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Upconversion fluorescent and X-ray-sensitive bifunctional nanoprobes for assessing the penetrability of inorganic nanoparticles in the digestive system†‡

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Nanotechnology is receiving increasing attention due to its fantastic advantages and potential applications in nanofood and nanomedicine. However, the safety of touching manufactured nanoparticles is still uncertain for human beings. Here, we track inorganic nanoparticles in the digestive system of the mouse through upconversion fluorescence and X-ray imaging, and try to demonstrate whether or not the inorganic nanoparticles will penetrate the digestive system to enter the blood system. Lanthanide-doped upconversion nanoparticles, which can convert infrared light to visible light and are simultaneously sensitive to X-rays, were selected as model inorganic nanoparticles. The investigation clarifies that even the ultrathin nanoparticles (~5 nm) could not penetrate the digestive tract to enter the bloodstream or surrounding tissues, but were gradually excreted out. Our results help assess the safety of inorganic nanoparticles potentially used in nanofood and nanomedicine.

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1. Introduction

Synthetic nanomaterials are increasingly applied in the fields of food, make-up, and clothes and so on.^{1–5} The safety of nanomaterials for human beings has received increasing attention with the rapid development of nanotechnology.^{6–8} The fear is whether the nanomaterials will penetrate the skin to enter the human body or cross the digestive system barriers to circulate in the blood system or other organs.^{9–12} On the cellular scale, much effort has been focused on the study of the penetration of nanomaterials across the cell membranes.^{13–17} It has gradually become clear that it is impossible for most synthetic nanomaterials to cross cell membranes.^{18–20} But, up to now, it has been determined that there are several kinds of synthetic nanomaterials, such as metal clusters, chemically functionalized carbon nanotubes,

and quantum dots (QDs), that are capable of penetrating cell membranes.^{21–24} They create pores in the cell membranes that can lead to cellular toxicity due to the destruction of the delicate concentration balance of intracellular *versus* extracellular ions, proteins, and other important macromolecules.^{25–27} Therefore, the penetration ability of a given nanomaterial towards cells or tissue should be individually determined and estimated.²⁸

Recently, nanomedicine has attracted extensive attention, owing to its unique advantages including low biological toxicity, precise targeting, solid-state properties and mobility, and higher circulation speed for improving drug delivery efficiency.^{29,30}

A series of inorganic nanoparticles including noble metal nanoparticles, quantum dots (QDs), and upconversion nanoparticles have been synthesized, modified and employed for diagnoses and phototherapy.^{31–34} The previous reports show that these nanoparticles can be used for background-free imaging, tracking drug delivery, targeting tumours, and photo-thermal and photochemical therapy.^{35–37} These inorganic nanomaterials were transported into the body through injection or ingestion. For the route of ingestion, it is necessary to determine the penetration of these inorganic nanoparticles in the digestive system, and whether these inorganic nanoparticles will enter into other organs beyond the digestive system or not, since these nanoparticles would lead to the breaching of the human organs if circulated in the organs for a long time.^{38,39} However, there are few reports so far.

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In this paper, lanthanide-doped upconversion nanoparticles were selected as model nanoparticles due to their merits of simultaneously realizing upconversion fluorescence and X-ray imaging, of which the merits of ultra-small size and strong luminescence can be used for imaging cells and clinical therapy. The strong upconversion luminescence is a process where low energy photons are always converted into higher energy photons by sequential absorption of two photons or multiphotons. We systematically evaluated the size-dependent and surface-dependent penetrability of upconversion nanoparticles towards the digestive system of the mouse through fluorescent signals and X-ray imaging (see Scheme 1).^{40–43} The upconversion luminescence (UCL) signals of upconversion nanoparticles (UCNPs) in the stomach, intestines, heart, liver, lung, and kidney of the mouse was tracked to address whether UCNPs can penetrate the digestive organs, such as stomach or intestine, and enter the blood system or surrounding tissues. Furthermore, the circulation of UCNPs in the mouse is demonstrated by *in vivo* X-ray computed tomography imaging (CT) due to the fact that the UCNPs are sensitive to X-ray. Transmission electron microscopy (TEM) was also carried out to characterize the existence of UCNPs in the blood.⁴⁴

2. Experimental

2.1 Materials

GdCl₃·6H₂O (99.9%), LuCl₃·6H₂O (99.9%), YbCl₃·6H₂O (99.9%), ErCl₃·6H₂O (99.9%), NaOH (≥98%), NH₄F (≥98%), methanol (99.5%), 1-octadecene (ODE) (90%), oleic acid (OA)

(90%), polyethylene glycol (PEG) and Nile red (≥98%) were purchased from Sigma Aldrich. Deionized water was used throughout. Unless otherwise noted, all chemicals were used directly without further purification.

