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Chelator-Free Labeling of Metal Oxide Nanostructures with Zirconium-89 for Positron Emission Tomography Imaging

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Abstract

Radiolabeling of molecules or nanoparticles to form imaging probes is critical for positron emission tomography (PET) imaging, which, with high sensitivity and the ability for quantitative imaging, has been widely used in the clinic. While conventional radiolabeling often employs chelator molecules, a general method for chelator-free radiolabeling of a wide range of materials remains to be developed. Herein, we uncovered that ten different types of metal oxide (M_xO_y , $M=Gd, Ti, Te, Eu, Ta, Er, Y, Yb, Ce, \text{ or } Mo$, $x=1-2$, $y=2-5$) nanomaterials with polyethylene glycol (PEG) modification could be labeled with ^{89}Zr , a PET tracer, *via* a simple yet general chelator-free radiolabeling method upon simple mixing. High labeling yields and good serum stabilities are achieved with this method, owing to the strong bonding between oxyphilic $^{89}Zr^{4+}$ with oxygen atoms on the M_xO_y surface. Selecting $^{89}Zr-Gd_2O_3-PEG$ as a multimodal imaging probe, we have successfully demonstrated *in vivo* PET imaging of draining lymph nodes, which are also visualized under magnetic resonance imaging, showing advantages over free ^{89}Zr in the mapping of draining lymph node networks. Our work describes a general and simple method for chelator-free radiolabeling of metal oxide nanostructures, which is promising for the development of multifunctional nanoprobe in biomedical imaging.

TOC

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Associated Content

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Figures S1–S12 and Table S1&S2 (PDF)

The authors declare no competing financial interest.

Keywords

Metal oxide nanomaterials; chelator-free labeling; ^{89}Zr ; labeling stability; lymph node PET imaging

Molecular imaging has become an important technology that is set to revolutionize our understanding, diagnosis, and prognosis of many diseases including cancer, neurological, and cardiovascular diseases.^{1–4} Among all diagnostic imaging methods, positron emission tomography (PET) has good advantages of high sensitivity, no tissue penetrating limit, and the ability to conduct the whole-body image.^{5–9} Radiolabeling of molecules or nanoparticles for PET imaging has been widely used not only to track the *in vivo* distribution of nanoparticles, but also to develop good nanotracers for imaging of specific targets of interest. To this point, most current radiolabeling methods involve the use of exogenous chelators, such as NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid), DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), or DFO-Bz-NCS (p-isothiocyanatobenzyl desferrioxamine B), which coordinate with radioisotope metal ions including ^{64}Cu and ^{89}Zr to form stable complexes.^{7, 10} However, such chelator-based radiolabeling methods present several well-known disadvantages, such as the limited chelator choices, transchelation with proteins, detachment of the molecular chelators, and the risk of significantly changing the surface properties of molecules or nanoparticles by the addition of chelators.

Recently, chelator-free radiolabeling, in which no chelator molecules are used, has emerged as a promising alternative approach to label nanoparticles in a facile way.¹¹ Using this strategy, the labeled nanoparticles can preserve their native pharmacokinetic profiles, and leave their surface functional groups intact for further modification or bio-conjugation. The most importantly, unlike traditional labeling procedures which depend on a limited number of chelators conjugated to the nanoparticles, various radioisotopes can be labeled on the surface of nanoparticles by this versatile chelator-free radiolabeling approach. Successful labeling of isotopes, such as copper-64 (^{64}Cu - MoS_2 , $t_{1/2}=12$ h),¹² arsenic-72 (^{72}As - Fe_3O_4 , $t_{1/2}=26$ h),¹³ germanium-69 (^{69}Ge - Fe_3O_4 , $t_{1/2}=39.1$ h),¹⁴ and zirconium-89 (^{89}Zr -MSN, $t_{1/2}=3$ days)^{11, 15}, has recently been achieved using this technique by several groups including ours, and applied for *in vivo* PET imaging. For instance, Shaffer *et al.* used silica nanoparticles as substrates for chelator-free labeling of various kinds of oxyphilic radioisotopes (^{89}Zr , ^{68}Ga , ^{111}In , ^{90}Y , and ^{177}Lu).¹¹ Chen *et al.* also used mesoporous silica nanoparticles (MSN) for chelator-free labeling of ^{89}Zr through deprotonated silanol groups.¹⁵ Recently, we precisely tuned the surface chemistry and composition of WS_2/WO_x nanoparticles and achieved optimal chelator-free ^{89}Zr radiolabeling yields.¹⁶ However, a

