Published in final edited form as:

Small. 2014 January 15; 10(1): 160–168. doi:10.1002/sml.201300828.

Synthesis of brightly PEGylated luminescent magnetic upconversion nanophosphors for deep tissue and dual MRI imaging

Hongyu Chen^{a,†}, Bin Qi^{b,†}, Thomas Moore^c, Daniel C. Colvin^d, Prof. Thomas Crawford^e, Prof. John C. Gore^d, Prof. Frank Alexis^c, Prof. O. Thompson Mefford^b, and Jeffrey N. Anker^{a,*}

^aDepartment of Chemistry, Center for optical materials science and engineering (COMSET), and environmental toxicology program, Clemson University, Clemson SC, 29634, USA

^bDepartment of Materials Science Engineering, Center for optical materials science and engineering (COMSET), Clemson University, Clemson, SC 29634, USA

^cDepartment of Bioengineering, 301 Rhodes Research Center, Clemson, SC 29634, USA

^dVanderbilt University Medical Center, AAA 3107 MCN, Nashville, TN 37232, USA

^eDepartment of Physics and Astronomy and Smart State Center for Experimental Nanoscale Physics, University of South Carolina, Columbia, SC

Abstract

We developed a novel method to fabricate monodispersed biocompatible Yb/Er or Yb/Tm doped β -NaGdF₄ upconversion phosphors using polyelectrolytes to prevent irreversible particle aggregation during the conversion of the precursor (Gd₂O(CO₃)₂•H₂O:Yb/Er or Yb/Tm) to β -NaGdF₄:Yb/Er or Yb/Tm. The polyelectrolyte on the outer surface of nanophosphors also provided an amine tag for PEGylation. This method is also employed to fabricate PEGylated magnetic upconversion phosphors with Fe₃O₄ as the core and β -NaGdF₄ as a shell. These magnetic upconversion nanophosphors have relatively high saturation magnetization (7.0 emu g⁻¹) and magnetic susceptibility (1.7×10⁻² emu g⁻¹ Oe⁻¹), providing them with large magnetophoretic mobilities. We have studied their magnetic properties for separation and controlled release in flow, their optical properties for cell labeling, deep tissue imaging, and their T₁ and T₂-weighted magnetic resonance imaging (MRI) relaxivities. The magnetic upconversion phosphors display both strong magnetophoresis, dual MRI imaging (r₁=2.9 mM⁻¹ s⁻¹, r₂=204 mM⁻¹ s⁻¹), and bright luminescence under 1 cm chicken breast.

*Corresponding-Author: Prof. Jeffrey N. Anker., Department of Chemistry, Center for optical materials science and engineering (COMSET), and environmental toxicology program, Clemson University, Clemson SC, 29634, USA. Tel:+1-864-656-1726, janker@clemson.edu.

[†]These authors contribute equally.

Supporting Information: Supporting Information, comprising the experimental methods, and characterization data via X-ray diffraction, electron microscopy images, FTIR spectroscopy, and thermogravimetric analysis, is available from the Wiley Online Library or from the author.

Keywords

upconversion nanophosphor; magnetic nanoparticle; magnetic capture and release; deep tissue imaging; MRI

1. Introduction

Rare earth doped upconversion nanophosphors are promising bioimaging agents because of their narrow and distinct spectral emission peaks, the long optical penetration depth of their near-infrared excitation and red/near infrared emission, the negligible autofluorescence backgrounds generated at these wavelengths, and the particles' excellent photo & chemical stability and low toxicity.^[1] The luminescence of upconversion nanophosphors is generated by a sequential absorption of multiple low energy photons (e.g. 980 nm near infrared light) followed by fluorescent emission of higher energy photons from the excited states. They have been applied in immunoassays,^[2] chemical sensing,^[3] cell labeling,^[4] and small animal imaging.^[1b]

Upconversion nanophosphors are primarily synthesized by hydrothermal and thermal decomposition methods.^[1b] Traditional hydrothermal synthesis methods usually generate relatively monodispersed particles with controlled size and shape by incubating rare-earth precursors with fluoride salts in aqueous solutions at high temperature (150~300 °C).^[5] The thermal decomposition method involves the decomposition of organometallic reagents (e.g. rare-earth trifluoroacetates) with hydrophobic organic ligands (e.g. oleic acid, oleylamine) in a high boiling point organic solvent.^[6] In order to apply these hydrophobic upconversion nanophosphors in bioimaging, their surface usually needs to be functionalized with hydrophilic ligands. This method also has been demonstrated to yield upconversion nanophosphors with well dispersed size and shape.^[7]

