Nd$^{3+}$-doped LiYF$_4$ nanocrystals for bio-imaging in the second near-infrared window†

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The fluorescent probes working in the near-infrared (NIR) window have unparalleled advantages of a high penetration depth and low interference from autofluorescence. In this paper we introduced a rare-earth doped nanoprobe working in this region with a narrow emission band, a high efficiency, and good bio-compatibility. The newly-developed host material LiYF$_4$ managed to strengthen the emission intensity and generate a fine structure of the emission peaks. After the optimization of the doping ratio of Nd$^{3+}$ and surface modification, the LiYF$_4$:5%Nd$^{3+}$ probe could reach a penetration depth of 3–5 mm and could be successfully utilized in lymphatic imaging, with a signal-to-noise ratio of 14.8 in vivo and a resolution of ~0.2 mm in circulatory imaging. This probe may be further used in bio-detection and the host material itself may replace the traditional NaYF$_4$ in some aspects of applications.

Introduction

The development of a luminescent probe, with its advantages of high sensitivity, high accuracy, fast feedback and the absence of radiation, has opened up a new area in bio-imaging. In bio-imaging applications, light may be absorbed or scattered easily at shorter wavelengths like the ultraviolet (UV) region and the visible region, which harms the efficiency of these probes. The autofluorescence from biological tissues also influences the final imaging results. Due to the aforementioned reasons, fluorescent probes working in the second near-infrared window (NIR II, 1000–1350 nm), where the interference from autofluorescence, absorption and scattering is minimal, are more propitious for intravital imaging, especially ones with excitation and emission wavelengths both inside the NIR II region.

However, the practical utilization of probes in the NIR II window requires expensive detectors based on indium gallium arsenide (InGaAs) or mercury cadmium telluride (HgCdTe) sensors, which are expensive and still not widely used. Meanwhile, the development of bio-compatible probes in the NIR II window for imaging, like single-walled carbon nanotubes (SWNTs), quantum dots (QDs) and polymer fluorophores, remains in nascent stages. The SWNTs usually have a relatively broad emission band and low quantum yield which restrict their usage in imaging, and the wide length distributions may affect their pharmacokinetic behavior, ultimately impeding their practical applications. For QDs, most of them are still affected by the potential problem of toxicity. The newly-developed silver chalcogenide QDs require upgrade in surface modification to ameliorate their quantum yield (QY). Therefore, it is meaningful to design a probe of a narrower emission band, a higher efficiency, a controllable size and lower bio-toxicity.

Rare earth metals are commonly used for synthesizing nanomaterials for bioapplications. Their abundant 4f electrons allow researchers to tune the luminance in a wide range from UV to NIR using different doping ions. Meanwhile, the emission peaks generated from rare earth ions are sharp and resistant to impact from the outside environment. Apart from the outstanding luminescent properties, no serious bio-toxicity has been reported. Recently, different kinds of rare-earth materials in the NIR II range have also been developed, like rare-earth fluorides and oxides for cancer targeting and in vivo imaging.

Herein, we introduce LiYF$_4$ to be the host material for our probe. As a member of rare earth fluorides, it possesses the merits of outstanding optical transparency and low phonon energy which can minimize the non-radiative energy loss. Nd$^{3+}$ was chosen to be the doping ion for its intrinsic NIR emissive luminescent properties. Numerous applications of Nd$^{3+}$ ions in the NIR region have been reported, from the widely-used Nd laser oscillators to the newly-designed Nd$^{3+}$-sensitized solar batteries. For bioapplications, the NIR emission of Nd has already been used in bio-imaging after subcutaneous injection or intravenous injection on a mouse model. It also showed great potential in intratumoral thermal detection and photothermal treatment. We combined it with LiYF$_4$ and designed a LiYF$_4$:Nd$^{3+}$ nanoparticle (NP) system which needs a few steps to be synthesized and has high luminescent efficiency.
Laser at 808 nm, bringing less heat effect,\textsuperscript{35,36} was applied to excite the Nd\textsuperscript{3+} ions and luminescent signals at 900 nm, 1050 nm and 1330 nm were designed as imaging signals. The as-prepared probe has relatively stronger emission compared to NaYF\textsubscript{4} NPs with the same doping ratio of luminescent ions, due to the special crystal structure of LiYF\textsubscript{4},\textsuperscript{37,38} and the desired superiorities of LiYF\textsubscript{4}:Nd\textsuperscript{3+} have been proved in the bio-imaging applications.