general method that can be employed to radiolabel a wide range of different nanomaterials in a chelator-free manner remains to be explored.

Metal oxide nanomaterials, due to their special optical, electrical, and magnetic properties, have attracted much attention in many different fields including nanomedicine.^{17–20} A key characteristic of metal oxide nanostructures is that the oxygen atoms arranged on the surface of metal ions are excellent electron donors. Many medically-relevant isotopes are chelated by electron donors (e.g., oxygen, sulfur, or nitrogen atoms), when these donors are arranged in the required asymmetry to form stable coordination complexes.²¹ For example, Desferrioxamine (DFO),²² a hexadentate ligand with three hydroxamate groups that provide six oxygen donors for metal binding, currently is the preferred chelator for labeling of ⁸⁹Zr, which, with a relatively long decay half-life, is highly suitable for long-term PET imaging.^{11, 15} In this regard, it is reasonable to hypothesize that metal oxide nanostructures could be used for intrinsic chelator-free radiolabeling. Herein, we report a general method for chelator-free radiolabeling of various kinds of metal oxide nanomaterials with ⁸⁹Zr for *in vivo* PET imaging (Figure 1a). Though a simple sol-gel method, ten kinds of metal oxide (M_xO_y, M=Gd, Ti, Te, Eu, Ta, Er, Y, Yb, Ce, and Mo, x=1–2, y=2–5) nanomaterials with different morphologies are successfully synthesized. After polyethylene glycol (PEG) modification, the obtained PEGylated M_xO_y nanostructures are stable in the physiological solutions. Due to the presence of oxygen atoms on the surface, all the synthesized M_xO_y nanostructures could be labeled with oxyphilic ⁸⁹Zr *via* a chelator-free method upon simple mixing, yielding ⁸⁹Zr-M_xO_y with high labeling yield and good serum stability. After intravenous administration into mice (M=Gd, Ti, Ta, and Y, four of M_xO_y nanomaterials for example), these ⁸⁹Zr-M_xO_y-PEG show no appreciable bone uptake over two weeks, indicating the good stability of the nanotracer in the body (as free ⁸⁹Zr homes to the bone quickly). *In vivo* draining lymph node networks imaging is also successfully conducted with ⁸⁹Zr-Gd₂O₃-PEG, which enables dual modal PET / magnetic resonance (MR) imaging of draining lymph nodes of mice.

Results and discussion

Metal oxide (M_xO_y) nanomaterials with different morphologies were synthesized through a one pot sol-gel method.²³ By slowly adding alkaline solution into the metal precursor, metal oxide hydrate complexes were formed at room temperature. After incubation in an oven to remove the hydrate, various kinds of metal oxide nanomaterials with different morphologies were successfully synthesized (Figure 1b). For Gd, Ti, Te, and Eu precursors, uniform nanorod morphologies were observed (Gd₂O₃, TiO₂, TeO₂, and Eu₂O₃). However, spherical morphologies existed in the other five metal oxides (Er₂O₃, Y₂O₃, Yb₂O₃, CeO₂, and MoO₃) with different sizes. For the tantalum precursor, the special hollow structure of Ta₂O₅ nanomaterials with an average diameter of 100 ± 12 nm was formed by this simple sol-gel method. Particularly, it is noteworthy that all of the metal oxide nanomaterials showed clear lattice fringes on high-resolution TEM images (Figure 1b), indicating the highly crystalline nature of the metal oxide nanomaterials formed by using this general and simple approach.