2.2 Synthesis of NaGdF₄ nanoparticles

The synthesis of NaGdF₄:Yb³⁺,Er³⁺ nanoparticles was carried out following a solvothermal method with modification. 2 mL aqueous solution of RECl₃·6H₂O (0.2 M, RE = Lu, Gd, Yb and Er) was added to a 50 mL flask containing ODE (12 mL) and OA (4 mL). The resulting mixture was heated to 160 °C with constant stirring to remove residual water and oxygen. After 30 min, the temperature was cooled to room temperature with a flow of argon gas through the reaction flask. Shortly thereafter, 5 mL methanol solution of NH₄F (1.5 mmol) and NaOH (1 mmol) was added and the resultant solution was stirred for another 30 min at 50 °C. After the methanol from the reaction mixture was evaporated, the solution was heated to 310 °C under an argon atmosphere for 60 min and then cooled down to room temperature naturally. The resulting nanoparticles were precipitated by addition of ethanol, collected by centrifugation at 10 000 rpm for 5 min, and washed with ethanol three times. Finally, the prepared nanocrystals could be re-dispersed in nonpolar organic solvents such as cyclohexane and chloroform.

2.3 Surface modification of OA-UCNPs with PEG

The surface modification with PEG was conducted according to previous protocols. In brief, a 500 μL cyclohexane solution

Scheme 1 Schematic illustration of the distribution of size-controlled upconversion nanoparticles (UCNPs) in the digestive system and blood circulation system (e.g., stomach, small intestine, large intestine, heart, liver, lung, kidney) after a mouse was fed with a mixture of fodder and UCNPs.

Fig. 1 TEM images of upconversion nanoparticles (a) NaGdF₄:2% Yb³⁺/0.5% Er³⁺; (b) NaGdF₄:18% Yb³⁺/2% Er³⁺; (c) NaGdF₄:28% Yb³⁺/2% Er³⁺; (d) NaLuF₄:10% Gd³⁺/18% Yb³⁺/2% Er³⁺. The images (e–h) show the corresponding high-resolution TEM images of (a–d), respectively. (i) Histogram of the size distribution of the OA-UCNPs from TEM images shown in (a–d). EDX spectra of (b) and (d) are shown in (j) and (k), respectively. The four kinds of nanocrystals are referred to as UCNPs-1, UCNPs-2, UCNPs-3 and UCNPs-4 in the following discussion.

of oleic acid coated NaGdF₄ nanoparticles was added to ethanol and acetic acid aqueous solution (3 v/v%) and kept for 30 min, and subsequently precipitated by centrifugation at 3500 rpm with the supernatant discarded. The collected nanoparticles were dispersed in aqueous solution with a drop (~0.1 ml) of PEG, then the precipitates were separated by centrifugation after vigorous stirring for 1 hour at 26 °C, rinsed with ethanol three times to remove the unreacted PEG, and readily dissolved in water. The prepared solution was filtered through a 0.22 μm syringe filter to remove larger aggregates. PEG-UCNPs were thus formed.

2.4 Characterization

The size and morphology of the prepared nanoparticles were measured using a H-7650c transmission electron microscope (TEM) operating at 80 kV and a JEM 3010 high-resolution transmission electron microscope (HRTEM) operating at 200 kV. Energy dispersive X-ray (EDX) analysis of the samples was also performed during HRTEM measurements. The photoluminescence (PL) emission spectrum was measured from 400 to 700 nm using a Hitachi F-2700 spectrophotometer equipped with a 980 nm laser as the excitation source. The photos of upconversion luminescence were obtained digitally by a Nikon multiple CCD camera.

2.5 *Ex vivo* upconversion fluorescence bioimaging

The mice were obtained from Changsha Laboratory Animal Center. Nude mice weighing 20 g were used at ages from 4 to 5 weeks. All animal operations were in accordance with institutional animal use and care regulations, animal care and handling procedures were in agreement with the guidelines of the Regional Ethics Committee for Animal Experiments. For investigating biological penetrability and bio-distribution of UCNPs between the digestion system and bloodstream, the mice were fed with various sizes of nanoparticles for different times; subsequently, organs of the mice were obtained by dissection after an intraperitoneal injection with chloral hydrate solution for about 10 minutes, and then *ex vivo* upconversion fluorescence images of the organs were collected digitally using a Nikon multiple CCD camera under the excitation of a 980 nm laser diode. UCL signals were collected at 540 ± 12 nm. All imaging experiments were performed under the same conditions.

