Talanta 184 (2018) 461–467

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

# Intensely red-emitting luminescent upconversion nanoparticles for deep-tissue multimodal bioimaging

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ARTICLE INFO

*Keywords:*
Nanoprobes
Red upconversion luminescence
Deep tissue
Multimodal bioimaging

ABSTRACT

Nowadays, in order to improve the diagnostic accuracy of the disease, more and more contrast agents have been applied in the clinical imaging modalities. A combination of nanotechnology with optical, computed X-ray tomography (CT), and magnetic resonance imaging (MRI) has great potential to improve disease diagnosis and therapy. Herein, we developed a novel multimodal contrast agent for deep-tissue bioimaging based on PEGylated $Mn^{2+}$ doped $NaLuF_4$:Yb/Er nanoparticles (PEG-UCNPs). The multimodal nanoprobes have revealed the intensely red upconversion luminescence emission for deep-tissue upconversion luminescence (UCL) imaging. Moreover, Owing to the high longitudinal relaxivity, the PEG-UCNPs can be used as $T_1$-weighted MRI contrast agents. Additionally, with the high X-ray mass absorption coefficient of $Lu^{3+}$, the novel nanoprobes are appropriate for CT imaging. With integration the high paramagnetic property, superior X-ray mass absorption coefficient and excellent upconversion luminescence in one system, the multimodal nanoprobes can provide a unique opportunity for MRI, CT and UCL imaging. More importantly, modification with PEG endows the novel nanoprobes with high biocompatibility, which would bring more opportunities for the biomedical applications in clinic.

## 1. Introduction

With the development of biomedicine, non-invasive medical techniques for early disease diagnosis, such as fluorescent imaging, magnetic resonance imaging (MRI) and computed X-ray tomography (CT) have attracted great attention [1–3]. Among these modern imaging methods, MRI is able to provide unsurpassed 3D soft tissue details and functional information with the high spatial resolution and deep tissue penetration [4,5]. CT can provide accurate and high-resolution information of the anatomic structure of tissues due to the diversity of X-ray absorption ability by tissues. Nevertheless, they suffer from insufficient sensitivity and their application is limited in some disease detection [6–9]. Fluorescence imaging has the superior resolution and high detectability. However, owing to the strong auto-fluorescence and weak tissue penetration, the application of fluorescence imaging in vivo is limited [10]. In order to defeat these restrictions of each modality, the multimodal imaging approach has emerged. The multimodal imaging approach can integrate the advantages of each modality and show more detailed information via the help of the multimodal imaging nanoprobes [11,12]. Therefore, it is highly desired to develop multimodal imaging nanoprobes for offering more complementary, effective and accurate information for diagnosis and prognosis.

In the past few years, with the development of nanotechnology, significant attention has been focused on developing lanthanide-doped upconvertion nanoparticles (UCNPs), which open a new gate for multimodal bioimagings [13–16]. Typically, $Gd^{3+}$, $Yb^{3+}$, and $Lu^{3+}$-based UCNPs have shown more excellent CT contrast efficiency and are used as CT contrast agents due to the large K-edge value and high X-ray mass absorption coefficient. Additionally, with unpaired f electrons, $Gd^{3+}$ and $Mn^{2+}$ doped UCNPs have been reported as contrast agents for MRI. Especially, UCNPs can be simultaneously doped with sensitizers ($Yb^{3+}$) and activators ($Er^{3+}$, $Tm^{3+}$, or $Ho^{3+}$), which can convert NIR light into the necessary light for fluorescent imaging through a LRET (upconversion luminescence resonance energy transfer) process. Due to the weak autofluorescence backgrounds, higher signal-to-noise ratio, resistance to photobleaching, and lower photodamage effect, UCNPs have been widely studied for bioimaging [17–25]. To date, Yb/Er or Yb/Ho co-doped UCNPs have been demonstrated as efficient NIR-to-visible (green or blue) nanoprobes for fluorescent imaging. However, a major drawback of UCNPs for in vivo fluorescent imaging is the strong light absorption and scattering of short-wave-length light below 600 nm by biological tissues. In order to overcome the obstacle, both the excitation

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https://doi.org/10.1016/j.talanta.2018.03.018
Received 3 January 2018; Received in revised form 28 February 2018; Accepted 7 March 2018
Available online 14 March 2018

and emission light should be located in the “optical window” the biological tissues, whose wavelength is in the red region and NIR spectral range (600–1100 nm). The light scatting, absorbance and autofluorescence of light in the “optical window” by the tissue are minimum [26–28]. Although Yb/Tm co-doped $NaYF_4$ showed an intense emission in the NIR region (about 800 nm), the other blue emission can't penetrate the deep tissue and may lead to the side effects for the normal tissue. Therefore, it is highly desired to construct the novel UCNPs whose all emission peaks are in the “optical window”.

Herein, for the first time, we reported the intensely pure red emission of $Mn^{2+}$ doped $NaLuF_4$:Yb/Er UCNPs for deep-tissue multimodal bioimaging. In our system, the upconversion luminescence of the $NaLuF_4$:Yb/Er UCNPs exhibited strong red emission (650–670 nm) through $Mn^{2+}$ ions doping. Because of the $Mn^{2+}$ ions have unpaired f electrons, the as-prepared UCNPs can be potentially employed as contrast agents for the $T_1$-weighted MRI. Additionally, Owing to the relatively large K-edge value and high X-ray mass absorption coefficient of $Lu^{3+}$, the novel UCNPs can be used as CT contrast agents. In a word, integration the paramagnetic behavior, high X-ray mass absorption coefficient and strong upconversion fluorescence in one system, the as-prepared nanoprobes can provide a unique opportunity for MRI, CT and upconversion luminescence imaging. More importantly, modification with PEG endows the novel nanoprobes with excellent biocompatibility, which brings more opportunities for transferring the novel nanoprobe to the potential biomedical applications in clinic.

