Temperature Sensing and Bio-imaging Applications Based on Polyethylenimine/CaF₂ Nanoparticles with Upconversion Fluorescence

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Abstract

Rare earth ion-doped upconversion nanoparticles (UCNPs) have become a research hotspot for their applications such as nano-thermometry and bio-imaging. Here we report one pot solvothermal synthesis of polyethylenimine (PEI) coated CaF₂: 1%Er³⁺, 2%Yb³⁺ UCNPs with good water-solubility and biocompatibility. The nanoparticles show a spherical shape with an average size of 40 nm and display intense green light (Yb³⁺ and Er³⁺) under the 980 nm irradiation. The maximum sensitivity of temperature sensing in the biological temperature range is obtained to be 0.00215 K⁻¹ using the fluorescence intensity ratio (FIR) technique. Furthermore, after the UCNPs are incubated with Hela cancer cells for 6 h, the obvious green sign from Er³⁺ can be observed under the excitation at 980 nm. All the experimental results imply that the PEI/CaF₂: 1%Er³⁺, 2%Yb³⁺ nanoparticles are excellent candidates for nano-thermometry and have good potential for biological applications.

Keywords: Optical temperature sensors; Nano-thermometry; Bio-imaging.

1. Introduction

The temperature plays an important role for the physical, chemical and biological processes [1, 2]. In particular, the rates of chemical reactions are affected strongly by

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the internal temperature of cells [3]. In general, the signals of cell states can be detected by the temperature based on the fact that some infected cells have higher temperature than the normal cells, urgently requiring the nano-thermometers for the biological assays [4, 5]. In addition, the cancer cells can be killed by increasing the temperature of nano-thermometry in cells [3].

In recent years, a lot of related materials have been used for nano-thermometry, including quantum dots (QDs), organic fluorophores and rare earth ion-doped luminescent materials [6-9]. Nevertheless, there is a limit for QDs in biological imaging due to the visible light as the sole pump source [10, 11]. Moreover, the QDs may have high toxicity for the existence of heavy metals. In contrast, the near-infrared (NIR) light can be used to excite the organic fluorophores, but they have low emission efficiency and are easy to undergo photobleaching [12]. The rare earth ion-doped luminescent materials, especially the upconversion nanoparticles (UCNPs), have great advantages in biological applications. At first, the NIR light fits the biological window (from 750 nm to 1100 nm) as a pump source, which achieves deeper penetration in biological tissues. Secondly, the UCNPs possess lower toxicity, autofluorescence and light scattering based on their excellent optical properties [10, 13]. Moreover, the UCNPs can be excited under low pump power, which produce less thermal energy and are less harmful to biological tissues [14]. In addition, the internal temperature of cells can be evaluated based on the fluorescence intensity ratio (FIR) from the transitions of thermal coupled levels (TCLs) of rare earth ions. Thus the UCNPs with high quantum yield can be regarded as great fluorescence nano-thermometers and promising bio-imaging probes.

Rare earth fluorides UCNPs have achieved a high upconversion luminescent efficiency by enhancing the radiation rate with the help of good physicochemical stability and low phonon energy. They are the most suitable materials for nano-thermometry and optical bio-imaging in comparison with the halides, sulfides, and oxides [15-19]. In particular, the rare earth ion-doped CaF_2 UCNPs have great fluorescence properties, such as high fluorescence efficiency under low NIR excitation density, which have been proven in previous reports [20-23].

In the present paper, $\text{Yb}^{3+}/\text{Er}^{3+}$ -doped CaF_2 UCNPs coated by polyethylenimine (PEI) are fabricated via solvothermal method. The strong fluorescence can be observed by naked eyes under the irradiation of 980 nm. The upconversion mechanism and process are proposed and the fluorescence properties are investigated in detail. In addition, the temperature sensing behavior of the sample by using the TCLs based on the FIR technique is studied. Furthermore, the biocompatibility and upconversion luminescence (UCL) bio-imaging of the sample are investigated, indicating that the $\text{Yb}^{3+}/\text{Er}^{3+}$ -doped CaF_2 UCNPs coated by PEI have great potential for their applications in temperature sensing and bio-imaging.

