Published in final edited form as:

Nanoscale. 2017 January 19; 9(3): 1299–1306. doi:10.1039/c6nr08468k.

Evaluation of PEG-coated iron oxide nanoparticles as blood pool tracers for preclinical Magnetic Particle Imaging

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Abstract

Superparamagnetic iron oxide (SPIO) nanoparticles with optimized and well-characterized properties are critical for Magnetic Particle Imaging (MPI). MPI is a novel *in vivo* imaging modality that promises to integrate the speed of CT, safety of MRI and sensitivity of PET. Since SPIOs are the source of MPI signal, both the core and surface properties must be optimized to enable efficient *in vivo* imaging with pharmacokinetics tailored for specific imaging applications. Existing SPIOs like Resovist (ferucarbotran) provide suboptimal MPI signal, and further limit MPI's *in vivo* utility due to rapid systemic clearance. An SPIO agent with a long blood half-life ($t_{1/2}$) would be a versatile MPI tracer with widespread applications. Here we show a long circulating polyethylene glycol (PEG)-coated SPIO tracer, *LS-008*, provides excellent colloidal stability and persistent intravascular MPI signal, showing potential as the first blood pool tracer optimized for MPI. We evaluated variations of the PEG coating and found that colloidal stability of tracers improved with increasing PEG molecular weight (keeping PEG loading constant). Blood circulation in mice, evaluated using Magnetic Particle Spectrometry (MPS), showed that $t_{1/2}$ of SPIO tracers varied with both PEG molecular weight and loading. *LS-008*, coated with 20 kDa PEG at 18.8% loading capacity, provided the most promising long-term colloidal stability with a $t_{1/2}$ of about 105 minutes in mice. *In vivo* MPI imaging with *LS-008* in a 7 T/m/ μ_0 3D x-space MPI mouse scanner revealed a prolonged intravascular signal (3-5 hours) post-injection. Our results show the optimized magnetic properties and long systemic retention of *LS-008* make it a promising blood pool MPI tracer, with potential to enable MPI imaging in cardio - and cerebrovascular disease models, and solid tumor quantification and imaging via enhanced permeation and retention.

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† Electronic Supplementary Information (ESI) available. Following movies in AVI format are included as supplementary material: time-lapse movie showing 2-D maximum intensity projection (MIP) MPI images of LS-008 circulating in mouse #3; Rotating 3-D MIP and volume rendered MPI images of mouse #3 at 15 minutes and 360 minutes after injection. See DOI: 10.1039/x0xx00000x

The authors APK, SJK, RMF and KMK have a financial interest in LodeSpin Labs, LLC. Authors PWG and SMC have a financial interest in Magnetic Insight, Inc.

Introduction

Magnetic Particle Imaging (MPI) is a promising *in vivo* imaging technology which exploits the nonlinear magnetic response of superparamagnetic iron oxide (SPIO) nanoparticles¹⁻³. It uses the combination of an alternating excitation magnetic field and a static magnetic field gradient, called the selection field, for real-time, quantitative and tomographic imaging of SPIOs distributed within an imaging volume. MPI provides exceptional contrast and signal-to-noise ratio because the signal is exclusively detected from SPIOs with no background signal from the subject; consequently, MPI by definition is a tracer imaging modality. The magnetic signal is also depth-independent, providing a quantifiable signal from the entire imaging volume. Moreover, the selection field can be rapidly and continuously scanned over the region of interest (ROI) for real-time imaging of SPIO distribution⁴. In short, MPI promises a biomedical imaging modality with the speed of x-ray computed tomography (CT), the safety of Magnetic Resonance Imaging (MRI)^{5,6} and the sensitivity of Positron-Emission Tomography (PET).