X-ray diffraction (XRD) was used to study the chemical composition of the obtained nanomaterials. All of the synthesized metal oxide nanomaterials (Supporting Figure S1&S2)

showed crystalline structures. X-ray photoelectron spectroscopy (XPS) characterization of the synthesized M_xO_y nanostructure was also performed. Taking Gd_2O_3 nanorods for example, two peaks centered at 143.6 eV and 149.6 eV could be assigned to $Gd_{3d5/2}$ and $Gd_{3d3/2}$ peaks of Gd^{3+} ions, respectively. The peaks located at 530.5 eV and 532.2 eV could be assigned to O_{1s} (Supporting Figure S3). No other peaks were detected for the Gd_2O_3 sample, indicating the high purity of the as-prepared Gd_2O_3 nanorods. HADDF-STEM-EDS elemental mapping also confirmed that the distributions of Gd and O elements were largely co-localized for Gd_2O_3 nanorods (Supporting Figure S4). The above characterization data confirmed the successful formation of Gd_2O_3 nanorods and other nine metal oxide nanomaterials.

To make the synthesized metal oxide nanomaterials water-soluble and biocompatible, we used polyethylene glycol (PEG) polymer to modify the metal oxide nanomaterials through a layer by layer (LBL) polymer-coating strategy (Supporting Figure S5a).²⁴ All of the synthesized metal oxide nanomaterials showed positive surface charge at the beginning of surface coating (Supporting Table S1), allowing coating by the negatively charged polymer poly (acrylic acid) (PAA, MW=1800) *via* electrostatic binding (Supporting Figure S5b). Amine-terminated PEG (MW=5 kDa) was then conjugated to surface carboxyl groups on Gd_2O_3 -PAA nanorods *via* amide formation. As the number of polymer layers increased during the LBL assembly process, the sizes of Gd_2O_3 nanorods measured by dynamic light scattering (DLS) were slightly increased. The final Gd_2O_3 -PEG nanorods showed an average hydrodynamic diameter of ~140 nm (Supporting Figure S5c). After PEGylation, the Gd_2O_3 -PEG nanorods exhibited obvious improved stability for several days' incubation in physiological solutions. For the other nine metal oxide nanomaterials (TiO_2 , TeO_2 , Eu_2O_3 , Er_2O_3 , Y_2O_3 , Yb_2O_3 , CeO_2 , MoO_3 , and Ta_2O_5), both zeta potential and DLS data showed similar changes to that of Gd_2O_3 -PEG nanorods modified using this method (Supporting Table S1), indicating the successful surface modification of those nanomaterials.

Inspired by our previous work about the intrinsic radiolabeling of ^{89}Zr onto mesoporous silica nanoparticles,¹⁵ we hypothesized that the presence of oxygen as electron donors on the surface of metal oxides could also be used for chelator-free radiolabeling of ^{89}Zr . ^{89}Zr produced according to our previous procedures was incubated with the above ten different types of PEGylated metal oxide nanomaterials for 2 h.²⁵ Thin-layer chromatography (TLC) was employed to determine the radiolabeling yields for different types of materials. High labeling yields were achieved after incubation at 75 °C (more than 80%), while incubation at 37 °C resulted in relatively low labeling yields (less than 20%, Figure 2a&b). Taking Gd_2O_3 -PEG nanorods for an example, after mixing ^{89}Zr -oxalate with Gd_2O_3 -PEG in HEPES buffer (0.1 M, pH 7–8) and shaking at 75 °C, the radiolabeling yield of ^{89}Zr - Gd_2O_3 -PEG was found to be over 78% within the first 15 min of incubation, and reached to as high as 90% after a 2 h incubation (Figure 2a, Supporting Table S2). To exclude the possibility of physical adsorption, DFO, a standard ^{89}Zr chelator, was used as a competitive chelation agent to remove any unstable ^{89}Zr loosely associated with the nanorods. A high labeling yield of > 80% was still obtained after the DFO competitive incubation at 75 °C (Supporting Figure S6a), suggesting that those ^{89}Zr ions were indeed strongly bound on the surface of metal oxide instead of loose physical adsorption. Similar high labeling yields (with over 80% ^{89}Zr retained) have been observed for the metal oxide nanostructures after the EDTA

competitive incubation (Supporting Figure S7). Notably, ^{89}Zr labeling on metal oxide nanomaterials was also found to be highly stable in mouse serum for up to two weeks even in a DFO competitive situation (Figure 2c, Supporting Figure S6b), further demonstrating the strong binding between ^{89}Zr and the metal oxide surface.

