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Fluorine-18-labeled $Gd^{3+}/Yb^{3+}/Er^{3+}$ co-doped $NaYF_4$ nanophosphors for multimodality PET/MR/UCL imaging

Jing Zhou [a], Mengxiao Yu [a], Yun Sun [a], Xianzhong Zhang [b], Xingjun Zhu [a], Zhanhong Wu [c], Dongmei Wu [d], Fuyou Li [a,*]

[a] Department of Chemistry & Advanced Materials Laboratory, Fudan University, 220 Handan Road, Shanghai 200433, PR China
[b] Key Laboratory of Radiopharmaceuticals, Ministry of Education & College of Chemistry, Beijing Normal University, 19 Xinjiekou Outer Street, Beijing 100875, PR China
[c] Department of Nuclear Medicine, Peking Union Medical College Hospital 1 Shuaifuyuan, Wangfujing, Beijing 100730, PR China
[d] Shanghai Key Laboratory of Magnetic Resonance, Physics Department, East China Normal University, Shanghai 200062, PR China

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ABSTRACT

Molecular imaging modalities provide a wealth of information that is highly complementary and rarely redundant. To combine the advantages of molecular imaging techniques, ^{18}F-labeled $Gd^{3+}/Yb^{3+}/Er^{3+}$ co-doped $NaYF_4$ nanophosphors (NPs) simultaneously possessing with radioactivity, magnetic, and upconversion luminescent properties have been fabricated for multimodality positron emission tomography (PET), magnetic resonance imaging (MRI), and laser scanning upconversion luminescence (UCL) imaging. Hydrophilic citrate-capped $NaY_{0.2}Gd_{0.6}Yb_{0.18}Er_{0.02}F_4$ nanophosphors (cit-NPs) were obtained from hydrophobic oleic acid (OA)-coated nanoparticles (OA-NPs) through a process of ligand exchange of OA with citrate, and were found to be monodisperse with an average size of 22 × 19 nm. The obtained hexagonal cit-NPs show intense UCL emission in the visible region and paramagnetic longitudinal relaxivity (r_1 = 0.405 $s^{-1}\cdot(mM)^{-1}$). Through a facile inorganic reaction based on the strong binding between Y^{3+} and F^-, ^{18}F-labeled NPs have been fabricated in high yield. The use of cit-NPs as a multimodal probe has been further explored for T_1-weighted MR and PET imaging *in vivo* and UCL imaging of living cells and tissue slides. The results indicate that ^{18}F-labeled $NaY_{0.2}Gd_{0.6}Yb_{0.18}Er_{0.02}$ is a potential candidate as a multimodal nanoprobe for ultra-sensitive molecular imaging from the cellular scale to whole-body evaluation.

1. Introduction

Molecular imaging is playing an increasingly important role in defining micro-quantitative molecular events critical to biomedical research and clinical diagnostics. Different molecular imaging methods, such as optical imaging, nuclear medicine and magnetic resonance imaging (MRI), have different spatial resolutions, imaging depths and areas of application [1,2]. Nuclear imaging, especially positron emission tomography (PET) [3,4], is an established clinical tool for whole-body imaging with detection sensitivity reaching below the picomolar range for functional imaging, albeit with a low (mm) spatial resolution. MRI provides an excellent spatial resolution (several tens of micrometers) and depth for *in vivo* imaging, and provides exceptional anatomical information, but suffers from limited sensitivity [5,6]. In addition, both PET and MRI are unsuitable for imaging living cells because of low planar resolution, but this can be resolved by fluorescent imaging. Fluorescent imaging provides the highest sensitivity and spatial resolution (several hundreds of nanometers) and is widely used for cell and tissue imaging in basic biomedical research [7,8]. However, fluorescent imaging lacks the full capability to obtain anatomical and physiological detail *in vivo* because of its limited penetration. Ideally, to bridge gaps in sensitivity, resolution, and depth of multi-level molecular imaging from cellular scale to whole body, radioactivity, magnetic, and fluorescence properties should be combined within one system to facilitate ultra-sensitive and multi-level molecular imaging. To date, however, few examples of tri-modality bioimaging, using one probe with radioactivity, magnetic, and fluorescence properties, have been reported [9,10].