**Experimental**

**Materials**

All the chemicals used were of analytical grade and were used without further purification. Deionized water was used throughout. Rare-earth oxides, RE\textsubscript{2}O\textsubscript{3} (99.999\%) (RE\textsuperscript{3+} = Y\textsuperscript{3+}, Nd\textsuperscript{3+}), NaOH, LiOH, H\textsubscript{2}O, NH\textsubscript{4}F, HCl, ethanol and cyclohexane were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. Oleic acid, 1-octadecene and nitrosonium tetrafluoroborate (NOBF\textsubscript{4}) were bought from Alfa Aesar Ltd. Dimethyl sulfoxide (DMSO) was bought from Sigma-Aldrich Co. Ltd. Ethylenediaminetetra(methylene phosphonicacid) (EDTMP) was gained from TCI (Shanghai) Development Co., Ltd. All the doping ratios of RE\textsuperscript{3+} were calculated in moles in our experiments.

**Synthesis of LiYF\textsubscript{4}:x\%Nd\textsuperscript{3+} (x = 1.0, 3.0, 5.0, 7.0, 10.0) NPs**

The rare-earth chlorides were synthesized by adding HCl into rare-earth oxides, followed by evaporating the remaining liquid to get solid powder. The NPs of LiYF\textsubscript{4} were synthesized using a specially-modified co-precipitation method.\textsuperscript{39} A mixture of 1 – x\% mmol YCl\textsubscript{3}, x\% mmol NdCl\textsubscript{3}, oleic acid (OA, 6 mL), 1-octadecene (15 mL) was added into a 100 mL three-necked flask at room temperature. The oxygen was moved out by vacuuming under vigorous magnetic stirring. Then, the system was heated to 130 °C to form a clear and transparent solution. After the solution was cooled to 70 °C naturally, 8 mL of methanol solution containing LiOH-H\textsubscript{2}O (0.106 g) and NH\textsubscript{4}F (0.148 g) were slowly added into the flask, and precipitates appeared in the solution. The mixture was stirred for 30 min and gradually got transparent again, guaranteeing the complete consumption of fluorides, and then it was heated to evaporate methanol for 15 min, followed by vacuuming for 15 min to ensure the removal of methanol and water. After that, the solution was heated to 300 °C under the protection of a nitrogen atmosphere. The solution was held at the final temperature for 90 min after which it was allowed to cool to the room temperature prior to precipitation with absolute ethanol. The mixture was separated by centrifugation and the precipitates were collected and further purified by cyclohexane/ethanol (v/v 1:2) twice. The resulted NPs were dispersed in cyclohexane (10 mL) for further use.

**Surface modification of LiYF\textsubscript{4}:Nd\textsuperscript{3+} NPs**

To make these NPs hydrophilic, the as-prepared cyclohexane solution (5 mL) of NPs and cyclohexane (5 mL) was added into a centrifugal tube and then dispersed evenly using the ultrasonic method. NOBF\textsubscript{4} was added into the solution until no more precipitate forms.\textsuperscript{40} The precipitates were separated with centrifugation and re-dispersed in water (5 mL), and then we added ethanol drop by drop until the solution got transparent again. The NPs were separated with centrifugation and washed with water several times. Then we dispersed them in water (5 mL). The bare NPs could be modified using different ligands. In our research we chose EDTMP as a surface ligand for further biological applications. The water solution of bare NPs was added into EDTMP solution and the precipitates were separated by centrifugation. The excessive ligands were washed away with water, and then re-dispersed the EDTMP-modified NPs with water for further use.