2. Experimental Section

2.1. Synthesis of $\text{Yb}^{3+}/\text{Er}^{3+}$ co-doped CaF_2

The CaF_2 : x Er^{3+} , y Yb^{3+} ((a) y=2%; x=1%, 2%, 3%; (b) x=1%; y=1%, 2%, 3%, 5%) (mole fraction) were synthesized via a solvothermal process. All the chemicals were commercially available and used without further purification. CaCl_2 (99.5%, Beijing Chemical Reagent Co., Ltd), $\text{YbCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{ErCl}_3 \cdot 6\text{H}_2\text{O}$ (99.99%, Sinopharm Chemical Reagent Co., Ltd) were dissolved in ethylene glycol solution containing branched PEI (Sigma-Aldrich, Mw~25000) and stirred for approximately 30 min under heating (60 °C). Subsequently, ethylene glycol solution with NH_4F (99.5%, Beijing Chemical Reagent Co., Ltd) was dropped in the initial solution containing the chlorides and stirred for 60 min. The resulting pale yellow solution was then placed in a 60 mL Teflon-lined autoclave and heated to 200 °C keeping for 12 h. The as-obtained sample was isolated from the ethylene glycol solution and washed with a solution containing distilled water and ethanol ($V_{\text{solution}}: V_{\text{water}}: V_{\text{ethanol}} = 1:1:1$). Finally, the UCNPs were dried at 60 °C under air for 12 h.

2.2. Biocompatibility of the UCNPs

The MTT (3 - (4, 5-dimethylthiazol-2-yl)-2, 5 -diphenyltetrazolium bromide) cell assay was needed on Hela cells for the evaluation of biocompatibility of the UCNPs, which the toxicity of sample can be reflected by the assay. Hela cells for MTT assay (5×10^3 each well) were seeded in a 96-well plate and incubated in 5% CO_2 at 37°C for

24 h to make them attach to the walls. Then $\text{Yb}^{3+}/\text{Er}^{3+}$ co-doped CaF_2 UCNPs were placed into the wells. The concentrations of the sample were 1.5625, 3.125, 6.25, 12.5, 25, 50, and 100 μM , respectively. Then the cells were cultured with them in 5% CO_2 at 37 °C for 24 h. The MTT was dissolved in phosphoric acidic buffer solution (PBS) to obtain the MTT solution (5 mM). Then the solution (20 μL) was placed into each well and the 96-well plate was induced in 5% CO_2 at 37 °C for 4 h. After that, the supernatant of all the wells were removed. The formazan crystals can be dissolved by dimethyl sulfoxide (DMSO), and every well was added in 150 μL of DMSO. Subsequently, the plate was placed into a microplate reader (Therom Multiskan MK 3) to shake for 5 min and test the absorbance under the irradiation of 490 nm.

2.3. UCL images of CaF_2 UCNPs

The instrument equipped with an inverted fluorescence microscope (Nikon Ti-S) was used for UCL images. Simultaneously a pump source of 980 nm laser focused on the wells. Hela cells (3×10^5 per well) were seeded in a 6-well plate and incubated in 5% CO_2 at 37 °C overnight for UCL images. Subsequently the CaF_2 UCNPs were placed into the wells and incubated with cells for 2 h and 6 h, respectively. Next, the cells were washed by PBS three times, fixed with 4% formaldehyde (3-5 mL) at 37 °C for 10 min, and washed with PBS three times again. Then 1mL PBS and 1 μL 4',6-diamidino-2-phenylindole (DAPI) were added into each well to dye nucleus for 3 min, then washed with PBS three times again. Finally, these cells were used for detection of UCL under 980 nm pump power.

2.4. Cellular uptakes

Hela cells (3×10^5 each well) were seeded in 6-well culture plates and incubated in 5% CO_2 at 37 °C overnight. Then the CaF_2 UCNPs were placed into the wells and incubated with cells for 12 h and 24 h, respectively. The cells were washed with PBS three times, trypsin digestion and centrifugation (3000 r, 5 min). Subsequently, the cells were placed in 37 °C, -20 °C, -80 °C for 15 min, respectively. And the process was repeated 7 times. Half of them were used for measured the amount of rare earth ions in cells by inductively coupled plasma-mass spectrometry (ICP-MS), the others were treated via bicinchoninic acid (BCA) protein assay to detect the content of

protein in cells. The standard curve was made by testing the absorbance under the irradiation of 570 nm using the dilutions of bovine serum albumin (BSA) as the template. Furthermore, the regression equation of the standard curve can be obtained by fitting.