2.6 *In vivo* CT imaging

In order to study the bio-distribution of UCNPs in the mouse organs at different time points, a mouse was first fed with a mixture of fodder and UCNPs for three hours, then anesthetized by intraperitoneal injection of 0.2 ml chloral hydrate

Fig. 2 (a) Upconversion fluorescence spectra of the oleic acid and polyethylene glycol (PEG) coated $\text{NaGdF}_4\text{:Yb}^{3+}\text{,Er}^{3+}$ nanoparticles, under the excitation of a 980 nm near infrared laser. Insets: Left picture demonstrates the good solubility of upconversion nanoparticles in cyclohexane, while the middle and right pictures show the emission of $\text{NaGdF}_4\text{:Yb}^{3+}\text{,Er}^{3+}$ nanoparticles with OA and PEG coatings, respectively. (b) FT-IR spectra of OA-UCNPs and PEG-UCNPs.

solution (5 wt%) for the first CT imaging. The mouse was anesthetized again at different time points (from 3 hours to 3 days) for CT imaging following revival after several hours. CT images were acquired using a ZKKS-MCT-Sharp (Chinese Academy of Sciences and Guangzhou Kaisheng Medical Technology Co., Ltd.) with the following parameters: thickness, 0.14 mm; pitch, 0.07; 60 kVp, 0.5 mA; large field view; gantry rotation time, 0.5 s; speed, 5 mm s^{-1} .

3. Results and discussion

3.1 Upconversion fluorescence properties of the resulting NaGdF_4 nanoparticles

In order to investigate size-dependent biological penetrability of upconversion nanoparticles in the digestive system of the mouse, four kinds of $\text{Yb}^{3+}\text{/Er}^{3+}\text{/Lu}^{3+}$ ion-doped NaGdF_4 nanoparticles with sizes ranging from ~5 to 100 nm were synthesized by a general solvothermal method. To reveal the phase and size control, these nanoparticles were characterized by TEM and HRTEM. Fig. 1a–d shows the TEM results for

$\text{NaGdF}_4\text{:Yb}^{3+}\text{/Er}^{3+}$ with various Gd^{3+} concentrations of 97.5%, 80%, 70%, and 10%, respectively, of which the average diameters were determined to be about 5.25 ± 1.2 , 21.5 ± 2.0 , 69.0 ± 8.2 , 102.5 ± 17.5 nm (Fig. 1i). The size of the NaGdF_4 nanoparticles gradually increased with decreasing content of Gd^{3+} ions. This phenomenon can be ascribed to the lower electron charge density accelerating the diffusion of F^- ions so that the particle size was increased when the concentration of Gd^{3+} ions was decreased. In Fig. 1a–c, the content of Gd^{3+} was reduced and that of Yb^{3+} was increased correspondingly. In Fig. 1d, Gd^{3+} was further decreased by introducing external Lu^{3+} , of which the aim was to avoid deleterious cross relaxation caused by a high concentration of Yb^{3+} or Er^{3+} . Moreover, the comparison of energy dispersive X-ray spectrometry (EDX) analysis (Fig. 1j and k) verified that Gd^{3+} was substituted partly by Lu^{3+} successfully, and showed the presence of Na, Gd, Lu, F, and doped Yb, Er elements. HRTEM was also performed, as shown in Fig. 1e–h. The obvious lattice fringes indicated that the upconversion nanoparticles have a highly crystalline nature and structural uniformity.

As shown in Fig. S1c,† the UCL spectrum of $\text{NaGdF}_4\text{:18% Yb/2% Er}$ consisted of four emission bands centered at 407 nm, 523 nm, 540 nm, and 655 nm, which can be assigned to $^4\text{F}_{5/2} \rightarrow ^4\text{I}_{15/2}$, $^2\text{H}_{11/2} \rightarrow ^4\text{I}_{15/2}$, $^4\text{S}_{3/2} \rightarrow ^4\text{I}_{15/2}$, and $^4\text{F}_{9/2} \rightarrow ^4\text{I}_{15/2}$ transitions of Er^{3+} , respectively (Fig. S1d,†), and appears as green light to the naked eye due to the green emission dominating the overall luminescence.