**Characterization**

The morphologies of as-prepared LiYF\textsubscript{4}:Nd\textsuperscript{3+} NPs were examined using a JEOL JEM-2010 low- and high-resolution (HR) transmission electron microscope (TEM) operating at 200 kV. The samples were dispersed in cyclohexane and dropped onto a copper grid for TEM tests. The energy-dispersive X-ray analysis (EDXA) was also performed on this microscope. X-ray powder diffraction (XRD) measurements were performed at room temperature on a Bruker D4 diffractometer at a scanning rate of 1° min\textsuperscript{-1} in the 2\theta range of 10–80°, with graphite monochromated Cu Ka radiation (\(\lambda = 0.15406 \text{ nm}\)). The NIR fluorescence spectrum was measured using an Edinburgh Instruments FLS920 fluorescence spectrometer, equipped with an external 0–3 W adjustable laser (808 nm, Beijing Hi-Tech Optoelectronic Co., China) as the excitation source at room temperature. The collected range was from 830 nm to 1400 nm. The quantum yield was measured at room temperature using a Photo Technology International QM40 fluorescence spectrometer, equipped with a xenon lamp as the excitation source. The cyclohexane solution of LiYF\textsubscript{4}:5%Nd\textsuperscript{3+} NPs and pure cyclohexane (blank) were excited at 808 nm and the emission was observed from 750 nm to 1150 nm. The numbers of absorption photons (N\textsubscript{abs}) and emission photons (N\textsubscript{em}) were recorded to calculate the QY using the following formula:

\[
\text{QY} \, (\%) = \left( \frac{N_{\text{em}}}{N_{\text{abs}}} \right) \times 100.
\]

**Intragastric administration on a mouse model**

All animal procedures were in agreement with the guidelines of the Institutional Animal Care and Use Committee. 0.20 mg (0.10 mL \times 2.0 mg mL\textsuperscript{-1}) of EDTMP-LiYF\textsubscript{4}:Nd\textsuperscript{3+} NPs were imbed to the stomachs of a nude mouse and a Kunming mouse, both purchased from the Second Military Medical University (Shanghai, China), by using gastric syringes. The NIR luminescence imaging was performed using the in vivo imaging system designed by our group.\textsuperscript{41} We used 808 nm lasers as the excitation source and Andor DU897 EMCCD as a signal collector. The quantum efficiency of the CCD is shown in Fig. S6 (ESI†). The excitation intensity of 808 nm irradiation was kept below 400 mW cm\textsuperscript{-2} and the exposure time was 1 s. Images of luminescent signals were analysed with Carestream MI SE.
The signals were collected at wavelength > 980 nm. The transmittance of the 980 nm long-pass edge filter (Semrock BLP01-980R Optical Filter) is shown in Fig. S7 (ESI†).

**Lymphatic mapping on a mouse model**

1.0 mg (0.10 mL × 10 mg mL⁻¹) of EDTMP–LiYF₄:Nd³⁺ NPs were injected into nude mice from paw and footpad respectively. The NIR luminescence imaging was performed using the in vivo imaging system designed by our group as mentioned above. 24 h after injection we collected the imaging results in vivo, then the mice were opened up at the sites of the lymph nodes and the lymph nodes were taken out for imaging afterwards. The excitation intensity of 808 nm irradiation was kept below 400 mW cm⁻² and the exposure time was 1 s. Images of luminescent signals were analysed with Carestream MI SE. The signals were collected at wavelength > 980 nm.