To understand the mechanism of ^{89}Zr -labeling of metal oxide nanomaterials, several control experiments were performed. Incubation of the same amount of ^{89}Zr with nanomaterials without surface oxygen atoms (for example, Au nanoparticles, tungsten disulfide nanosheets (WS_2), and metal diselenide ($\text{FeSe}_2/\text{Bi}_2\text{Se}_3$)) led to no apparent ^{89}Zr labeling (Figure 3, Supporting Figure S8), indicating the high labeling specificity of ^{89}Zr for metal oxide nanomaterials. Furthermore, negative control experiments involving the mixture of free ^{89}Zr and surface coating reagents alone (PAA or PEG) showed nearly zero labeling yields (Supporting Figure S8), confirming that ^{89}Zr was attached on M_xO_y nanomaterials instead of the coating polymers. For further validation, we also checked the activity of the bottom precipitate and the upper supernatant solution of each ^{89}Zr -labeled sample separated by centrifugation. Supporting Figure S9 summarizes the labeling yields of ten kinds of M_xO_y -PEG nanomaterials and negative controls (Au, WS_2 , and $\text{FeSe}_2/\text{Bi}_2\text{Se}_3$). All of the metal oxide nanomaterials showed much higher labeling yields than oxygen-free metal compounds and surface coating polymers, in agreement with the prior TLC labeling yield analysis. However, lots of factors could affect the radiolabeling yields, such as the geometry and size of the metal oxide nanostructure, the crystal planes of the nanostructures, and surface defects. This helps explain the slight differences across the many nanoparticles explored here.

Based the above conclusions, we explained a mechanism for the labeling of ^{89}Zr to various metal oxide compounds. ^{89}Zr is a hard Lewis acid and thus prefers to bind with hard Lewis bases such as metal oxides ($-\text{M}-\text{O}-$) that act as electron donors. Therefore, metal oxide nanomaterials would be ideal nanoplatfroms for chelator-free labeling by ^{89}Zr with high labeling yields and good stabilities, promising for *in vivo* PET imaging.

Many cancer deaths can be attributed to the metastatic spread of tumor cells.^{26–28} Sentinel draining lymph nodes (SLNs) are usually the primary sites of tumor metastases, especially at early stages. Therefore, it is important to identify the exact locations of draining LNs to remove them along with the primary solid tumor during surgery and prevent further spreading of tumor cells. For conventional SLN mapping, after injection of colored dyes or carbon black into the solid tumor, surgical resection is then performed as guided by the color of draining LNs.^{29–31} However, the sensitivity of this method is quite limited. With high spatial resolution and sensitivity, PET imaging holds promise for imaging SLNs. As a proof-of-concept, we demonstrated the possibility of using PET imaging with $^{89}\text{Zr}-\text{M}_x\text{O}_y$ -PEG probes for highly sensitive *in vivo* LN mapping (Figure 4a–4e). $^{89}\text{Zr}-\text{M}_x\text{O}_y$ -PEG ($^{89}\text{Zr}-\text{Gd}_2\text{O}_3$ -PEG, $^{89}\text{Zr}-\text{TiO}_2$ -PEG and $^{89}\text{Zr}-\text{Ta}_2\text{O}_5$ -PEG for example) nanostructures showed *in vivo* good stability after i.v. injection for 2 weeks as evidenced by the little bone uptake (less than $4.2 \pm 1.3\%$ ID/g uptake) of ^{89}Zr (Figure 4f–4h), which is a general behavior for free ^{89}Zr . Upon local injection of $^{89}\text{Zr}-\text{Gd}_2\text{O}_3$ -PEG nanorods ($\sim 30\ \mu\text{L}$, $50\ \mu\text{Ci}$) into the right rear footpad of mice, serial PET scans were performed for those mice (Figure 5a). An extensive draining lymphatic network containing five deep-seated LNs (PO, popliteal LN;

