Synthesis and characterization of Nd^{3+}: Yb^{3+} co-doped near infrared sensitive fluorapatite nanoparticles as a bioimaging probe

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ABSTRACT

Trivalent Nd and Yb co-doped rod shaped hexagonal phase fluorapatite (FAP) nanoparticles of length and width about 32 and 13 nm, respectively were prepared by hydrothermal method and investigated the ability for 980 nm emission *via* Nd^{3+} → Yb^{3+} energy transfer with the objective of utilizing them in biomedical imaging. Nd^{3+} → Yb^{3+} energy transfer in FAP was studied as a function of both Nd^{3+} and Yb^{3+} concentrations and found that when Yb^{3+} concentration was 10 mol% the FAP phase has partially turned in to YbPO$_4$ phase. The Yb^{3+} emission intensity at 980 nm significantly increased up to 5 mol% Yb^{3+} doping and then reduced drastically for further increase in its concentration. Nd^{3+} → Yb^{3+} energy transfer rates were evaluated from the decay curves and found that a transfer rate of 71% for 2 mol% Nd^{3+} co-doped with 5 mol% Yb^{3+}. The cytocompatibility test with fibroblast like cells using MTT assay revealed that the nanoparticles are compatible with the cells.

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1. Introduction

The rapid advancement of bioimaging field demands the development of biocompatible bioprobes [1,2]. Compared with the conventional biological labels, such as organic dyes and quantum dots, rare earth doped nanoparticles possess several advantages like, lower toxicity, photo stability, high thermal and chemical stabilities, high luminescence quantum yield and sharp emission bands [3–5]. Furthermore, surface modification does not significantly affect their optical properties, as their luminescence arises from the electronic transitions of the lanthanide ion. But the main challenge lies at making biocompatible lanthanide based bioprobes at nanometer scale. Rare earths can be doped in a variety of host matrixes such as phosphates, fluorides, glasses, *etc.* [6–9]. Among the phosphate host, hydroxyapatite nanoparticles (Ca$_{10}$(PO$_4$)$_6$(OH)$_2$, HAP) have attracted extensive interest for potential applications in the biomedical field such as biological probes, drug delivery and tissue engineering because of their low cost and biocompatibility [10–13]. Rare earth ions (Eu^{3+}, Tb^{3+}, *etc.*) doped HAP nanoparticles can be endowed with fluorescent properties and used as novel fluorescence probes for cell imaging and drug delivery applications [14–20].

The fluorescence quenching effect of excited state of the rare earth ions in HAP by the hydroxyl groups of HAP can be alleviated by replacing the hydroxyl groups with fluorine. FAP (Ca$_{10}$(PO$_4$)$_6$F$_2$) is structurally very similar to HAP and has improved thermal and chemical stability, crystallinity and corrosion resistance. The low vibrational energies of FAP favor the rare earth fluorescent transition that makes it suitable for biomedical imaging [21–23]. When compared to HAP, rare earth doped FAP nanoparticles show improved luminescence and it can be considered as a better contrast agent than HAP in medical imaging applications [24,25].

For bioimaging applications, when compared to UV excitation, NIR excitation is preferred due to circumvention of several drawbacks of the UV based techniques. Recently Yb^{3+} based up and down conversion luminescent nanoparticles have been investigated by co-doping with Ho^{3+} and Er^{3+} [26,27]. However, the major shortcomings of Yb^{3+} sensitized up and down conversion process for *in vivo* biological applications are poor tissue penetration and absorption induced local heating [28,29].

Nd^{3+} doped down conversion luminescence materials excited with 800 nm are gaining a lot of attention recently. Nd^{3+} has large absorption cross section of 1.2×10^{-19} cm^2 compared to that of

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Yb^{3+} absorption cross section $1.2 \times 10^{-20} \, cm^2$ and the biological tissues have more penetration depth at 800 nm [29–31]. Nd^{3+} doped nanoparticles are excited in the first (650–900 nm) biological window which results in three emission bands that falls in first and second (1000–1350 nm) biological windows [32–35]. This multiple band emission feature of Nd^{3+} is a limiting factor in achieving highly efficient single channel luminescent bioprobes for bioimaging applications. Hence it is essential to identify the means of tuning single emission with high intensity. The intense 980–1000 nm single band of Yb^{3+} that has the additional significance of longer decay times and absence of cross-relaxation is preferred over Nd^{3+}. This may be attained by $Nd^{3+} \rightarrow Yb^{3+}$ energy transfer mechanism through co-doping and these materials may be very efficient candidates for luminescent bioprobes, luminescent nano thermometers and photo dynamics therapy [28,36–39]. Nd^{3+} and Yb^{3+} co-doped systems are reported in different host materials such as hexagonal phase LaF_3, $NaGdF_4$ and monoclinic phase $LiLaP_4O_{12}$ with quantum efficiency up to 70% [29,36,37,40]. A system based on Nd^{3+} and Yb^{3+} co-doped FAP seems to be a better choice. The aim of the present work is to investigate the possibility of achieving 980 nm emission in biocompatible FAP host matrix by $Nd^{3+} \rightarrow Yb^{3+}$ energy transfer for NIR imaging applications.

2. Experimental

2.1. Material synthesis

Pure, Nd^{3+} doped and Nd^{3+}:Yb^{3+} co-doped FAP nanoparticles were synthesized by hydrothermal technique. Reagent grade neodymium nitrate hexahydrate [$Nd(NO_3)_3 \cdot 6H_2O$, 99.9%, Aldrich], ytterbium nitrate pentahydrate [$Yb(NO_3)_3 \cdot 5H_2O$, 99.99%, Sigma], calcium nitrate tetra hydrate [$Ca(NO_3)_2 \cdot 4H_2O$, 98%, Merck], disodium hydrogen phosphate [Na_2HPO_4, 98%, Merck], ammonium fluoride [NH_4F, 95%, Merck] and ammonia solution [NH_4OH, Merck] were used as the precursors for the synthesis.