**Blood vessel imaging on a mouse model**

1.0 mg (0.20 mL × 5.0 mg mL⁻¹) of EDTMP–LiYF₄:Nd³⁺ NPs were injected into a nude mouse intravenously to test the resolution of blood vessel imaging. 2.0 mg (0.20 mL × 10 mg mL⁻¹) of EDTMP–LiYF₄:Nd³⁺ NPs were injected into a Kunming mouse intravenously to view the bio-distribution. The excitation intensity of 808 nm irradiation was kept below 400 mW cm⁻² and the exposure time was 1 s. Images of luminescent signals were analysed with AndorSolis, Carestream MI SE and origin 8.0⁶. The signals were collected at wavelength > 980 nm.

**Cytotoxicity assay**

The in vitro cytotoxicity was measured using the methyl thiazolyltetrazolium (MTT) assay using the HeLa cell line. Cells growing in the log phase were seeded into a 96-well cell-culture plate (Corning Costar, Cambridge, MA, USA) and then incubated under 5% CO₂ in RPMI-1640 (Invitrogen, Grand Island, NY, USA), supplemented with 10% heat-inactivated fetal bovine serum (FBS; Invitrogen), 50 IU mL⁻¹ penicillin and 50 mg mL⁻¹ streptomycin (Invitrogen). Solutions of 0–600 μg mL⁻¹ EDTMP–LiYF₄:Nd³⁺ NPs were added to the well of the treatment group, and RPMI-1640 to the negative control group. The cells were incubated for 24 h at 37.5 °C under 5% CO₂. For a colorimetric MTT assay, DMSO was added to solubilize formazan and absorbance was measured at 562 nm. The following formula was used to calculate the viability of cell growth:

\[
\text{Cell viability (\%)} = \left( \frac{\text{mean of Abs value of treatment group}}{\text{mean of Abs value of control}} \right) \times 100.
\]

**Histology of animal organs**

Kunming mice were intravenously injected with 1.5 mg (0.15 mL × 10 mg mL⁻¹) of EDTMP–LiYF₄:Nd³⁺ NPs. Organs were harvested 1 day and 7 days after injection, fixed using paraformaldehyde, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E). Organs from untreated animals were analysed as control.

**Results and discussion**

**Synthesis and characterization of LiYF₄:Nd³⁺ NPs**

The synthesis of LiYF₄ nanocrystals in relatively large quantities has not been widely reported due to their special characteristics. Compared with the widely-used NaYF₄ nanocrystals, they also belong to rare earth fluorides, and possess a similar rigid crystal lattice and ordered structure. The LiYF₄ structure is isomorphous with CaWO₄, with Li⁺ at the spots of W⁴⁺ and Y³⁺ at the spots of Ca²⁺. The tetragonal LiYF₄ synthesized here showed an octahedral shape (Fig. 1a and b, Fig. S1a–e, ESI†), which was also consistent with former research studies.⁶ The XRD patterns confirmed the special crystal structure and the high crystallinity of the nanocrystals. As shown in Fig. 1c and Fig. S1g (ESI†), the planes could be smoothly fit to the standard PDF card (JCPDS 17-0874) of tetragonal LiYF₄. The peaks were broadened due to the small sizes of nanomaterials. The EDXA analysis proved the existence of Nd³⁺ (Fig. S2, ESI†). With an increase in the doping ratio of Nd³⁺, the NPs grew larger from 18 to 30 nm at length and from 15 to 25 nm at width (Fig. S1a–e, ESI†), with a standard deviation of around 1–2 nm. And the phase of crystals did not show obvious change. The growing trend was possibly caused by the relatively large radius of Nd³⁺ compared to that of Y³⁺, which distorted the rigid arrangement of atoms in the lattice, since after implementing different reaction conditions this trend in size remained stubbornly.
The luminescent properties of LiYF₄: Nd³⁺ NPs were characterized with fluorescence spectroscopy. Since the NPs were synthesized with the control of OA, they were ultimately covered with OA ligands on the surface. As a result, they were dispersible in organic solvents like cyclohexane, and the fluorescence spectra were measured in cyclohexane solution. As shown in Fig. 2, the emission was ascribed to the f-f electronic transitions of Nd³⁺. The Nd³⁺ ions were excited from 4I⁹/₂ to 4F⁵/₂ by the laser of 808 nm, and then the transitions of 4F⁵/₂→4I⁹/₂ (900 nm), 4F⁵/₂→4I¹₁/₂ (1050 nm) and 4F⁵/₂→4I¹₃/₂ (1330 nm) occurred and generated these emission peaks. The luminescence intensity increased when the doping ratio of Nd³⁺ was raised from 1% to 5%. However, after reaching the peak emission intensity, it gradually fell at the doping ratios of 7% and 10%, which was possibly caused by the cross-relaxation among Nd³⁺ ions, between which the distance lowered as well. The QY of LiYF₄:5%Nd³⁺ NPs was tested by the cross-relaxation among Nd³⁺ ions, between which the emission intensity and the split of emission peaks was more delicate changed by the crystal field, leading to stronger emission and more refined split. 36,37

