Published in final edited form as:

Adv Mater. 2014 August 13; 26(30): 5119–5123. doi:10.1002/adma.201401372.

Intrinsically Germanium-69 Labeled Iron Oxide Nanoparticle: Synthesis and *In Vivo* Dual-modality PET/MR Imaging

Rubel Chakravarty,

Department of Radiology, University of Wisconsin - Madison, WI, USA

Isotope Applications and Radiopharmaceuticals Division, Bhabha Atomic Research Centre, Mumbai, India

Hector F. Valdovinos,

Department of Medical Physics, University of Wisconsin - Madison, WI, USA

Feng Chen,

Department of Radiology, University of Wisconsin - Madison, WI, USA

Christina M. Lewis,

Department of Medical Physics, University of Wisconsin - Madison, WI, USA

Paul A. Ellison,

Department of Medical Physics, University of Wisconsin - Madison, WI, USA

Haiming Luo,

Department of Radiology, University of Wisconsin - Madison, WI, USA

M. Elizabeth Meyerand,

Department of Medical Physics, University of Wisconsin - Madison, WI, USA

Robert J. Nickles, and

Department of Medical Physics, University of Wisconsin - Madison, WI, USA

Weibo Cai

Department of Radiology, University of Wisconsin - Madison, WI, USA

Department of Medical Physics, University of Wisconsin - Madison, WI, USA

University of Wisconsin Carbone Cancer Center, Madison, WI, USA

Weibo Cai: wcai@uwhealth.org

Keywords

Germanium-69; Iron oxide nanoparticle; Chelator-free radiolabeling; PET/MR imaging; Multimodality imaging

Correspondence to: Weibo Cai, wcai@uwhealth.org.

Dr. R. Chakravarty, H. F. Valdovinos and Dr. F. Chen contributed equally to this work

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Germanium-69 (^{69}Ge , $t_{1/2} = 39.05$ h, 21% β^+ , $E_{\text{max}} = 1205$ keV) is a novel positron emitting radionuclide whose potential is yet to be explored for positron emission tomography (PET) imaging.^[1] The adequately long half-life of ^{69}Ge , makes it an ideal choice for immunoPET imaging using ^{69}Ge -labeled antibodies. Also, the production route of this radioisotope is simple and cost-effective.^[1] However, the major limitation in the use of this novel radioisotope is its complex coordination chemistry in aqueous medium and the unavailability of suitable radiolabeling techniques for the preparation of ^{69}Ge -based radiopharmaceuticals. It has been reported that the predominant germanium species in aqueous media are $\text{Ge}(\text{OH})_4$, $[\text{GeO}(\text{OH})_3]^-$, $[\text{GeO}_2(\text{OH})_2]^{2-}$, and $[[\text{Ge}(\text{OH})_4]_8(\text{OH})_3]^{3-}$ with the distribution of each species depending on the total concentration and the pH of the medium,^[2] making it a major challenge for radiolabeling with ^{69}Ge using traditional chelator-based methods.

Fortunately, the incorporation of another germanium isotope, ^{68}Ge ($t_{1/2} = 279$ d), into metal oxides such as TiO_2 , ZrO_2 , CeO_2 , SnO_2 , Fe_2O_3 , Fe_3O_4 , Al_2O_3 , etc., has long been reported for the preparation of clinically useful $^{68}\text{Ge}/^{68}\text{Ga}$ generators.^[3] In these generators, ^{68}Ge remains selectively adsorbed in a chromatographic column containing metal oxide sorbent, and the daughter isotope ^{68}Ga ($t_{1/2} = 68$ min) can be regularly eluted out using a suitable solvent for a prolonged period of time (generally ~ 1 y). The strong binding affinity of germanium ions to these metal oxides has also been demonstrated by the fact that the breakthrough of ^{68}Ge from the generator column is less than 0.01 % on multiple ^{68}Ga -elutions over the period of 1 y.^[3a, 4]

Inspired by these, we hypothesized that using a suitable water soluble metal oxide as a platform, ^{69}Ge -labeled metal oxides could be synthesized simply by mixing metal oxide with ^{69}Ge ions, forming intrinsically radiolabeled particles without the assistance of any chelators (Figure 1a). Here in this communication, superparamagnetic iron oxide nanoparticle (SPION) was selected for this purpose also considering the possibility to use the same nanoplatform for simultaneous PET and magnetic resonance imaging (MRI).^[5] The synergistic combination of PET (with high sensitivity) and MRI (with high resolution and exquisite soft tissue contrast) has attracted tremendous interest over the last decade, and PET/MRI scanners are now been used in clinical practices.^[6] The future of PET/MRI scanning will greatly benefit from the use of dual-modality PET/MRI probes, such as the one presented in this work.

