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Controllable synthesis of NaYF₄ : Yb,Er upconversion nanophosphors and their application to *in vivo* imaging of *Caenorhabditis elegans*[†]

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Abstract

β -NaYF₄ : Yb,Er upconversion nanoparticles (UCNPs) can emit bright green fluorescence under near-infrared (NIR) light excitation which is safe to the body and can penetrate deeply into tissues. The application of UCNPs in biolabeling and imaging has received great attention recently. In this work, β -NaYF₄ : Yb,Er UCNPs with an average size of 35 nm, uniformly spherical shape, and surface modified with amino groups were synthesized by a one-step green solvothermal approach through the use of room-temperature ionic liquids as the reactant, co-solvent and template. The as-prepared UCNPs were introduced into *Caenorhabditis elegans* (*C. elegans*) to achieve successful *in vivo* imaging. We found that longer incubation time, higher UCNP concentration and smaller UCNP size can make the *in vivo* fluorescence of *C. elegans* much brighter and more continuous along their body. The worms have no apparent selectivity on ingestion of the UCNPs capped with different capping ligands while having similar size and shape. The next generation of worms did not show fluorescence under excitation. In addition, low toxicity of the nanoparticles was demonstrated by investigating the survival rates of the worms in the presence of the UCNPs. Our work demonstrates the potential application of the UCNPs in studying the biological behavior of organisms, and lays the foundation for further development of the UCNPs in the detection and diagnosis of diseases.

Introduction

Biolabeling and optical imaging techniques have been widely used in the fields of medicine and life science, which results in the rapid development of bio-marking materials. Generally, organic dyes and fluorescent proteins are recognized as traditional bio-makers, which were, for their own disadvantages such as poor stability and high photobleaching rate, limited regarding further development.¹ In 1998, the groups of Alivisatos² and Nie³ developed different modification methods to make quantum dots (QDs) hydrophilic and biocompatible,

[†]Electronic supplementary information (ESI) available: The experimental section for the hydrothermal synthesis of bare NaYF₄ : Yb,Er UCNPs, three additional figures showing TEM image, fluorescence spectrum and XRD pattern of the hydrothermally synthesized nanoparticles; A video showing the movement of the *Caenorhabditis elegans* following ingestion of NaYF₄ : Yb,Er UCNPs.

which opened up a new emerging class of biological labels. QDs possess many advantages such as strong luminescence, excellent photostability, tunable luminescence colors upon changing the size and composition, and narrow emission spectra making multiplexed imaging available,^{4,5} which has resulted in their extensive applications in biolabeling.^{6–10} However, there are also some concerns about the toxicity of QDs because they contain highly toxic metals,^{11,12} and autofluorescence of biological tissues resulting from the use of ultraviolet (UV) radiation cannot be avoided. Upconversion nanoparticles (UCNPs) have thus received great attention, and research into their synthesis and biomedical applications has become a new focus.^{13–18} UCNPs can absorb longer wavelength radiation such as near-infrared (NIR) light and then upconvert to emit a shorter wavelength fluorescence such as green light.¹⁹ In comparison with organic dyes, fluorescent proteins and QDs, UCNPs can be excited by NIR light which allows for tissue penetration to a depth of centimetres^{20,21} and the avoidance of autofluorescence from the biological tissue,²² leading to improved detection sensitivity. Moreover, UCNPs have high chemical stability, high quantum yields, large Stokes shifts, and low toxicity, and their emitting colors can be tuned by changing the host matrix and lanthanide dopants.^{23,24} In recent years, UCNPs were increasingly used as excellent bio-labels and achieved prominent applications in cell labeling and fluorescent imaging.^{25–29} However, to the best of our knowledge, there are only a few articles about using UCNPs for *in vivo* imaging.^{25,27,30–32} Therefore, using UCNPs as a probe for *in vivo* labeling and imaging to monitor the existence, distribution and expression of biomolecules or cells in an organism, is of significant importance for studying the metabolism of organisms and diagnosing diseases.

