensity was largest when the particles were oriented parallel to the sample plane and presented the largest scattering area. The scattering intensity decreased when the particles were oriented parallel to the optical axis. Fig. 6C shows the magnetically modulated optical scattering by the nanoeyes. This rotational modulation cannot be observed for spherical particles which have isotropic scattering properties.

In addition to magnetic rotation and separation, the nanoeyes display fluorescence under 480 nm excitation light and radioluminescence under X-ray excitation (Fig. 7). The main emission peak of the $\text{Gd}_2\text{O}_3\cdot\text{Eu}$ scintillator shell with cubic structure was observed at 610 nm, which corresponds to a red emission from the $^5\text{D}_0 \rightarrow ^7\text{F}_2$ Eu^{3+} transition. The fluorescence and radioluminescence of the solid core nanorice were hardly detected. The luminescence quenching is likely due to inner filter effects as the 10 nm silica shell spacer and hollow regions in the core would inhibit resonant energy transfer. Previous approaches to address this quenching problem by separating the luminescent shell from the absorptive core using a coating thick silica shell (>50 nm) also increase the particle size.^[16, 20] By contrast, the current method of partial dissolving the iron oxide core reduces the quenching while maintaining the total nanoparticle size and volume. We expect that carefully controlling dissolution time will allow optimization of the optical and magnetic properties. In addition, a hollow space is formed around the iron oxide core in the nanoeyes which could serve to encapsulate drugs for drug delivery applications (See Fig. S4B, ESI). We expect that low molecular weight drugs can be loaded into the hollow space of the particles.

Although the hollow nanorice obtained by completely dissolving the iron oxide are paramagnetic, brightly luminescent, and have a large internal space for encapsulation, their magnetic moment is weak compared to the solid nanorice and nanoeyes, especially at low fields. As a result, they separate only very slowly with typical permanent magnet-generated magnetic fields and field gradients. The nanorice particles with a solid iron oxide core respond rapidly to magnetic fields but display only weak fluorescence and negligible radioluminescence. In addition, there is not much space within the core for dye and drug encapsulation. By partially dissolving the iron oxide core, nanoeyes are formed which display magnetophoresis and radioluminescence. Their bright luminescence under UV (365 nm) and X-ray irradiation is shown in Fig. 8 Compared with the UV fluorescence, the X-ray luminescence of the nanoeyes provides a background-free image which can be used for deep tissue imaging. The hollow regions in the nanoeyes could also be used for indicator dye and drug encapsulation for theranostic applications. To test whether the pores in the Gd_2O_3 shell

were large enough for small molecules to diffuse through, we incubated the nanoparticles in a solution of bromocresol blue dye, encapsulated the dye and particle with ~ 10 nm silica layer, and washed and separated the particles via centrifugation. The dye could indeed be encapsulated (see Fig. 4S, ESI), while a control, the solid core particles could not encapsulate dye. We are currently developing coatings to control the dye release rate.

Magnetic luminescent nanoparticles as T_2 contrast agent

It is useful to have contrast agents that provide both radioluminescence and MRI contrast in order to validate measurements, and to provide complimentary information (e.g. molecular information with functional XLT spectroscopy using indicators and tissue contrast with MRI). Our magnetic radioluminescent particles are expected to serve as T_2 and T_2^* contrast agents because of their strong magnetic moment in static MRI fields. This is consistent with recent work showing that gadolinium oxide nanoparticles have higher relaxivities compared with gadolinium chelates.^[27, 45–46]

Fig. 9 shows T_2 and T_2^* weighted images after 4 ms and 1.5 ms, respectively. The decrease with echo time was fit to an exponential in order to calculate the relaxation rate, $R_2 = 1/T_2$ and $R_2^* = 1/T_2^*$ at each concentration. These relaxation rates are shown as a function of concentration in Fig. 10 and Fig. S5 (see Table S1 for the molar concentration of Gd and Fe in 0.8, 0.4, 0.1, and 0.05 mg/ml of solution, ESI). The curves are approximately linear, although there is some evidence of saturation at the highest concentration, 0.8 mg/ml. Fitting the points up to 0.4 mg/mL (2.6–3 mM), the relaxivities, r_2 and r_2^* are $69 \text{ mM}^{-1}\text{s}^{-1}$ and $274 \text{ mM}^{-1}\text{s}^{-1}$ respectively for the nanorice; $58 \text{ mM}^{-1}\text{s}^{-1}$ and $120 \text{ mM}^{-1}\text{s}^{-1}$ for the nanoeyes, and $46 \text{ mM}^{-1}\text{s}^{-1}$ and $112 \text{ mM}^{-1}\text{s}^{-1}$ for the hollow nanorice. The relaxivity is calculated based on the total molar concentration of both Gd^{3+} and Fe^{3+} . For comparison with other literature we have also calculated r_2 and r_2^* using the molar concentrations of just Gd^{3+} and Fe^{3+} , and the total mass concentration in mg/mL (see Table S2, ESI). For all particles, r_2^* is larger than r_2 because r_2 includes contributions from local static field inhomogeneities caused by the magnetic moment of the particles. The difference between r_2 and r_2^* may provide more specificity towards the contrast agents, especially for the nanorice which display a factor of 4 increased relaxivity. The iron oxide core significantly increased the relaxivities, with the solid core providing the highest relaxivity and the hollow core the least.