IL, iliac LN; RE, renal LN; IN, inguinal LN; AX, axillary LN) was visualized through PET imaging at as early as 0.5 h post injection of ^{89}Zr - Gd_2O_3 -PEG. The signals in those LNs remained strong 24 h after local injection of ^{89}Zr - Gd_2O_3 -PEG and were observable for 3 days (Figure 5a&5b). In marked contrast, after local injection with free ^{89}Zr (~50 μCi in 30 μL HEPES/PBS), only the first draining LN (PO) could be seen at the first time point (0.5 h p.i.). Later on, free ^{89}Zr gradually diffused throughout the mouse body and showed considerable uptake in the bone (Figure 5a&5c), similar to the *in vivo* distribution profile of free ^{89}Zr after *i.v.* injection. Although various types of nanoparticles have been investigated for LN imaging, most of them are only capable of imaging a few draining LNs near the injection sites.^{32–34} Our ^{89}Zr - Gd_2O_3 -PEG nanorods appear to be an ideal PET agent for mapping of the more complete draining lymphatic network with deep-seated lymph nodes.

Magnetic resonance (MR) imaging is another extensively used clinical imaging technique with high spatial resolution and soft tissue contrast.^{1, 35, 36} Many kinds of MR contrast agents including paramagnetic metal complexes and superparamagnetic nanoparticles have been widely reported.^{37–40} In particular, Gd^{3+} complexes with 7 unpaired 4f electrons are known to be effective T1 MR contrast agents. We thus explored the utility of our Gd_2O_3 nanorods for MR imaging applications. T1-weighted MR images of Gd_2O_3 -PEG solution revealed a concentration-dependent brightening effect. The transverse relaxivity (r_1) of Gd_2O_3 -PEG was calculated to be $16.89 \text{ mM}^{-1}\text{S}^{-1}$ (Supporting Figure S10), which was much higher than that of magnevist, a commercial Gd^{3+} reagent ($8.96 \text{ mM}^{-1}\text{S}^{-1}$),⁴¹ indicating the promise of Gd_2O_3 -PEG as a strong T1-MR contrast agent. *In vivo* T1-weighted MR imaging was then carried out after the local injection of Gd_2O_3 -PEG nanorods (~50 μL , 2 mg/mL) into the right rear footpad of mice (Figure 5d&5e). Obviously brightened T1 MR signals appeared in the LNs located in the inner knees of mice, matched well with the above PET imaging, indicating that our Gd_2O_3 -PEG nanoprobe could be used as a good contrast agent for PET / MR dual-modal LN mapping.

We also investigated dual-modal imaging based on the synthesized ^{89}Zr labeled nanomaterials in a cancer metastasis model.³⁰ Notably, the SLNs on the popliteal site clearly appeared in the PET imaging at 30 min after the injection of the ^{89}Zr - Gd_2O_3 -PEG nanorods, and the signal increased over time (Supporting Figure S11), indicating the ^{89}Zr - Gd_2O_3 -PEG nanorods moved from the primary tumor to the SLNs likely *via* lymphatic drainage. Consistent with PET imaging, *in vivo* MR imaging also revealed the LNs accumulation of Gd_2O_3 -PEG nanorods after injection (Supporting Figure S12). Due to lack of access to an integrated micro PET-MRI scanner, the dual modality imaging of ^{89}Zr - Gd_2O_3 -PEG nanorods was achieved separately here. However, our work proved the concept of these promising candidates for simultaneous PET/MRI imaging. Combining these two imaging modalities (PET/MRI) with high sensitivity and high resolution together, it would be better for physicians to design the best cancer treatment approach.