The synthesis of high quality UCNPs is a prerequisite for their use in biolabeling. The hexagonal-phase NaYF₄ is regarded as one of the excellent hosts³³ and β -NaYF₄ UCNPs doped with Yb–Er ion couples are the most efficient infrared-to-visible upconversion phosphors.³⁴ Up to now, only a few articles have reported the synthesis of β -NaYF₄ : Yb,Er UCNPs with small size, uniformly spherical shape, good hydrophilicity and biocompatibility, and strong fluorescent intensity.^{15,35,36} In this work, we employed a method of one-step synthesis to produce β -NaYF₄ : Yb,Er UCNPs with surfaces presenting amino groups, which were introduced by coating polyethylenimine (PEI) on the nanoparticle surface. Cationic polyelectrolyte PEI is a well known gene-delivery vector because its high cationic charge can effectively condense DNA and it can escape from endosomes through the “proton sponge” effect.³⁷ The toxicity of PEI, arising from its high charge, can be greatly reduced when used in a certain range of reasonable concentrations.^{36–38} The PEI capped UCNPs will be beneficial to biological applications because of the electrostatic attraction between the net positive charge of PEI and the negative charge of cells, which can facilitate cell-binding affinity, shorten absorption time and increase labeling efficiency. Moreover, the amino groups on the PEI can be further conjugated to targeting ligands, such as antibodies, to enable the further labeling of the targets. PEI was also chosen as the capping ligand due to its excellent solubility in water, which can improve the colloidal dispersity and stability of UCNPs. In addition, we integrated the green chemistry concept into a solvothermal method to prepare β -NaYF₄ : Yb,Er UCNPs. In this solvothermal approach, room-temperature ionic liquids (RTILs) were used as a “green” co-solvent due to their negligible vapor pressure, chemical stability and non-flammability,^{39,40} and also served as a reactant and template in the green synthesis.³⁵ The ionic liquid used in this work was based on 1-butyl-3-methyl-imidazolium tetrafluoroborate ([Bmim][BF₄]). Finally, we found that the synthesized UCNPs were spherical in shape with an average diameter of 35 nm, and well dispersed in water due to the presence of amino groups on their surface. Transmission electron microscopy (TEM), X-ray diffraction (XRD), photo-luminescence spectroscopy (PL) and Fourier transform infrared spectroscopy (FT-IR) were used to characterize the UCNPs. The UCNPs synthesized by the above green solvothermal method, the green hydrothermal approach (for details, see ESI†), and those prepared previously by our group⁴¹

were used for the *in vivo* imaging of *Caenorhabditis elegans* (*C. elegans*). The *in vivo* imaging was studied under different conditions including the concentration, size, and surface ligands of nanoparticles as well as the time of nanoparticle incubation with the *C. elegans*. The toxicity of NaYF₄ : Yb,Er@PEI UCNPs was assessed by investigating the survival rates of *C. elegans* which were exposed to various concentrations of the colloidal solutions under different incubation periods. *C. elegans* were chosen for the *in vivo* imaging because they are cheap and their tissues can be examined easily under the microscope due to their transparent body. Moreover, *C. elegans* have rapid growth and short life cycles, making them an ideal model animal for research.^{42,43}

Experimental

Materials

Rare-earth oxides (Y₂O₃, Yb₂O₃, Er₂O₃) were of 99.99% purity and purchased from Grilem Advanced Materials Co., Ltd. (Beijing, China). [Bmim][BF₄] was purchased from Shanghai Chengjie Chemical Co., Ltd., China (99%). The other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All of the reagents were used as received without further purification. Triple-distilled water was used throughout the experiments.

Characterization

The dimension and morphology characterizations of the solvothermally-prepared UCNPs were performed by a TECNAI-20 transmission electron microscope (TEM, FEI Ltd., USA), using an acceleration voltage of 200 kV. Powder X-ray diffraction (XRD) patterns were recorded by an X'Pert Pro diffractometer (PANalytical Co., Holland) at a scanning rate of 4° min⁻¹ in the 2 θ angle range from 10° to 65°, with graphite monochromatized Cu-K α radiation (λ = 0.15406 nm). Upconversion fluorescence spectra were obtained on a LS-55 fluorescence spectrophotometer (Perkin Elmer Co., USA), using an external 980 nm laser (Beijing Hi-Tech Optoelectronic Co., China) as the excitation source, instead of the internal equipped lamp. Fourier transform infrared (FT-IR) spectra were recorded using a Spectrum One (B) spectrometer (Perkin Elmer Co., USA).