Nd^{3+} concentration was fixed at 1 mol% and 2 mol% while Yb^{3+} was varied as 0, 1, 2, 5 and 10 mol% for each concentration of Nd^{3+}. Appropriate amount of $Ca(NO_3)_2 \cdot 4H_2O$, $Nd(NO_3)_3 \cdot 6H_2O$ and $Yb(NO_3)_3 \cdot 5H_2O$ were dissolved in deionized water keeping $Ca + Nd + Yb$ concentration equal to 1 M. To this 0.6 M of Na_2HPO_4 and 0.2 M of NH_4F were added dropwise under constant stirring. The pH of the mixture was maintained above 10 using ammonia solution. The resultant white mixture was transferred into teflon lined stainless steel autoclave reactor and treated at 120 °C for 150 min. After cooling to room temperature naturally, the white precipitate was collected by centrifugation and washed several times with water. The obtained precipitate was dried using freeze dryer (Boyikang laboratory instruments, China).

Pure FAP was also synthesized by the above mentioned method for comparison. All the synthesized samples were heat treated using a muffle furnace (NSW103 model) at 900 °C for 1 h. Here after pristine and Nd^{3+}: Yb^{3+} doped FAP samples of ratios (1:0), (2:0), (1:1), (2:1), (1:2), (2:2), (1:5), (2:5), (1:10) and (2:10) will be referred to as FAP, N1, N2, N1Y1, N2Y1, N1Y2, N2Y2, N1Y5, N2Y5, N1Y10 and N2Y10, respectively.

2.2. Characterization

The crystalline phases present in the as prepared and heat treated samples were identified by powder X-ray diffraction (PXRD) using a Rigaku MiniFlex II powder X-ray diffractometer with a Ni-filtered $CuK\alpha$ radiation (1.5406 Å). The morphology of the samples was examined using high resolution transmission electron microscopy (HRTEM, JEOL JEM-2100, Japan). Fourier transform infrared (FT-IR) spectra of as prepared and heat treated samples

were recorded in the region of 4000–400 cm^{-1} with a 4 cm^{-1} resolution using a Perkin Elmer RXI FT-IR spectrometer by KBr pellet technique. Optical absorption spectra of the heat treated samples were recorded on a Perkin Elmer Lambda 19 spectrophotometer. Fluorescence measurements of the heat treated samples were done by the NIR 800 nm power tunable fiber coupled Fabry Perot continous laser diode (Thorlab, Model LM14S2) and the emission of the sample was recorded by the Quanta Master 51 spectrofluorimeter (Photon Technology International Inc. NJ, U.S.) with an InGaAs detector (Teledyne Judson Technologies, 062–8451, U.S.).

Photoluminescence decay curves of 1060 and 980 nm emission were measured on a Quanta Master 40 system (Photon Technology International Inc. NJ, U.S.) using the single shot transient digitizer technique with a Nitrogen pumped dye laser (Photon Technology International part GL-3300 + GL-302) as excitation source. The Nitrogen laser, with 800 ps pulse width, pumps a high resolution dye chamber to give 523 ± 0.04 nm light. The collected decay curve was analyzed by Origin 8 software (Origin lab, U.S.). For the quantum yield (QY) measurements, we employed a barium sulfate coated 203 mm diameter integrating sphere (Oriel, Model 70451) mounted on the side of the spectrofluorimeter sample chamber and excited by the 800 nm laser.

The cytocompatibility of FAP and N2Y5 samples were tested with fibroblast like L929 cells using MTT assay. The cells were grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS) with 100 U per ml penicillin-streptomycin at 37 °C under humidified atmosphere of 95% air and 5% CO_2. Maintained cultures were passaged every week and the culture medium was changed twice a week. The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with a medium containing 5% FBS to give a final density of 1×10^5 cells per ml. 100 µl per well of cell suspension were seeded into 96 well plates at a plating density of 10000 cells per well and incubated at 37 °C in 5% CO_2, 95% air and 100% relative humidity. After 24 h incubation, samples FAP and N2Y5 were added to the culture medium at different dosages (25, 50, 100, 250 and 500 µgml^{-1}). The plates were further incubated for 48 h at 37 °C in 5% CO_2, 95% air and 100% relative humidity [40].

After 48 h incubation, 15 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution (5 mgml^{-1} in phosphate buffer saline (PBS)) was added into each well and the plate was further incubated for 4 h in the incubator. After discarding the supernatants, the dark blue formazan crystals were dissolved in 100 µl dimethyl sulfoxide (DMSO) and the optical density was measured using a Synergy H4 micro plate reader at 570 nm. The mean and the standard deviation were obtained from sums of three different experiments. The cell viability was calculated by the following equation

$$Cell \ viability(\%) = \left(\frac{OD_{sample}}{OD_{control}} \right) \times 100$$

where OD_{sample} and $OD_{control}$ represent the optical density (OD) values of cells cultured with the sample and without the sample, respectively.

3. Results and discussion

The PXRD pattern of the as prepared and heat treated samples are shown in Fig. 1. The XRD patterns of as prepared samples are in good agreement with the standard JCPDS data for FAP (JCPDS file no. 15–0876) which indicates that the as prepared sample contained no other phases except FAP. The characteristic diffraction