Intra-arterial infusion of PEGylated upconversion nanophosphors to improve the initial uptake by tumors in vivo†

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An alternative method to the intravenous administration of upconversion nanoparticles (UCNPs) was highlighted by the use of an unconventional blood pathway, the artery. The uptake of NaYF₄:Yb₃⁺,Tm³⁺ nanoparticles modified with polyethylene glycol (PEG-UCNPs) by MCF-7 tumors following intra-arterial (i.a.) injection was nearly three-fold higher than that obtained with intravenous (i.v.) injection. This result suggests a new method for administering UCNPs in vivo to achieve more efficient tumor targeting and therapy.

Recent years have witnessed the rapid development of lanthanide-based upconversion nanophosphors (UCNPs) as superior luminescent probes in bioimaging because of their unique capability to emit higher energy visible or near-infrared (NIR) light under continuous-wave excitation of lower energy photons typically at a wavelength of 980 nm.¹–³ Unlike many other fluorescent materials, both the excitation and the emission of UCNPs co-doped with Yb³⁺ and Tm³⁺ are in the NIR region. This has many advantages such as minimal autofluorescence from biological samples, high penetration depths in biological tissues, and enhanced sensitivity.⁴–⁷ With these merits, UCNPs are ideal candidates for high contrast small-animal imaging. Therefore, there is increased interests in developing biological luminescence labels based on UCNPs.⁸–²⁸

In most reported studies, the administration of such UCNPs to realize tumor targeting in mice was by intravenous (i.v.) infusion through the tail vein. This method resulted in a significant uptake of the UCNPs by the liver and spleen.⁴,⁵,²² By removing the nanoparticles from systemic circulation, the liver and spleen quickly lower the concentration of the nanoparticles that can be delivered and taken up by the tumor, which undermines the targeting or therapeutic effect of these nanomaterials. Intra-arterial (i.a.) infusion has been investigated for a long time as an alternative pathway for the administration of drugs.²⁹–³¹ By comparing i.a. and i.v. infusions using positron emission tomography (PET), Tyler et al. showed an increased delivery of ¹¹C-labelled 1,3-bis-(2-chloroethyl)-l-nitrosourea (BCNU), a chemotherapy drug, to a tumor site in the brain when the drug was injected into a selected artery.³² Based on reported studies, it can be reasonably proposed that, with intra-arterial infusion, nanoparticles initially run into the main bloodstream, and then are distributed to the capillaries, thus branching out into tissues and organs. This means that nanoparticles will have more possibilities to enter the tumor site before being exposed to filtration by the liver or spleen. However, until now, there are no reports of the biodistribution of UCNPs injected intra-arterially into mice.

In this present study, a core–shell upconversion nano-composite NaYF₄:Yb³⁺,Tm³⁺@SiO₂ conjugated with PEG (denoted as PEG-UCNPs) was synthesized. The methods of intra-arterial (i.a.) and intravenous (i.v.) infusion by using PEG-UCNPs are shown in Scheme 1. The PEG-UCNPs were injected into the MCF-7 tumor-bearing mice intravenously and intra-arterially to compare the tumor uptake efficiency by these two pathways.

The NaYF₄:Yb³⁺,Tm³⁺ upconversion nanoparticles were synthesized by a well-established solvothermal method.³³ As shown in Fig. 1a, the NaYF₄:Yb³⁺,Tm³⁺ nanoparticles were spherical, with a diameter of about 20 nm. The powder X-ray diffraction (XRD) pattern of the NaYF₄:Yb³⁺,Tm³⁺ nanoparticles (Fig. 2a) showed that the characteristic diffraction peaks were in good agreement with the hexagonal phase of NaYF₄ (JCPDS card no. 16-0334). Moreover, the lattice fringes of the HR-TEM image indicated the high crystallinity of the nanoparticles. The distance between the adjacent lattice fringes was 0.50 nm (Fig. 1a inset), corresponding to the spacing for the (100) lattice planes in the hexagonal NaYF₄ structure. The obtained nanoparticles were hydrophobic which were capped with oleate.