To further investigate variation of the photon excitation process when increasing the extent of Gd^{3+} in nanoparticles, the corresponding power dependence of the green and red emission bands were measured and are shown in Fig. S1a and b.† The slope value (n) of $\log(I)$ versus $\log(P)$ is obtained by linear fitting for $\text{NaGdF}_4\text{:Yb}^{3+}\text{,Er}^{3+}$ nanoparticles, where I is the UCL intensity and P is the pump power, which represents the number of photons needed to produce one UCL photon and can be calculated according to Auzel's theory as follows:⁴⁵

$$I \propto P^n$$

It can be seen crisply from Fig. S1a and b.† that the n value of the green and red emission is equal to (1.60, 1.32, 1.47, and 0.29) and (1.90, 1.67, 1.73, and 0.62) for the nanocrystals with 10%, 70%, 80%, and 97.5% Gd^{3+} ions, respectively. It is noted that the n value decreased gradually with increasing the content of the Gd ions. This can be demonstrated as the cross relaxation among Er^{3+} ions being promoted with increasing the concentration of Gd^{3+} . As a result, the populating electrons in the $^4\text{I}_{11/2}$ energy level of Er^{3+} ion will be largely increased so that the saturation effect happens at extremely high concentrations of Gd^{3+} ions in doped upconversion nanoparticles.

In the majority of biological applications, upconversion nanoparticles were transformed into biocompatible and hydrophilic nanoparticles through oleic acid ligand reduction

Fig. 3 Time-dependent upconversion luminescence (UCL) imaging of the isolated organs from mice fed with various sizes of nanoparticles (e.g., UCNPs-1, UCNPs-2, UCNPs-3 and UCNPs-4, dose: 16.5 mg/20 g) with a coating of oleic acid for different times, using a green band pass filter (nanoparticles dispersed in the fodder). The major imaged organs: stomach, small intestine, large intestine, heart, liver, lung, and kidney. The bright field and UCL images of organs are shown in column 1 and column 2–6, respectively.

and subsequent coating with PEG with slight attenuation of UCL intensity (Fig. 2a). The Fourier transform infrared (FTIR) spectra were measured to verify the presence of PEG in the ligand, and are shown in Fig. 2b. The FTIR spectrum of PEG-modified UCNPs showed two absorption bands at 1096 and 2921 cm^{-1} (the C–O bond and the CH_2^- stretching vibration of PEG chains, respectively) and an absorption band at 1637 cm^{-1} corresponding to the stretching vibration of C=O, indicating the successful PEGylation of the UCNPs.

3.2 Biological penetrability assessment of upconversion nanoparticles

Biological penetration mainly includes the following categories: free diffusion, facilitated diffusion, and active transfer, which are very significant ways for water, inorganic salts, and nutrients (e.g., glucose, amino acids, and fatty acids) to enter into the blood circulation system and lymphatic vessels through the epidermal cells of the digestive system in order to maintain life activity.⁴⁶ The inorganic nanoparticles may penetrate the digestive system to enter the blood system and other organs, and then not be excreted out of the body, and

further lead to fatal injuries to the organs. Therefore, it is very critical and valuable to assess the biological penetrability of the upconversion nanoparticles towards the digestive system.

In order to evaluate the biological penetrability, the upconversion nanoparticles with different particle size (UCNPs-1, UCNPs-2, UCNPs-3, and UCNPs-4) were first prepared and mixed with the fodder. The concentration of the upconversion nanoparticles was fixed at 16.5 mg/20 g. Then the mice were fed with this mixed fodder through oral administration in the standard environment. The biological penetrability of nanoparticles can be assessed by monitoring the changes in *ex vivo* UCL imaging of harvested organs (e.g., heart, liver, lung, and kidney) after different incubation times.