Since the emergence of MPI, optimized SPIO tracer development has been recognized as critical for its clinical translation. It is known that physicochemical properties of SPIOs and the surrounding solvent environment can influence their magnetic response to external fields^{7,8}. Properties of SPIO cores such as core size and size distribution, iron oxide phase and crystallinity dictate the magnetic response and must be optimized to generate images with both high signal intensity and high resolution. Colloidal properties such as hydrodynamic size, zeta potential and surface hydrophobicity dictate stability, pharmacokinetics and MPI response of SPIOs in the dynamic *in vivo* environment. In order to clinically realize the wide range of potential applications of MPI, SPIO tracers with both superior signal and pharmacokinetics are needed. Unfortunately, traditional SPIOs used for MRI contrast consist of polydisperse magnetic cores and are suboptimal for MPI. Eberbeck *et al* studied the effect of size distribution on MPI performance and discovered that only 30% of the bimodal T2-MRI contrast agent Resovist[®], consisting of ~24 nm agglomerates formed due to clustering of smaller SPIO cores, contributed to the MPI signal⁹. The remaining 70% was practically unresponsive to MPI. This limitation was in fact noted in the first MPI paper, where Gleich *et al* remarked that the iron mass of the signal-generating particles in Resovist represented only 3% of the total iron mass¹. In our own work, we showed that single core SPIOs with a narrow size distribution around 25 nm provided three times greater signal intensity and two times better spatial resolution (1.6 mm at 7 T/m/ μ_0 field gradient), compared with Resovist, in MPI images of phantoms¹⁰. Additionally, Resovist has a blood half-life ($t_{1/2}$) of ~5 minutes, as it is designed to rapidly accumulate in the liver¹¹. This poor half-life limits its use for MPI and demonstrating applications in cardiovascular, stroke and tumor imaging.

It is widely known that poly(ethylene glycol) (PEG) coatings can prolong the blood half-life of various intravenously administered agents, including SPIOs¹²⁻¹⁴. In this work, we focus on the role of PEG surface coatings in affecting the colloidal stability, pharmacokinetic behavior and *in vivo* imaging of MPI-optimized SPIO tracers. We studied the pharmacokinetics of PEG-coated SPIOs in mice using *ex vivo* Magnetic Particle Spectroscopy (MPS) measurements and *in vivo* MPI imaging. MPS measures the

magnetization response of SPIO cores in an AC field, which is quantifiable and provides critical information about SPIO concentration in collected blood volumes¹⁵. SPIO tracers were further imaged *in vivo*, in healthy mice, using a MPI scanner to compare with the MPS-measured $t_{1/2}$, and evaluate their imaging capability.

Experimental

Materials

1-Octadecene (tech. 90%), oleic acid (tech. 90%), and iron trichloride hexahydrate (ACS, 97.0-102.0%) were obtained from Alfa Aesar. Sodium oleate (>97%) was obtained from Tokyo Chemical Industry CO, LTD. Sodium hydroxide, sodium sulfate (anhydrous), potassium permanganate (99.2%), *n*-heptane, ethyl acetate and methylene chloride (HPLC grade) were obtained from Fisher Scientific. Hexane (mixture of isomers), chloroform, and acetone were HPLC grade and obtained from Sigma-Aldrich. Ethanol (200 proof) was obtained from Decon Labs. Phosphoric acid (85%), sulfuric acid and concentrated hydrochloric acid were obtained from Macron. 1% oxygen in argon was obtained from Praxair. Water used in any experiment was purified at 18.2 MOhm-cm. The DigiTrol II was obtained from Sigma-Aldrich. SUBA-SEAL[®] septum were obtained from Chemglass. Poly(maleic anhydride-alt-1-octadecene) (average Mn 30,000-50,000 Da) was obtained from Sigma-Aldrich. Methoxy-poly(ethylene glycol)-amine, HCl salt was purchased from JenKem Technology USA.

Synthesis of SPIO cores

SPIO nanoparticles were synthesized using the thermolysis reaction of iron(III)-oleate¹⁶⁻¹⁸. A comprehensive report of our modified synthesis method, which includes an in situ oxidation step and additional reaction controls, is published elsewhere¹⁹. Here we describe the method pertaining to particles used in this study. Iron(III)-oleate (40 mmol of Fe) was mixed with 6.7-fold molar equivalent of oleic acid (75.7 g, 268 mmol) and 1-octadecene (200 g) which was evacuated for 25 minutes at 40 °C. The reaction mixture was heated (5 °C/min) to 318 °C under constant argon purging (40 mL/min). After reaching 318 °C, argon purging was stopped, and approximately 130 minutes after that, 1% oxygen in argon was flowed into the reaction mixture at a rate of 140 mL/min for 2 hours and then above the reaction mixture at 25 mL/min for 21 hours. The reaction mixture was allowed to cool to approximately 50 °C and transferred to a 500 mL bottle for storage. Washing of nanoparticles for analysis or phase transfer was performed as follows: the crude nanoparticle solution (2 mL) was diluted with ethyl acetate (8 mL) and the particles separated magnetically. The washing was repeated four times with a mixture of hexane and ethyl acetate (1:4). The washed SPIOs were dried for 1 hour under high vacuum to provide about 10 mg of nanoparticles. Core size was determined from images acquired at 19,500 magnification in a 200 kV Transmission Electron Microscope (FEI Tecnai G2 F20). The ImageJ open source image analysis software was used to determine core diameter from a minimum of 2000 particles imaged from four different regions on the TEM grid. The spinel phase of the SPIO cores was confirmed using Selected Area Electron Diffraction (SAED) in TEM.

