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Synthesis of ligand-functionalized water-soluble [^{18}F]YF₃ nanoparticles for PET imaging†

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Abstract

We report a simple, efficient synthesis of novel ^{18}F -labeled imaging agents based on YF₃ nanoparticles. Targeting ligands and antitumor drug molecules can be introduced onto the YF₃ nanoparticles in a one-pot synthesis. The ^{18}F -labeling reaction proceeds in aqueous solutions at room temperature with excellent radiolabeling yields (> 80%) in very short time (5–10 min). ^{18}F -labeled YF₃ nanoparticles displayed high stability in mouse & human serum, and their application for mapping lymph nodes in live rats after local injection has also been demonstrated.

Rare-earth fluoride nanoparticles have attracted tremendous interest over the last few years because of their unique luminescence properties.^{1–4} Among all the studied rare-earth fluoride nanoparticles, yttrium trifluoride (YF₃) nanoparticles have received much attention due to their potential applications as new laser materials and up-conversion imaging labels.^{5–7} Li *et al.* reported the preparation and up-conversion luminescence properties of fullerene-like and rice-like Ln³⁺-doped YF₃ nanoparticles.^{8,9} Wang *et al.* studied the multicolor up-conversion emission in Yb³⁺/Tm³⁺, Yb³⁺/Ho³⁺ and Yb³⁺/Er³⁺ doped YF₃ particles.¹⁰ Up-conversion YF₃:Tm³⁺/Yb³⁺ nanocrystals were reported to emit from blue to magenta.¹¹ These YF₃ nanoparticles synthetic procedures often led to particles with irregular shapes, relatively large sizes or hydrophobic surface coatings. Nanoparticles with a smaller size, uniform shape, and good aqueous solubility are preferred for biolabeling and imaging applications.

On the other hand, optical imaging has limited tissue penetration in comparison to other imaging modalities such as positron emission tomography (PET). Biomolecules have been commonly radiolabeled for PET imaging studies of the activity of target receptors in living subjects.^{12–16} Fluorine-18 (^{18}F) is often used for PET imaging due to its ease in production in high quantities on a medical cyclotron and an ideal half-life of 110 min, but its introduction to the target molecule generally requires multiple synthetic steps often under harsh conditions and tedious purification processes.^{17–19} Recently, the reaction between

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fluoride and rare-earth metal ions has been applied to label NaYF₄ nanoparticles with ¹⁸F.^{20–22} These NaYF₄ nanoparticles were made at high temperatures (320 °C) or in organic environments (in oleyl amine), and had to be converted into biocompatible nanoparticles through additional synthetic processes such as a ligand-exchange process of oleic acid with citrate. Generally, such multi-step strategies are associated with some intrinsic limitations, such as relatively high cost, and complicated preparation and post-treatment procedures. In this work, we describe a simple, efficient approach to synthesize ¹⁸F-labeled imaging agents based on YF₃ nanoparticles (Scheme 1), the whole process including nanoparticles synthesis and ¹⁸F-labelling are performed in aqueous solution. Targeting ligands and even drug molecules were introduced to the nanoparticles in a one-pot synthesis. We also demonstrated their application for mapping lymph nodes in rats.

Citric acid stabilized YF₃ nanoparticles were synthesized by a modified coprecipitation procedure (ESI).^{23,24} The size and shape of synthesized nanoparticles are dependent on the ligand concentration, reaction time, and temperature. For example, when the concentration of citric acid decreased from 1 mmol to 0.5 mmol, the size range of Cit-YF₃ nanoparticles increased from a range of 30–40 nm (Fig. 1A) to 30–80 nm (Fig. 1B). Increasing both reaction temperature and time led to larger Cit-YF₃ nanoparticles; for example, by increasing the reaction temperature from 75 °C to 90 °C and the reaction time from 15 min to 60 min, the average size of Cit-YF₃ nanoparticles increased from 30–40 nm to 50–70 nm (Fig. 1C).