Green solvothermal synthesis of β -NaYF₄ : Yb,Er UCNPs

In a typical procedure for the green solvothermal synthesis of β -NaYF₄ : Yb,Er UCNPs, the rare-earth stearate precursor (C₁₇H₃₅COO)₃RE (RE = Y_{0.78}Yb_{0.20}Er_{0.02}) was prepared according to our previously reported method.⁴⁴ Then, 0.9577 g of the rare-earth stearate precursor was added to a beaker containing 0.5645 g of [Bmim][BF₄], 1.6998 g of NaNO₃, 6 mL of water, 15 mL of ethanol and 4 mL of PEI (average molecular weight 20000, 50%). After stirring for about 5 min, the homogeneous mixture was poured into a Teflon-lined autoclave and subsequently heated to 180 °C for 24 h. The nanoparticles were collected by centrifugation, followed by washing with distilled water and ethanol three times. They were then dried and collected prior to further use.

Labeling of *C. elegans* and the effect of experimental conditions on the *in vivo* imaging

N2 wild type *Caenorhabditis elegans* (*C. elegans*) hermaphrodite were grown on nematode growth medium (NGM) culture plates at 20 °C, which were covered with *E. coli* strain OP50. Twenty suitable *C. elegans* were selected and then transferred to the 5.0 mg mL⁻¹

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colloidal solution in the culture dish. The colloidal solution was prepared by dispersing 10 mg of solvothermally synthesized NaYF₄ : Yb,Er@PEI UCNPs in 2.0 mL of M9 buffer solution (15.12 g of Na₂HPO₄·12H₂O, 3.0 g of KH₂PO₄, 5.0 g of NaCl, 0.25 g of MgSO₄·7H₂O, 1000 mL of deionized water) under sonication. Then the culture dish was shaken gently at a constant temperature (20 °C) for two different uptake times (6 and 20 h) to investigate the effect of incubation time on the *in vivo* imaging of *C. elegans*. In addition, we transferred the *C. elegans* with UCNPs ingested to the NGM agar plate seeded with *E. coli* strain OP50 to eject the UCNPs and then observe the action of the *C. elegans*. Then, twenty suitable *C. elegans* were also incubated with solvothermally synthesized NaYF₄ : Yb,Er@PEI colloidal solutions with different concentrations (0.5 and 2 mg mL⁻¹) for 20 h uptake to investigate the effect of the concentration of nanoparticles on the *in vivo* imaging of *C. elegans*. To investigate the effects of capping ligand and UCNP size on the *in vivo* imaging of *C. elegans*, twenty suitable *C. elegans* were incubated with UCNPs synthesized by another two different methods for 6 h uptake. One is the 5.0 mg mL⁻¹ of NaYF₄ : Yb,Er@oleic acid (OA) colloidal solution, which was prepared by dispersing 10 mg of NaYF₄ : Yb,Er@OA UCNPs synthesized by the method reported by us previously⁴¹ in 2.0 mL of M9 buffer solution under sonication. The other is the 5.0 mg mL⁻¹ of bare NaYF₄ : Yb,Er colloidal solution prepared by dispersing 10 mg of hydrothermally synthesized bare NaYF₄ : Yb,Er UCNPs (Fig. S1, ESI[†]) with a diameter of approximately 150~200 nm in 2.0 mL of M9 buffer solution under sonication. The NaYF₄ : Yb,Er@OA nanoparticles synthesized by our group previously,⁴¹ having a mean diameter of about 35 nm and an oleic acid capped surface, were used as a control for the comparison in the *in vivo* labeling and imaging. Control experiments, where twenty *C. elegans* at a similar stage as above were incubated with 2.0 mL of M9 buffer solution in the absence of UCNPs for 6 and 20 h, were also performed.

Toxicity test of NaYF₄ : Yb,Er@PEI UCNPs

The toxicity of the NaYF₄ : Yb,Er@PEI UCNPs was evaluated by studying the survival rates of the *C. elegans* after ingestion of UCNPs with various concentrations at different times. Specifically, solutions of NaYF₄ : Yb,Er@PEI UCNPs at three different concentrations (1, 2.5 and 5 mg mL⁻¹) were made by mixing appropriate amounts of NaYF₄ : Yb,Er@PEI UCNPs with 2 mL of M9 buffer solution under sonication. In every concentration, fifty *C. elegans* at the same stage of development were incubated with UCNPs. The culture dishes contained the above colloidal solutions and worms were shaken gently at 20 °C for 3 and 24 h, respectively. Finally, the number of surviving worms under each condition was counted. Each experiment was done in duplicate and control experiments without UCNPs were also performed.

