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Intrinsically Zirconium-89 Labeled $\text{Gd}_2\text{O}_3\text{:Eu}$ Nanoprobes for In Vivo Positron Emission Tomography and Gamma-Ray-Induced Radioluminescence Imaging

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Graphical Abstract

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Here we report the engineering of a novel dual-modality imaging probe by intrinsically labeling zirconium-89 (^{89}Zr , a positron emission radioisotope with a half-life of 78.4 h) to PEGylated $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}$ nanophosphors, forming $[^{89}\text{Zr}]\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@\text{PEG}$ for *in vivo* PET/Radioluminescence lymph node mapping

Keywords

Radioluminescence Imaging; Intrinsic Radiolabeling; $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}$ Nanoparticles; Positron Emission Tomography

Radioluminescence imaging is an emerging innovative optical imaging modality that utilizes ionizing radiation of high energy rays (such as X-rays, gamma rays or beta particles) to excite nanophosphors to visualize biological features with an improved signal-to-noise ratio and a deeper tissue penetration depth (when compared with conventional optical imaging approaches).^[1–6] Radioluminescence nanophosphors have recently shown great promise for biomedical imaging due to their unique optical properties, including exceptional photochemical stability, tunable emission spectrum with large Stokes shifts, negligible photobleaching, and bright radioluminescence.^[4, 7–9] Positron Emission Tomography (PET) is an attractive quantitative imaging modality which possesses remarkable detection sensitivity but with a limited spatial resolution ($\sim\text{mm}$). Photoluminescence imaging is capable of providing the highest spatial resolution, however, lacks the capability to obtain anatomical and physiological detail *in vivo* owing to the limited penetration depth of light in tissues. The combination of these two imaging modalities into one nanosystem could potentially hold balance in sensitivity, resolution, and penetration depth, enabling excellent visualizing (cancer) cells from the cellular scale to whole-body non-invasive imaging. Here we reported the engineering of a novel dual-modality imaging probe by intrinsically labeling zirconium-89 (^{89}Zr , a positron emission radioisotope with a half-life of 78.4 h) to PEGylated $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}$ nanophosphors, forming $[^{89}\text{Zr}]\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@\text{PEG}$ for *in vivo* PET/Radioluminescence lymph node mapping (Figure 1a).

A co-thermal decomposition of precursors method was used for the synthesis of $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}$ nanoparticles. Oleic acid (OA) and oleylamine (OL) were used as the combined surfactants

for controlling the growth of nanoparticles. As shown in Figure 1b, cube-shaped $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}$ with an average size of 25 nm was obtained. The successful synthesis of $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}$ nanoparticles was confirmed by the X-ray diffraction (XRD) study (with the patterns match well with the standard $\text{Gd}_2\text{O}_2\text{S}$ reflection, JCPDS No. 026-1422; Figure S1), and the strong red emission (from the doped Eu^{3+} ions) under UV light excitation (Figure 1b, inset). A well-established phospholipids surface modification strategy was introduced to transfer hydrophobic OA/OL-capped $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}$ nanoparticles from the organic solvent to the water phase.^[10] Commercially available phospholipids (*i.e.* DSPE-PEG_{5k}-Mal) with a molecular weight of 5 kDa were used. As-synthesized nanoparticles became water-dispersible *via* the hydrophobic van der Waals interactions between the hydrophobic tail of the phospholipids and the primary oleate ligands on the particles surface. Neither obvious morphology change nor severe particle aggregation was observed after the surface modification, as shown in Figure 1c and Figure S2. As expected, we saw a slight optical quenching effect and reduced fluorescent intensity in the water soluble $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@\text{PEG}$ nanoparticles (Figure 1c, inset), possibly due to the optical quenching in aqueous solution. To characterize the optical properties of the $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}$, excitation, and emission spectra were collected (Figure S3). The excitation spectrum is composed of an excitation bands ranging from 200 to 400 nm, which can be ascribed to the charge transfer band (CTB) between the anions and the Eu^{3+} ions.^[11] The peak around 330 nm is attributed to the transition from the S^{2-} to Eu^{3+} .^[12] Upon excitation at 330 nm, the significant emission peaks at 615 and 625 nm were observed due to the forced electric-dipole $^5\text{D}_0 \rightarrow ^7\text{F}_2$ transitions of the Eu^{3+} ions.^[13] Emission at 703 nm (resulting from $^5\text{D}_0 \rightarrow ^7\text{F}_4$ transitions within the so-called “biological window”) was also observed.^[14]

Zirconium-89 is an attractive radioisotope which decays by positron emission (23%) and electron capture (77%) to the stable isotope yttrium-89 (^{89}Y). To demonstrate the existing of $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}$ radioluminescence by using the gamma energy derived from the ^{89}Zr radioactive decay, a pilot study was first performed. Eppendorf tubes that contained free ^{89}Zr (~40 μCi), $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}$ (1 mg/mL), $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@\text{PEG}$ (1 mg/mL) and PBS control were placed close to each other, and imaged in IVIS Spectrum optical imaging system (*with excitation filters all closed, and emission filter open at 620 nm*). As shown in Figure 2a, both $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}$ (in cyclohexane) and $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@\text{PEG}$ (in water) showed clear luminescence at around 620 nm (where free ^{89}Zr only shows very low Cerenkov luminescence background). While no obvious luminescence can be detected from neither the free ^{89}Zr nor the PBS control, clearly indicating the gamma energy-induced radioluminescence.