2.5. Characterization

The X-ray diffraction (XRD) patterns of samples were measured by a D8 Focus diffractometer (Bruker) equipped with Cu K α radiation ($\lambda=0.15405$ nm) at a scanning rate of 15°/min in the 2 θ range from 10° to 80°. A field emission-scanning electron microscope (FE-SEM, S4800, Hitachi) was used for the measurement of the morphology and the size of the material. Transmission electron microscopy (TEM) images were detected by utilizing FEI Tecnai G2 S-Twin with a field emission gun operating at 200 KV. Fourier-transform infrared (FT-IR) spectra were measured on a Vertex PerkinElmer 580 BIR spectrophotometer (Bruker) by KBr pellet technique. Thermogravimetric (TG) Analysis was detected on Netzsch Thermoanalyzer STA 409 instrument in an atmospheric environment with a heating rate of 10 °C /min from room temperature to 800 °C. The UCL spectra were measured by an Andor Shamrock SR-750 fluorescence spectrometer. A CCD detector combined with a monochromator was used for signal collection from 400 to 750 nm. A diode laser (980 nm) was regarded as the pump source. The diode was coupled with a fiber (the core diameter 200 μ m, numerical aperture 0.22). The luminescence spectra were recorded by utilizing an Andor SR-500i spectrometer (Andor Technology Co, Belfast, UK). The Yb³⁺/Er³⁺ co-doped CaF₂ UCNPs were filled in an iron sample cell and the temperature of the samples were heated by resistive wire elements in the range of 303-573 K. The temperature of material is detected by a copper-constant thermocouple. A confocal laser scanning microscope (Olympus, FV 1000) was used for confocal laser scanning microscopy (CLSM) images. ICP was detected on a Thermo Electron X Series II ICP apparatus.

3. Results and discussion

3.1. Morphology and component of UCNPs

The FE-SEM and TEM images are used to investigate the morphology of Yb³⁺/Er³⁺

co-doped CaF_2 UCNPs, which are shown in Fig.1. It can be seen that the UCNPs are uniform in size and the average diameter is 40 nm. The black spots in the images result from PEI absorbed on the surface of the UCNPs, which are burnt out under exposure to the accelerated electron beam during the TEM measurement. The XRD pattern is used to confirm the structure and phase of $\text{Yb}^{3+}/\text{Er}^{3+}$ co-doped CaF_2 UCNPs, which is shown in Fig. 2. It can be seen that all of the diffraction peaks can be indexed to the CaF_2 , which is perfectly consistent with the JCPDS card (No. 65-0535), indicating that the rare earth ions have entered into the host lattices by occupying the chemical substitutional sites. FT-IR spectroscopy of $\text{Yb}^{3+}/\text{Er}^{3+}$ co-doped CaF_2 UCNPs coated by PEI is detected, as shown in Fig. 3(a). The broad absorption band centered at 3267 cm^{-1} is indexed to the stretching vibration of N-H and O-H, the peak appearing at 2935 cm^{-1} is indexed to the stretching vibration of C-H bonds in $-\text{CH}_2-$, and the absorption band centered at 1465 cm^{-1} can be indexed to the deformation vibration of C-H bonds, implying the existence of PEI and ethylene glycol. In addition, the FT-IR band assignments are shown in Table 1. The TG experiment of CaF_2 UCNPs is displayed in Fig. 3(b). PEI loses weight in the temperature range of $250 \sim 400^\circ\text{C}$, we can see that the sample loses weight ($\Delta W=1.4\%$) in corresponding temperature range from Fig. 3(b). The result shows that although the amount of PEI is little, the CaF_2 UCNPs have been coated already by PEI.

3.2. Photoluminescence properties and upconversion mechanism

Lanthanide-doped CaF_2 UCNPs have been paid more attention due to their high upconversion fluorescence efficiency [24]. Fluorescence spectrum of the $\text{Yb}^{3+}/\text{Er}^{3+}$ co-doped CaF_2 UCNPs under the 980 nm irradiation is presented in Fig. 4. The spectrum exhibits two characteristic emission bands located at 522, 538 and 655 nm, resulting from $^2\text{H}_{11/2}/^4\text{S}_{3/2} \rightarrow ^4\text{I}_{15/2}$ (green emissions) and $^4\text{F}_{9/2} \rightarrow ^4\text{I}_{15/2}$ (red emission), respectively. The insets are the UC emission images of the sample in ethylene glycol and PBS without and with the 980 nm irradiation, respectively. Especially, the strong green emission can be observed, indicating that the CaF_2 UCNPs are well-dispersed in ethylene glycol and PBS to form colloidal solutions based on the good solubility of PEI in water and many organic solvents. The result demonstrates that the sample has

great potential in bio-related applications.