Synthesis of poly(maleic anhydride-alt-1-octadecene) (PMAO) with polyethylene glycol (PEG)

In a round bottom flask equipped with a magnetic stir bar, PMAO (average $M_n \sim 30$ -50 kDa) and mPEG-NH₂ ($M_n = 5$ kDa, 10 kDa or 20 kDa) were dissolved in methylene chloride at 100 mg PEG/ml, with either a 57, 43 or 29-fold molar excess of PEG per PMAO. The amount of mPEG-NH₂ used was based on the loading desired, which was calculated as a percentage of the 228 carboxylic acids available per PMAO molecule. For example, for 25% loading the molar excess mPEG-NH₂ per PMAO was 57-fold, 43-fold for 18.8%, and 29-fold for 12.5%. In order to test the effect of PEG M_n and loading, we prepared five variants of PMAO-PEG: PMAO loaded at 25% capacity with either 5 kDa, 10 kDa or 20 kDa mPEG-NH₂, PMAO loaded with 18.8% 20 kDa mPEG-NH₂, and PMAO loaded with 12.5% 20 kDa mPEG-NH₂. Triethylamine (6 mmol per PEG amine mmol) was added to the polymer mixture, which was stirred for 72 hours at room temperature. The formation of the PMAO-PEG product was monitored using gel permeation chromatography (GPC) using CHCl₃ as eluent and was considered complete when the peak corresponding to mPEG-NH₂ disappeared, while a new peak corresponding to PMAO-PEG appeared at a retention time substantially shorter than both mPEG-NH₂ and PMAO peaks. The mixture was concentrated using a rotary evaporator, dried using high vacuum for 2 hours and dissolved in a 1% sodium bicarbonate solution. The mixture was stirred overnight to ensure complete hydrolysis of unreacted anhydrides and then dialyzed against DI water in a 50,000 MWCO cellulose ester membrane. The dialyzed product was lyophilized and stored at room temperature.

Phase transfer of SPIO nanoparticles to water

Each of the five PMAO-PEG variants were used to coat SPIO cores, generating a total of five phase transferred formulations. In a typical phase transfer using PMAO loaded with 25% 20 kDa mPEG-NH₂, 10 mg of purified and dried 25 nm SPIOs were dispersed in 1 ml of chloroform by sonication. In a separate vial, 100 mg of PMAO-PEG was dissolved in 9 ml of chloroform. Note that the amount of PMAO-PEG used was based on a ratio of 200 polymer units per SPIO core, and thus varied with PEG molecular weight and loading. The PMAO-PEG solution was added to the SPIO dispersion and sonicated for 1 hour. The mixture was then stirred for 48 hours to allow sufficient time for the amphiphilic PMAO-PEG polymer to coordinate with the hydrophobic oleic acid surface of SPIOs. Chloroform was evaporated using a rotary evaporator and the concentrate was dried for 1 hour under high vacuum. The dried concentrate, consisting a uniform dispersion of SPIOs in a polymer matrix, was sonicated for 90 minutes in 10 ml of deionized (DI) water. The PMAO-PEG coated SPIOs were completely dispersed in DI water and passed through a 200 nm nylon syringe filter to remove any large agglomerates. Excess polymer was removed by running the SPIO dispersion through a S-200 Sephacryl gel column (GE Healthcare) equilibrated with 1x phosphate buffered saline (PBS, pH = 7.4). Hydrodynamic diameter and zeta potential were measured in a Dynamic Light Scattering (DLS) instrument (Malvern Zetasizer Nano ZS). Any small agglomerates detected in DLS were removed via centrifugation for 10-15 minutes at 4,000 rcf. The final solution in PBS was concentrated using a 100,000 MWCO centrifugal filter and stored at 4°C. Iron concentration was determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, Perkin Elmer). Saturation magnetization was measured at room temperature in a Vibrating

Sample Magnetometer (VSM, LakeShore). The MPS signal is proportional to the time-varying magnetization response of SPIOs to an AC field, or differential susceptibility, $\chi_{diff} = dm/dH$. χ_{diff} was measured in a home-built MPS operating at $f = 25$ kHz and $H_{max} = 20$ mT/ μ_0 sinusoidal excitation field.