Water soluble poly(acrylic acid) (PAA) modified SPION (SPION@PAA) was first prepared by reacting oleate-coated SPION with PAA in diethylene glycol.^[7] Transmission electron microscopy (TEM) images of SPION before and after PAA modification are shown in Figure 1b and c, respectively, which showed no obvious change in morphology. Dynamic light scattering (DLS) analysis of water soluble SPION@PAA in phosphate buffered saline (PBS, pH 7.4) solution indicated a diameter of ~ 23 nm (Figure S1), slightly larger than the core size (~ 10 nm) observed by TEM, which is expected due to the PAA coating and hydration layer on SPION surface. Enhanced T_2^* MR contrast imaging property of as-synthesized water soluble SPION@PAA has been demonstrated previously,^[7a] which showed the decrease of T_2 time (from 484.4 ± 95.9 ms to 5.4 ± 0.4 ms) with the increase of Fe concentrations (from zero to 1.94 mM, as indicated in Table S1). The T_2 relaxivity (r_2) of

SPION@PAA was estimated to be $93.8 \text{ mM}^{-1}\text{s}^{-1}$ in the 4.7 T microMRI scanner. As-synthesized SPION@PAA was found to be highly stable in many different biological solutions (e.g. cell culture medium, saline) and mouse serum, with no visible aggregation even after storage for 6 months.^[7a]

Germanium-69 was produced by $^{69}\text{Ga}(\text{p},\text{n})^{69}\text{Ge}$ reaction using the University of Wisconsin GE PETtrace Cyclotron.^[1] Subsequently, it was chemically separated from the bulk target material and co-produced extraneous radioisotopes with N,N,N',N'-tetra-n-octyldiglycolamide (DGA) extraction resin (Figure S2). The overall yield of ^{69}Ge was $\sim 111 \text{ MBq}$ (after a $20 \mu\text{A}\cdot\text{h}$ irradiation), 60% of which could be effectively separated in $600 \mu\text{L}$ of deionized water. The γ -spectra of ^{69}Ge before and after the purification process were obtained using a high purity germanium detector (HPGe) coupled to a multichannel analyzer. The analysis of the γ -spectra showed peaks at 319 keV, 574 keV, 872 keV, 1107 keV and 1337 keV (Figure S3a), which are the characteristic peaks of ^{69}Ge .^[1] The unpurified ^{69}Ge sample contained significant levels of radionuclidic impurities, such as $^{197\text{m}}\text{Hg}$ (134 keV) and ^{55}Co (931 keV), which could be completely removed after the purification process (Figure S3b). The radionuclidic purity of ^{69}Ge was determined to be $>99.99\%$.

The pH-dependent sorption behavior of germanium ions to iron oxide has been well documented with the optimum range found to be pH 4–8.^[8] Here, radiolabeling of SPION@PAA with ^{69}Ge was carried out by adjusting the pH of the ^{69}Ge solution to ~ 7 , not only to maintain the stability of ^{69}Ge -SPION@PAA but also to achieve the best labeling efficiency. Radiolabeling yield of ^{69}Ge -SPION@PAA was then determined at different time intervals using radio-thin layer chromatography (radio-TLC), where ^{69}Ge -SPION ($R_F = 0$) could easily be distinguished from free ^{69}Ge ($R_F = \sim 0.95$) (Figures 1d, 1e and S4). Radiolabeling yield of ^{69}Ge -SPION@PAA was found to be $>75\%$ after 3 h incubation at 37°C , and gradually increased to $\sim 90\%$ after 24 h of incubation (specific radioactivity $\sim 3.7 \text{ MBq}/\mu\text{mol}$ of Fe) (Figure 1d, green line). Negative control experiment with the mixture of free ^{69}Ge and water showed nearly zero labeling yield, confirming the successful labeling of ^{69}Ge to SPION@PAA (Figure 1d, red line).