Magnetic nanoparticles are another attractive material for bioimaging and actuation because they can be manipulated using external magnetic fields. They play an important role in biomedical applications such as bio-magnetic separation, drug delivery, MRI, and hyperthermia.^[8] Much effort has been applied to designing and fabricating multifunctional magnetic upconversion nanophosphors for multimodal imaging, separation, and sensing. Previous work on magnetic upconversion nanophosphor fabrication usually involved chemically conjugating, or physically attaching, magnetic particles to upconversion nanophosphors. In 2010, Shen and coworkers fabricated magnetic upconversion phosphor by conjugating iron oxide nanoparticles on the surface of upconversion phosphors by organic ligands.^[9] However, the magnetic nanophosphors synthesized in this work were relative large (~200 nm). In 2012, Zhang and coworkers reported using magnetic upconversion nanophosphors ($\text{Fe}_3\text{O}_4@ \alpha\text{-NaYF}_4\text{:Yb/Er}$, 115 $\pm$ 20 nm) as drug carrier for doxorubicin.^[10] However, the upconversion nanoshell is cubic phase $\text{NaYF}_4\text{:Yb/Er}$ ($\alpha\text{-NaYF}_4\text{:Yb/Er}$) which exhibits lower upconversion luminescent efficiency than hexagonal phase nanophosphors ($\beta\text{-NaYF}_4\text{:Yb/Er}$)^[11]. Cai *et. al.* reported a type magnetic upconversion nanophosphor (~80 nm) with a Fe_3O_4 core and $\beta\text{-NaGdF}_4\text{:Yb/Er}$ shell to serve as a drug carrier by attaching upconversion phosphors onto iron oxide nanoparticles.^[12] However, the iron oxide core needed to be coated with a thick porous

silica shell in order to load small α -NaGdF₄:Yb/Er nanoparticles into the porous silica shell. Meanwhile, calcination at 400 °C in H₂ atmosphere was required to convert α -NaGdF₄:Yb/Er to brighter luminescent β -NaGdF₄:Yb/Er. Liu and co-workers recently report a method to obtain Fe₃O₄@ β -NaGdF₄:Yb/Er by thermal decomposition of organometallic reagents on iron oxide nanoparticles.^[13] The obtained magnetic upconversion nanoparticles have a narrow size distribution and good size range ($\sim$ 30 nm) to achieve long blood circulation time. However, the non-water soluble surface limits its bio-applications. To obtain biocompatible magnetic upconversion nanoparticles with long blood circulation time, the size of particles should be large enough ($>$ 5 nm) to avoid renal filtration but small enough to minimize opsonization and reticuloendothelial system (RES) clearance.^[14] It remains a challenge to appropriately sized (5-100 nm) monodispersed multifunctional nanophosphors with both magnetic (e.g. Fe₃O₄) and brightly luminescent components (β -NaYF₄:Yb/Er or β -NaYF₄:Yb/Tm) because a thick upconversion nanoshell or a separation layer (e.g SiO₂) is needed to compensate luminescence absorption and quenching by magnetic core.

Herein, we report a novel method to fabricate monodispersed PEGylated hexagonal phase nanophosphors (β -NaGdF₄:Yb/Er and β -NaGdF₄:Yb/Tm). This novel method is further applied to synthesize magnetic upconversion nanophosphors with iron oxide core and hexagonal phase nanophosphors (β -NaGdF₄:Yb/Er and β -NaGdF₄:Yb/Tm). The resultant magnetic upconversion nano-phosphors display bright upconversion luminescence with low quenching by the iron oxide core. In addition, a novel technique was used to magnetically capture these upconversion nanophosphors from flowing solution onto a magnetized surface and release them with a rotating magnetic field for drug eluting stent applications. Furthermore, the magnetic upconversion nanophosphors were successfully used as cell labeling reagents and imaged through 1 cm chicken breast with low dose (0.2 ml, 1 mg/ml) under 980 nm laser excitation. Last but not least, these magnetic nanophosphors with gadolinium and iron contents are shown good dual-modal (T₁ and T₂-weighted) MRI contrast agents.

2. Results and discussion

2.1. Synthesis and optical properties of PEGylated upconversion nanophosphors

Upconversion nanophosphors were fabricated according to the scheme shown in Figure 1A. The Yb/Er and Yb/Tm doped precursor (Gd₂O(CO₃)₂•H₂O) of upconversion nanophosphor was first synthesized by precipitating rare earth ions by urea in aqueous solution. This precursor is highly positively charged, with a zeta potential of +98.5 mV for Gd₂O(CO₃)₂•H₂O:Yb/Er and +101.5 mV for Gd₂O(CO₃)₂•H₂O:Yb/Tm. Negatively charged sodium alginate (AL) and positively charged polyethyleneimine (PEI) were then alternately coated onto the nanophosphors layer-by-layer in order to prevent aggregation during conversion to NaGdF₄:Yb/Er or Yb/Tm in a teflon-lined autoclave. The PEI also provided amine groups for further functionalization. After the negative charged sodium alginate was coated on the surface of the precursor the zeta potential was changed to -62.9 mV and then shifted to +41.9 mV after the positive charged PEI coating. The polymer coated precursors were then converted to GdF₃ during the decomposition of NH₄F. The final step involves

converting the GdF_3 to $\beta\text{-NaGdF}_4$ in a 25 mL Teflon-lined autoclave in presence of NaF at 200 °C for 8 h. The powder X-ray diffraction (XRD) patterns in Figure S1 (Supporting information) show the amorphous structure of precursor ($\text{Gd}_2\text{O}(\text{CO}_3)_2 \cdot \text{H}_2\text{O}:\text{Yb/Er}$) and the structure of $\text{GdF}_3:\text{Yb/Er}$ before and after treatment with NH_4F . $\beta\text{-NaGdF}_4:\text{Yb/Er}$ can be clearly identified by the typical peaks after the $\text{GdF}_3:\text{Yb/Er}$ were incubated in autoclave with NaF 200 °C for 8 h.