Conclusion

In summary, a general method has been reported in this work for chelator-free radiolabeling of various kinds of metal oxide nanomaterials with ^{89}Zr for *in vivo* PET imaging. Ten types of metal oxide (M_xO_y , $\text{M}=\text{Gd}$, Ti , Te , Eu , Ta , Er , Y , Yb , Ce , and Mo , $x=1-2$, $y=2-5$)

nanomaterials with different morphologies were successfully synthesized and modified by PEG. Interestingly, the fabricated M_xO_y nanostructures could be labeled with oxyphilic ^{89}Zr *via* a simple chelator-free method, yielding $^{89}\text{Zr}-M_xO_y$ with high labeling yields and good serum stabilities. As a proof-of-concept, deep-seated draining lymph node networks were visualized by PET imaging with $^{89}\text{Zr}-\text{Gd}_2\text{O}_3$ -PEG nanorods, which in the meanwhile also offered strong contrast in MR imaging. Our work thus presents a general and simple method for chelator-free labeling of metal oxide nanomaterials, which may be promising nanoprobe in multimodal molecular imaging.

Methods and experiments

Materials

GdCl_3 , TiCl_4 , TeCl_4 , EuCl_3 , ErCl_3 , YCl_3 , YbCl_3 , CeCl_4 , MoCl_6 , and TaCl_5 were obtained from Sigma-Aldrich. NaOH was obtained from Sinopharm Chemical Reagent Co. Deionized water used in all experiments was obtained from a Milli-Q water system.

Synthesis of metal oxide (M_xO_y) nanostructures

In a standard experiment, 10 μmol of metal chloride precursor was dissolved in distilled water and stirred at room temperature. Then a solution containing an equal mole of NaOH was added dropwisely. The solution changed from colorless to turbid gradually, indicating the formation of hydrated metal oxide nanomaterials. After reaction for 4 h, hydrated metal oxide nanomaterials were collected by centrifugation, and repeatedly washed with distilled water at least three times. The sample was then incubated in a 100 °C oven to remove the hydrate from the hydrated metal oxide to obtain the metal oxides.

PEGylated M_xO_y nanomaterials

PEGylated M_xO_y nanomaterials were synthesized through an LBL method according to the previous study (Supporting Information).²⁴ After the PEGylation, the yielded M_xO_y -PEG (Gd_2O_3 -PEG, TiO_2 -PEG, TeO_2 -PEG, Eu_2O_3 -PEG, Er_2O_3 -PEG, Y_2O_3 -PEG, Yb_2O_3 -PEG, CeO_2 -PEG MoO_3 -PEG, and Ta_2O_5 -PEG) solution was stored at 4 °C for future use.

Characterization

The phase and crystallography of the M_xO_y nanomaterials were characterized by using a PANalytical X-ray diffractometer equipped with Cuka radiation ($\lambda=0.15406$ nm). A scanning rate of 0.05°s^{-1} was applied to record the pattern in the 2θ range of $10\text{--}80^\circ$. Transmission electron microscopy (TEM) images of the nanostructures were obtained using an FEI Tecnai F20 transmission electron microscope. X-ray photoelectron spectroscopy (XPS) was performed on an SSI S-Probe XPS Spectrometer. Zetasizer Nano-ZS (Malvern Instruments, UK) was used to check the DLS. Inductively coupled plasma optical emission spectroscopy (ICP-OES, Thermo) was used to determine the concentration of the metal ion.

^{89}Zr labeling

^{89}Zr was produced with an onsite cyclotron (GE PETrace) at the University of Wisconsin-Madison.¹⁵ $^{89}\text{ZrCl}_4$ (150 MBq) was diluted in 300 μL of 1 mM HEPES solution (pH ~ 7)

and mixed with 100 μL of M_xO_y -PEG nanomaterials (1 mg/mL). The reaction was conducted at 75°C or 37°C for 2 h with constant shaking. Thin-layer chromatography (TLC) using 0.05 M EDTA as the mobile phase was used to check the labeling yields at different time points. For the challenge study, the EDTA (0.5mM) or DFO (p-isothiocyanatobenzyl desferrioxamine B, 1 mM) was added into the ^{89}Zr - M_xO_y solution and shaken for 5 min before the TLC analysis. The resulting ^{89}Zr - M_xO_y -PEG could be easily collected by centrifugation (at 15000 rpm for 5 min).