The specific labeling of ^{69}Ge to SPION was further demonstrated by 1) blocking the SPION surface with a layer of dense silica (dSiO_2), and 2) using non-metal oxide nanoparticles (e.g. copper sulfide [CuS]) as nanoplateforms. TEM images of both SPION@ dSiO_2 and CuS were also shown in Figure S5. As expected, very low radiolabeling yield (i.e. $<15\%$) was observed when mixing SPION@ dSiO_2 or CuS with free ^{69}Ge under the same conditions (Figure 1d, blue and black lines, respectively). In order to rule out the possibility of ^{69}Ge uptake by the PAA, ^{69}Ge labeling of ligand-free SPION (bare SPION without any surface coating) and free PAA were carried out under similar conditions (Figure S6). Results showed that the ^{69}Ge radiolabeling to ligand-free SPION ($\sim 2.0 \text{ mg/mL}$) was $>95\%$ at the end of 24 h, which was slightly higher than what was observed in the case of ^{69}Ge -SPION@PAA (Figure S7). Moreover, there was hardly any uptake of ^{69}Ge by PAA (Figure S7), clearly indicating the successful labeling of ^{69}Ge to SPION@PAA is due to the specific binding of ^{69}Ge with SPION, and non-specific absorption of ^{69}Ge to PAA is negligible. All these systematic studies amply illustrate the specific affinity of ^{69}Ge to SPION.

In order to increase the *in vivo* stability of ^{69}Ge -SPION, PEGylation step was introduced for coating ^{69}Ge -SPION with a layer of poly(ethylene glycol) (PEG). Enhanced *in vitro* serum stability was also observed when comparing ^{69}Ge -SPION@PEG with non-PEGylated ^{69}Ge -SPION (Figure 1f). ^{69}Ge -SPION@PEG was found to be highly stable (intact ^{69}Ge ~75%) even when incubated in excess volume of whole mouse serum at 37 °C (310 K) for 24 h (Figure 1f, black line). In contrast, non-PEGylated ^{69}Ge -SPION was significantly less stable in mouse serum with intact ^{69}Ge found to be <60 % after 6 h of incubation. Before the *in vivo* PET imaging, ^{69}Ge -SPION@PEG was purified using PD-10 column with phosphate buffered saline (PBS) as the mobile phase. The radioactivity fractions (which typically elute between 3.0 and 4.0 mL) of ^{69}Ge -SPION@PEG were collected. Free unlabeled ^{69}Ge was eluted out after 6 mL, which allowed complete and baseline separation of ^{69}Ge -SPION from ^{69}Ge . A typical size exclusion column chromatography profile is also provided in Figure S8.

In vivo stability and biodistribution pattern of ^{69}Ge -SPION@PEG was then assessed by PET imaging studies in normal BALB/c mice. Dominant liver uptake of ^{69}Ge -SPION@PEG was observed at different time points after intravenous (i.v.) injection (Figure 2a), as expected for most nanoparticles. Quantitative data obtained from region-of-interest (ROI) analysis of these PET images are also shown in Figure S10. In stark contrast, free ^{69}Ge exhibited rapid renal clearance and nearly no accumulation in the liver (Figure 2a). Such difference in biodistribution pattern is a direct evidence of the fact that ^{69}Ge -SPION@PEG could retain its integrity *in vivo* and thus demonstrates its suitability as a PET imaging probe. The liver uptake of ^{69}Ge -SPION@PEG was further confirmed by *in vivo* MRI, which clearly showed the darkening of the liver and no detectable signal change from the kidney (or bladder) after i.v. injection of ^{69}Ge -SPION@PEG (Figure 2b). It may be noted that ‘cold’ ^{69}Ge -SPION@PEG was prepared under the similar conditions for MRI since radioactive materials (e.g. ^{69}Ge -SPION@PEG) are not allowed in the microMRI facility of our University. The biodistribution of ^{69}Ge -SPION@PEG was also investigated at 36 h post-injection (p.i.) after the final PET scan by measuring the tissue radioactivity. As shown in Figure S11, dominant uptake of ^{69}Ge -SPION@PEG in the liver and spleen was observed, which validated that serial non-invasive PET imaging (Figure 2a) accurately reflected the biodistribution pattern of ^{69}Ge -SPION@PEG in mice. All these studies demonstrate the feasibility of ^{69}Ge -SPION@PEG for dual-modality PET/MR imaging.