The size of the nanophosphor is continuously tunable from 50 nm to 300 nm by controlling the size of precursor synthesized (Figure S2, supporting information). In order to apply these nanophosphors in bioimaging, amine groups were covalently bonded to PEG by DCC/NHS chemistry.^[16] The TEM images shown in Figure 1 indicate the obtained PEGylated upconversion nanophosphors were spherical and monodispersed with mean diameter of 50 +/- 5 nm (the size distribution is shown in Figure S3, supporting information). The AL and PEI coating were shown to be very effective at preventing aggregation. The obtained phosphors without polyelectrolyte coating prior to hydrothermal treatment were irregular microsized crystals (Figure S4, supporting information). The presence of PEG coating was confirmed by zeta potential (-21.0 mV) and FTIR (Figure S5, supporting information).

The upconversion nanophosphors display bright luminescence under 980 nm laser excitation (Figure 1D, E). The main emission peaks of the PEGylated $\beta\text{-NaGdF}_4:\text{Yb/Er}$ were observed at 537 and 654 nm, which corresponds to the Er^{3+} transition $^4\text{S}_{3/2} \rightarrow ^4\text{I}_{15/2}$ and $^4\text{F}_{9/2} \rightarrow ^4\text{I}_{15/2}$, respectively. The Yb/Tm doped nanophosphors ($\beta\text{-NaGdF}_4:\text{Yb/Tm}$) displayed very intense luminescence at 800 nm which was assigned to the $^3\text{H}_4 \rightarrow ^3\text{H}_6$ transition of Tm^{3+} . These near-infrared exciting and emitting upconversion phosphors are promising for cell labeling and deep tissue imaging applications.

2.2 Synthesis and optical properties of magnetic upconversion nanophosphors

In order to demonstrate the flexibility of this controlled synthesis method, magnetic upconversion nanophosphors were synthesized with a magnetic core and upconversion shell. As illustrated in Figure 2, hydrophobic oleic acid capped Fe_3O_4 nanoseeds were first treated with NaIO_4 to make their surface hydrophilic.^[17] The strong oxidizer- NaIO_4 breaks the double bond in oleic acid and generates a carboxyl group at the end of the ligand (zeta potential: -23.5 mV). The precursor of the upconversion nanophosphor ($\text{Gd}_2\text{O}(\text{CO}_3)_2 \cdot \text{H}_2\text{O}:\text{Yb/Er}$ or Yb/Tm) was deposited on the surface of iron oxide nanoparticles sequentially by a modified protocol from literature.^[15b, 15d] The rest of the protocol, i.e. polymer coating, NH_4F etching, autoclave incubation with NaF, and PEGylation are similar to the previously described single-component upconversion nanoparticle synthesis.

The TEM image in Figure 2B shows the monodispersed hydrophilic Fe_3O_4 nanoseeds with mean size of 17.5 ± 3 nm in diameter after surface modification. The core shell structure of magnetic upconversion phosphor precursor ($\text{Fe}_3\text{O}_4@\text{Gd}_2\text{O}(\text{CO}_3)_2\cdot\text{H}_2\text{O}:\text{Yb/Er}$) with an iron oxide core and ~ 25 nm upconversion phosphor precursor shell ($\text{Gd}_2\text{O}(\text{CO}_3)_2\cdot\text{H}_2\text{O}:\text{Yb/Er}$) is clearly distinguished in Figure 2C. After the $\text{Gd}_2\text{O}(\text{CO}_3)_2\cdot\text{H}_2\text{O}:\text{Yb/Er}$ was converted to $\beta\text{-NaGdF}_4:\text{Yb/Er}$ and the surface was functionalized with PEG, monodispersed PEGylated $\text{Fe}_3\text{O}_4@\beta\text{-NaGdF}_4:\text{Yb/Er}$ was obtained with an average size of 65 ± 17 nm. (Figure S6B, supporting information) The interface between Fe_3O_4 and $\beta\text{-NaGdF}_4:\text{Yb/Er}$ is not distinguishable in the TEM image due to low contrast between the materials of magnetic core (Fe_3O_4) and luminescent shell ($\beta\text{-NaGdF}_4:\text{Yb/Er}$). The PEGylated $\text{Fe}_3\text{O}_4@\beta\text{-NaGdF}_4:\text{Yb/Tm}$ nanophosphors were synthesized by the same method with an average diameter of 68 ± 18 nm in diameter. (Figure 2E, Figure S6B, supporting information). However, the Fe_3O_4 core still can be identified from its XRD patterns in Figure S7 (supporting information). Figure S7a and b display the XRD patterns of the Fe_3O_4 seeds and magnetic luminescent precursor ($\text{Fe}_3\text{O}_4@\text{Gd}_2\text{O}(\text{CO}_3)_2\cdot\text{H}_2\text{O}:\text{Yb/Er}$), respectively. After the amorphous $\text{Gd}_2\text{O}(\text{CO}_3)_2\cdot\text{H}_2\text{O}:\text{Yb/Er}$ shell was converted to $\beta\text{-NaGdF}_4:\text{Yb/Er}$, the XRD patterns corresponding to Fe_3O_4 core in Figure S7c (Supporting information) are relative weak because the cores have a small volume percentage and cross-section compared to the $\text{NaGdF}_4:\text{Yb/Er}$. The presence of AL, PEI, and PEG was confirmed by FTIR (Figure S8, Supporting information).