Alternatively, lanthanide materials may be ideal building blocks for multimodal bioimaging probes, because lanthanide ions having unique luminescent and magnetic properties [11–39]. For example, Gd^{3+}, having seven unpaired electrons, shows high paramagnetic relaxivity, and Gd^{3+}-containing materials are usually T_1-positive-contrast agents [11–13]. Some lanthanide ions-co-doped

* Corresponding author. Tel.: +86 21 55664185; fax: +86 21 55664621.
E-mail address: fyli@fudan.edu.cn (F. Li).

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nanoparticles show a unique upconversion luminescence (UCL) [14,15]. For example, $NaYF_4$ nanoparticles doped with Yb^{3+} and Er^{3+} display upconversion luminescence with maxima at 520 nm and 650 nm, under continuous-wave (CW) excitation at 980 nm [17–39]. By combining Gd^{3+}, Yb^{3+}, and Er^{3+} in one system, it is easy to fabricate magnetic/luminescent difunctional materials for dual-modal bioimaging [11–13]. Very recently, our group reported Tm^{3+}/Er^{3+}/Yb^{3+} co-doped $NaGdF_4$ with UCL and magnetic resonance properties for T_1-positive MR and UCL *in vivo* dual-modality whole-body imaging of small animals [13].

In this present study, we have further developed a radioactive, magnetic upconversion nanophosphor based on lanthanide nanocrystals of Gd^{3+}/Yb^{3+}/Er^{3+} co-doped $NaYF_4$ simultaneously labeled with the most widely used radionuclide, ^{18}F, for multimodality PET, MR, and UCL imaging. In our design strategy of a tri-modality probe, Gd^{3+}(60%)/Yb^{3+}(18%)/Er^{3+}(2%) co-doped $NaYF_4$ nanoparticles provide a basis for MR and UCL imaging, and ^{18}F radiolabeling enables PET imaging. Furthermore, the effectiveness of ^{18}F-labeled Gd^{3+}/Yb^{3+}/Er^{3+} co-doped $NaYF_4$ nanoparticles as a multifunctional nanoprobe has been demonstrated by *in vitro* UCL imaging and *in vivo* MR/PET bioimaging.

2. Experimental section

2.1. Materials

Rare-earth oxides Y_2O_3 (99.999%), Gd_2O_3 (99.999%), Yb_2O_3 (99.999%), and Er_2O_3 (99.999%) were purchased from Beijing Lansu Co. China. Rare earth chlorides ($LnCl_3$, Ln: Y, Gd, Yb, Er) were prepared by dissolving the corresponding metal oxide in 10% hydrochloric solution at elevated temperature and then evaporating the water completely. Oleic acid (>90%) and 1-octadecene (90%) were purchased from Alfa Aesar Ltd. NaOH, NH_4F, citric acid monohydrate, methanol, ethanol, cyclohexane, chloroform, toluene and hydrochloric solution were purchased from Sinopharm Chemical Reagent Co. China. All the other chemical reagents were of analytical grade and were used directly without further purification. Deionized water was used throughout.

2.2. Synthesis of citrate-coated upconversion nanoparticles (cit-NPs)

Oleic acid-coated $NaY_{0.2}Gd_{0.6}Yb_{0.18}Er_{0.02}F_4$ nanoparticles (namely OA-NPs) were synthesized according to previous method [17]. Citrate-capped $NaY_{0.2}Gd_{0.6}Yb_{0.18}Er_{0.02}F_4$ nanoparticles (namely cit-NPs) were obtained from OA-NPs through a ligand-exchange process of OA with citrate [37].

2.3. Characterization

Sizes and morphologies of the OA-NPs and cit-NPs were determined at 200 kV using a JEOL JEM-2010F high-resolution transmission electron microscope (HR-TEM). Energy-dispersive X-ray analysis (EDXA) of the cit-NPs was also performed during HR-TEM measurements. Powder X-ray diffraction (XRD) measurement was measured with a Bruker D4 X-ray diffractometer (Cu Kα radiation, $\lambda = 0.15406$ nm). Fourier-transform infrared (FTIR) spectra were measured using an IRPRESTIGE-21 spectrometer (Shimadzu) from samples in KBr pellets. Upconversion luminescence spectrum of the cit-NPs was measured with an Edinburgh LFS-920 fluorescence spectrometer by using an external 0–800 mW adjustable laser (980 nm, Beijing Hi-Tech Optoelectronic Co., China) as the excitation source instead of the Xenon source in the spectrophotometer.