In vivo imaging of *C. elegans*

In order to image the organism under NIR irradiation, the *C. elegans* that were incubated with and had ingested the UCNPs were transferred onto precleaned glass slides which were spread by agar solution with a mass fraction of 1%, then 20 µL of M9 buffer solution was dripped onto each glass slide. Subsequently, cover slips were put onto the glass slides. Imaging of the *C. elegans* was performed on an Olympus IX51 inverted fluorescence microscope equipped with a 980 nm NIR laser (the power was set as 1500 mW) and a Nikon CCD camera. A filter in front of the CCD camera was used to cut off the excitation light. A video showing the fluorescence of the organism can be found in the ESI.[†]

Results and discussion

Green solvothermal synthesis of β -NaYF₄ : Yb,Er UCNP

Similar to the liquid–solid–solution (LSS) reaction mechanism,⁴⁵ in the green solvothermal synthesis, rare-earth stearate was dispersed in a water–ethanol system to form a solid phase; NaNO₃ and [Bmim][BF₄] were dissolved in a water–ethanol–PEI mixture to form a liquid phase. When the temperature was elevated, RE³⁺ ions were released from rare-earth stearate, and when the reaction temperature exceeded the boiling temperature of [Bmim][BF₄], the ionic liquid decomposes, and the [BF₄][−] underwent fast hydrolysis producing F[−] ions.⁴⁶ Then RE³⁺ ions reacted with F[−] and Na⁺ ions at the solid–liquid interface to form NaYF₄ particles. In the reaction process, high temperature and long reaction times provide enough energy to overcome the energy barrier for cubic-to-hexagonal phase transition.⁴⁷ The imidazolium cations in RTILs were wrapped around NaYF₄ particles, preventing the NaYF₄ nucleation centers from growth and aggregation. Hence, the RTILs actually acted as the reactant, co-solvent and template.³⁵ Scheme 1 illustrates the reaction mechanism for the formation of NaREF₄. The PEI acted as a ligand capping the surface of nanoparticles, further preventing the NaYF₄ nucleation centers from growth and aggregation. Meanwhile the amino groups of PEI rendered the UCNP hydrophilic.

The TEM image, fluorescence spectrum, XRD pattern and FT-IR spectrum of the UCNP are shown in Fig. 1. The TEM image (Fig. 1a) shows that the particles are roughly spherical in shape with a mean diameter of about 35 nm. Fig. 1b shows that there are three emission peaks at 520 nm, 540 nm and 654 nm, which are assigned to the ²H_{11/2} → ⁴I_{15/2}, ⁴S_{3/2} → ⁴I_{15/2} and ⁴F_{9/2} → ⁴I_{15/2} transitions of Er³⁺ ions, respectively. The inset of Fig. 1b is the photograph of an aqueous solution of the UCNP under the 980 nm excitation. The XRD spectrum (Fig. 1c) is in very good agreement with the standard pattern of the hexagonal phase NaYF₄ (bottom plot, JCPDS No. 028-1192), suggesting the pure hexagonal phase and high crystallinity of the nanoparticles. Fig. 1d presents the FT-IR spectrum of the nanoparticles. The strong band at around 3436 cm^{−1} corresponds to the O–H and/or N–H stretching vibration, while the bands at 1638 and 750 cm^{−1} are related to the bending vibration of the N–H bond in PEI. The weak bands at around 2930 and 2861 cm^{−1} can be assigned to the asymmetric and symmetric stretching vibrations of the –CH₂ in PEI, respectively. The peaks attributed to the vibrations of the imidazole ring can be seen at 1726 and 1500 cm^{−1}, while the band at 1169 cm^{−1} is ascribed to the C–N stretching vibration of the ring. These data indicated that the UCNP are capped with a layer of PEI, rendering the nanoparticles hydrophilic and readily dispersible in water.