Previous work reported that when the phosphor is in direct contact with the iron oxide, the fluorescence or luminescence will be quenched. The common method to prevent the quenching is to coat a silica or polymer spacer layer (>50 nm thick) between iron oxide and luminescent shell.^[12, 18] Even though our magnetic nanophosphors do not have a spacer layer, they still display bright luminescence under 980 nm laser (Figure 2F, G). Compared with the upconversion nanosphers without magnetic component (Figure 1), both the Er^{3+} and Tm^{3+} doped magnetic upconversion nanophosphors show a low luminescent quenching effect as a result of the magnetic core. The emission peaks at shorter wavelength are attenuated more than the emission peaks at longer wavelength which is consistent with the extinction spectrum of iron oxide nanocores (Figure 2H).^[19]

2.3 Magnetically controlled capture and release of magnetic upconversion nanophosphors

The presence of iron oxide can be clearly observed in the magnetic hysteresis loop of PEGylated $\text{Fe}_3\text{O}_4@\beta\text{-NaGdF}_4:\text{Yb/Er}$ compared with the hysteresis loop of $\beta\text{-NaGdF}_4:\text{Yb/Er}$. The room temperature magnetic hysteresis loop of oleic acid capped iron oxide nanoparticles shows the saturation magnetization was 44 emu/g which is lower than reported result (~ 84 emu/g)^[20] on bulk magnetite due to the large amount (33% w/w, Figure S9, supporting information) of oleic acid absorbed on the surface of iron oxide. The magnetic hysteresis loop of $\beta\text{-NaGdF}_4:\text{Yb/Er}$ at 300 K indicates the upconversion phosphor is paramagnetic (Figure 3), with a magnetic susceptibility of 1.1×10^{-4} emu/g/Oe. After the iron oxide was encapsulated into the paramagnetic $\beta\text{-NaGdF}_4:\text{Yb/Er}$, the magnetic hysteresis curve includes a ferrimagnetic component with a saturation magnetization of 7.0 emu/g (64 emu/g normalized by the mass of Fe_3O_4), a coercivity of 20 Oe, and a magnetic susceptibility (initial slope) of 1.7×10^{-2} emu/g/Oe. The saturation magnetization of the

magnetic nanophosphors ($\text{Fe}_3\text{O}_4@ \beta\text{-NaGdF}_4\text{:Yb/Er}$) was significantly smaller than the saturation magnetization of bulk magnetite, which is 84 emu/g.^[20] The lower measured saturation magnetization can be attributed in large part to the rather small size of the iron oxide nanoparticles^[21] and the added mass of the organic ligands and upconversion nanoshell ($\beta\text{-NaGdF}_4\text{:Yb/Er}$). Hysteresis is observed in the dry sample because the 17 nm magnetite cores are thermally blocked at room temperature, but in solution the particles will rotate with a Brownian relaxation time of $\sim 120 \mu\text{s}$, and will be superparamagnetic at longer time scales.^[22] The aqueous solution of magnetic upconversion nanophosphors can be separated by a magnet within 2 min.

Magnetic particles can be used as direct drug carriers to local sites in the human body, such as stents,^[23] tumors,^[24] the inner ear,^[25] the retina of the eye,^[26] and other regions. The magnetic force on these magnetic particles is given by (in cgs units):

$$\vec{F} = \nabla(\vec{m}_{particle}(\vec{H}) \bullet \vec{H})$$

Where $\vec{F}$ is the magnetophoretic force (dyn), $\vec{m}_{particle}$ is the magnetic moment of the particle (emu), and $\vec{H}$ is the external applied magnetic field (Oe). In solution, the particle magnetic moment aligns with the external field and experiences a force towards the region of highest field, with a force proportional to the gradient.^[27] Because the external magnetic field decays roughly with the radius of the magnetic source, there is an inherent tradeoff between magnetic field uniformity and field gradient such that the strongest field gradients occur in close proximity to small magnetic field sources. Implanted magnetic materials such as stents offer a method to generate strong local fields and field gradients to capture particles. Stents are widely used to expand blood vessels in treatment of coronary heart disease, peripheral artery disease, renal artery stenosis, and carotid artery disease but create risk of potentially fatal thrombosis.^[28] Drug eluting polymer coatings have been developed to reduce inflammation, but it's difficult to control drug release over long periods and cannot be replenished. Magnetizable stents have been used to capture endothelial cells^[29] or drugs such as paclitaxel^[23] and sirolimus^[30] by magnetic nanoparticles to prevent stent thrombosis^[28] and restenosis,^[28] relying on external fields to magnetize the stents in order to create large field gradients. Alternatively, to capture particles in the absence of an external field, permanent magnetic materials may be used such as duplex steel.^[29] For all these applications, it is important to have magnetic carriers that can be controlled and imaged in order to study accumulation and release. We show here that our particles can be captured via a permanently magnetized film, and that the particles can be released after capture using an external magnetic field.

