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NIR to NIR upconversion in KYb₂F₇: RE³⁺ (RE = Tm, Er) nanoparticles for biological imaging

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Abstract

Until recently, many contrast agents widely used in biological imaging have absorbed and emitted in the visible region, limiting their usefulness for deeper tissue imaging. In order to push the boundaries of deep tissue imaging with non-ionizing radiation, contrast agents in the near infrared (NIR) regime, which is not strongly absorbed or scattered by most tissues, are being sought after. Upconverting nanoparticles (UCNPs) are attractive candidates since their upconversion emission is tunable with a very narrow bandwidth and they do not photobleach or blink. The upconversion produced by the nanoparticles can be tailored for NIR to NIR by carefully choosing the lanthanide dopants and dopant ratios such as KYb₂F₇: RE³⁺ (RE = Tm, Er). Spectroscopic characterization was done by analyzing absorption, fluorescence, and quantum yield data. In order to study the toxicity of the nanoparticles Monkey Retinal Endothelial Cells (MREC) were cultivated in 24 well plates and then treated with nanoparticles at different concentrations in triplicate to obtain the optimal concentration for in vivo experiments. It will be shown that these UCNPs do not elicit a strong toxic response such as quantum dots and some noble metal nanoparticles. 3-D optical slices of nanoparticle treated fibroblast cells were imaged using a confocal microscope where the nucleus and cytoplasm were stained with DAPI and Alexa Fluor respectively. These results presented support the initial assumption, which suggests that KYb₂F₇: RE³⁺ would be excellent candidates for NIR contrast agents.

Keywords

Upconverting nanoparticles; biomedical imaging; rare-earth nanoparticles; near infrared

Introduction

Upconverting materials have drawn much attention due to the long list of potential applications. Some of these applications are in security inks¹⁻⁴, display lighting⁵⁻⁸, energy harvesting⁹⁻¹³, and biomedical imaging¹⁴⁻²⁴. Upconversion is a very useful multi-photon process in which a rare-earth ion absorbs two or more lower energy photons and emits one higher energy photon, usually taking place from the near infrared to the visible or ultraviolet regime. Due to the fact that most upconverting materials used in biomedical imaging operate in the visible region, the efficacy is not optimal due to the scattering and absorption of soft tissue and water in this region. By utilizing some rare-earth elements, such as thulium and erbium, it is possible to produce upconversion within the biological window, located between

700 and 1100 nm^{23,25,26}, where the absorption and scattering of biological tissue is lower, resulting in deeper tissue penetration. Erbium and Thulium were chosen because of their strong and sharp NIR to NIR transitions. Ytterbium has a very strong absorption cross section for 980 nm radiation which then is transferred to an erbium or thulium ion which was excited by 980nm leading to the emission of a lower energy level. Current upconverting nanoparticles with dimensions of less than 30 nm have a quantum yield, which is defined as the ratio of the photons emitted to the photons absorbed, and dependent on the crystal structure, size of the nanoparticle, and dopant ratio, less than 1%^{27,28}. By creating a material which has a better quantum yield the nanoparticles can be excited at lower power densities decreasing the likelihood of tissue damage. In order to obtain the material with the highest quantum yield a dopant ratio study was conducted.

The most common transition in a ytterbium-erbium codoped system is from the infra red to the visible, resulting in higher attenuation and shallow penetration, yet some upconverting nanoparticles have been used to successfully image tumors and other tissues^{22, 28, 29}. The nanoparticles have to be nontoxic in order to become candidates for any biological application. The addition of a polymer, in this case polyethylene glycol, during the synthesis process will serve both as a nucleating agent for the nanoparticle formation and growth, as well as a capping agent ensuring water solubility, biocompatibility, smaller size for cellular uptake, and ease of functionalization if needed. In order to test the cytotoxic response from the nanoparticles cultivated cells were treated with nanoparticles then counted to determine cell viability percentage. Some cells were fixed, stained, and treated with nanoparticles then imaged using a confocal microscope to check the cellular uptake of the nanoparticles. Although KYb₂F₇ is a known and studied as a bulk material^{30, 31}, little is known about its properties as a nanocrystal.