As is shown in Fig. 3, the biological penetrability of UCNPs-4 of about 100 nm diameter was investigated first. Three hours after the mouse ingested UCNPs-4, the highest intensity luminescence in the stomach can be clearly observed compared with the small and large intestines; meanwhile, no UCL signal was detected in the heart, liver, lung, and kidney. When the incubation time was extended to 1 and 2 days, the signal is much stronger in large intestine than

Fig. 4 Left column: Histogram of the integral luminescence intensity in isolated organs of the mouse fed with various sizes of nanoparticles (e.g., UCNPs-1, UCNPs-2, UCNPs-3 and UCNPs-4, dose: 16.5 mg/20 g) for different time. Error bars were based on triplet integral calculations. Right column: The corresponding integral luminescence intensity ratios of $UCL_{\text{small intestine}}/UCL_{\text{stomach}}$ and $UCL_{\text{large intestine}}/UCL_{\text{stomach}}$.

that in the stomach and small intestine, which is due to the digestion of the mixtures containing UCNPs; meanwhile, no signal was observed in the organs of the blood system such as heart, liver, lung and kidney. The incubation time was further prolonged to 3 and 5 days, most of UCNPs were excreted, while no UCL signal can be seen in the heart, liver, lung and kidney. These results indicated that the ~ 100 nm UCNPs-4 can not penetrate the digestion system to enter the bloodstream through the epidermal cells of the digestion system even if the incubation time reached up to 5 days. Similar to the process of UCNPs-4, the UCNPs-2 and UCNPs-3 with diameters of ~ 20 nm and ~ 70 nm were also tested. The organs such as stomach, small intestines, and large intestines presented similar UCL signal evolution tendencies, but the UCL intensity was weaker over a long period of incubation, which can be interpreted being due to the size reduction of the nanoparticles. More interestingly, the nanoparticles (UCNPs-2, UCNPs-3) with smaller sizes can not penetrate either by observing the luminescence signal. For further investigation, we tried to feed the mouse with nanoparticles on

the scale of nutrients. The ultrathin UCNPs with a diameter of ~ 5 nm were synthesized, mixed with the fodder, and ingested by the mouse. It is clear in Fig. 3 that the fluorescent signals from the ultrathin nanoparticles appeared in the stomach and intestines but not in the heart, liver, lung and kidney, which can be ascribed to the immunity and recognition by epidermal cells, so that the ultrathin nanoparticles with similarities to nutrients could not enter bloodstream, but were excreted from the body after several days. Moreover, the nanoparticles were not found in the blood of the mouse fed with UCNPs-1 by TEM (Fig. S3†). The prohibition of ultrathin nanoparticles provides a security of guarantee in nanomedicine when using the nanoparticles as diagnostic agents and therapeutic drugs.

The UCL signals on the *ex vivo* fluorescence images were quantified to study the distribution of upconversion nanoparticles in the isolated organs from mice fed with various upconversion nanoparticles (left column of Fig. 4).^{47,48} The results showed that the nanoparticles in the digestive tract were completely excreted in a short time. Fluorescence

Fig. 5 Middle column: Time-dependent upconversion luminescence imaging of the isolated organs from mice fed with UCNPs-2 coated with polyethylene glycol (PEG) dispersed in fodder for different times (from 3 hours to 5 days). Dose of UCNPs-2 is 10.0 mg/20 g. Left column: Conventional optical bio-imaging of organs using bright field. Right column: The overlay of the left and middle columns with green band pass filter (GBPF). 1: stomach; 2: small intestine; 3: large intestine; 4: heart; 5: liver; 6: lung; 7: kidney.

intensity ratios of intestines to stomach were determined and are shown in the right column of Fig. 4. They indicate that the fluorescence intensity first increased in the intestines, which is consistent with the fodder moving from the stomach to the intestines. Then, the fluorescent signal intensity decreased in the intestines which indicated that the nanoparticles were gradually excreted from the large intestine.

The oleic acid (OA)-coated UCNPs with various sizes from 5 nm to 100 nm showed no biological penetrability towards epidermal cells of the digestive track. However, biological penetrability of the UCNPs coated with hydrophilic PEG is still not clear. Furthermore, UCNPs-2 as model nanoparticles were modified and coated with PEG for investigating the influence of the surface properties on the biological penetrability (Fig. S4†).

Similarly, mice were first fed with the mixture of PEG-modified UCNPs-2 and fodder for different incubation times

(3 hours, 1 day, 3 days and 5 days), and then anesthetized, dissected, and imaged, as shown in Fig. 5. Three hours after ingestion, strong UCL signals were exhibited in stomach and intestines while the signal was not observed in the organs of the blood system such as the heart, liver, lung, and kidney. With increasing the incubation time from 1 day to 5 days, it is very clear that the intensity of fluorescence signals in the stomach and intestines decreased gradually, and finally vanished owing to the metabolism of the digestion system, but no fluorescence from UCNPs-2 was observed in the heart, liver, lung, and kidney during the process. These results revealed that both hydrophobic OA- and hydrophilic PEG-coated UCNPs could not penetrate epidermal cells of the digestive system to get into the blood system and the surrounding tissues.