## 2. Materials and methods

### 2.1. Chemicals

All chemicals were purchased from reagent company and directly applied without further purification. $Ln(NO_3)_3{\cdot}xH_2O$ ($Lu(NO_3)_3{\cdot}6H_2O$, $Yb(NO_3)_3{\cdot}6H_2O$, $Er(NO_3)_3{\cdot}6H_2O$), $MnCl_2{\cdot}4H_2O$, oleic acid were purchased from Aladdin Chemical Reagent Co. (Shanghai, China). NaF and dehydrated ethanol was obtained from Beijing Chemical Factory (Beijing, China). The amphiphilic phospholipid functionalized poly (ethylene glycol) (DSPE-PEG 5000) was purchased from Paige Biotechnology Co. (Changchun, China). The Milli-Q water system (Millipore, Bedford, MA, USA) supplied the deionized water using in the experiment.

### 2.2. Synthesis of the $Mn^{2+}$ doped $NaLuF_4$:Yb/Er upconversion nanoparticles (UCNPs)

UCNPs were synthesized by a hydrothermal method according to the previous report with some modification [20]. Firstly, NaOH solution was prepared by dissolving the 0.3 g NaOH into the deionized water (1.5 mL). Then, oleic acid (5 mL) and ethanol (10 mL) respectively were dissolved in the NaOH solution with strong mechanical agitation. Next, $MnCl_2$ (0.2 mmol), $Lu(NO_3)_3$ (0.6 mmol), $Yb(NO_3)_3$ (0.18 mmol) and $Er(NO_3)_3$ (0.02 mmol) were dissolved in the 2 mL deionized water and put in the above colloidal solution with strong mechanical agitation for 1 h. Following, NaF solution (4 mM, 2 mL) was dropwise dissolved in the above solution and stirred for 1 h. Subsequently, the above mixture was put in the stainless Teflon-lined autoclave (50 mL) and reacted for 8 h at 200 °C. Finally, the as-prepared nanoparticles were collected by centrifugation, washed with hexane and ethanol for 3 times for removing remained reagents. The powder samples were dried at 60 °C for 12 h.

### 2.3. Synthesis of the PEGylated UCNPs (PEG-UCNPs)

In a typical synthesis procedure, 10 mg UCNPs and 20 mg DSPE-PEG 5000 were added into the 3 mL aliquot of chloroform. Then, the colloidal solution was sonicated for 10 min. Then, the mixture was stirred for 1 h. Next, the resulting mixture was evaporated to obtain the powder samples. The powder samples were dispersed in water by sonication and were washed with water for 3 times for eliminating the remained remnants. The products were dried overnight at 60 °C in vacuum.

### 2.4. Cell cultures

The cells in the experiment were purchased from ATCC (American Type Culture Collection). The cell cultures assay was according to our previous report [12]. A549 and HEK 293 cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 100 U/mL penicillin, 100 U/mL streptomycin, and 10% FBS (fetal bovine serum) at 37 °C and 5% $CO_2$ in a incubator. Before plating, A549 and HEK 293 cells were digested by trypsin and were resuspended in fresh medium.

### 2.5. Cytotoxicity studies

The cytotoxicity of PEG-UCNPs was studied with the MTT reduction assays. In a typical procedure, A549 and HEK 293 cells ($5 \times 10^3$ cells/well) were seeded and cultured in 96-well assay plates for 24 h. Then, serial dilutions of nanoparticles were put in the above medium. The redundant PEG-UCNPs were removed after 24 h incubation. Then, MTT was added into the plates and cultured with cells for 4 h. Following, DMSO (dimethyl sulfoxide) was added in the plates for dissolving the formazan crystals. The absorbance values of formazan at 490 nm (corrected for background absorbance at 630 nm) were determined by a microplate reader (Bio-Rad model-680). Five replicates were treated in each group.

### 2.6. UCL imaging

For UCL imaging in vivo, mice were first intraperitoneally injected with chloral hydrate solution (10 wt%) for anesthetization. Then, PEG-UCNPs solution (2 mg/mL) was injected into the abdominal area of the mice. The in vivo UCL images of mice were performed by an animal UCL imaging system.

### 2.7. $T_1$-weighted MR imaging

The different concentrations of PEG-UCNPs were dispersed in PBS including 1% agarose. Then, the samples were scanned by a 1.5 T MRI instrument (Siemens). For in vivo experiments, the PEG-UCNPs in PBS buffer (1 mg/mL, 1 mL) were intravenously administrated with the anesthetized mouse. The mouse was scanned with the 1.5 T MRI instrument.

### 2.8. CT imaging

To study in vitro CT imaging, the different concentrations of PEG-UCNPs were dispersed in PBS including 1% agarose. Then, the samples were scanned by a CT imaging system (Philips 256-slice CT Scanner). For assessing CT contrast efficacy in vivo, 1 mL PEG-UCNPs solution (50 mg/mL) was administrated intravenously into the anesthetized mouse. The CT images of the mouse were scanned by the Philips CT imaging system.

### 2.9. Hematology studies

The PEG-UCNPs (5 mg/kg) were intravenously administrated for the five healthy Kunming mice as treatment group. As the control group, 0.9 wt% NaCl (1 mL) was administrated intravenously into five mice. After 30 days injection, the blood of mice in each group was collected for hematology studies.