The numbers of photons are needed to know for upconversion mechanism, the emission intensity and the pump power can be linked with the relation $I_{up} \propto (P_{pump})^n$, where n stands for the numbers of pump photons populated to the upper level [25-27]. Fig. 5(a) reveals that the UCL intensities increase linearly with the rise of excited power, which can be fitted with a line. The slopes n for green emissions (${}^2\text{H}_{11/2} \rightarrow {}^4\text{I}_{15/2}$, ${}^4\text{S}_{3/2} \rightarrow {}^4\text{I}_{15/2}$) are approximately 2.47 and 2.53, respectively. The result suggests that two NIR photons are absorbed simultaneously a visible photon is produced for the as-obtained sample under the 980 nm irradiation [25, 28].

Er^{3+} ions have great advantage for UCL based on TCLs (${}^2\text{H}_{11/2}/{}^4\text{S}_{3/2}$) and the UC mechanisms are widely discussed [29-32]. Compared to single doping (Yb^{3+} or Er^{3+}), the fluorescence intensity from Yb^{3+} and Er^{3+} co-doping is strong based on the energy transfer between the Yb^{3+} and Er^{3+} , which can be observed by naked eyes. In addition, the main processes of transitions and energy transfers are shown in Fig. 5(b). The Yb^{3+} ions at ${}^2\text{F}_{7/2}$ state absorb a photon of 980 nm and populate to ${}^2\text{F}_{5/2}$ state (${}^2\text{F}_{7/2} \rightarrow {}^2\text{F}_{5/2}$) based on ground absorption (GSA) process. Then the population of ${}^4\text{I}_{15/2}$ (Er^{3+}) state is populated to ${}^4\text{I}_{11/2}$ (Er^{3+}) state by an energy transfer (ET), simultaneously the population of ${}^2\text{F}_{5/2}$ (Yb^{3+}) state returns to ${}^2\text{F}_{7/2}$ (Yb^{3+}) state. Then the ${}^4\text{I}_{11/2}$ (Er^{3+}) state is shifted to ${}^4\text{F}_{7/2}$ (Er^{3+}) state based on the second ET ($\text{Yb}^{3+} \rightarrow \text{Er}^{3+}$), the excited state absorption (ESA) also has contribution to it: ${}^4\text{I}_{11/2} + h\nu \rightarrow {}^4\text{F}_{7/2}$. Subsequently, the ${}^4\text{F}_{7/2}$ state populates to ${}^2\text{H}_{11/2}/{}^4\text{S}_{3/2}$ state via multiphonon relaxation (MPR). After the first-level excitation, the MPR happens from ${}^4\text{I}_{11/2}$ to ${}^4\text{I}_{13/2}$ state, the population of ${}^4\text{I}_{13/2}$ state is populated to ${}^4\text{F}_{9/2}$ state by ESA and ET. Then the transitions of ${}^2\text{H}_{11/2}({}^4\text{S}_{3/2}) \rightarrow {}^4\text{I}_{15/2}$ and ${}^4\text{F}_{9/2} \rightarrow {}^4\text{I}_{15/2}$ take place [33, 34].

3.3. Temperature Sensing Behavior

The ${}^2\text{H}_{11/2}/{}^4\text{S}_{3/2}$ levels of Er^{3+} can be regarded as TCLs to investigate the temperature sensing since the UCL is strongly affected by the temperature. In fact, the ${}^2\text{H}_{11/2}$ and ${}^4\text{S}_{3/2}$ levels are so close that the population of them can be described by Boltzmann type population distribution [35, 36]. The UC emission spectra of sample