Targeting ligands may be introduced during the synthesis. Folate was used together with citric acid as co-ligands to synthesize folate-conjugated YF₃ nanoparticles (FA-YF₃) for potentially targeting tumors over expressing folate receptors.² TEM showed that the average diameter of FA-YF₃ nanoparticles slightly decreased to 20–30 nm (Fig. 1E), and no obvious change in size was observed when the concentration of folate increased from 0.1 mmol to 0.2 mmol. High resolution TEM showed that the YF₃ nanoparticles are composed of several smaller nanocrystals of ~ 5 nm (Fig. 1D&F). The energy-dispersive X-ray analysis (EDXA) pattern of FA-YF₃ samples confirmed the presence of Y, F, O and N in the nanoparticles, with the additional Cu peak being readily attributed to the copper grid used (Fig. S1).

In addition to the targeting ligands, anticancer drug molecules such as doxorubicin (DOX) could be loaded onto the surface of FA-YF₃ nanoparticles via electrostatic²⁵ and coordination²⁶ interactions to produce DOX-FA-YF₃ (Scheme 1). After DOX conjugation, slight aggregation was observed from TEM images (Fig. S2). Dynamic Light Scattering (DLS) showed that lognormal size distribution of nanoparticles increased from 36 nm to 73 nm after DOX conjugation (Fig. S3). FA-YF₃ nanoparticles exhibited an absorption peak at 281 nm pertaining to folate, and DOX-FA-YF₃ nanoparticles exhibited two additional absorption peaks at 231 nm and 478 nm pertaining to DOX besides the folate absorption peak at 281 nm (Fig. 2A). These absorption peaks can be applied to determine the amount of folate and DOX loaded on the YF₃ nanoparticles, which was about 4 ng/μg and 0.1 μg/μg, respectively (Fig. S4&5).

In vitro cytotoxicity of YF₃ nanoparticles was measured using the MTT assay against human breast cancer cell line MDA-MB-468 (Fig. 2B). Both Cit-YF₃ and FA-YF₃ showed low cytotoxicity: their cellular viability was estimated to be 73.8% and 79.4%, respectively, using the same concentration of 200 μg/mL nanoparticles. However, the viability of MDA-MB-468 cells incubated with DOX-FA-YF₃ nanoparticles at the same concentration was estimated to be 20.3%, demonstrating that DOX was successfully loaded onto the YF₃ nanoparticles and the loaded DOX remained cytotoxic. In comparison, free DOX exhibited a slightly higher toxicity, with 11.9% cellular viability at the same drug concentration.

^{18}F -labeling was carried out by simply mixing ^{18}F]KF solution with aqueous solutions of YF_3 nanoparticles at room temperature followed by 5 to 10 min incubation, and free ^{18}F was easily removed by centrifugation. YF_3 nanoparticles with different surface ligands, including citric acid (Cit- YF_3), folate (FA- YF_3), DOX (DOX-FA- YF_3) and PEG linkage (PEG₂₀₀₀-Cit- YF_3) were ^{18}F -labeled using this method. Excellent radiolabeling yields were observed, generally in the range of 80–95% (decay corrected to end of bombardment) (Fig. 3A). The concentration of nanoparticles and increased reaction time did not lead to significant increases in the radiolabeling yields. For example, the ^{18}F -labeling yield for Cit- YF_3 nanoparticles was 96%, 95%, and 84% with just 5 min reaction time at the concentration of 1, 0.5, and 0.2 mg/mL, respectively, and no obvious change in the radiolabeling yield was observed when the incubation time increased to 20 min (Fig. S6). We examined the serum stability of ^{18}F -labeled YF_3 nanoparticles by measuring dissociated ^{18}F] fluoride in the supernatant. Only 6% of ^{18}F]fluoride was released from Cit- ^{18}F]YF₃ nanoparticles during first 30 min incubation in mouse and human serum, which might be due to the loss of fluoride non-specifically absorbed on the nanoparticles, and nearly no further ^{18}F] fluoride dissociation was observed in prolonged incubations of 2–6 h (Fig. S7).