Figure 4 shows the principle of magnetic upconversion nanophosphors capture and release by a film of permanent magnetic materials. The magnetic film is a refrigerator magnet with an adhesive backing (McMasterCarr, Inc., Atlanta, GA) which is composed of an array of alternating magnetic regions 1 mm wide (Figure 4A); this geometry is designed to generate strong field gradients over small distances in order to attach strongly to the iron in refrigerator doors. The magnetic field gradients are strong enough to capture upconversion

nanophosphors from a flowing solution of nanophosphors in PBS (Figure 4B). The captured magnetic upconversion nanophosphors can be imaged under 980 nm light, during capture and holding (Figure 4C). In order to release the particles, an external magnetic field is applied in order to rearrange the particles into chains aligned with this field. The formation of chains pushes the particles into the fluid, where they are carried by the flow in the tube (Figure 4D). The flow velocity near the surface to be essentially static, but increases quadratically towards the center of the tube. Permanent magnetic arrays materials have been used to assemble magnetic particle arrays,^[31] to transport them across a magnetic array surface in rotating magnetic fields,^[32] and to push magnetic fluids into the inner ear.^[25, 33] To our knowledge, however, this is the first report of nanoparticle capture and release from a permanent magnetic structure. Compared to conventional approaches of capturing particles with soft magnetic materials by applying a field and release by removing the field, the advantage of our approach is that for biomedical applications, capture often occurs over long periods where application of a magnetic field is inconvenient, and release is often desired to be rapid and controlled.

Figure 5 A and B shows a photograph of the magnetic upconversion nanophosphors during captured from a 1 mg/mL solution in PBS fluid with a flow rate of 0.5 mL/s in a 5 mm inner diameter tube. The captured particles form a dashed-line pattern when they are attracted to the high field gradient regions between the alternating magnetic domains in the fridge magnet (Figure 5B and C). The pattern of particles is most clearly observed under 980 nm irradiation (Figure 5E). This bright upconversion luminescence can be used to track the particles in deep tissue due to the near infrared emission. After applying a rotating external magnetic field, the magnetic upconversion nanophosphors are released into the fluid (Figure 5D and F). This is significant because it shows that particles can be captured and retained on magnetized surfaces for long periods, and subsequently released using external magnetic fields. Future work will investigate the amount released as a function of external field strength, pulse duration, and flow rate. We will also functionalize particles with anti-inflammatory and antimicrobial drugs.

2.4 *In vitro* cytotoxicity test and bio-imaging by upconversion nanophosphors

To test whether the synthesized upconversion nanophosphors would be endocytosed by cells, we incubated MCF-7 breast cancer cells with PEGylated magnetic upconversion phosphors ($\text{Fe}_3\text{O}_4@ \beta\text{-NaGdF}_4\text{:Yb/Er}$, 250 $\mu\text{g/ml}$) for 24 hrs. The MCF-7 breast cancer cells were then washed six times to eliminate nanocapsules from the cell culture media. As shown in Figure 6, the cells incubated with magnetic nanophosphors became brightly luminescent under 980 nm excitation, while there was no observed luminescence from the unlabeled control sample.

In order to study the potential cytotoxicity of the magnetic upconversion nanophosphors, an *in vitro* cytotoxicity test of magnetic upconversion nanophosphors was performed on MCF-7 breast cancer cells (Figure 7). The viability of the untreated cells was set as 100% to calculate the viability of the cells treated by different concentration of upconversion nanophosphors. Cell viability is not significantly affected by the magnetic upconversion nanophosphor up to concentration of at least 1000 $\mu\text{g/ml}$.

Rare earth elements are toxic in their free ionic form.^[34] For example, gadolinium MRI contrast agents can reduce neuromuscular transmission and interfere with cell membranes and intracellular enzymes because Gd^{3+} can prevent the transportation of calcium through ion channels of muscle cells and nerve tissue cells if the chelator is not sufficiently stable.^[35] To test the chemical stability of the magnetic upconversion $Fe_3O_4@β-NaGdF_4:Yb/Er$ particles, we incubated a solution of particles (1 mg/ml) in nitrate acid (0.5 M) at 37 °C for 24 hr. ICP tests for free Gd^{3+} and Fe^{3+} showed no detectable dissolution of magnetic core and luminescent shell ($Fe < 0.1$ ppm, $Gd < 0.1$ ppm), while in a control with a mixture of hydrophilic Fe_3O_4 nanoparticles and $β-NaGdF_4:Yb/Er$ nanoparticles, the Fe_3O_4 nanoparticles completely dissolved. These results indicate that upconversion phosphors ($β-NaYF_4:Yb/Er$) are highly chemically stable and prevent dissolution of magnetic core in acid environments (e.g. lysosomes and endosomes). The intense luminescent signal and chemical stability of magnetic nanophosphors is useful in cell labeling and magnetic guidance targeting.

2.5 Deep tissue and MRI imaging of magnetic nanophosphors

To demonstrate the feasibility of the magnetic nanophosphors for imaging through deep tissue (e.g. for locating tumors), PEGylated $Fe_3O_4@β-NaGdF_4:Yb/Tm$ nanophosphors with near infrared emission at 800 nm were injected into chicken breast. These magnetic nanophosphors display extremely bright luminescence without background signal under 1 cm thickness of chicken breast at a dose of 1 mg/ml (200 $μ$ l), see Figure 8.