As a proof-of-concept, non-invasive PET/MR dual-modality sentinel lymph nodes (SLNs) mapping using ^{69}Ge -SPION@PEG was also demonstrated. Typically, SLNs are the first sites of primary tumor metastasis and therefore successful imaging of SLNs has been considered as an important strategy for tracking cancer metastasis in the clinical context.^[9] Upon subcutaneous injection of ^{69}Ge -SPION@PEG solution (40 μL , ~370 kBq) into the left footpad of normal BALB/c mice, serial PET scans were performed. Accumulation of ^{69}Ge -SPION@PEG in the popliteal lymph node could clearly be seen at 0.5 h, 2 h and 20 h p.i. (Figure 3a, as pointed by green arrows), with uptake of ^{69}Ge -SPION@PEG found to be 7.5 ± 2.5 , 12.5 ± 3.1 and 28.0 ± 5.2 percentage injected dose per gram of tissue (%ID/g), respectively ($n = 3$; Figure 3b). The accumulation of ^{69}Ge -SPION@PEG in one of the lymph nodes (dashed green circle in Figure 3c) could also be clearly visualized by MRI, which

showed gradual and prominent darkening of the lymph node after injection of Ge-SPION@PEG (40 μ L, 7.77 mM of Fe). As an internal control, the contralateral lymph node (dashed red circle in Figure 3c) showed no contrast enhancement at all-time points examined. The accumulation of ^{69}Ge -SPION@PEG in lymph nodes after injection was primarily due to the small size (~ 23 nm) of these nanoparticles, which is well suited to uptake by the lymphatics.^[10]

Although dual-modality PET/MR imaging could only be achieved separately in our current work due to the lack of an integrated microPET/microMRI scanner, this work may serve as an important proof-of-concept to establish ^{69}Ge -SPION@PEG as a promising candidate for future simultaneous PET/MR imaging. With the growing interests in using clinical and preclinical PET/MR scanners for integrated and simultaneous whole-body PET/MR imaging worldwide,^[11] further improvement of both the PET/MR scanners and the development of novel PET/MR imaging agents (e.g. ^{69}Ge -SPION@PEG) would benefit clinical cancer patient management in the future (e.g. diagnosis, staging, etc.). The chelator-free strategy reported here could also be effectively utilized for preparation of multifunctional theranostic agents for integrated imaging and therapy by incorporating therapeutically relevant isotopes (e.g. ^{77}Ge [$t_{1/2} = 11.3$ h, 100% β^- , $E_{\beta\text{max}} = 2.2$ MeV]) into SPION.^[1] With the presence of carboxyl groups at the surface of ^{69}Ge -SPION, further conjugation of specific targeting ligands, such as proteins, antibodies, or peptides,^[12] could also be readily achieved, which would make this class of agents even more powerful for future cancer-targeted imaging and therapy.

In conclusion, using SPION as a nanoplatform, we reported the synthesis and *in vivo* dual-modality PET/MR imaging of ^{69}Ge -SPION@PEG. To the best of our knowledge, this is the first example on the successful utilization of ^{69}Ge -based agent for PET/MR imaging. The key challenge involved in the preparation of ^{69}Ge -radiopharmaceuticals has been circumvented by incorporating ^{69}Ge onto SPION *via* a fast and highly specific chelator-free approach. The biodistribution pattern and the feasibility of ^{69}Ge -SPION@PEG for *in vivo* dual-modality imaging and lymph-node mapping have also been investigated.

Experimental Section

Production and purification of ^{69}Ge

Germanium-69 was produced by bombarding a Ga/Ni alloy target with 11 MeV protons at the University of Wisconsin GE PETtrace Cyclotron Facility (Figure S2). For this purpose, 16 MeV proton beam from the cyclotron was degraded down to 11 MeV using a 254 μm thick Mo foil. The target for irradiation was prepared by electrodeposition of ~ 40 mg of Ga/Ni alloy (in 3:1 ratio) onto 1 cm^2 of a gold substrate. The target was mounted on a water-cooled target holder for irradiation and was able to sustain currents up to 20 μA . The activity of ^{69}Ge produced at the end of bombardment was 6.8 ± 1.3 MBq/ $\mu\text{A}\cdot\text{h}$ ($n = 4$). After irradiation, the target was allowed to decay overnight to avoid unnecessary radiation exposure from the short-lived radiocopper products that originate from the nickel target material. The target was dissolved in concentrated nitric acid. The separation of ^{69}Ge from the bulk target material and other radionuclidic impurities [mainly $^{197\text{m}}\text{Hg}$ from ^{197}Au (p, n) and ^{55}Co from ^{58}Ni (p, α)] was carried out in nitric acid medium using 200 mg of DGA

extraction resin packed in a 5 mm diameter column. On passing the radioactive solution through the chromatographic column, ^{69}Ge was trapped in the resin which could subsequently be eluted in 600 μL of deionized water.

