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Rare Earth doped nanoparticles in imaging and PDT

Brian G. Yust^{*,a}, Dhiraj K. Sardar^a, Lawrence C. Mimun^a, Ajith K. Gangadharan^a, and Andrew T. Tsin^b

^aDept. of Physics & Astronomy, University of Texas at San Antonio, San Antonio, Tx, USA 78249

^bDept. of Biology, University of Texas at San Antonio, San Antonio, Tx, USA 78249

Abstract

Nanoparticles doped with rare earth ions for biomedical imaging and infrared photodynamic therapy (IRPDT) have been synthesized, characterized, and compared. Specifically, these nanoparticles utilize two primary modalities: near infrared excitation and emission for imaging, and near infrared upconversion for photodynamic therapy. These nanoparticles are optimized for both their infrared emission and upconversion energy transfer to a photoactive agent conjugated to the surface. Finally, these nanoparticles are tested for toxicity, imaged in cells using the near infrared emission pathway, and used for selective killing of cells through the upconversion driven IRPDT.

Keywords

Rare earths; upconversion; infrared photodynamic therapy; biomedical imaging

1. INTRODUCTION

Rare earth doped nanoparticles have become an increasingly popular topic of study due to their many applications in lighting, displays, biomedicine, sensing, and security, particularly because of their unique optical properties. Rare earth ions are attractive as emitters because, when doped into a host structure, they can exhibit extremely sharp absorption and emission lines and do not photobleach.^{1,2} For biomedical imaging and therapy, these properties make rare earth based nanoparticles extremely advantageous over conventional fluorescent dyes, and current work involving them has shown great promise.^{3,4} In the case of these nanoparticles, the absorbing and emitting rare earth ions, being doped into a nanocrystalline structure, are also well shielded from the outside environment meaning that there is little to no quenching due to the biological milieu. Also, the sharp emission lines of different rare earths allow for multiplexing where one can test for or image multiple objects or molecules of interest and distinguish them spectroscopically.

One particular type of rare earth based nanoparticle utilizes excited state absorption to take two infrared photons and convert them into visible emission. This phenomenon, known as

upconversion, is possible because of the electronic energy levels of certain rare earths such as erbium and ytterbium. Upconverting nanoparticles can be very useful for biomedical applications because biological tissues do not absorb or scatter near-infrared (NIR) light very strongly; therefore, NIR excitation sources can be used at higher powers than visible or ultraviolet sources without adversely affecting or damaging the tissues.^{5,6} Also, the low amount of scattering means that NIR light from excitation sources can effectively travel through more tissue before becoming too diffuse. Upconverting nanoparticles are not only useful for biomedical imaging but also photodynamic therapy.^{7,8} By conjugating a photosensitive molecule whose absorption overlaps with the emission lines of the nanoparticle, energy may be transferred resonantly from the emitter to the photosensitive molecule. This molecule then may create reactive oxidative species (ROS) such as single oxygen when in an aqueous environment which can kill cells (Fig. 1). In this way, the nanoparticles can be used to selectively kill cells such that only those cells with nanoparticles that are exposed to the excitation source are killed.

The work presented here involves the synthesis of different sodium and potassium metal fluoride nanoparticles doped with erbium and ytterbium. Spectroscopic and morphological analyses were carried out for each nanoparticle type. Upconverting nanoparticles were also implemented in *in vitro* imaging and photodynamic therapy experiments. Ultimately, these nanoparticles may be useful for targeted near-infrared imaging and photodynamic therapy of cancers and other diseases.

2. EXPERIMENTAL METHODS & MATERIALS

Sodium and potassium metal fluoride nanoparticles (NaMF_3 and KMF_3) doped with erbium and ytterbium were synthesized using a one-pot solvothermal method.^{2,9} Sodium fluoride (NaF) or potassium fluoride (KF) as well as the desired metal ($\text{M} = \text{Ba}, \text{Mg}, \text{Mn}, \text{Ni}, \text{Co}, \text{Fe}, \text{Cr}, \text{Y}, \text{Gd}$) and rare earth nitrates ($\text{Er}(\text{NO}_3)_3$, $\text{Yb}(\text{NO}_3)_3$) were dissolved in a solution of water, ethanol, and polyethylene glycol (PEG), transferred to a Teflon lined autoclave and heated to 200°C for 2 to 24 hours. The nanoparticles were washed thoroughly with water and ethanol and collected through centrifugation. The polymer (PEG) acts as a nucleating agent during the growth phase of the synthesis and leaves a final polymer coating on the nanoparticles so that they are immediately water-soluble after synthesis. This alleviates the need for post-processing of the nanoparticles to make them water-soluble, such as when they are synthesized in hydrophobic ligands like oleic acid. Emission spectra of each nanoparticle type were collected with a SPEX 1250M monochromator while under 980 nm excitation from a laser diode. SEM images were obtained for each sample using a Hitachi S-5500. Nanoparticles in fibroblast cells were imaged with a two photon microscope after incubation with the nanoparticles for 24 hours. The cells were prepared with a nuclear DAPI stain and Alexa flour 647 phalloidin stain.