Serum Stability Studies

For serum stability studies, ^{89}Zr - M_xO_y -PEG nanomaterials were incubated in mouse serum at 37°C for up to 7 days. Portions of the solution were sampled at different time points and filtered through 100 kDa cut-off filters. The filtrates were collected and their radioactive contents were measured. The percentage of retained (*i.e.*, intact) ^{89}Zr on the M_xO_y -PEG nanomaterials were calculated using the following equation:

$$(\text{Total radioactivity} - \text{Radioactivity in filtrate}) / \text{Total radioactivity}$$

To demonstrate the stability of ^{89}Zr in M_xO_y -PEG nanomaterials, DFO was also added into 250 μL of ^{89}Zr - M_xO_y -PEG ($\sim 300 \mu\text{Ci}$) HEPES solution (pH=7) at 37°C under constant shaking (600 rpm) for 7 days. The final DFO concentration was fixed to be 0.1 mM. At each time point, 25 μL of the mixture was taken out and re-suspended in 100 μL of HEPES buffer solution. A 100 kDa filter was used to separate ^{89}Zr -DFO from ^{89}Zr -labeled nanomaterials. The ^{89}Zr -DFO and ^{89}Zr -labeled nanomaterial radioactivity were measured by a gamma counter (PerkinElmer).

In Vivo PET Imaging

All animal studies were conducted under a protocol approved. For *in vivo* lymph node mapping with PET, 30 μL of ^{89}Zr - Gd_2O_3 -PEG ($\sim 2.5 \text{ MBq}$) or free ^{89}Zr with the same dose was injected into the right footpad of the mouse and imaged using a microPET/microCT Inveon rodent model scanner (Siemens Medical Solutions USA, Inc.). Time points of 0.5, 2, 4, 12, 24, 48, and 72 h post-injection were chosen for PET scans. Data acquisition, image reconstruction, and ROI analysis of the PET data were performed as described previously.²⁵

In vivo MR imaging

MR imaging was conducted using a 3.0-T clinical MR scanner (GE Healthcare, USA) equipped with a special coil for small animal imaging. For *in vivo* MR lymph node imaging, 30 μL of Gd_2O_3 -PEG ($\sim 50 \mu\text{L}$, 2 mg/mL) was locally injected into the right footpad of the mouse. T1-weighted images were acquired using the following parameters: TR 2000 ms; TE 106.4 ms; slice thickness 2.0 mm; slice spacing, 0.2 mm; matrix, 224×192 ; FOV 10 cm $\times$ 10 cm. After acquiring T1-weighted MR images, the signal intensities were measured within a manually drawn region of interest (ROI) for each mouse.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Characterization of different types of metal oxide (M_xO_y) nanostructures. **(a)** A schematic illustration showing the chelator-free labeling of a metal oxide (M_xO_y) with ^{89}Zr . **(b)** TEM and magnified TEM images of M_xO_y nanomaterials including Gd_2O_3 nanorods, TiO_2 nanorods, Te_2O_3 nanorods, Eu_2O_3 nanorods, Ta_2O_5 nanospheres, Er_2O_3 nanoparticles, Y_2O_3 nanoparticles, Yb_2O_3 nanoparticles, CeO_2 nanoparticles, and MoO_3 nanoparticles.

Figure 2.