Synthesis of ^{69}Ge -SPION@PEG

In order to label SPION with ^{69}Ge , the pH of the radioactive ^{69}Ge solution was carefully adjusted to ~ 7 by addition of ~ 100 μL of 2 M Na_2CO_3 solution. The radioactive solution containing 37 MBq (1 mCi) of ^{69}Ge was then added to 500 μL of SPION@PAA (3.1 mM Fe, dispersed in 1 mM sodium hydroxide solution, pH 7–8) in an Eppendorf tube. The mixture was kept under constant shaking at 500 rpm at 37 $^\circ\text{C}$. After the radiolabeling procedure, unlabeled free ^{69}Ge was separated from ^{69}Ge -SPION@PAA by passing through a PD-10 column, preconditioned with PBS.

For PEG modification, 500 μL of ^{69}Ge -SPION@PAA in PBS was mixed with suitable amount of EDC/S-NHS (62.5 μL , 2 mg/mL) and activated for 15 min at pH 4–5. Two mg of NH_2 -PEG_{5k}-Mal and Na_2CO_3 (2 μL , 0.1 M) were then added and reacted at room temperature for 2 h (pH 7–8). As-synthesized ^{69}Ge -SPION@PEG was subsequently concentrated by using a centrifugal filter (molecular weight cut off: 10 kDa), and re-dispersed in PBS before injection into mice.

In vivo PET imaging and biodistribution studies

For *in vivo* PET imaging and biodistribution studies, 200 μL of ^{69}Ge -SPION@PEG (~ 1.85 MBq) was i.v. injected into normal BALB/c mice ($n = 3$) and PET scans were performed at different time intervals. After the last PET scans at 36 h p.i., biodistribution studies were carried out to confirm that the %ID/g values based on PET imaging truly represented the radioactivity distribution in mice. For *in vivo* lymph node mapping with PET, 40 μL of ^{69}Ge -SPION@PEG (~ 0.74 MBq) was subcutaneously injected into the left footpad of mouse. The time points of 0.5 h, 2 h and 20 h post-injection were chosen for serial PET scans.

In vivo MR imaging

All MRI experiments were performed using a 4.7 T small animal scanner (Agilent Technologies, Santa Clara, CA). *In vivo* imaging was carried out before contrast agent administration and at several time points post-injection of 200 μL (or 40 μL) of Ge-SPION@PEG (7.77 mM Fe) in PBS solution for liver imaging or lymph node mapping. To detect SPION accumulation, *in vivo* T_2^* -weighted images were collected using a multi-slice gradient echo sequence with the following parameters: TR = 500 ms; TE = 12 ms; flip angle = 20° ; FOV = 40×40 mm²; matrix 256×256 ; NEX = 8; slice thickness = 1 mm for axial liver images and 0.5 mm for coronal lymph node mapping.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work is supported, in part, by the University of Wisconsin - Madison, the National Institutes of Health (NIBIB/NCI 1R01CA169365, P30CA014520, T32CA009206, and T32GM08349), the Department of Defense (W81XWH-11-1-0644), the American Cancer Society (125246-RSG-13-099-01-CCE), and the Fulbright Scholar Program (1831/FNPDR/2013).