The dose of the nanoparticles is an essential parameter when employed as diagnostic agents and therapeutic drugs,

Fig. 6 *In vivo* X-ray CT imaging of the mouse before and after oral administration of NaGdF₄:Yb/Er (UCNPs-2 with OA coating) (25 mg) for different time periods, which can directly and roughly track the nanoparticles in the digestive system. Left panel: maximum intensity projection (MIP); middle panel: the corresponding 3D volume-rendered (VR), *in vivo* CT images of the mouse; right panel: lateral view of 3D VR CT images.

because the excess of nanoparticles has side effects on the body.^{49–51} Moreover, according to the diffusion principle of biological penetrability, the nanoparticles in the digestion system may enter the bloodstream, and then be distributed in the organs such as heart, liver, lung, and kidney. Based on the principle of penetration, we investigated the distribution of nanoparticles in the isolated organs of the mouse after feeding with a mixture of UCNPs and fodder for different times (from 1 to 4 times, dose: 10.0 mg/20 g), the feeding period was once a day. As shown in Fig. S5,† after feeding once, obvious UCL was present in the stomach and intestines, while fluorescence from the organs of the blood system was not observed. When the frequency of feeding was increased from 2 to 4 times, almost the same luminescence intensity was observed from stomach and intestines, which was as-

cribed to the balance between ingestion and excretion. There was still no UCL signal in heart, liver, lung, and kidney. This indicates there are no nanoparticles penetrating the digestive system, even at high doses.

In order to further confirm the conclusion that the nanoparticles cannot penetrate the epidermal cells of the digestive tract to get into the bloodstream and then remain in the organs (heart, liver, lung, and kidney), but are excreted completely from the body of the mouse, the time-dependent distribution of the UCNPs in a mouse was tracked by *in vivo* CT imaging after oral ingestion. Due to the high X-ray absorption coefficient of Gd and Yb, the UCNPs-2 of NaGdF₄:Yb/Er mixed with fodder were used for feeding the mouse, which was then imaged *in vivo* by X-ray CT at different post-ingestion times (Fig. 6). As shown in the pre-ingestion

images, no signals can be observed in the chest and abdomen except for those from the hard bones. Three hours after ingestion, extremely strong signals of the organs (stomach and intestines) in the abdomen can be observed, but we cannot see any signal in the organs such as the heart, liver, lung, and kidney. With further increasing the time from 1 day to 3 days, the gradual attenuation of the signals in the abdomen can be seen owing to the metabolism of UCNPs-2 in the digestion system. As expected, no signal can be detected in the organs of the blood system (heart, liver, lung, and kidney) during the whole incubation time from 3 hours to 3 days, indicating that the UCNPs-2 could not penetrate the digestive system to get into bloodstream, but are excreted from the body.

Therefore, the UCNPs, which have been shown to be harmless and have no residue, show huge application potential in nanomedicine, such as in the detection of chemicals *in vivo*. The detection of Nile red by employing the upconversion nanoparticles as fluorescent probes is discussed in the ESI† (Part I), and the concentration of Nile red can be easily determined according to fluorescence signal ratios, while the lowest detection concentration is $10.0 \mu\text{g mL}^{-1}$.

4. Conclusions

In the present study, we first use lanthanide-doped upconversion nanoparticles, with upconversion fluorescence and simultaneous sensitivity to X-rays, for assessing the biological penetrability of inorganic nanoparticles. Lanthanide-doped upconversion nanoparticles, also called upconversion nanoparticles, with uniform particle size and tunable emission were synthesized by a hydrothermal method. They can absorb infrared light to emit visible light, and have received increasing attention due to their application in background-free biological imaging. In comparison to other inorganic nanoparticles, UCNPs have the following advantages: narrow emission band, high emission efficiency, background-free visible light signals under the excitation of infrared light, easily controlled particle size, and good stability to the irradiation. In addition, NaGdF₄ upconversion nanoparticles in our experiments are paramagnetic and are sensitive to X-rays. Here, we track the nanoparticles in the digestive system, benefiting from the background-free fluorescence signals and the sensitivity to X-rays of the upconversion nanoparticles.