Imaging of *C. elegans* and effects of incubation time and UCNP concentration on the *in vivo* imaging

Control worms incubated for 6 and 20 h in the absence of UCNP (Fig. 2) were observed without autofluorescence. Subsequently, when the *C. elegans* were incubated with the colloidal solution of nanoparticles, the UCNP can be clearly seen in the intestines (Fig. 3a) because the *C. elegans* are transparent and UCNP can emit strong green fluorescence under the 980 nm NIR laser. The phosphors were distributed along the rectum presenting green fluorescence. This fact indicated that the NaYF₄ : Yb,Er@PEI UCNP were not rejected by the *C. elegans* but had been successfully introduced into *C. elegans*. (A video showing the movement of the *C. elegans* after ingestion of NaYF₄ : Yb,Er UCNP is given in the ESI†). When the *C. elegans* with UCNP ingested were deprived of food over a period of 12 h, the *in vivo* fluorescence showed little change (data not shown), which suggested that the metabolism of the *C. elegans* was slowed due to the lack of food, and the NaYF₄ : Yb,Er UCNP were biostable. What's more, when the NaYF₄ : Yb,Er UCNP were ejected from *C. elegans* after being fed with *E. coli* strain OP50, the worms still exhibit normal behaviour,

manifesting that the radiation of the 980 nm laser and the uptake of the NaYF₄ : Yb,Er UCNPs did not cause an apparent negative influence on *C. elegans*. Particularly, a little green luminescence was observed in the intestinal cells around the gut cavity (shown by the arrows in Fig. 3a) and a possible reason for this phenomenon may be the endocytosis by the cells, resulting from the electrostatic attraction between the NaYF₄ : Yb,Er@PEI UCNPs and the cells. Meanwhile, internalization may also occur due to the good dispersity of NaYF₄ : Yb,Er@PEI UCNPs in the solution and even in the gut cavity of *C. elegans*.⁴⁸

It can be seen from Fig. 3 that, under the same concentration of solution, the longer the incubation period, the larger the amount of NaYF₄ : Yb,Er@PEI UCNPs ingested by *C. elegans*. It is obvious that the green fluorescence in *C. elegans* in Fig. 3b is stronger than that in Fig. 3a under the same output power of the laser. Notably, the *C. elegans* displayed no apparent signs of abnormal behaviour after being incubated with colloidal solutions of high concentration for a long time period (20 h). Comparing Fig. 4 with Fig. 3b, we can see that the green fluorescence in *C. elegans* became more continuous and brighter upon the increase of UCNP concentration under the same incubation time. As shown in Fig. 4, the hatched larvae can be seen clearly beside the adult hermaphrodite, indicating that the NaYF₄ : Yb,Er@PEI UCNPs did not cause any side effects on the oogenesis and embryonic development. The next generation of the *C. elegans* in this experiment did not show fluorescence under excitation, suggesting that the transfer of NaYF₄ : Yb,Er@PEI UCNPs did not reach the embryos of the adult hermaphrodite. Namely, if the fluorescent materials can be delivered to the reproductive organs of the *C. elegans*, the larvae of the next generation can have fluorescence under the excitation.⁴⁸

Effects of surface ligands on the *in vivo* imaging

A comparison between Fig. 5 and Fig. 3a reveals that, when other conditions were the same, the *C. elegans* ingested two kinds of NaYF₄ : Yb,Er UCNPs with different capping ligands (OA and PEI, respectively), and presented similar continuous *in vivo* fluorescence, indicating that the capping ligands of UCNPs have little influence on the *in vivo* imaging of *C. elegans* when the UCNPs are of similar shape and size. However, the NaYF₄ : Yb,Er@OA UCNPs can not be seen in the intestinal cells in Fig. 5, indicating that endocytosis by the intestinal cells may be sensitive to the capping ligands.

Effects of UCNP size on the *in vivo* imaging

From Fig. 6 it can be seen that the *C. elegans* ingested little bare NaYF₄ : Yb,Er UCNPs synthesized by the hydrothermal method; the resultant UCNPs have irregular shape, larger size and their unmodified surface causes them to aggregate (see ESI†). This fact suggested that the UCNPs with a uniformly spherical shape, smaller size and better dispersion in solution are more suitable for the *in vivo* imaging of *C. elegans*. Besides, no transfer of bare NaYF₄ : Yb,Er UCNPs between gut cavity and intestinal cells can be observed, and we thought this may because of the large aggregation of UCNPs in the gut cavity, preventing endocytosis by the intestinal cells.⁴⁸

Toxicity test of NaYF₄ : Yb,Er@PEI UCNPs

The survival rates of the *C. elegans* treated with different concentrations of NaYF₄ : Yb,Er@PEI colloidal solutions for 0, 3 and 24 h can be seen in Fig. 7. The results indicate that NaYF₄ : Yb,Er@PEI UCNPs have no significant biological toxicity except when used at high concentrations and under a long incubation period. What's more, we believe the observed toxicity of UCNPs at high concentrations may result from the capping ligand PEI, which can cause membrane damaging effects because of its high positive charge.