Zinc phthalocyanine (ZnPc), a well-known photosensitive molecule, was adsorbed onto the surface of some of the nanoparticles to form an infrared photodynamic therapy (IRPDT) platform. First, ZnPc was dissolved in an aqueous/ethanol solution containing a polymer under vigorous stirring. Next, rare earth doped nanoparticles were added to the solution which was then stirred for 30 minutes to 24 hours. The polymers used for this work were

PEG, chitosan, and HEMA. These composite nanoparticles were washed thoroughly with ethanol and water and collected through centrifugation. To test the efficacy of these nanoparticles as potential IRPDT agents, they were first incubated with fibroblast cells at a concentration of 100 $\mu\text{g/mL}$ for 24 hours. Then, the cells were washed three times with a buffer solution to remove any excess nanoparticles not taken up by the cells. Then, the cells were exposed to 700 mW of 980 nm radiation from a diode laser for 5 minutes. Afterwards, the cells were given a 2 hour recovery period, washed, trypsinized, stained with trypan blue, and counted. Negative and positive controls were kept by exposing some cells without nanoparticles to the laser radiation and by not exposing some cells with nanoparticles to the laser radiation.

3. RESULTS

By varying the metal of the host nanocrystalline structure, a change in the upconversion emission profile can be achieved (Fig. 2 – 3). This shift in relative intensities for the different emission manifolds is due to a change in the crystal field which the absorbing and emitting rare earth ion experiences as well as the energy levels of the different metals composing the host structures. Therefore, for a system such as a nanoparticle codoped with erbium and ytterbium, one can tailor the upconversion emission profile to be almost completely green or red depending on the application. In fact, by taking the area under the curve for each spectrum, the percent of total upconversion emission for each emission manifold was calculated and can be found in Table 1. From this data, it is clear that NaYF_4 gives the greatest amount of green upconversion fluorescence which is consistent with findings from the literature that it is one of the most efficient hosts for the upconversion process. It is also clear that the samples containing Mg and Mn have some of the strongest red and NIR upconversion emission out of the samples examined which is why they were chosen to be included in the imaging and photodynamic therapy experiments as well.

Fibroblast cells incubated with KMgF_3 : ErYb and NaGdF_4 : ErYb nanoparticles were imaged under a two photon microscope (Fig 7 – 9). From these images we can clearly see that the PEG coated upconverting nanoparticles are taken up by the cells and appear within the cytoplasm and nucleus without any further surface functionalization. Figure 9 shows colocalization channels of the upconversion emission with the fluorescent emission from the DAPI and Alexa fluor stains which indicates that the nanoparticles being detected are within the nucleus or cytoplasm. It should also be noted that although these nanoparticles were imaged using a pulsed Ti:Sapphire laser within the two photon microscope system, the power source does not need to be either pulsed or particularly high power for the upconversion emission to be easily detectable. In fact, while imaging the cells with nanoparticles, we were using less than 1% of the total peak output power of the laser at 980 nm.

Finally, fibroblast cells were incubated with NaYF_4 : ErYb, KMnF_3 : ErYb, and KMgF_3 : ErYb nanoparticles conjugated with ZnPc by PEG, chitosan, or HEMA as described earlier and treated by laser for 5 minutes. The total cell populations were counted as a metric of the killing efficacy of these composite nanoparticles, and the results may be found in Table 2. It is evident that even with a single 5 minute exposure, enough ROS were produced to

effectively reduce the cell populations. Meanwhile, the control culture with no nanoparticles which was exposed to the laser as well did not decrease significantly. Furthermore, it should also be noted that the upconversion emission of the Mg and Mn containing nanoparticles overlaps a more absorptive region for the ZnPc which should allow for more efficient energy transfer from the emitting ions to the photosensitizer (Fig. 10). Therefore, using these nanoparticles with a NIR excitation source at 980 nm seems to be a very effective and promising route to selectively killing cells either for biological experiments or cancer therapies.

4. CONCLUSIONS

It has been shown that rare earth doped water-soluble upconverting nanoparticles can be easily synthesized by a solvothermal process. By changing the composition of the host nanocrystalline structure, the relative intensities of the emission profile can be altered. Of particular interest for biomedical applications are the cases of those hosts composed of either manganese or magnesium since these exhibited very strong red upconversion. These nanoparticles may prove to be very useful for imaging, sensing, and assays, especially if the upconversion emission can be further engineered to be truly single color. Furthermore, the red and infrared emission from these nanoparticle overlaps a spectral region of greater absorption for ZnPc which explains the more effective photodynamic treatment. These nanoparticles have also been imaged with a two photon microscope and shown to effectively be taken up by fibroblast cells without specific surface modification. Experiments are planned to image the uptake of these nanoparticles in real time without having to fix the cells to better understand the uptake mechanism and how the nanoparticles without surface modification interact with cells. Furthermore, imaging of live cells with ZnPc loaded nanoparticles is also planned to better understand the actual mechanism of killing the cells by photodynamic action. Finally, more experiments are planned to further optimize the conjugation of ZnPc to the nanoparticles and determine the best the appropriate nanoparticle dosage and laser treatment needed.