2.4. Synthesis of ^{18}F-labeled-cit-NPs (^{18}F-cit-NPs)

$^{18}F^-$ (~148 MBq, $t_{1/2} = 109.7$ min) in 100 μL saline and 200 μL distilled water were added to the as-prepared cit-NPs (100 μL of 5 mg/mL in distilled water). The reaction mixture was incubated for 10 min at room temperature and centrifuged to move ^{18}F-cit-NPs from unreacted $^{18}F^-$. The ^{18}F-cit-NPs were washed three times in distilled water by sonication and centrifugation. Radio-TLC analysis demonstrated >99% radiochemical purity of ^{18}F-cit-NPs (~74 MBq).

2.5. Relaxivity measurement in vitro

The T_1-weighted MR images were obtained using a 3 T S Magnetom Trio running on Siemens' Syngo software version B15 (Siemens Medical Systems), in conjunction with an 8 array Loop coil (Siemens Medical Systems). Dilutions of cit-NPs (0.4, 0.2, 0.1, 0.04 mM) in deionized water were placed in a series of 1.5 mL tubes for T_1-weighted MR imaging and T_1-weighted contrast enhancement. The following parameters were adopted: a standard inversion recovery (IR) spin-echo sequence: a repetition time (TR) of 6500 ms, an echo time (TE) of 7.6 ms, and ten inversion recovery times (TI = 23, 100, 200, 400, 600, 800, 1000, 1200, 1500, 2000 and 3000 ms). Flip angle = 120 deg, slice thickness = 3.0 mm, FOV read = 190 mm, base resolution = 320. The MR signal intensity in tubes was ascertained by the average intensity in the defined regions of interests (ROIs). T_1 values of each tube were calculated using the following formula: $S(TI) = S_0 \times [1\text{-}2\exp(-TI/T_1)]$ to fit the T_1 recovery curve in the circular regions of interest for the sample. The resulting T_1 values were averaged over the region of interest and plotted as $1/T_1$ (R_1) *vs* molar concentration of cit-NPs. The slope of the line provides the molar relaxivity r_1.

2.6. Cell culture and cytotoxicity

A human nasopharyngeal epidermal carcinoma cell line KB cell was provided by Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences (CAS, China). The KB cells were grown in RPMI 1640 (Roswell Park Memorial Institute's medium) supplemented with 10% FBS (fetal bovine serum) at 37 °C and 5% CO_2.

In vitro cytotoxicity was measured by performing methyl thiazolyl tetrazolium (MTT) assays on the KB cells. Cells were seeded into a 96-well cell culture plate at 5×10^4/well, under 100% humidity, and were cultured at 37 °C and 5% CO_2 for 24 h; different concentrations of cit-NPs (0, 100, 200, 300 and 400 μg/mL, diluted in RPMI 1640) were then added to the wells. The cells were subsequently incubated for 4 h or 24 h at 37 °C under 5% CO_2. Thereafter, MTT (10 μL; 5 mg/mL) was added to each well and the plate was incubated for an additional 4 h at 37 °C under 5% CO_2. After the addition of 10% sodium dodecyl sulfate (SDS, 100 μL/well), the assay plate was allowed to stand at room temperature for 12 h. The optical density OD_{570} value (*Abs.*) of each well, with background subtraction at 690 nm, was measured by means of a Tecan Infinite M200 monochromator-based multifunction microplate reader. The following formula was used to calculate the inhibition of cell growth: Cell viability(%) = (mean of *Abs.* value of treatment group/mean *Abs.* value of control) × 100%.

2.7. Magnetic resonance imaging (MRI) in vivo

Animal procedures were in agreement with the guidelines of the regional ethic committee for animal experiments. *In vivo* experiments were performed on anesthetized mice ($n = 20$ g) with 10% chloral hydrate 100 μL. MRI was conducted on a 3 T S Magnetom Trio, using a T_1-mapping sequence (TR = 15.0 ms,