under different temperature (303 K, 373 K, 473 K and 573 K) are shown in Fig. 6(a), and the pump power is set at 1.0W. It can be observed that the UCL intensity of the sample decreases with the increasing temperature, which the non-radiation transition is strong at high temperature [35]. Nevertheless, the FIR between ${}^2\text{H}_{11/2}$ and ${}^4\text{S}_{3/2}$ levels rises with the increasing temperature, which can be explained by thermalization process. The population of ${}^4\text{S}_{3/2}$ state can be populated to ${}^2\text{H}_{11/2}$ state effectively with the rise of temperature due to a quasi-thermal equilibrium based on Boltzmann distribution. Then the 522 nm emission (${}^2\text{H}_{11/2} \rightarrow {}^4\text{I}_{15/2}$) rises with the reduction of 538 nm emission (${}^4\text{S}_{3/2} \rightarrow {}^4\text{I}_{15/2}$). Furthermore, the FIR between TCLs (${}^2\text{H}_{11/2}/{}^4\text{S}_{3/2}$) can be given as follow [37-39]:

$$FIR = \frac{N({}^2\text{H}_{11/2})}{N({}^4\text{S}_{3/2})} = \frac{I_{522}}{I_{538}} = \frac{g_2 \sigma_{2j} \omega_{2j}}{g_1 \sigma_{1j} \omega_{1j}} \exp\left(\frac{-\Delta E}{K_B T}\right) = B \exp\left(\frac{-\Delta E}{K_B T}\right), \quad (1)$$

Where

$$B = \frac{g_2 \sigma_{2j} \omega_{2j}}{g_1 \sigma_{1j} \omega_{1j}}$$

N denotes the populations of the upper (${}^2\text{H}_{11/2}$) and lower (${}^4\text{S}_{3/2}$) states, g represents the degeneracy, σ denotes emission cross-section, ω symbols angular frequency. I_{522} and I_{538} are the integrated intensities of the ${}^2\text{H}_{11/2} \rightarrow {}^4\text{I}_{15/2}$ and ${}^4\text{S}_{3/2} \rightarrow {}^4\text{I}_{15/2}$, respectively. The energy separation (${}^2\text{H}_{11/2}/{}^4\text{S}_{3/2}$) can be labeled by ΔE , K_B represents the Boltzmann constant and T represents the absolute temperature [32]. The evolution of FIR (I_{522}/I_{538}) with temperature is presented in Fig. 6(b) and corresponding data can be fitted by Eq. (1). Interestingly, the Eq. (1) can be rearranged as follow:

$$\ln(FIR) = \ln B - \frac{\Delta E}{K_B T}, \quad (2)$$

where all the terms have been mentioned in the Eq. (1). Thus the experiment data about FIR (I_{522}/I_{538}) and inverse absolute temperature can be fitted by a straight line, which is shown in Fig. 6(c). The slope ($\Delta E / K_B = 1084.14$) and intercept ($\ln B = 1.76$) are obtained, respectively. The result suggests that the internal temperature of

biological systems can be estimated by the ratio of the I_{522} and I_{538} in the related temperature range.

As optical temperature sensors, the sensitivity is an important parameter for their application, which suggests the rate of temperature change by using the FIR (I_{522}/I_{538}). In particular, the sensitivity S can be defined as follow [40-42]:

$$S = \frac{dFIR}{dT} = FIR \left(\frac{\Delta E}{K_B T^2} \right) = B \frac{\Delta E}{K_B T^2} \exp \left(\frac{-\Delta E}{K_B T} \right), \quad (3)$$

Fig. 6(d) represents the sensitivity of $\text{Yb}^{3+}/\text{Er}^{3+}$ co-doped CaF_2 UCNP's under different temperature. The maximum sensitivity (0.0029 K^{-1}) is obtained at the temperature of 448 K. In the biological temperature range (292-335 K), the maximum sensitivity is achieved approximately 0.00215 K^{-1} at 335 K, showing that the CaF_2 UCNP's have potential to act as nano-thermometers.

3.4. The study of biocompatibility and cytotoxic effect

It is important to explore the biocompatibility and cytotoxic of UCNP's for its application in biology and medicine. And the standard MTT assay can be used to it [43], which is showed in Fig. 7. We can find that the viability of HeLa cells culturing with $\text{Yb}^{3+}/\text{Er}^{3+}$ co-doped CaF_2 UCNP's for 24 h is more than 90% at 100 μM (Fig. 7(a)), comparing with the viability of DOX is only about 40% at 10 μM (Fig. 7(b)). The above results show that the UCNP's possess good biocompatibility and low cytotoxic in vitro.