Inspired by our previous work on the intrinsic radiolabeling of ^{89}Zr to mesoporous silica nanoparticles (with over 2 million $-\text{Si}-\text{O}^-$ groups in each particle),^[15] we hypothesize that the presence of oxygen donors at the surface of water-soluble $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@\text{PEG}$ can also be used for chelator-free radiolabeling of ^{89}Zr . The labeling was performed by mixing ^{89}Zr -oxalate with $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@\text{PEG}$ in HEPES buffer (0.1 M, pH 7–8) and kept shaking at 75 °C. Radiolabeling yield of [^{89}Zr] $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@\text{PEG}$ was found to be over 75% within the first 30 min of incubation, and kept nearly consistent during the 180 min incubation time (Figure 2b, **black line**, Figure S4a). Successful ^{89}Zr -labeling can also be achieved under lower labeling temperature with a lower labeling yield (~50%) after 3 h incubation (Figure

2b, **red line**, Figure S4b). A pH-dependent labeling yield was also observed with a significantly reduced labeling yield (<15% at 75 °C for 3 h) when labeling at pH 2–3, mostly likely due to the protonation of surface oxygen donors (Figure 2b, **blue and green lines**, Figure S4c,d).^[15] Radioluminescence of [⁸⁹Zr]Gd₂O₂S:Eu@PEG was found to be dependent on both the radioactivity of ⁸⁹Zr in [⁸⁹Zr]Gd₂O₂S:Eu@PEG and the concentration of Gd₂O₂S:Eu@PEG nanoparticles, as clearly shown in Figure 2c and 2d.

Before *in vivo* PET/Radioluminescence dual-modality imaging, the short-term biocompatibility of Gd₂O₂S:Eu@PEG was studied both *in vitro* and *in vivo*. No obvious cytotoxicity of Gd₂O₂S:Eu@PEG to normal cells (HEK-293) and tumor cells (HUN-7) was observed at a studied concentration (from 0.2 to 1 mg/mL) up to 24 h and 48 h (Figure S5). Serum biochemistry assays were then conducted to assess the potential toxicity after the injection of Gd₂O₂S:Eu@PEG (dose: 450 mg/kg). The primary liver function markers including aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TBIL) as well as kidney function markers including serum urea (UREA) and serum creatinine (CREA) were all measured. Mice injected with PBS were served as a control group (n=3). Our results show no obvious hepatic or kidney disorder of mice on both Day 7 and Day 14 post-injection. On Day 14, major organs (*i.e.* heart, liver, spleen, lungs, kidneys) were sliced and stained by H&E for histology analysis. The results in Figure 3b further revealed that there was no noticeable tissue damage in all major organs of mice. More systematic long-term *in vivo* toxicity study is needed to better understand the potential toxicity of Gd₂O₂S:Eu@PEG nanoparticles.

We then study the radiostability and PET/Radioluminescence dual-modality imaging of [⁸⁹Zr]Gd₂O₂S:Eu@PEG *in vivo*. As shown in Figure S6, over 98% of ⁸⁹Zr remained intact in both cases after incubating at 37 °C for 48 h. As synthesized [⁸⁹Zr]Gd₂O₂S:Eu@PEG was then intravenous (*i.v.*) injected in healthy BABL/C mice (n=3) and imaged using microPET. Due to the relatively large hydrodynamic size (>60 nm, Figure S2), dominant liver (35.3 ± 7.9 %ID/g) and spleen (39.3 ± 8.7 %ID/g) uptake of [⁸⁹Zr]Gd₂O₂S:Eu@PEG was observed based on *in vivo* PET/Radioluminescence dual-modality imaging (Figure 4a,b), and confirmed in *ex vivo* quantitative biodistribution studies (Figure S7). Considering that detached free ⁸⁹Zr is an osteophilic cation and will be accumulated in mouse bones quickly,^[16] high bone uptake of ⁸⁹Zr-labeled nanoparticles usually suggests their low radiostability *in vivo*. In our study, only less than 1%ID/g bone uptake of ⁸⁹Zr was observed in bones (Figure 4a and Figure S7) at 48 h post-injection, clearly indicating a high radiostability of [⁸⁹Zr]Gd₂O₂S:Eu@PEG *in vivo*.