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To make the nanoparticles hydrophilic and to modify them with polyethylene glycol (PEG) ligands, a reverse microemulsion method was used first to form a hydrophilic silica layer on the surface of NaYF₄:Yb³⁺,Tm³⁺ nanoparticles. Then, ethoxy silane functionalized polyethylene glycol (PEG–siloxane) was added into the microemulsion solution to form NaYF₄:Yb³⁺,Tm³⁺@SiO₂–PEG nanoparticles (PEG-UCNPs). After the surface modification, the nanoparticles were approximately 36 nm in size according to the TEM image (Fig. 1b). The broad peak in the XRD pattern of the PEG-UCNPs (Fig. 2a) indicates the amorphous silica coating on the NaYF₄:Yb³⁺,Tm³⁺ nanoparticles.

FTIR characterization was conducted to confirm the existence of PEG moieties in the PEG-UCNPs. The signal at 3440 cm⁻¹ is a characteristic vibration of the O–H group. The signal at 1730 cm⁻¹ was identified as the characteristic vibration of carbonyl groups that are red shifted. Another signal characteristic of PEG–siloxane is the C–O–C stretching that occurs around 1100 cm⁻¹ (ESI, Fig. S1†). Based on the detected vibrations and the characteristic signals of PEG, it can be confirmed that PEG–siloxane was covalently bonded to the surface of UCNPs, thus forming PEG-UCNPs.

It is worth noting that PEG was selected as the surface ligand because of its ability to prolong the presence of molecules in the blood plasma as reported in the literature.²⁴,²⁵,²⁸,³⁷ Longer circulation times are important for tumor targeting and drug delivery because of the high level of angiogenesis and poor lymphatic drainage by tumors, which also enables the UCNPs to exploit the enhanced permeability and retention (EPR) effect. This study was designed to examine whether i.a. or i.v. infusion is able to exploit the EPR effect in the MCF-7 tumor in nude mice, to increase the uptake of the nanoparticles by the tumor and decrease the amount that is cleared by the liver and spleen. Dynamic light scattering (DLS) data of PEG-UCNPs in water showed that PEG-UCNPs possess good dispersity and stability in aqueous solutions, with a mean diameter of 64.7 nm (see Fig. 2b).

The upconversion luminescence spectrum of Yb³⁺/Tm³⁺ co-doped NaYF₄ in Fig. 3 was taken to confirm the anti-Stokes luminescence that occurs with these co-doped UCNPs. When excited by 980 nm light, PEG-UCNPs in solution emitted blue light. The emissions at wavelengths of 450 and 475 nm can be
attributed to the $^1D_2 \rightarrow ^3F_4$ and the $^1G_4 \rightarrow ^3H_6$ transitions. The emission at 650 nm is a result of the $^1G_4 \rightarrow ^3F_4$ transition. The strong infrared emission at 800 nm is caused by the $^3H_4 \rightarrow ^3H_6$ electron transition. These shifts are characteristic of Yb$^{3+}$ and Tm$^{3+}$ co-doped UCNPs and confirms that the synthesis was as expected.

Since PEG-UCNPs are designed to be used as bioimaging probes, it is essential to evaluate the cytotoxicity and cell permeability characteristics of these nanoparticles. The cytotoxicity of PEG-UCNPs in MCF-7 cells was tested with dosages in the range 0–500 μg mL$^{-1}$ by measuring the reduction of the activity of methyl thiazolyl tetrazolium (MTT) (Fig. 4a). PEG-UCNPs had low cytotoxicity; as much as 90% cell viability was observed after 24 h at a concentration of 500 μg mL$^{-1}$. The application of PEG-UCNPs in the upconversion luminescence (UCL) imaging of living cells was investigated, by utilizing a laser scanning upconversion luminescence microscope (LSUCLM) under CW excitation at 980 nm. As illustrated in Fig. 4b, an obvious intracellular luminescence can be observed after incubation with 200 μg mL$^{-1}$ PEG-UCNPs. Bright field measurements after treatment with PEG-UCNPs corroborated that the cells are viable throughout the imaging experiments. The overlay of the bright field and UCL images further confirmed that luminescence was deposited in the intracellular region, suggesting that the PEG-UCNPs were internalized into the cells rather than merely staining the membrane surface.