3.5. The UC fluorescence imaging

Except the nano-thermometry, the $\text{Yb}^{3+}/\text{Er}^{3+}$ co-doped CaF_2 UCNP's can also be used for bio-imaging, and the experiment, which HeLa cells are incubated with them

(100 μM) for 2 h and 6 h at 37°C, has been done. The green emission from Er^{3+} can be observed under the 980 nm irradiation in the Fig. 8. The nucleus can be stained by DAPI, which emits the blue light. In addition, the blue and green emissions come from the same location in the overlays images of the bright-field, nuclei and UCL after incubated for 6 h, suggesting that the UCNPs have been taken in HeLa cells and have good luminescence property for bio-imaging. In addition, the fluoride possesses high UC efficiency, which achieving strong bio-imaging under low excitation density. The result indicates that the fluoride has great potential for its application in bio-imaging. Moreover, we can find that a little fluorescence sign can be detected after incubated for 2 h, and the green emission becomes stronger for 6 h. The result indicates that the UCNPs are little in cells for 2 h and the enough time is needed for cells uptake. This may be related to the size and type of UCNPs and the way of UCNPs entering to cells also has contribution to it [24]. In fact, the NIR light is harmless to tissues at low excitation density and the background lights have little effect on signal collection, comparing quantum dots and organic dyes in bio-imaging, which the down conversion (UV) or visible light are acted as pump light. Furthermore, the NIR light has deeper penetration in bio-imaging. Thus the $\text{Yb}^{3+}/\text{Er}^{3+}$ co-doped CaF_2 UCNPs appear to be great potential for it.

3.6. Protein assays

In order to further prove that the $\text{Yb}^{3+}/\text{Er}^{3+}$ co-doped CaF_2 UCNPs have entered into Hela cells, the experiment of cellular uptakes has been done. The standard curve of protein concentration is obtained by BCA protein assay, which reflecting the

relationship between protein concentration and light absorption value of working reagent at 570 nm. And the curve is fitted via a straight line with the coefficient $R^2 = 0.998$ (Fig. 9(a)). At the same time, the samples are treated with same method and we obtain the protein concentration of cells through standard curve equation, which the cells are incubated with the samples for different time (12 h and 24 h). The result is shown in Fig. 9(b). Fig. 9(c) represents the ion concentrations ($\text{Yb}^{3+}/\text{Er}^{3+}$) in cells, which are incubated with the samples for different time (12 h and 24 h) and the concentrations are obtained by ICP-MS. The result suggests that the rare earth ions have been engulfed by cells. Finally, the ion concentrations of each mg of protein are obtained in by Fig. 9(b) and Fig. 9(c), which is shown in Fig. 9(d). Meanwhile, it can be observed that the concentration of Yb^{3+} is always twice the concentration of Er^{3+} at same time, implying that the rare earth ions come from UCNPs rather than impurity. And the ion concentrations for 24 h are higher a lot than ion concentrations of 12 h. The result indicates that the $\text{Yb}^{3+}/\text{Er}^{3+}$ ions have entered into the internal of cells by incubated with the sample (CaF_2 : 1% Er^{3+} , 2% Yb^{3+}). Furthermore, the cells have cytophagy capacity for the $\text{Yb}^{3+}/\text{Er}^{3+}$ co-doped CaF_2 UCNPs and it is a slow process, which is result from large size of sample and the way of cells endocytic. The result is consistent with the UCL imaging experiment.

4. Conclusions

The CaF_2 : 1% Er^{3+} , 2% Yb^{3+} UCNPs coated by PEI are prepared via a solvothermal route, which can be dispersed in aqueous solution based on the good solubility of PEI in water and many organic solvents. The intense green emissions from the transitions

of TCLs ($^2\text{H}_{11/2}/^4\text{S}_{3/2}$) of Er^{3+} are observed clearly under the 980 nm laser irradiation, which can be used for biology temperature sensing based on the FIR technique and the maximum sensitivity is obtained (0.00215 K^{-1}) in the biological temperature range. In addition, the UCNPs with good biocompatibility can be ingested slowly by cells, which the UCL emitted from cells incubated with UCNPs for 6 h can be observed clearly. The above results indicate that the material is an excellent candidate as nano-thermometer and has good potential for bio-imaging.