Our results certificated that even ultrathin inorganic nanoparticles (~5 nm) could not penetrate the digestive system of the mouse to enter the blood system or surrounding tissues. For investigating the effects of size, surface functional groups, and dose of nanoparticles on biological penetrability, a batch of mice were first fed with these nanoparticles. It was determined based on the UCL signals that the ultra-small nanoparticles (5 nm) with similarity to nutrients cannot penetrate the epidermal cells of the digestive tract to enter the bloodstream or surrounding tissues, but were excreted completely after a sufficient incubation time, which is ascribed to the immunity and recognition of epidermal cells.

These results were also confirmed by the *in vivo* X-ray CT imaging of a mouse after being fed with the UCNPs/fodder mixture carried out at different incubation periods. No CT signal was observed beyond the digestive system in the whole process of metabolism,

The detailed and systematic investigation indicates that the inorganic upconversion nanoparticles can be completely excreted and cause no obvious injury to the digestive system of the mice.

Our results help assess the safety of inorganic nanoparticles potentially used in nanofood and nanomedicine.

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Notes and references

- 1 B. A. Magnuson, T. S. Jonaitis and J. W. Card, *J. Food Sci.*, 2011, **76**, R126.
- 2 G. Oberdörster, A. Maynard, K. Donaldson, V. Castranova, J. Fitzpatrick, K. Ausman and S. Olin, *Part. Fibre Toxicol.*, 2005, **2**, 1.
- 3 A. M. Nyström and B. Fadeel, *J. Controlled Release*, 2012, **161**, 403.
- 4 Y. Xia, P. Yang and Y. Sun, *Adv. Mater.*, 2003, **15**, 353.
- 5 D. B. Trout and P. A. Schulte, *Toxicology*, 2010, **269**, 128.
- 6 M. P. Holsapple, W. H. Farland, T. D. Landry, N. A. Monteiro-Riviere, J. M. Carter, N. J. Walker and K. V. Thomas, *Toxicol. Sci.*, 2005, **88**, 12.
- 7 J. S. Tsuji, A. D. Maynard and P. C. Howard, *Toxicol. Sci.*, 2006, **89**, 42.
- 8 G. G. Onishchenko, A. I. Archakov and V. V. Bessonov, *Gig. Sanit.*, 2006, **6**, 3.
- 9 R. Landsiedel, L. Ma-Hock, A. Kroll, D. Hahn, J. Schnekenburger, K. Wiench and W. Wohlleben, *Adv. Mater.*, 2010, **22**, 2601.
- 10 S. Sharifi, S. Behzadi, S. Laurent, M. L. Forrest, P. Stroeve and M. Mahmoudi, *Chem. Soc. Rev.*, 2012, **41**, 2323.
- 11 M. Kendall and I. Lynch, *Nat. Nanotechnol.*, 2016, **11**, 206.
- 12 A. D. Maynard, R. J. Aitken, T. Butz, V. Colvin, K. Donaldson, G. Oberdörster and S. S. Tinkle, *Nature*, 2006, **444**, 267.
- 13 A. Verma, O. Uzun, Y. Hu, Y. Hu, H. S. Han, N. Watson and F. Stellacci, *Nat. Mater.*, 2008, **7**, 588.
- 14 F. F. Larese, F. D'Agostin, M. Crosera, G. Adami, N. Renzi, M. Bovenzi and G. Maina, *Toxicology*, 2009, **255**, 33.
- 15 J. Lin, H. Zhang, Z. Chen and Y. Zheng, *ACS Nano*, 2010, **4**, 5421.
- 16 B. Baroli, M. G. Ennas, F. Loffredo, M. Isola, R. Pinna and M. A. López-Quintela, *J. Invest. Dermatol.*, 2007, **127**, 1701.
- 17 K. Simons and E. Ikonen, *Nature*, 1997, **387**, 569.
- 18 A. Verma and F. Stellacci, *Small*, 2010, **6**, 12.
- 19 B. D. Chithrani, A. A. Ghazani and W. C. Chan, *Nano Lett.*, 2006, **6**, 662.