To show qualitatively and quantitatively the differences in the uptake by tumors in vivo, the intra-arterial and intravenous injections of PEG-UCNPs were compared using UCL imaging and biodistribution determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). There was a significantly larger signal detected in the UCL images of tumors of intra-arterially injected mice than the intravenously injected mice (Fig. 5c and f). After 20 min post-injection, the animals were sacrificed and their tissues were analyzed using ICP-AES (Fig. 5g). The intra-arterial injection resulted in a three-fold higher concentration of PEG-UCNPs in tumor tissue than the intravenous injection. The increased concentration of PEG-UCNPs in the tumor can be attributed to an increased initial concentration.

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**Fig. 3** Room temperature upconversion luminescence (UCL) spectrum of PEG-UCNPs dissolved in water (5 mg mL$^{-1}$) and excited with a CW 980 nm laser (power ~800 mW). The visual photograph (inset) of PEG-UCNPs in water and the total luminescence photograph (inset) of PEG-UCNPs appears blue under CW excitation at 980 nm (powder ~800 mW).

**Fig. 4** (a) Cell viability values (%) estimated by the MTT proliferation tests versus concentrations of the PEG-UCNPs incubated with cells. MCF-7 cells were cultured in the presence of 100–500 mg mL$^{-1}$ PEG-UCNPs at 37 °C for 4 and 24 h, respectively. (b) Upconversion luminescence (UCL) image and (c) the overlay of the UCL and bright-field images of MCF-7 cells incubated with 200 mg mL$^{-1}$ PEG-UCNPs for 1 h at 37 °C.

**Fig. 5** In vivo UCL images of the tumor-bearing nude mice in bright field (a and d), dark field (b and e) and overlay (c and f) images, following intravenous infusion (a−c) and intra-arterial (aorta abdominalis) infusion (d−f) of PEG-UCNPs (200 μL, 5 mg mL$^{-1}$). (g) Bio-distribution of nanoparticles in organs of a mouse bearing an MCF-7 tumor, sacrificed 20 min after intravenous and intra-arterial injection of PEG-UCNPs. The number of mice used in each injection method is 5. The columns labelled with asterisk have significant difference to the counterpart with vein injection.

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of PEG-UCNPs in the systemic circulation. In intra-arterial injections, the PEG-UCNPs enter the bloodstream and then are distributed to the capillaries that branch out into the tissues and organs. This means that, following the injection into the aorta abdominalis, which was made in the abdomen below the liver, the PEG-UCNPs were able to disperse into the systemic bloodstream and target the tumor before being exposed to filtration by the liver or spleen. The latter is what appears to be the problem with intravenous injections. When injecting into the tail vein, the PEG-UCNPs must be sent back through the heart and lungs to be oxygenated, and then be exposed to filtration, thus lowering the concentration of the PEG-UCNPs in the blood before it can target the tumor. Although a relatively large amount of nanoparticles were accumulated in the liver of intra-arterially injected mice, about 1.6-fold more nanoparticles were in the liver of intravenously injected mice. The decrease in the concentration of PEG-UCNPs in the liver can be accounted for by the longer retention time of the PEG-UCNPs in the heart of intra-arterially injected mice than intravenously injected mice, which could be attributed to the longer retention time of the PEG-UCNPs in the liver. Based on these results, it can be concluded that intra-arterial injections of PEG-UCNPs results in an increased initial concentration in tumors in vivo.

We have successfully shown that an increase in the initial uptake of PEG-UCNPs by tumors in vivo can be achieved by changing the blood pathway through which the infusion is administered. By increasing the concentration of the nanoparticles taken up by tumors, assays can be run with a higher efficiency, and this could play a role in the therapeutic treatment of tumors. To further solidify the findings of this experiment, future studies can be run using a different artery for administering the infusion, the injection into the artery can be made through a catheter to prolong the life of the mice, thus enabling multiple questions to be tested at one time. Although intra-arterial injections are not a novel technique in clinical practice, the application of this technique for infusions of PEG-UCNPs or similar nanoparticles can play a role in the enhancement of bioimaging and tumor therapeutics.

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Notes and references