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Figure and Table Captions

Table 1. The table of characteristic absorption peak and infrared group.

Fig. 1. FE-SEM images with different resolution of $\text{CaF}_2: \text{Yb}^{3+}, \text{Er}^{3+}$ UCNPs: (a) 300 nm, (b) 500 nm, (c) 1 μm ; TEM images with different resolution of $\text{CaF}_2: \text{Yb}^{3+}, \text{Er}^{3+}$ UCNPs: (d) 20 nm, (e) 50 nm, (f) 100 nm.

Fig. 2. XRD pattern for $\text{Yb}^{3+}/\text{Er}^{3+}$ co-doped CaF_2 UCNPs. The standard data for CaF_2 (JCPDS card No. 65-0535) is shown as reference.

Fig. 3. FT-IR spectrum (a) and TG experiment (b) of the $\text{Yb}^{3+}/\text{Er}^{3+}$ co-doped CaF_2 coated by PEI.

Fig. 4. The UCL spectra of $\text{CaF}_2: 1\% \text{Er}^{3+}, 2\% \text{Yb}^{3+}$ under the excitation of 980 nm laser. The insets are the UC emission images of sample in ethylene glycol (a)/(c) and PBS (b)/(d) without and with 980 nm irradiation, respectively.

Fig. 5. (a) The log-log plots of UC emission intensity of $\text{CaF}_2: 2\% \text{Yb}^{3+}, 1\% \text{Er}^{3+}$ as a function of 980 nm laser pump power; (b) The energy level diagrams of $\text{Yb}^{3+}/\text{Er}^{3+}$ co-doped CaF_2 under the excitation of 980 nm laser and possible UC process.

Fig. 6. Temperature sensing property of the $\text{Yb}^{3+}/\text{Er}^{3+}$ co-doped CaF_2 UCNPs. (a) UC emission spectra at different temperature; (b) The evolution of FIR (I_{522}/I_{538}) with temperature; (c) monolog plot of the FIR as a function of inverse absolute temperature; (d) sensor sensitivity as a function of the temperature.

Fig. 7. (a) The viability of HeLa cells after incubated with different concentrations of $\text{Yb}^{3+}/\text{Er}^{3+}$ co-doped CaF_2 UCNPs for 24 h; (b) The viability of HeLa cells after incubated with different concentrations of DOX for 24 h.

Fig. 8. Fluorescence images of Hela cells incubated with $\text{Yb}^{3+}/\text{Er}^{3+}$ co-doped CaF_2 UCNPs for 2 h and 6 h at 37°C 5% CO_2 . Each series can be classified as the nuclei, UCL images, the overlay of both and the overlay of above and bright-field images.

Fig. 9. (a) Standard curve for BSA in modified BCA. The modified BCA assay is used to assess the absorbance of BSA standards. The value of coefficient R^2 is obtained about 0.998; (b) the protein concentration of cells with different incubation time; (c) the $\text{Yb}^{3+}/\text{Er}^{3+}$ concentrations of cells with different incubation time; (d) the $\text{Yb}^{3+}/\text{Er}^{3+}$ concentrations of each mg of protein.

Graphical abstracts

The polyethylenimine (PEI) coated $\text{CaF}_2: 1\%\text{Er}^{3+}, 2\%\text{Yb}^{3+}$ UCNPs are great candidates for nano-thermometry and have good potential for bio-imaging.

Table 1. The table of characteristic absorption peak and infrared group.

Frequency range/cm ⁻¹	Group	Vibration
3700-3000	OH,NH,C≡C-H	νX-H
3100-3000	Ar-H,=CH,CH ₂ -X	νAr-H,ν=CH
3000-2700	CH ₃ ,CH ₂ ,CH,-CHO	Alkane, Aldehyde
2400-2000	C≡C,C≡N,C=C=C,O=C=O	Triple bond, cumulated double bonds
1900-1650	C=O	Carbonyl,
1675-1500	C=C,C=N,NH	νC=C,νC=N, Benzene ring,δNH
1500-1100	CH ₃ ,CH ₂ ,CH,C-C,C-O,C-N	νC-O,νC-N,δCH
1000-650	Ar-H,=CH,OH,NH,C-X(X: Halogen)	ωAr-H,ω=CH,ωOH,ωNH,νC-X

ν: Stretch vibration

δ: Deformation vibration

ω: Bending vibration