Previous work showed that ultrasmall Gd_2O_3 nanoparticles (1–10 nm)^[26, 28–29, 47] and hollow nanoparticles with thin Gd_2O_3 shell (~10 nm)^[30] served as good T_1 contrast agents and moderate T_2 contrast agents. In contrast, our spindle-shaped nanoparticles with a ~40 nm $\text{Gd}_2\text{O}_3\text{:Eu}$ shell worked better as a T_2 contrast agents. This may be due to low water exchange efficiency inside of the porous Gd_2O_3 nanoparticles, which is consistent with earlier work by using the porous Gd_2O_3 nanoparticles (~200 nm in diameter).^[30] Compared to FDA-approved iron oxide nanoparticle contrast agents such as Ferumoxtran (Resovist®, $65 \text{ mM}^{-1}\text{s}^{-1}$), cross-linked iron oxide particle (CLIO-Tat, $62 \text{ mM}^{-1}\text{s}^{-1}$), and water soluble iron oxide (WSIO, $78 \text{ mM}^{-1}\text{s}^{-1}$), our nanorice, nanoeyes, and hollow nanorice had similar T_2 relaxivities.^[48–51] However, these T_2 relaxivities were several times higher than typical gadolinium chelates (Gd-DTPA, $4.9 \text{ mM}^{-1}\text{s}^{-1}$)^[52] and Gd_2O_3 nanoparticles ($14.1\text{--}16.9 \text{ mM}^{-1}\text{s}^{-1}$).^[52–54] Most importantly, the contrast is sufficient for many drug delivery applications. For example, 5–10 mg/mL is a typical magnetic particle concentration in tumors for magnetic hyperthermia,^[55] while our particles have several times faster relaxation rates than the gel even at the lowest concentrations of $50 \mu\text{g/mL}$ (R_2 is 37.7 s^{-1} for the nanorice, 29.3 s^{-1} for nanoeyes, and 20.0 s^{-1} for the hollow nanorice, compared to $5.1 \pm 0.1 \text{ s}^{-1}$ for the unloaded gel).

Nanoparticle Cytotoxicity Assay

Previous studies toxicity of ~80 nm iron oxide nanoparticles found no acute or subacute toxic effects by histologic or serologic studies in rats or beagle dogs which received a total of 3 mmol Fe/kg.^[56] In order to study the potential cytotoxicity of the rare earth elements in the magnetic imaging probes (nanorice, nanoeyes, and hollow nanorice), an *in vitro* cytotoxicity test of hollow nanorice was performed on MCF-7 breast cancer cells (Fig. 11). The viability of the untreated cells was set as 100% in order to calculate the viability of the cells treated by different concentration of X-ray scintillators. Cell viability is not significantly affected by the Gd₂O₃:Eu nanoparticles up to concentration of at least 250 µg/ml (24 hr exposure).

Conclusion

We successfully prepared magnetic luminescent nanoparticles using silica coated hematite as a template. The silica coating plays a critical role on the growth of scintillator shell on the surface of the nanocores. After synthesis, the cores were made magnetic via high temperature reduction in H₂ and oxidization in air. The magnetic core size could be controlled while preserving the luminescent shell by dissolving the core in oxalic acid. Intermediate core sizes displayed both magnetic and luminescent properties. These novel magnetic luminescent nanoparticles are advantageous because they allow for magnetic separation and multimodal imaging with MRI, fluorescence and radioluminescence. We expect that such nanocomposites, combining the advantages of magnetism and luminescence, will find extensive applications in drug delivery and bioimaging. For example, the hollow structure in the partially dissolved iron oxide scintillator can be loaded with small dyes and drug molecules. Our synthesis technique is attractive because multifunctional particles can be made by coating the core templates with multiple layers of materials, each with a controlled thickness and composition. Different sizes, shapes (spherical and non-spherical), and structures (hollow and non-hollow) can be obtained by varying the shape and size of the hematite templates. Future work will study the effect of size and shape on magnetic, luminescent, and bio-distribution properties.