Blood half-life determination using MPS

Female CD-1 mice (Charles River Laboratories) from 7-9 weeks old were used for all animal experiments. SPIO samples were dispersed in sterile 1x PBS and filtered with 0.2 μ m nylon filters prior to intravenous administration. Mice were injected with 5 mgFe/kg SPIO as a bolus in the lateral tail vein using a MonoJect™ insulin syringe equipped with a 28 gauge 5/8" needle. Blood was collected from the retro-orbital sinus at predetermined time points; each mouse was used to obtain two time points with $n = 3$ for each time point. Prior to each blood collection, mice were anesthetized with 4-5% isoflurane in an induction chamber. Approximately 150 μ l of blood per time point was collected in EDTA-coated tubes and stored at 4 °C. Immediately after the second bleed, mice were euthanized with CO₂ exposure and cervical dislocation as secondary confirmation. For an MPS measurement, 100 μ l of whole blood was transferred to a 0.6 ml microcentrifuge tube and inserted in the coaxial transmit and receive coil assembly of the MPS. The χ_{diff}^{max} signal from SPIOs present in 100 μ l of blood was measured and quantified based on a linear calibration curve. All animal procedures were performed according to the protocol approved by IACUC at the University of Washington.

In vivo image acquisition in a 7 T/m/ μ_0 3D X-space MPI scanner

SPIOs were injected as a bolus at 5 mgFe/kg in the lateral tail-vein of mice ($n = 3$). Mice were initially anesthetized with 4-5% isoflurane and then maintained at 1-2% for the duration of the imaging period. Imaging was performed with respiratory gating in a $3.5 \times 3.5 \times 7$ T/m/ μ_0 imager, with $f = 20.05$ kHz and $H_{max} = 20$ mT/ μ_0 drive field in the z -direction, and a $3.5 \text{ cm} \times 3.5 \text{ cm} \times 8.5 \text{ cm}$ FOV²⁰. Scans (~7 min. duration) were done at 30-minute intervals with the longest time point at 6 hours after injection. In order to monitor the clearance of SPIOs from the blood vessels, we selected the neck region for signal analysis due to the presence of major blood vessels and its relative isolation from perfused tissues in the abdomen and head regions. For each MPI mouse image, at each time point an ROI was drawn over the jugular vein using a region-growing algorithm seeded with a single maximum pixel and a cut-off threshold of 5% of maximum. By using the maximum intensity pixel to seed the center of the ROI, this method becomes insensitive to small shifts in animal position between scans. The quoted MPI signal was an average signal in an ROI over the imaged vessels.

Results and Discussion

Nanoparticle characterization and colloidal stability

Figure 1a shows a representative TEM image of SPIO nanoparticles synthesized using the iron(III)-oleate thermolysis reaction. Using the PSA r12 size analysis plugin in ImageJ, it was determined that SPIOs had a median core diameter (d_c) of 25.1 nm with $\sigma = 0.06$, where σ is standard deviation of the number-weighted lognormal distribution. SAED confirmed

nanoparticles were primarily composed of the spinel phase of iron oxide (*Figure 1b*). The oleic acid capped SPIOs were subsequently transferred to water using PMAO-PEG (*Figure 1c*)^{21,22}, which was loaded with 5 kDa, 10 kDa or 20 kDa mPEG-NH₂. *Table 1* summarizes the surface properties of phase transferred SPIOs used in this study. PEG loading was 25% of the total grafting capacity, except for samples LS-008 and NP-4, which were coated with 18.8% and 12.5% mPEG-NH₂ loading, respectively.