Materials

The upconverting nanoparticles were synthesized through a hydrothermal method where KF, Yb(NO₃)₃, Tm(NO₃)₃ or Er(NO₃)₃, were dissolved in a solution of 28 mL of DI water, 42 mL of ethanol, and 3mL of polyethylene glycol. After all the precursors were fully dissolved the solution was put in a Teflon container and heated to 200 °C and held for 24 hours. The nanoparticles were then washed with ethanol and water to remove excess materials and finally freeze dried. The nanoparticles were then characterized starting with X-Ray Diffraction, which showed the particles to have an orthorhombic crystal structure, Figure 1. The upconverting nanoparticles were then taken to a scanning and transmission electron microscopes revealing the size of the nanoparticles to be between 5 and 15 nm, with an elemental distribution confirming the expected contents of the nanoparticles, Figure 1 and Figure 2.

Results

Absorption and emission spectra of KYb₂F₇:Tm³⁺, Er³⁺ of different samples varying in dopant ratios was collected using a PTI monochromator, showing the upconversion emission from 980 nm, Fig. 5. The two transitions are the thulium ³H₄ → ³H₆ (980 - 800 nm) and from the ⁴F_{9/2} → ⁴I_{15/2} (980 – 660 nm) transition from erbium. Quantum yield was

conducted at different power densities, 6.29, 12.58, 18.86, and 25.15 W/cm², revealing that the highest efficiency is from the lowest concentration and at 18.86 W/cm², for thulium and erbium, Figure 3. The reason for the high quantum yield from lowest dopant concentration is due to the fact that at high concentrations the energy is being quenched.

To study the way the nanoparticles in a biological setting, monkey retinal endothelial cells were cultivated in MEM- α with 1% streptomycin, on a 24-well plate for 24 hours after which a nanoparticle treatment was given at different concentrations (10, 100, and 500 μ g/mL) in triplicate to ensure reliability. A 24-hour uptake period was given followed by a HBSS. Trypan blue was then used to stain the membrane of live cells and then a hemocytometer and an inverted microscope were used to count live cells, Fig. 4. The nanoparticles did not induce a toxic response below or at the 10 μ g/mL concentration, moderate toxicity at 100 μ g/mL, and high toxicity at 500 μ g/mL. To further study biocompatibility fibroblast cells were fixed, treated with nanoparticles and then stained with DAPI for the nuclei, and Alexa Fluor was used for the cytoskeleton. The nanoparticle-treated cells were then taken to a confocal microscope to obtain 3D optical slices and determine if the nanoparticles enter the cell. The images show that the nanoparticles enter the cell and sometimes even reach the inside of the nucleus. Imaris software was used to analyze the images and to obtain colocalization images, Fig 5, where light blue indicates the colocalization of the nucleus and nanoparticle channels, and light green the colocalization between the cytoskeleton and nanoparticle channels confirming that the nanoparticles are effectively internalized by the cells. Given that the particles were not functionalized or capped to ensure internalization and yet their emission is observed from inside the cells, further demonstrates validates their use in a biological environment.

Conclusion

KYb₂F₇:Tm³⁺, Er³⁺ upconverting nanoparticles are viable candidates to be used as contrast agents because of their ability to upconvert within the biological window and have a higher quantum yield than current upconverting nanoparticles. Their null cytotoxicity at low concentrations coupled with their ability to enter into the cells reaffirms their ability to perform well in biomedical applications. Future work will include the study of the uptake mechanism and the interaction of the cells and the upconverting nanoparticles as well as *in vivo* studies.

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Figure 1.

a) X-Ray Diffraction of $\text{KYb}_2\text{F}_7:\text{Er}^{3+}$ confirming that the particles are in an orthorhombic crystal structure. b), c) Transmission Electron Microscope images of $\text{KYb}_2\text{F}_7:\text{Tm}^{3+}$ with sizes ranging between 5 – 15 nm.

Figure 2. a) Electron diffraction and b) Scanning Electron Microscope image of KYb₂F₇:Er³⁺

Figure 3.

Upconversion emission of a) KYb₂F₇:Tm³⁺ and b) KYb₂F₇:Er³⁺ nanoparticles at different power levels under 980 nm excitation. Quantum yield of c) KYb₂F₇:Tm³⁺ nanoparticles and d) KYb₂F₇:Er³⁺ nanoparticles at different power densities and dopant ratios under 980 nm excitation source.

Figure 4.
Cell viability of MREC 24 hours after the nanoparticle treatment at different concentrations.

Figure 5. Confocal microscope images of stained fibroblast cells where blue indicates the nucleus, red the cytoplasm, light blue the colocalization of the nucleus and the nanoparticles, and yellow the colocalization of the cytoplasm and the nanoparticles.