- 20 F. Zhao, Y. Zhao and Y. Liu, *Small*, 2011, 7, 1322.
- 21 J. Yu, S. A. Patel and R. M. Dickson, *Angew. Chem.*, 2007, 119, 2074.
- 22 P. R. Leroueil, S. A. Berry, K. Duthie, G. Han, V. M. Rotello, D. Q. McNerny and M. M. Banaszak Holl, *Nano Lett.*, 2008, 8, 420.
- 23 X. Michalet, F. F. Pinaud, L. A. Bentolila, J. M. Tsay, S. J. J. L. Doose, J. J. Li and S. Weiss, *Science*, 2005, 307, 538.
- 24 H. Duan and S. Nie, *J. Am. Chem. Soc.*, 2007, 129, 3333.
- 25 T. Xia, M. Kovochich, J. Brant, M. Hotze, J. Sempf, T. Oberley and A. E. Nel, *Nano Lett.*, 2006, 6, 1794.
- 26 A. Magrez, S. Kasas and V. Salicio, *Nano Lett.*, 2006, 6, 1121.
- 27 M. Auffan, J. Rose and M. R. Wiesner, *Environ. Pollut.*, 2009, 157, 1127.
- 28 Z. Li, J. C. Barnes, A. Bosoy, J. F. Stoddart and J. I. Zink, *Chem. Soc. Rev.*, 2012, 41, 2590.
- 29 V. Wagner, A. Dullaart, A. K. Bock and A. Zweck, *Nat. Biotechnol.*, 2006, 24, 1211.
- 30 E. Boisselier and D. Astruc, *Chem. Soc. Rev.*, 2009, 38, 1759.
- 31 X. Wang, C. Wang, L. Cheng, S. T. Lee and Z. Liu, *J. Am. Chem. Soc.*, 2012, 134, 7414.
- 32 A. P. Alivisatos, *Science*, 1996, 271, 933.
- 33 Z. Chen, X. Wu, S. Hu, P. Hu, H. Yan, Z. Tang and Y. Liu, *J. Mater. Chem. C*, 2015, 3, 153.
- 34 Y. Liu, D. Wang, J. Shi, Q. Peng and Y. Li, *Angew. Chem., Int. Ed.*, 2013, 52, 4366.
- 35 Z. Chen, X. Wu, S. Hu, P. Hu, H. Yan, Z. Tang and Y. Liu, *J. Mater. Chem. C*, 2015, 3, 6067.
- 36 M. Liong, J. Lu, M. Kovochich, T. Xia, S. G. Ruehm, A. E. Nel and J. I. Zink, *ACS Nano*, 2008, 2, 889.
- 37 C. Sun, J. S. Lee and M. Zhang, *Adv. Drug Delivery Rev.*, 2008, 60, 1252.
- 38 A. H. Faraji and P. Wipf, *Med. Chem.*, 2009, 17, 2950.
- 39 L. Sandiford, A. Phinikaridou, A. Protti, L. K. Meszaros, X. Cui, Y. Yan and P. J. Blower, *ACS Nano*, 2012, 7, 500.
- 40 F. Wang, J. Wang and X. Liu, *Angew. Chem.*, 2010, 122, 7618.
- 41 S. Cui, H. Chen, H. Zhu, J. Tian, X. Chi, Z. Qian and Y. Gu, *J. Mater. Chem.*, 2012, 22, 4861.
- 42 Q. Liu, T. Yang, W. Feng and F. Li, *J. Am. Chem. Soc.*, 2012, 134, 5390.
- 43 Y. Liu, K. Ai, J. Liu, Q. Yuan, Y. He and L. A. Lu, *Angew. Chem., Int. Ed.*, 2012, 51, 1437.
- 44 F. Wang, R. Deng, J. Wang, Q. Wang, Y. Han, H. Zhu and X. Liu, *Nat. Mater.*, 2011, 10, 968.
- 45 D. L. Dexter and J. H. J. Schulman, *Chem. Phys.*, 1954, 22, 1063.
- 46 O. H. Griffith, P. J. Dehlinger and S. P. Van, *J. Membr. Biol.*, 1974, 15, 159.
- 47 D. E. Owens and N. A. Peppas, *Int. J. Pharm.*, 2006, 307, 93.
- 48 I. Roy, T. Y. Ohulchanskyy, H. E. Pudavar, E. J. Bergey, A. R. Oseroff, J. Morgan and P. N. Prasad, *J. Am. Chem. Soc.*, 2003, 125, 7860.
- 49 A. Bagheri, H. Arandiyan and C. Boyer, *Adv. Sci.*, 2016, 3.
- 50 A. Gnach, T. Lipinski and A. Bednarkiewicz, *Chem. Soc. Rev.*, 2015, 44, 1561.
- 51 Z. Gu, L. Yan and G. Tian, *Adv. Mater.*, 2013, 25, 3758.