Magnetic properties were measured in water and averaged from five separate phase transfers of the same nanoparticle cores. Saturation magnetization (M_s) was ~ 370 kA/m, or 80% the bulk saturation value of magnetite (450 kA/m)²³. The differential susceptibility of SPIOs, χ_{diff} , which is a time-varying magnetization response to an ac field, was measured in our home-built MPS. The critical features of a χ_{diff} plot that directly influence MPI imaging quality are the peak intensity per unit mass of iron, χ_{diff}^{max} (m³/gFe), and the Full Width at Half Maximum, χ_{diff}^{fwhm} (mT/ μ_0). The former corresponds to the signal or brightness in MPI, and the latter corresponds to the spatial resolution. Additional details about MPS signal and the physics of image reconstruction can be found in several publications focusing on MPI imaging physics^{24,25}.

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(−5.5 mV), also potentially due to insufficient carboxylate shielding at the 12.5% loading. In addition to surface characteristics, there were differences in phase transfer yields. Hydrodynamic size distribution of unfiltered SPIOs coated with 5 kDa PEG showed signs of aggregation, indicated by a shoulder in the volume-weighted distribution. The presence of agglomerates can adversely affect both MPI performance and nanoparticle pharmacokinetics and were removed by filtration and centrifugation steps, which inevitably affects yield. Typical yields with 5 kDa PEG were in the 10-20% range, compared to the high 90% range with 10 kDa and 20 kDa PEG.

We further evaluated the colloidal stability of PMAO-PEG coated SPIOs during long-term storage. Often during prolonged storage at 4°C in 1x PBS solution, we observed certain formulations underwent visible sedimentation. In order to accelerate testing and quantify stability issues, we dispersed SPIOs in high salt concentration and acidic solutions – both conditions promoted aggregation (PMAO-PEG coated SPIOs showed good stability in alkaline solutions). SPIOs were dispersed in 5% KH_2PO_4 (pH $\approx$ 4.3) or 0.26% (w/v) hydrochloric acid with 0.2% (w/v) sodium chloride solution (pH $\approx$ 1.2), and the change in both d_H and saturation magnetic moment (m ; Am^2) was monitored for 7 days at room temperature (Figure 2). Ranking samples in order of decreasing colloidal stability, LS-008 showed the smallest change in d_H , demonstrating the best colloidal stability, followed by NP-3, then NP-2, and finally NP-1 (NP-4 not tested). Samples also exhibited relatively poor colloidal stability in 5% KH_2PO_4 (Figure 2a) compared to the lower pH solution (Figure 2c), possibly due to the PEG coating undergoing a salting out effect in the 5% KH_2PO_4 solution. Compared to d_H , the change in m was significantly small (Figure 2b and Figure 2d). The magnetic moment, m , generally trended lower relative to the initial value for all samples likely due to gradual acidic digestion of the iron oxide core. The average decrease in m as a percentage of the initial value was 2.5% ($\pm$ 2.1%) at pH $\approx$ 4.3 and 12.1% ($\pm$ 4.2%) at pH $\approx$ 1.2, which were considered significantly different (p-value = 0.006; unpaired t-test). As expected, the rate of SPIO core digestion was slightly higher in the more acidic environment.

SPIO pharmacokinetics from ex vivo MPS measurements

Circulation data of each PMAO-PEG coated SPIO sample (Figure 3) injected intravenously in female CD-1 mice was obtained from *ex vivo* MPS measurement of whole blood volumes collected at various time points (n = 3 mice/time point). Pharmacokinetic parameters, summarized in Table 2, were obtained after fitting clearance data to the standard first-order elimination model. R^2 -values (between 0.94 and 0.99) indicate good fitting to the first-order model. Sample NP-1, coated with the 5 kDa PEG, had the smallest d_H and showed the longest blood half-life ($t_{1/2}$) of about 155 minutes. For the same PEG loading density (25%), $t_{1/2}$ trended lower with increasing PEG molecular weight – from 100 minutes for NP-2 (10 kDa PEG) to 58 minutes for NP-3 (20 kDa PEG). LS-008 (20 kDa PEG at 18.8% loading) had the longest $t_{1/2}$ of 105 minutes among the set of samples coated with 20 kDa PEG at different loading densities; NP-4 (12.5% loading) had the shortest at 26 minutes. In order to explain observed differences in circulation time it is important to consider the major SPIO clearance mechanisms. Organs of the mononuclear phagocytic system are primarily responsible for clearing SPIOs out from systemic circulation. The spleen has an open