The magnetic upconversion nanophosphors may also potentially serve as T_1 and T_2 -weighted MRI contrast agents due to the single domain ferrimagnetic Fe_3O_4 core and paramagnetic shell ($β-NaGdF_4$). In order to measure their relaxivity, different concentrations of $Fe_3O_4@β-NaGdF_4:Yb/Tm$ nanophosphors were prepared in 1 wt% agarose gel and imaged with a 4.7 T MRI instrument. Figure 9 clearly shows the positive T_1 and negative T_2 contrast effects of the magnetic nanophosphors: the T_1 -weighted images become brighter with increased particle concentration, while the T_2 -weighted images become darker with increased particle concentration. The T_1 relaxivity coefficient (r_1) for the magnetic nanophosphor ($Fe_3O_4@β-NaGdF_4:Yb/Tm$) could also be calculated from the curve of $1/T_1$ vs. concentration of gadolinium (Figure 9A). The data shows that r_1 is $2.9 \text{ mM}^{-1} \text{ s}^{-1}$ which is similar to the relaxivity ($2.8 \text{ mM}^{-1} \text{ s}^{-1}$) reported on $NaGdF_4:Yb/Er$ ^[36]. However, the calculated r_2 is as high as $204 \text{ mM}^{-1} \text{ s}^{-1}$ which is much larger than 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, $78 \text{ mM}^{-1} \text{ s}^{-1}$).^[37]

3. Conclusion

We successfully developed a novel method to synthesize PEGylated upconversion nanophosphors with controlled monodispersed sizes, and prevented aggregation via polyelectrolyte coating of the precursors. The approach is versatile and was used to prepare PEGylated magnetic upconversion nanophosphors with an iron oxide core and a luminescent shell of uniform thickness. We expect that such bifunctional nanocomposites, combining the advantages of magnetism and luminescence, will find extensive applications in magnetic

delivery and MRI/luminescence bioimaging. Future work will focus on the magnetic field guided *in vivo* tumor targeting and stent functionalization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported in part by NIH award 1R15EB014560 to HC and JNA, an NSF award CMMI-1100684 to BQ and OTM, and an NSF award CMMI-1130636 to TC. Electron microscopy images were acquired at the Clemson University Electron Microscope Facility and use of this facility was funded by The Center of Biomaterials for Tissue Regeneration (CBTR) funded under NIH grants 5P20RR021949 and 8P20GM103444.

References

1. a) Mader HS, Kele P, Saleh SM, Wolfbeis OS. *Current Opinion in Chemical Biology*. 2010; 14:582. [PubMed: 20829098] b) Zhou J, Liu Z, Li F. *Chemical Society Reviews*. 2012; 41c) Wang F, Banerjee D, Liu Y, Chen X, Liu X. *Analyst*. 2010; 135:1839. [PubMed: 20485777]
2. Soukka T, Rantanen T, Kuningas K. *Annals of the New York Academy of Sciences*. 2008; 1130:188. [PubMed: 18596348]
3. Achatz, D.; Ali, R.; Wolfbeis, O. *Luminescent Chemical Sensing, Biosensing, and Screening Using Upconverting Nanoparticles*. Prodi, L.; Montalti, M.; Zaccheroni, N., editors. Vol. 300. Springer Berlin Heidelberg; 2011. p. 29
4. Wang F, Liu X. *Chemical Society Reviews*. 2009; 38:976. [PubMed: 19421576]
5. a) Wang L, Li Y. *Chemistry of Materials*. 2007; 19:727.b) Li Z, Zhang Y. *Nanotechnology*. 2008; 19:345606. [PubMed: 21730655]
6. a) Mai HX, Zhang YW, Si R, Yan ZG, Sun LD, You LP, Yan CH. *Journal of the American Chemical Society*. 2006; 128:6426. [PubMed: 16683808] b) Teng X, Zhu Y, Wei W, Wang S, Huang J, Naccache R, Hu W, Tok AIY, Han Y, Zhang Q, Fan Q, Huang W, Capobianco JA, Huang L. *Journal of the American Chemical Society*. 2012; 134:8340. [PubMed: 22506543]
7. a) Yi GS, Chow GM. *Advanced Functional Materials*. 2006; 16:2324.b) Chen G, Ohulchanskyy TY, Kumar R, Ågren H, Prasad PN. *ACS Nano*. 2010; 4:3163. [PubMed: 20509664]
8. a) Hu FQ, Wei L, Zhou Z, Ran YL, Li Z, Gao MY. *Advanced Materials*. 2006; 18:2553.b) Chen FH, Zhang LM, Chen QT, Zhang Y, Zhang ZJ. *Chemical Communications*. 2010; 46:8633. [PubMed: 20941412] c) Yu MK, Jeong YY, Park J, Park S, Kim JW, Min JJ, Kim K, Jon S. *Angewandte Chemie International Edition*. 2008; 47:5362.d) Jun, Yw; Huh, YM.; Choi, Js; Lee, JH.; Song, HT.; KimKim; Yoon, S.; Kim, KS.; Shin, JS.; Suh, JS.; Cheon, J. *Journal of the American Chemical Society*. 2005; 127:5732. [PubMed: 15839639] e) Katagiri K, Imai Y, Koumoto K, Kaiden T, Kono K, Aoshima S. *Small*. 2011; 7:1683. [PubMed: 21567941] f) Guardia P, Di Corato R, Lartigue L, Wilhelm C, Espinosa A, Garcia-Hernandez M, Gazeau F, Manna L, Pellegrino T. *ACS Nano*. 2012; 6:3080. [PubMed: 22494015]
9. Shen J, Sun LD, Zhang YW, Yan CH. *Chemical Communications*. 2010; 46:5731. [PubMed: 20585692]
10. Zhang F, Braun GB, Pallaoro A, Zhang Y, Shi Y, Cui D, Moskovits M, Zhao D, Stucky GD. *Nano Letters*. 2011; 12:61. [PubMed: 22133237]
11. He M, Huang P, Zhang C, Hu H, Bao C, Gao G, He R, Cui D. *Advanced Functional Materials*. 2011; 21:4470.
12. Gai S, Yang P, Li C, Wang W, Dai Y, Niu N, Lin J. *Advanced Functional Materials*. 2010; 20:1166.
13. Zhong C, Yang P, Li X, Li C, Wang D, Gai S, Lin J. *RSC Advances*. 2012; 2:3194.
14. Yoo JW, Chambers E, Mitragotri S. *Current pharmaceutical design*. 2010; 16:2298. [PubMed: 20618151]