Radiolabeling and serum stability of ^{89}Zr labeled M_xO_y nanomaterials. (a) Thin-layer chromatography (TLC) plates of various types of M_xO_y nanomaterials at different time points after mixing ^{89}Zr with M_xO_y nanomaterials at 75 °C: Gd_2O_3 , TiO_2 , Te_2O_3 , Eu_2O_3 , Ta_2O_5 , Er_2O_3 , Y_2O_3 , Yb_2O_3 , CeO_2 , and MoO_3 . UL: Unlabeled ^{89}Zr ; L: Labeled ^{89}Zr . (b) Time-dependent ^{89}Zr labeling yields of M_xO_y -PEG nanomaterials (Gd_2O_3 , TiO_2 , Te_2O_3 , Eu_2O_3 , Ta_2O_5 , Er_2O_3 , Y_2O_3 , Yb_2O_3 , CeO_2 , and MoO_3) at different temperatures (37 °C and 75 °C). (c) Stability test of ^{89}Zr labeling on M_xO_y nanomaterials after incubation in serum at 37 °C for various periods of time (M_xO_y : Gd_2O_3 , TiO_2 , Te_2O_3 , Eu_2O_3 , Ta_2O_5 , Er_2O_3 , Y_2O_3 , Yb_2O_3 , CeO_2 , and MoO_3).

Figure 3.

Hypoxic nanomaterials labeled with ^{89}Zr . (a–c) TEM images of the synthesized (a) Au nanoparticles; (b) WS_2 nanosheets; (c) $\text{FeSe}_2/\text{Bi}_2\text{Se}_3$ nanocomposites. (d–f) TLC plates of various nanomaterials at various time points after mixing ^{89}Zr : (d) Au nanoparticles; (e) WS_2 nanosheets; (f) $\text{FeSe}_2/\text{Bi}_2\text{Se}_3$ nanocomposites. (g–i) Quantified labeling yields of ^{89}Zr on those nanomaterials at various time points after incubation (n=3): (g) Au nanoparticles; (h) WS_2 nanosheets; (i) $\text{FeSe}_2/\text{Bi}_2\text{Se}_3$ nanocomposites.

Figure 4.

In vivo radio-stability study using PET imaging. (a–e) *In vivo* Maximum intensity projections (MIPs) of mice after *i.v.* injection of ^{89}Zr - M_xO_y -PEG nanomaterials: (a) ^{89}Zr - Gd_2O_3 -PEG; (b) ^{89}Zr - TiO_2 -PEG; (c) ^{89}Zr - Ta_2O_5 -PEG; (d) ^{89}Zr - Y_2O_3 -PEG, and (e) Free ^{89}Zr at different time points. (f&g) Quantitative region of interest (ROI) analysis of the dynamic uptake of ^{89}Zr after *i.v.* injection of ^{89}Zr - Gd_2O_3 -PEG (f) or free ^{89}Zr (g) in bone and liver. (h) Biodistribution of ^{89}Zr - Gd_2O_3 -PEG and free ^{89}Zr measured at 14 days p.i. Data is presented as the percentage of injected dose per gram of tissue (% ID/g). **Sk**: Skin; **Mu**: Muscle; **B**: Bone; **Lu**: Lung; **L**: Liver; **K**: Kidney; **Sp**: Spleen; **In**: Intestine. Error bars are based on the standard error of the mean (SEM) of triplicate samples.

Figure 5.

In vivo lymph node (LN) mapping after local injection. (a) *In vivo* MIP of lymph node (LN) imaging with PET after local injection of $^{89}\text{Zr-Gd}_2\text{O}_3$ -PEG nanorods or free ^{89}Zr into the right footpad of each mouse. PO, popliteal LN; IL, iliac LN; RE, renal LN; IN, inguinal LN; AX, axillary LN. (b) Quantification of $^{89}\text{Zr-Gd}_2\text{O}_3$ -PEG nanorods uptake in the LNs. (c) Quantification of $^{89}\text{Zr-Gd}_2\text{O}_3$ -PEG nanorods and free ^{89}Zr uptake in the liver and bone. (d) *In vivo* MR imaging for LN mapping using Gd_2O_3 -PEG nanorods. MR images were

taken before (left) and after (right) injection of Gd₂O₃-PEG nanorods. (e) Quantification of Gd₂O₃-PEG nanorods in the popliteal LNs (PO) based on MR imaging.

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