circulation system that plays a critical role in filtering senescent red blood cells and large nanoparticles^{27,28}. Blood entering the spleen is filtered by interendothelial slits (IES) present in the marginal zone, and must percolate its way through phagocytic cells before reentering circulation. In principle, the spacing between IES, which can range from 200-500 nm²⁹, puts a physiological upper limit to hydrodynamic diameter for nanoparticle circulation³⁰. Large nanoparticle agglomerates may not be present initially, but can form after *in vivo* administration due to non-specific binding with plasma proteins. Agglomerates are often quickly removed from circulation as they get trapped in the marginal zone. Thus, non-aggregating SPIOs with the smallest average d_H should circulate longest as they are more readily able to pass through the IES. On the other hand, liver vasculature is highly fenestrated and consists of sinusoidal capillaries lined with resident macrophages called Kupffer cells³¹. Resovist, which has a carboxydextran coating that promotes uptake in phagocytic cells³², has a short circulation time and is rapidly taken up in the liver. PEG coatings prolong circulation time by evading Kupffer cell recognition and phagocytic uptake¹⁴. But insufficient coverage due to low PEG loading, as might be the case in sample NP-4, can lead to non-specific binding with plasma proteins and recognition by Kupffer cells. This does not however explain why sample NP-3 (20 kDa PEG at 25% loading) had a shorter $t_{1/2}$ than LS-008 (20 kDa at 18.8% PEG loading). The mechanistic framework outlined here, combined with past studies of PEG-coated nanoparticles, provides a general explanation for the observed differences in circulation times and quantitative analysis of each sample's distribution in MPS organs will be needed to confirm the relative roles of the liver and spleen in clearance. Another pharmacokinetic parameter of particular significance is the distribution volume, V_d —it is the apparent space or compartment within which SPIOs distribute during circulation. A relatively low V_d (~0.06-0.07 L/kg) across all samples suggests SPIOs were confined primarily to the intravascular space in the early stages of circulation³³. Based on colloidal stability, phase transfer yield and pharmacokinetic data of samples tested in this work, we determined that LS-008 was the most suitable blood pool MPI tracer.

In order to evaluate blood pool imaging capability, we imaged a total of three mice with LS-008 in a custom-built 7 T/m/μ₀ 3D x-space MPI mouse scanner (Figure 4a). Figure 4b shows a series of 2D maximum intensity projection (MIP) images acquired from mouse 3. About 15 minutes after the bolus injection, LS-008 had distributed uniformly in the intravascular system. At this point the heart and lungs are distinguishable due to differences in their blood volume. The intravascular signal gradually decreases and the liver and spleen signal increase over time, confirming their role in clearance of LS-008. A time-lapse movie showing the clearance of LS-008 is provided in supplementary material. Movies demonstrating MPI's tomographic imaging capability (3-D volume rendering and rotating maximum intensity projection) are also included in supplementary material. Since SPIOs are the only source of signal in MPI, intensity from each voxel, which is linear with SPIO concentration, represents the amount of SPIO-associated iron (μg Fe)^{1,2,4,34}. By adding all the voxels in the ROI from each temporally discrete image, we were able to quantify SPIOs circulating through the neck region (Figure 5).

The average $t_{1/2}$ from MPI image signal quantification, obtained from fitting data to a first-order elimination model, was 103 ± 45 minutes. While the primary purpose of the imaging

study was to obtain a preliminary evaluation of blood pool imaging capability with *LS-008*, larger animal numbers are needed to improve statistics.

Nevertheless, SPIO quantification from MPI image signal highlights subject-to-subject variability because a single mouse provides the entire clearance profile without the need for blood collection and *ex vivo* evaluation. The imaging results demonstrated excellent blood pool imaging capability with *LS-008*, confirmed by the intravascular signal persisting for over 3.5 hours.