Then we chose to use LiYF₄:5%Nd³⁺ to conduct the following biological experiments. The surfaced ligands of NPs were washed using the reported method and then made hydrophilic with EDTMP. 40 The rare-earth nanoparticles capped with oleic acid will transform in the acidic physiological fluids, like lysosomes, resulting in pro-inflammatory effects and quenching of fluorescence. The coating of EDTMP could passivate the surfaces of the particles to lessen the transformation behaviour and lower the cytotoxicity, as well as preserve the fluorescence quality. Due to the strong coordination interaction between the central rare-earth atom and the ligand, it could be easily functionalized on the surface of bare LiYF₄: Nd³⁺ NPs. 21 The ligand exchange was successful as proven by the result of infrared (IR) spectroscopy (Fig. 3a). The OA-capped NPs showed the stretching vibration frequency at around 3000 cm⁻¹ of the carbonic double bond and methene, and these peaks were noticeably reduced in the EDTMP-capped NPs. The EDTMP-capped NPs showed the specific absorption of P–O in EDTMP at 1109 and 1007 cm⁻¹, which was also substantiated by the strong absorbance around these wavelengths in the IR spectroscopy of the pure EDTMP sample. The zeta-potential of EDTMP-capped NPs was −23.1 mV. These results confirmed the substitution of OA with EDTMP at the surface of the NPs. 21,44 The hydrophilic NPs were of perfect dispersibility and transparency as is shown in Fig. 3c. The hydrodynamic sizes of the water-phase NPs were also tested and no aggregation was observed (Fig. S5, ESI†). After the ligand exchange process, the emission intensity decreased by about 50 percent while the position of the emission peaks did not change (Fig. 3b). It was possibly caused by the quenching of the hydrophilic ligand and aqueous solution.

Intragastric administration
To evaluate the penetration depth of imaging applications with EDTMP–LiYF₄: Nd³⁺ NPs, we conducted the experiment of intragastric administration on both nude mouse and Kunming mouse as shown in Fig. 4, using common Si-CCD as a detector. The depth of mice’s stomachs was around 3–5 mm for nude mice and 5–7 mm for Kunming mice, and we could still collect signals of high levels with the amount of injection of 0.2 mg (0.1 mL × 2 mg mL⁻¹). The skin of nude mouse was relatively thinner, so that the imaging quality did not improve a lot after

Fig. 2 (a) Fluorescence spectra of cyclohexane solution of OA–LiYF₄: x% Nd³⁺ NPs, x = 1.0, 3.0, 5.0, 7.0, 10.0. The inset shows the intensity of emissions at 1045 nm and 1051 nm as a function of Nd³⁺ ion concentration. (b) Fluorescence spectra of cyclohexane solution of OA–LiYF₄: 5% Nd³⁺ NPs and OA–NaYF₄: 5% Nd³⁺ NPs.
removal of tissues above. For Kunming mouse covered by thick furs and skin, the fluorescence was low in vivo, so we had to raise the gain parameter of the CCD detector to twice of that applied to the nude mouse. However, it shall be noticed that with the inevitable impact caused by thick tissues, we were still able to collect signals strong enough for imaging and analysis. The fluorescence intensity increased significantly after opening up the mouse, by approximately an order of magnitude. The results showed the great penetration capability of this NIR II probe and laid solid foundations for further imaging in lymphatic and vascular systems.