Supplementary Material

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Fig. 1.

(A) Schematic illustration of the synthesis of nanoeyes ($\gamma\text{-Fe}_2\text{O}_3@\text{SiO}_2@\text{Gd}_2\text{O}_3:\text{Eu}$), SEM (B) and TEM (C) image of nanoeyes. The inserted figure in Fig.1B is the size distribution of nanoeyes.

Fig. 2. TEM images of nanoeyes incubated in 0.5 M oxalic acid at 60 °C for (A) 8.5 h, (B) 9.5 h, (C) 10 h.

Fig. 3. XRD patterns of (a) precursor of nanoeye ($\alpha\text{-Fe}_2\text{O}_3@\text{SiO}_2@\text{Gd}(\text{OH})\text{CO}_3:\text{Eu}$), (b) nanoeye $\gamma\text{-Fe}_2\text{O}_3@\text{SiO}_2@\text{Gd}_2\text{O}_3:\text{Eu}$ (iron oxide core was incubated in oxalic acid for 9.5 h), (c) nanoeye (iron oxide core was incubated in oxalic acid for 9.5 h) with thin $\text{Gd}_2\text{O}_3:\text{Eu}$ shell (~10 nm). ($\blacktriangledown$: $\alpha\text{-Fe}_2\text{O}_3$, $\bullet$: $\text{Gd}_2\text{O}_3:\text{Eu}$, $\blacklozenge$: $\gamma\text{-Fe}_2\text{O}_3$)

Fig. 4.
TEM image of nanorice (A) and hollow nanorice (B).

Fig. 5.

The magnetic hysteresis loops of magnetic probes. (A) nanorice with maghemite as the core, (B) nanoeyes (iron oxide core was incubated in oxalic acid for 9.5 h), (C) nanorice with hematite as the core, and (D) hollow nanorice. Bottom inset shows the hysteresis loops of nanorice with hematite as the core and hollow nanorice. Top inset show rapid (3 min) magnetophoretic separation of a solution of nanoeyes in a 1 cm pathlength cuvette.

Fig. 6. Schematic presentation of magnetic modulation of scattering of light (A, B) by nanoeyes (iron oxide core was incubated in oxalic acid for 9.5 h), (C) Intensity time series for nanoeyes under darkfield microscope.

Fig. 7. (A) Radioluminescence spectra of nanoeyes (iron oxide core was incubated in oxalic acid for 9.5 h) and nanorice excited by X-ray, (B) Fluorescence spectra of nanoeyes (iron oxide core was incubated in oxalic acid for 9.5 h) and nanorice excited by 480 nm light

Fig. 8.
Photograph of hollow nanorice under (A) UV light (365 nm) and (B) X-ray luminescence.

Fig. 9.

T_2 and T_2^* -weighted images of nanorice, nanoeyes (iron oxide core was incubated in oxalic acid for 9.5 h), and hollow nanorice at echo time of 4 ms and 1.5 ms, respectively. Group A: T_2 -weighted images of nanorice with concentration of 0.8 mg/ml, 0.4 mg/ml, 0.1 mg/ml, and 0.05 mg/ml. Group B: T_2 -weighted images of nanoeyes with concentration of 0.8 mg/ml, 0.4 mg/ml, 0.1 mg/ml, and 0.05 mg/ml. Group C: T_2 -weighted images of hollow nanorice with concentration of 0.8 mg/ml, 0.4 mg/ml, 0.1 mg/ml, and 0.05 mg/ml. Group A*: T_2 -weighted images of nanorice with concentration of 0.8 mg/ml, 0.4 mg/ml, 0.1 mg/ml, and 0.05 mg/ml. Group B*: T_2^* -weighted images of nanoeyes with concentration of 0.8 mg/ml, 0.4 mg/ml, 0.1 mg/ml, and 0.05 mg/ml. Group C*: T_2 -weighted images of hollow nanorice with concentration of 0.8 mg/ml, 0.4 mg/ml, 0.1 mg/ml, and 0.05 mg/ml.

Fig. 10. The relaxation rate curves as a function of concentration. ■: nanorice, ▲: nanoeyes (iron oxide core was incubated in oxalic acid for 9.5 h), ● hollow nanorice. Error bars represent the standard deviation.

Fig. 11.
Cytotoxicity test for hollow nanorice (Gd₂O₃:Eu).