To evaluate the targeting effect of folate-conjugated nanoparticles, the uptakes of FA- ^{18}F]YF₃ nanoparticles in PyMT (polyoma middle T oncoprotein mouse breast cancer, folate receptor positive)²⁷ and MDA-MB-468 cell lines (folate receptor negative)²⁸ were investigated. FA- ^{18}F]YF₃ showed 2–3 fold higher cellular uptake efficiency in PyMT cells compared with that in MDA-MB-468 cells at 45 min, 1 h, and 2 h, respectively (Fig. 3B).

Finally labeled ^{18}F]YF₃ nanoparticles were applied to map lymph nodes *in vivo*. The lymphatic system plays a crucial role in immune responses to foreign antigens and tumors, and in tumor metastasis in human and rodent models.²⁹ Two different sizes of Cit- ^{18}F]YF₃ nanoparticles, one in the range of 30–40 nm in diameter and the other at 50–70 nm, were evaluated by intradermal injections into the right and left front footpads of rats. Both axillary lymph nodes could be easily visualized non-invasively by microPET/CT imaging at 15 min post injection (Fig. 4 and Fig. S8). The axillary lymph node uptake of smaller nanoparticles was 6.7 times greater than that of larger nanoparticles. Similarly, an 8.4 times higher uptake of smaller nanoparticles in the popliteal lymph nodes was observed when these Cit- ^{18}F]YF₃ nanoparticles were injected into the rear footpads of rats (Fig. S9). The size of nanoparticles exhibits a strong effect on the migration time during lymph node mapping, and nanoparticles with a hydrodynamic diameter of 10–50 nm were reported to exhibit rapid uptake into the lymphatic system and considered optimal for lymph node mapping^{30,31} Our study further confirmed this result.

In conclusion, we have reported a simple, efficient procedure to synthesize water dispersible ^{18}F -labeled YF_3 nanoparticles, which avoided high temperatures, high pressures, and organic solvents. Targeting and antitumor drug molecules can be introduced onto the nanoparticles for ^{18}F -labeling. The labeling reaction proceeds in aqueous solutions at room temperature with excellent radiolabeling yields (> 80%) in very short time (5–10 min). Labeled YF_3 nanoparticles showed high serum stability and were able to map lymph nodes in live rats after local injection. This simple, efficient ^{18}F -labeling strategy should enable the use of rare-earth nanoparticles for both PET and optical imaging *in vivo*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. The transmission electron microscopy (TEM) images of the YF3 samples. (A) Cit-YF3: citric acid 1 mmol, 75 °C, 15 min, (B) Cit-YF3: citric acid 0.5 mmol, 75 °C, 15 min, (C) Cit-YF3: citric acid 1 mmol, 90 °C, 1 h, (D) the high resolution TEM of Cit-YF3 sample, (E) FA-YF3: citric acid 1 mmol, folate 0.1 mmol, 75 °C, 30 min, (F) the high resolution TEM of the FA-YF3 sample.

Fig. 2.

(A) The UV-Vis absorbance spectra of the synthesized YF₃ samples. (B) Cell viability values (%) estimated by MTT proliferation test versus incubation concentrations of nanoparticles and free DOX, respectively. MDA-MB-468 cells were cultured in the presence of nanoparticles (0–200 μg/mL) and free DOX (0–20 μg/mL) at 37 °C for 24 h, respectively.

Fig. 3.
 (A) ^{18}F labeling yield of synthesized YF₃ nanoparticles. (B) Comparison of uptakes of FA-[^{18}F]YF₃ nanoparticles in PYMT and MDA-MB-468 cell lines.

Fig. 4. Imaging lymph nodes in rats with [^{18}F]YF3 nanoparticles after 15 min injection at front foot pads (indicated by dotted arrows); axillary lymph nodes are indicated by solid arrows.

Scheme 1.
 Synthesis of $[^{18}\text{F}]\text{YF}_3$ nanoparticles.