The luminance of mouse food in the NIR II window has been reported. Control experiments have also been performed under the same condition (Fig. S8, ESI†). There did exist autofluorescence of the mice and mouse food, but the signals were very low (below 1500 counts) compared to the fluorescence of the materials (usually 2000–ca. 60 000 counts).

There were several other imaging experiments based on the fluorescence of Nd in the NIR II window after intravenous18 or subcutaneous injection, which have realized the imaging on mouse model on a basic level.

**Lymphatic imaging**

The lymphatic system is one of the common ways of tumor metastasis, so the imaging of the lymphatic system is of great significance in the diagnosis and treatment of cancer. Herein, we injected the EDTMP–LiYF₄:Nd³⁺ NPs into the nude mice. In Fig. 5a–c, the NPs were injected through paw and the axillary lymph node (pointed out with yellow arrows) was lightened. The emission intensity was already satisfying in in vivo imaging, which proved the outstanding detection depth, and we could almost see no autofluorescence, which was another advantage of NIR II imaging. The signal-to-noise ratio (SNR) reached 14.8 (Table S1, ESI†). When the skin and fat tissue upon the lymph node were removed, it showed stronger emission exceeding the detection range of the CCD (shown as white color block, Fig. 5b), and better SNR no less than 18.9. After that we found the lymph node and took it out, to avoid damaging the CCD detector, we lowered the gain parameter by half and collected signals of almost the same intensity (Fig. 5c). The observation result was further confirmed by histological analysis that lightened tissues were lymph nodes indeed (Fig. S11a, ESI†). The injection at footpad was also carried out and two of the sentinel
lymph nodes (pointed out with yellow arrows) were lightened (Fig. 5d–f), though the SNR was relatively lower (12.8, in vivo). The results were confirmed by histological analysis as well (Fig. S11b and c, ESI†). Compared to other research studies in NIR II lymphatic imaging,2 our materials were distributed mainly in the lymphatic system and maintained a good imaging quality 24 h after injection, which was beneficial for potential usage in long-term tracing, meanwhile implying the extraordinary bio-stability and bio-compatibility.

**Blood vessel imaging**

Angiography is another important way of cancer diagnosis, since the development of tumor will inevitably involve the growth of new blood vessels.45 In order to test the potential in practical applications, we assessed the feasibility of EDTMP–LiYF₄:Nd³⁺ NPs in blood vessel imaging. Fig. 6 displays the imaging results of blood vessels of a nude mouse. At vascular bifurcations, fine vessels could be seen separately, demonstrating intuitively the high resolution of this probe. It was further verified by the intensity profiles along the red-dashed lines, in which different peaks of the signals of blood vessels could be easily identified in Fig. 6a, c and d, and fit to Gaussian functions (marked with red-dashed curves) to extract the width. Due to the inevitable absorbance and scattering of the tissues, we used the full width at half maximum (FWHM) to represent the widths of the vessels. Almost all the calculated widths were consistent with the expected values around 0.1–2.0 mm,⁴⁶ except one of them shown in Fig. 6d, reaching the width of 2.80 mm. This result was still reasonable, as this blood vessel could be seen much wider than all the other ones around it with the naked eye in the bright-field picture of it (Fig. 6e). These results proved that our probe could show the width of vessels veritably, from ~0.2 mm to ~3.0 mm, with little interference from biological tissues, reaching a commensurate resolution limit of Micro-CT.⁴⁶ Furthermore, in Fig. 6a, c and d, all the Gaussian fit was restricted to the artificially selected data range. To put it another way, the signal peaks could not be identified by common data processing software like origin⁴⁶ in a relatively large range. That might be caused by the narrow width of the peak composed of only a few data points (4–5), which were not enough for the software to identify the peaks automatically, and this problem could not be solved now because of the limited resolution of the CCD detector. Only limited pixels could be collected on a line of fixed length, so we tried to find the vessels wide enough to contain more data points to prove the ability of this probe to distinguish separate blood vessels. The ones in Fig. 6f were both the extension of arteriacarotis, and the signal peaks could be identified automatically from the background. The fitting result was also satisfying and the FWHM could also be directly read from the diagrams. It should be pointed out that though the apparent resolution was not as high as those of the reported SWNTs⁴⁶ and Ag₂S QDs,² the CCD detector used in our imaging experiment was Si-CCD, and the quantum efficiency was about 40 percent lower than that of InGaAs-CCD at the working wavelength.³ The image was still clear enough to see the fine blood vessels of nude mice. Considering the high price of an InGaAs-CCD, this probe can be easily promoted for ordinary animal imaging requiring lower cost. The imaging quality might be improved by changing the activator ions and the imaging system in further research. 