15. a) Åhrén M, Selegård La, Klasson A, Söderlind F, Abrikosova N, Skoglund C, Bengtsson Tr, Engström M, Käll PO, Uvdal K. *Langmuir*. 2010; 26:5753. [PubMed: 20334417] b) Chen H, Colvin DC, Qi B, Moore T, He J, Mefford OT, Alexis F, Gore JC, Anker JN. *Journal of Materials Chemistry*. 2012; 22:12802. [PubMed: 24520183] c) Petoral RM, Söderlind F, Klasson A, Suska A, Fortin MA, Abrikosova N, Selegård La, Käll PO, Engström M, Uvdal K. *The Journal of Physical Chemistry C*. 2009; 113:6913. d) Chen H, Moore T, Qi B, Colvin DC, Jelen EK, Hitchcock DA, He J, Mefford OT, Gore JC, Alexis F, Anker JN. *ACS Nano*. 2013
16. Chul Cho K, Hoon Jeong J, Jung Chung H, Joe CO, Wan Kim S, Gwan Park T. *Journal of Controlled Release*. 2005; 108:121. [PubMed: 16139916]
17. Wang M, Peng M, Cheng W, Cui Y, Chen C. *Journal of Nanoscience and Nanotechnology*. 2011; 11:3688. [PubMed: 21776755]
18. Yu X, Shan Y, Li G, Chen K. *Journal of Materials Chemistry*. 2011; 21:8104.
19. a) Schlegel A, Alvarado SF, Wachter P. *Journal of Physics C: Solid State Physics*. 1979; 12:1157. b) Tang J, Myers M, Bosnick KA, Brus LE. *The Journal of Physical Chemistry B*. 2003; 107:7501.
20. O'handley, RC. *Modern magnetic materials: principles and applications*. Wiley New York: 2000.
21. Goya GF, Berquo TS, Fonseca FC, Morales MP. *Journal of Applied Physics*. 2003; 94:3520.
22. Rosensweig RE. *Journal of Magnetism and Magnetic Materials*. 2002; 252:370.
23. Chorny M, Fishbein I, Yellen BB, Alferiev IS, Bakay M, Ganta S, Adamo R, Amiji M, Friedman G, Levy RJ. *Proceedings of the National Academy of Sciences*. 2010; 107:8346.
24. Fortin-Ripoche JP, Martina MS, Gazeau F, Ménager C, Wilhelm C, Bacri JC, Lesieur S, Clément O. *Radiology*. 2006; 239:415. [PubMed: 16549622]
25. Sarwar A, Lee R, Depireux DA, Shapiro B. *Magnetics, IEEE Transactions on*. 2013; 49:440.
26. Yanai A, feli UO, Metcalfe AL, Soema P, Addo L, Gregory-Evans CY, Po K, Shan X, Moritz OL, Gregory-Evans K. *Cell Transplantation*. 2012; 21:1137. [PubMed: 22405427]
27. Cherry EM, Maxim PG, Eaton JK. *Medical Physics*. 2010; 37:175. [PubMed: 20175479]
28. Kempe H, Kempe M. *Biomaterials*. 2010; 31:9499. [PubMed: 20732712]
29. Tefft BJ, Gooden JY, Uthamaraj S, Harburn JJ, Klabusay M, Holmes DR, Simari RD, Dragomir-Daescu D, Sandhu GS. *Magnetics, IEEE Transactions on*. 2013; 49:463.
30. Chong PH, Cheng JW. *The Annals of Pharmacotherapy*. 2004; 38:661. [PubMed: 14766994]
31. Yellen B, Friedman G, Feinerman A. *Journal of Applied Physics*. 2003; 93:7331.
32. Yellen BB, Erb RM, Son HS, Hewlin JR, Shang H, Lee GU. *Lab on a Chip*. 2007; 7:1681. [PubMed: 18030387]
33. Guzzella L, Colaneri P, Banaszuk A. *IEEE Control Systems Magazine*. 2012
34. a) Arsenault TM, King BF, Marsh JW, Goodman JA, Weaver AL, Wood CP, Ehman RL. *Mayo Clinic Proceedings*. 1996:1150. [PubMed: 8945485] b) Spencer AJ, Wilson SA, Batchelor J, Reid A, Pees J, Harpur E. *Toxicologic pathology*. 1997; 25:245. [PubMed: 9210255]
35. Perazella MA. *Current drug safety*. 2008; 3:67. [PubMed: 18690983]
36. Johnson NJJ, Oakden W, Stanisz GJ, Scott Prosser R, van Veggel FCJM. *Chemistry of Materials*. 2011; 23:3714.
37. a) Wang Y, Hussain S, Krestin G. *European radiology*. 2001; 11:2319. [PubMed: 11702180] b) Josephson L, Tung C, Moore A, Weissleder R. *Bioconjugate Chemistry*. 1999; 10:186. [PubMed: 10077466] c) Jun Y, Huh Y, Choi J, Lee J, Song H, Kim K, Yoon S, Kim K, Shin J, Suh J, Cheon J. *Journal of the American Chemical Society*. 2005; 127:5732. [PubMed: 15839639]
38. Park J, An K, Hwang Y, Park JG, Noh HJ, Kim JY, Park JH, Hwang NM, Hyeon T. *Nat Mater*. 2004; 3:891. [PubMed: 15568032]