Sentinel lymph nodes (SLNs) is a common route for tumor metastasis, and successful identification of SLNs has been considered as an important way for the prediction of cancer metastasis as well as assessing tumor prognosis in clinical treatment.^[7] Upon subcutaneous injection of [⁸⁹Zr]Gd₂O₂S:Eu@PEG nanoparticles (~50 µCi) into the left footpad of normal BALB/C mice, serial PET scans were performed. Accumulation of [⁸⁹Zr]Gd₂O₂S:Eu@PEG in the popliteal lymph node could clearly be seen at 0.5, 2 and 6 h post-injection (Figure 4c), with the uptake estimated to be 8.6 ± 4.1, 13.4 ± 3.3 and 4.4 ± 1.7 %ID/g, respectively (n=3). Such accumulation could also be visualized by radioluminescence imaging (Figure 4d), and confirmed with *ex vivo* PET/Radioluminescence imaging (Figure S8) and histology

studies (Figure S9). *Ex vivo* biodistribution of [^{89}Zr]Gd₂O₂S:Eu@PEG was also investigated at 6 h post-injection where showed a dominant uptake of nanoparticles in the lymph node (Figure S10).

In conclusion, we have developed a facile synthesis and efficient chelator-free radiolabeling process to produce water-soluble [^{89}Zr]Gd₂O₂S@PEG. To the best of our knowledge, this is the first example of the successful utilization of [^{89}Zr]Gd₂O₂S@PEG for PET and gamma-ray-induced radioluminescence. This study might provide a new paradigm for combined PET/Radioluminescence imaging in preclinical and clinical applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Synthesis and characterizations of $[^{89}\text{Zr}]\text{Gd}_2\text{O}_2\text{S:Eu@PEG}$

(a) A schematic illustration showing the chelator-free synthesis of $[^{89}\text{Zr}]\text{Gd}_2\text{O}_2\text{S:Eu@PEG}$ nanoparticles. TEM images of $\text{Gd}_2\text{O}_2\text{S:Eu}$ before (b) and after PEGylation (c). Insets in (b) and (c) show the digital photos of $\text{Gd}_2\text{O}_2\text{S:Eu}$ nanoparticles under the excitation of UV light.

Figure 2. Intrinsic ^{89}Zr labeling and *ex vivo* radioluminescence imaging

(a) Radioluminescence imaging of $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}$ and $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@ \text{PEG}$ excited by gamma energy derived from the free ^{89}Zr radioactive decay. A PBS control was also used. (b) Time-dependent ^{89}Zr labeling yields of $[^{89}\text{Zr}]\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@ \text{PEG}$ with varied pH and temperature. (c) Radioluminescence imaging of $[^{89}\text{Zr}]\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@ \text{PEG}$ with the same $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@ \text{PEG}$ concentration (1 mg/mL) but varied radiation dose (ranging from 10 to 70 μCi) (Ex: Closed, Em: 620 nm). (d) Radioluminescence imaging of $[^{89}\text{Zr}]\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@ \text{PEG}$ with the same radiation dose (40 μCi) but varied $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@ \text{PEG}$ concentration (ranging from 0.25 to 1 mg/mL) (Ex: Closed, Em: 620 nm).

Figure 3. Biocompatibility study of $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@\text{PEG}$ in healthy mice

(a) Serum biochemistry of liver and kidney. Healthy BALB/c mice were intravenously injected with $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@\text{PEG}$ (dose: 450 mg/kg), and sacrificed on Day 7 and Day 14 post-injection (n=3). (b) H&E study of major organs (including heart, liver, spleen, lung and kidney) from mice after injecting $\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@\text{PEG}$ (dose: 450 mg/kg) for 14 days. Healthy mice treated with PBS were used as the control.

Figure 4. *In vivo* PET/Radioluminescence imaging of $[^{89}\text{Zr}]\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@ \text{PEG}$
(a) Serial *in vivo* PET imaging of $[^{89}\text{Zr}]\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@ \text{PEG}$ in mice at different post-injection time points. **(b)** *In vivo* radioluminescence imaging of $[^{89}\text{Zr}]\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@ \text{PEG}$ in mice at different post-injection time points (*Ex: Closed, Em: 620 nm*). **(c)** Serial *in vivo* PET-based lymph node mapping of $[^{89}\text{Zr}]\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@ \text{PEG}$ in mice at different post-injection time points. **(d)** *In vivo* radioluminescence-based lymph node mapping of $[^{89}\text{Zr}]\text{Gd}_2\text{O}_2\text{S}:\text{Eu}@ \text{PEG}$ in mice at different post-injection time points (*Ex: Closed, Em: 620 nm*). Lymph nodes were marked with white arrows.