References

1. Mirzadeh S, Lambrecht RM. J. Radioanal. Nucl. Chem. 1996; 202:7.
2. a) Park H-J, Tavlarides LL. Ind. Eng. Chem. Res. 2009; 48:4014.b) Neirinckx RD, Davis MA. J. Nucl. Med. 1979; 20:1075. [PubMed: 231643]
3. a) Roesch F, Riss PJ. Curr. Top. Med. Chem. 2010; 10:1633. [PubMed: 20583984] b) Chakravarty R, Dash A. J. Nanosci. Nanotechnol. 2013; 13:2431. [PubMed: 23763118] c) Chakravarty R, Shukla R, Ram R, Tyagi AK, Dash A, Venkatesh M. Nucl. Med. Biol. 2011; 38:575. [PubMed: 21531295] d) Chakravarty R, Shukla R, Ram R, Venkatesh M, Dash A, Tyagi AK. ACS Appl. Mater. Interfaces. 2010; 2:2069. [PubMed: 20568699]
4. Chakravarty R, Chakraborty S, Ram R, Dash A, Pillai MR. Cancer Biother. Radiopharm. 2013; 28:631. [PubMed: 23745686]
5. a) Guo J, Yang W, Wang C. Adv. Mater. 2013; 25:5196. [PubMed: 23996652] b) Hao R, Xing R, Xu Z, Hou Y, Gao S, Sun S. Adv. Mater. 2010; 22:2729. [PubMed: 20473985]
6. Afaq A, Syed R, Bomanji J. Br. Med. Bull. 2013; 108:159. [PubMed: 24227824]
7. a) Chen F, Ellison PA, Lewis CM, Hong H, Zhang Y, Shi S, Hernandez R, Meyerand ME, Barnhart TE, Cai W. Angew. Chem. Int. Ed. Engl. 2013; 52:13319. [PubMed: 24166933] b) Zhang T, Ge J, Hu Y, Yin Y. Nano Lett. 2007; 7:3203. [PubMed: 17854231]
8. a) Kopecký P, Mudrová B. Int. J. Appl. Radiat. Isot. 1974; 25:263.b) Benhong C, Zongquan L, Yongxian W. J. Radioanal. Nucl. Chem. 1998; 238:175.
9. Proulx ST, Detmar M. Exp. Cell Res. 2013; 11:1611. [PubMed: 23499738]
10. a) Thorek DL, Ulmert D, Diop NF, Lupu ME, Doran MG, Huang R, Abou DS, Larson SM, Grimm J. Nat. Commun. 2014; 5:3097. [PubMed: 24445347] b) Moghimi SM, Hunter AC, Murray JC. FASEB J. 2005; 19:311. [PubMed: 15746175] c) Ravizzini G, Turkbey B, Barrett T, Kobayashi H, Choyke PL. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 2009; 1:610. [PubMed: 20049820]
11. Herzog H, Pietrzyk U, Shah NJ, Ziemons K. Neuroimage. 2010; 49:2072. [PubMed: 19853045]
12. Yen SK, Padmanabhan P, Selvan ST. Theranostics. 2013; 3:986. [PubMed: 24396508]

Figure 1.

(a) A schematic illustration of chelator-free synthesis of ^{69}Ge -metal oxides. (b) TEM image of SPION. Inset shows a digital photo of SPION in cyclohexane solution. (c) TEM image of SPION@PAA. Inset shows a digital photo of SPION@PAA in aqueous solution. (d) Time-dependent ^{69}Ge labeling yield of SPION and other nanoparticles. (e) Autoradiograph of TLC plates of ^{69}Ge -SPION (top) and free ^{69}Ge (bottom). (f) Serum stability study of PEGylated (black line) and non-PEGylated ^{69}Ge -SPION (red line) in whole mouse serum at 37 °C.

Figure 2.

(a) Serial *in vivo* PET images of ^{69}Ge -SPION@PEG (top) and free ^{69}Ge (bottom) after i.v. injection into mice. (b) *In vivo* T_2^* -weighted MR images of mice before and after i.v. of Ge-SPION@PEG (in PBS). Transaxial images are presented to show the liver uptake of Ge-SPION@PEG, as well as lack of accumulation or contrast enhancement in the kidneys.

Figure 3.

(a) *In vivo* lymph node imaging with PET after subcutaneous injection of ^{69}Ge -SPION@PEG into the left footpad of the mouse. Lymph nodes and paws were indicated by green and red arrows, respectively. (b) Quantification of the ^{69}Ge -SPION@PEG uptake by the lymph node and the mouse paw (n = 3). (c) *In vivo* lymph node mapping with MRI before and after injection of Ge-SPION@PEG into the left footpad of the mouse. Obvious darkening of the lymph node could be seen (dashed green circle), whereas no contrast enhancement was observed for the contralateral lymph node (dashed red circle).