Figure 1.

(A) Schematic figure showing the synthesis route of PEGylated β -NaGdF₄: Yb/Er and β -NaGdF₄: Yb/Tm nanophosphors. (B) TEM image of PEGylated β -NaGdF₄: Yb/Er (C) TEM image of PEGylated β -NaGdF₄: Yb/Tm nanophosphors. Upconversion luminescence spectra for (D) PEGylated β -NaGdF₄: Yb/Er and (E) PEGylated β -NaGdF₄: Yb/Tm nanophosphors. The insets show photographs of the luminescence from the respective particle solutions in a 1 cm plastic cuvette during irradiation with a 980 nm laser.

Figure 2.

(A) Schematic figure showing the synthesis route of PEGylated Fe₃O₄@β-NaGdF₄: Yb/Er or Yb/Tm nanophosphors. TEM image of (B) Fe₃O₄ seeds after surface modification, (C) Fe₃O₄@Gd₂O(CO₃)₂·H₂O:Yb/Er@AL/PEI, (D) PEGylated Fe₃O₄@NaGdF₄:Yb/Er, (E) PEGylated Fe₃O₄@NaGdF₄:Yb/Tm. Upconversion luminescent spectra and images for (F) PEGylated Fe₃O₄@β-NaGdF₄:Yb/Er, (G) PEGylated Fe₃O₄@β-NaGdF₄:Yb/Tm nanophosphor, (H) Extinction spectrum of a 0.18 mg/mL colloidal solution of oleic acid-coated magnetite cores in a 1 cm path length cuvette.

Figure 3.

Magnetic hysteresis loop of solid powders of oleic acid capped Fe_3O_4 , PEGylated $\beta\text{-NaGdF}_4:\text{Yb/Er}$, Fe_3O_4 , and PEGylated $\text{Fe}_3\text{O}_4 @ \beta\text{-NaGdF}_4:\text{Yb/Er}$. Inset figure A shows magnetic upconversion nanoparticles in a 1 cm path length cuvette, while inset figure B shows the clear solution remaining after application of a magnetic field (the cylindrical magnet) for 2 minutes. Note, the brown reflection on the cylindrical magnet is from particles on the right edge of the cuvette.

Figure 4. Schematic showing the magnetic capture and release of magnetic upconversion nanophosphors.

Figure 5.

Photographs of (A) magnetic upconversion nanophosphors solution in a glass tube with a magnetic film in the tube (black rectangle) at $t=0$ min, (B) magnetic upconversion nanophosphors fluid with captured in an array pattern the magnetic film ($t=10$ min), (C) captured magnetic upconversion nanophosphors held on magnetic film during rinsing PBS solution, (D) magnetic film after the magnetic upconversion nanophosphors were released by the external field, (E) Upconversion luminescence of captured magnetic upconversion nanophosphors on magnetic film during rinsing with PBS solution (the size of luminescent area depends on the beam size of the 980 nm laser), (F) Upconversion luminescence of magnetic film after magnetic upconversion phosphors were released by external magnetic field.

Figure 6.

Photographs of MCF-7 breast cancer cell suspensions in 1.5 mL centrifuge tubes. Cells incubated with PEGylated $\text{Fe}_3\text{O}_4@ \beta\text{-NaGdF}_4\text{:Yb/Er}$ were viewed either under room light (A) or 980 nm irradiation (B). Control cells without incubation with PEGylated $\text{Fe}_3\text{O}_4@ \beta\text{-NaGdF}_4\text{:Yb/Er}$, were viewed either under room light (C) or 980 nm irradiation (D).

Figure 7.
Cytotoxicity study of PEGylated β -NaGdF₄:Yb/Er and PEGylated Fe₃O₄@ β -NaGdF₄:Yb/Er.

Figure 8.

Deep tissue imaging of magnetic upconversion nanophosphors after 200 μ l of (A) PBS solution and (B) 1 mg/ ml PEGylated $\text{Fe}_3\text{O}_4@ \beta\text{-NaGdF}_4\text{:Yb/Tm}$ were injected into chicken breast at 1 cm depth. The concentric cylindrical object at the right of each image is an SMA905 terminus of an optical fiber used to illuminate the tissue with 980 nm light. The black and white chicken breast image was acquired under overhead illumination, and the pseudo-colored upconversion luminescence image was superimposed.

Figure 9.

T_1 and T_2 -weighted MRI images and relaxivities for PEGylated magnetic upconversion nanophosphors ($\text{Fe}_3\text{O}_4@ \beta\text{-NaGdF}_4\text{:Yb/Er}$).