**Fig. 5** NIR II imaging of the lymph node 24 h after injection at the right paw of the nude mouse (a) in vivo, (b) after the removal of skin and fat tissues above. (c) The removed axillary lymph node. NIR II imaging of lymph nodes 24 h after injection at the right footpad of the nude mouse (d) in vivo, (e) after removal of skin and fat tissues above the lymph nodes. (f) The removed lymph nodes around leg. Scale bar = 0.5 cm. All the lymph nodes are notified with yellow arrows and the injecting site is marked with white arrows. The regions of interest (ROI) are marked using blue areas. The mean intensity of ROI 1 (specific uptake), ROI 2 (nonspecific uptake) and ROI 3 (background) was collected for the signal-to-noise ratio (SNR) calculation (see Table S1, ESI†).
Bio-distribution after intravenous injection

The distribution of this probe after intravenous injection was also tested. As shown in Fig. S9 (ESI†), the nanocrystals mainly aggregated in the liver of the mouse, showing a strong signal (SNR = 12.8, in vivo). It involved the elimination process of the nanomaterials from blood. It was corresponding to the result of the histology test (Fig. S10, ESI†) that the NPs did not accumulate in lung, so that the possibility of it causing damage in lung was low, while the risk of lung injury was often high when it comes to other nanomaterials.47,48

Test of bio-toxicity

One of the most important characteristics of an in vivo imaging probe is bio-compatibility. The possible bio-toxicity of EDTMP–LiYF₄:Nd³⁺ was assessed using MTT assay in the HeLa cell line (Fig. 7). The cell viability did not decrease with the increasing concentration of EDTMP–LiYF₄:Nd³⁺ NPs. Instead, it showed a growing trend as if the NPs exerted a positive influence on the cells. The result is explicable since rare-earth fluorides can promote the growth of living things at low concentration.49 So we may at least conclude that the NPs did not have apparent cytotoxicity.
Fig. 7 Cytotoxicity of EDTMP–LiYF₄:Nd³⁺ NPs determined for HeLa cells after 24 h of incubation.

The histology test was also conducted and the H&E stained tissue section did not show obvious lesions in heart, liver, spleen, lung and kidney tissues as well (Fig. S10, ESI†), which proved the good bio-compatibility of EDTMP–LiYF₄:Nd³⁺ NPs.

Conclusions

In brief, we successfully designed a simple and efficient NIR II probe for lymphatic and circulatory imaging. The newly-adopted host material, LiYF₄, enhanced the intensity and the split of emission peaks and was of great bio-compatibility. The activator ion, Nd³⁺, enabled excitation at 808 nm which gave out emission in the NIR II window, enjoying the merits of high penetration depth and low autofluorescence. The quantum yield of the nanomaterial provided great opportunity in bio-imaging and bio-detection, and could be widely promoted at relatively low cost. Further research on transferring traditional rare-earth doped systems to this new host material is also promising.

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Notes and references