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REFERENCES

1. Mai HX, Zhang YW, et al. High-Quality Sodium Rare-Earth Fluoride Nanocrystals: Controlled Synthesis and Optical Properties. *Journal of the American Chemical Society*. 2006; 128(19):6427–6436.
2. Wang M, Mi CC, et al. One-step synthesis and characterization of water-soluble NaYF₄:Yb, Er/ Polymer nanoparticles with efficient up-conversion fluorescence. *Journal of Alloys and Compounds*. 2009; 485:L24–L27.
3. Xiong L, Yang T, et al. Long-term in vivo biodistribution imaging and toxicity of polyacrylic acid-coated upconversion nanophosphors. *Biomaterials*. 2010; 31:7078–7085. [PubMed: 20619791]
4. Deng M, Ma Y, et al. Monodisperse Upconversion NaYF₄ Nanocrystals: Syntheses and Bioapplications. *Nano Res*. 2011; 4(7):685–694.
5. Wang J, Wang F, et al. Single-Band Upconversion Emission in Lanthanide-Doped KMnF₃ Nanocrystals. *Angew. Chem. Int. Ed*. 2011; 50:10369–10372.

6. Zhou J, Zhuang L, Fuyou L. Upconversion nanophosphors for small-animal imaging. *Chem. Soc. Rev.* 2012; 41:1323–1349. [PubMed: 22008740]
7. Bechet D, Couleaud P, Frochot C, Viriot M, Guillemin F, Barberi-Heyob M. Nanoparticles as vehicles for delivery of photodynamic therapy agents. *Trends in Biotechnology.* 2008; 26(11):612–621. [PubMed: 18804298]
8. Cui S, Chen H, Zhu H, Tian J, Chi X, Qian Z, Achilefu S, Gu Y. Amphiphilic chitosan modified upconversion nanoparticles for in vivo photodynamic therapy induced by near-infrared light. *J. of Mat. Chem.* 2012; 22:4861–4873.
9. Chen Z, Chen H, et al. Versatile Synthesis Strategy for Carboxylic Acid-functionalized Upconverting Nanophosphors as Biological Labels. *Journal of the American Chemical Society.* 2008; 130(10):3023–3029. [PubMed: 18278910]

Figure 1.
Upconversion and photodynamic energy scheme for erbium – ytterbium codoped systems.

Figure 2.
Emission of various ErYb codoped upconverting nanoparticles under 980 nm excitation.

Figure 3.
Emission of various ErYb codoped upconverting nanoparticles under 980 nm excitation.

Figure 4.
SEM image of NaYF₄:ErYb nanoparticle.

Figure 5.
SEM Image of $\text{KMgF}_3:\text{ErYb}$ nanoparticle.

Figure 6.
SEM Image of $\text{NaMgF}_3:\text{ErYb}$ nanoparticle.

Figure 7.
Two photon image of fibroblast cells (blue = nucleus, red = cytoskeleton) with NaGdF₄:
ErYb nanoparticles (green = upconversion emission).

Figure 8.
Two photon image of fibroblast cells (blue = nucleus, red = cytoskeleton) with KMgF₃:
ErYb nanoparticles (green = upconversion emission).

Figure 9. Colocalization image of fibroblast cells (red = cytoskeleton) with $\text{KMgF}_3:\text{ErYb}$ nanoparticles (green = upconversion emission from within cytoplasm, light blue = emission from within nucleus).

Figure 10.

Overlay of upconversion emission from NaYF_4 and NaMgF_3 with the absorption spectrum from ZnPc .

Table 1

Percent of total upconversion emission by manifold for each nanoparticle type.

Sample	550 nm emission	660 nm emission	800 nm emission
NaYF ₄ :ErYb	39.67%	55.91%	1.47%
KYF ₄ :ErYb	21.36%	65.92%	4.00%
NaNiF ₃ :ErYb	21.68%	57.67%	4.50%
NaMgF ₃ :ErYb	14.82%	81.11%	1.01%
NaBaF ₃ :ErYb	13.74%	62.58%	21.50%
NaCrF ₃ :ErYb	8.91%	67.01%	16.30%
NaMnF ₃ :ErYb	9.79%	61.32%	27.62%
NaCoF ₃ :ErYb	7.12%	62.46%	8.42%
NaFeF ₃ :ErYb	6.85%	62.94%	6.06%
KGdF ₄ :ErYb	6.76%	61.15%	10.40%
KMnF ₃ :ErYb	6.38%	82.14%	7.03%
KMgF ₃ :ErYb	6.28%	73.17%	16.39%
KCoF ₃ :ErYb	4.74%	86.94%	4.56%

Table 2

Percentage of live cell populations after 980 nm illumination for 5 minutes as compared with control.

NP type	Control	PEG@KMgF ₃ :ErYb	PEG@KMnF ₃ :ErYb	PEG@NaYF ₄ :ErYb	Chitosan@NaYF ₄ :ErYb	HEMA@NaYF ₄ :ErYb
% Viability	87.7%	32.9%	43.5%	42.8%	66.7%	64.2%