Conclusions

In this work, β -NaYF₄: Yb,Er UCNP were synthesized by a one-step green solvothermal method through the use of RTILs as the reactant, co-solvent and template. The synthesized UCNP were of small size, uniformly spherical shape and were covered by a layer of PEI which could render the UCNP hydrophilic and impart colloidal stability. Then the NaYF₄: Yb,Er UCNP were successfully introduced into *C. elegans*, and the *in vivo* imaging of *C. elegans* was successfully realized. It was observed that increasing the incubation time and the concentration of the UCNP colloidal solution can make the green fluorescence in *C. elegans* stronger and more continuous, which is favorable for *in vivo* imaging. The *C. elegans* are more apt to ingest UCNP with smaller size, uniform shape and good dispersibility. Moreover, a small number of NaYF₄: Yb,Er@PEI UCNP in the gut cavity were internalized into the intestinal cells *via* endocytosis, which might arise from the electrostatic attraction between the cells and UCNP, and this phenomenon may also be related to the good dispersity of UCNP in the gut cavity. The uptake of UCNP by *C. elegans* did not exhibit apparent selectivity for those covered by different capping ligands when the UCNP are of similar size and shape. The *C. elegans* did not show obvious exclusion of NaYF₄: Yb,Er UCNP during the feeding process and the worms still displayed a normal behaviour when UCNP were ejected out of the gut cavity after they were fed with food. The toxicity test showed low toxicity of UCNP. This study demonstrated that the β -NaYF₄: Yb,Er UCNP can be used as an excellent labeling material for *in vivo* imaging due to their unique advantages such as low toxicity and strong fluorescence intensity. They can be extensively applied to biolabeling and bioimaging, which lays the foundation for further development of the UCNP in the field of biomarkers. We believe that the NaYF₄: Yb,Er UCNP, when conjugated with proper proteins and tumor-homing peptides, can be used for the detection and diagnosis of cancers by *in vivo* imaging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Characteristics of solvothermally synthesized $\text{NaYF}_4 : \text{Yb,Er}$ upconversion nanoparticles. (a) TEM image. (b) Upconversion fluorescence spectrum. Inset is the photo of the UCNPs aqueous colloidal solution under excitation with 980 nm. (c) Powder XRD pattern (top plot) and the calculated line pattern (bottom plot) for $\beta\text{-NaYF}_4$ (JCPDS No. 028-1192). (d) FT-IR spectrum.

Fig. 2.

Images of *C. elegans* incubated with M9 buffer solution for (a) 6 h, (b) 20 h; the left columns are images in dark field, and the right columns are images in bright field.

Fig. 3. Fluorescent images of *C. elegans* incubated with 5 mg mL^{-1} of solvothermally synthesized $\text{NaYF}_4 : \text{Yb,Er@PEI}$ nanoparticles colloidal solution for (a) 6 h, (b) 20 h; the left columns are images in dark field, and the right columns are images in bright field.

Fig. 4. Fluorescent images of *C. elegans* incubated with (a) 0.5 mg mL^{-1} , (b) 2 mg mL^{-1} of solvothermally synthesized $\text{NaYF}_4 : \text{Yb,Er@PEI}$ nanoparticles colloidal solution for 20 h; the left columns are dark field images, and the right columns are bright field images.

Fig. 5. Fluorescent images of *C. elegans* incubated with 5 mg mL^{-1} of $\text{NaYF}_4 : \text{Yb,Er@OA}$ nanoparticles colloidal solution for 6 h; the left column is the dark field image; the right column is the bright field image.

Fig. 6. Fluorescent images of *C. elegans* incubated with 5 mg mL^{-1} of hydrothermally synthesized $\text{NaYF}_4 : \text{Yb,Er}$ nanoparticles solution for 6 h; the left column is the dark field image; the right column is the bright field image.

Fig. 7.

The survival rates of the *C. elegans* exposed to various concentrations of NaYF₄:Yb,Er@PEI colloidal solution for different periods.

Scheme 1.

Scheme of the formation of the $\text{NaYF}_4 : \text{Yb,Er}$ upconversion nanoparticles synthesized by the green solvothermal method.