Conclusions

MPI is a revolutionary imaging modality that enables tomographic imaging and quantification of SPIO tracers distributed in a 3D space. In this study we evaluated the colloidal stability and pharmacokinetic properties of PEG-coated SPIOs to lay the foundation for developing blood pool tracers for MPI. Monodisperse SPIO cores optimized for high MPI signal were coated with PMAO – an amphiphilic polymer – that was pre-PEGylated with mPEG-NH₂ of varying molecular weight and loading capacity. The MPI tracer *LS-008*, coated with 20 kDa mPEG-NH₂ at 18.8% of loading capacity, was the most stable in high salt concentration and acidic solutions compared to other samples tested in this study. In mouse studies, *LS-008* had a blood half-life of 105 (±10) minutes and a low distribution volume characteristic of an intravascular agent (0.07 ml/kg). *In vivo* MPI imaging of mice showed an intravascular signal persisting for up to 3.5 hours after injection and systemic clearance mediated primarily by the liver and spleen. These results suggest *LS-008* is a versatile tracer for blood pool MPI, with potential applications in cardio- and cerebrovascular imaging, and imaging and volume quantification of solid tumors via enhanced permeation and retention (EPR). In future work, surface modification to alter the pharmacokinetics of MPI-optimized SPIO cores contained in *LS-008* is expected to enable new MPI applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The work at LSL and UW was supported by NIH 2R42-EB013520-02A1 and NIH 1R24-MH109085 grants. The work at UCB and MI was supported by NIH 1R01-EB013689, NIH 1R24-MH106053, NIH 1R01-EB019458, CIRM RT2-01893, Keck Foundation 009323, and ACTG 037829 grants.

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Figure 1. (a) Bright field TEM image of 25 nm SPIO cores and (b) SAED confirming the spinel structure. (c) Schematic (not to scale) depicting SPIOs coated with PMAO-PEG polymer. Hydrophobic interactions between the alkyl chains in the PMAO backbone and the oleic acid layer adheres the polymer near the SPIO surface. The hydrophilic PEG layer extends outward, imparting solubility in aqueous solvents.

Figure 2.

Stability of PEG-coated SPIO nanoparticles dispersed in acidic solutions. d_H and saturation magnetic moment (m) were monitored over time in 5% KH₂PO₄ solution (pH ≈ 4.3), (a) and (b), and 0.26% (w/v) HCl with 0.2% (w/v) NaCl (pH ≈ 1.2), (c) and (d), respectively.

Figure 3.

Clearance of SPIO samples from *ex vivo* MPS measurements; $n = 3$ per time point.

Pharmacokinetic parameters, summarized in *Table 2*, were obtained after fitting data to a first-order elimination model.

Figure 4.

(a) Custom-built 7 T/m/μ₀ 3D x-space MPI mouse scanner at UC-Berkeley with animal stage. (b) MPI images of mouse 3 intravenously injected with *LS-008* at 5 mgFe/kg. Images are maximum intensity projections showing the dorsal view at several time intervals after injection. The neck region (dashed box at 15 min.) was selected to monitor the intravascular MPI signal over time. The vascular network in the neck and chest regions was visible over 3.5 hours after injection, demonstrating blood pool imaging capability with *LS-008*.

Figure 5.

Clearance of *LS-008* from MPI imaging. Signal from blood vessels in the neck ROI was measured to quantify intravascular iron [μgFe]. The $t_{1/2}$, averaged from three mice, was 103 ± 46 minutes.

Table 1

Surface characteristics of SPIOs coated with PMAO grafted with varying PEG molecular weight and loading.

Sample	PEG- M_n ; loading % ^a	d_H [nm]; PDI ^b	ζ [mV]
NP-1	5 kDa; 25%	60.7; 0.12	-14.2
NP-2	10 kDa; 25%	64.5; 0.09	-9.7
NP-3	20 kDa; 25%	81.0; 0.10	-5.5
NP-4	20 kDa; 12.5%	67.6; 0.09	-19.3
LS-008	20 kDa; 18.8%	78.3; 0.08	-4.5

^aloading % approximated based on the molar ratio of PEG to carboxylic acid groups in PMAO

^b d_H is the z-average diameter from DLS measurement in a Malvern ZetaSizer Nano ZS and PDI is the polydispersity index

Table 2

Pharmacokinetic parameters (n = 3 mice/time point) obtained from *ex vivo* MPS measurements of blood specimens collected at various time intervals.

Sample	λ [hr ⁻¹] ^a	R ² value of fit	C_0 [μ gFe/ml]	V_d [L/kg] ^b	$t_{1/2}$ [min]
NP-1	0.27	0.94	84 $\pm$ 17	0.060	155 $\pm$ 2
NP-2	0.42	0.96	86 $\pm$ 4	0.058	100 $\pm$ 12
NP-3	0.71	0.98	71 $\pm$ 2	0.070	58 $\pm$ 6
NP-4 ^c	1.62	0.97	90 $\pm$ 3	0.067	26 $\pm$ 3
LS-008	0.39	0.99	73 $\pm$ 8	0.068	105 $\pm$ 10

^a λ is first-order elimination constant in $C(t) = C_0 \exp(-\lambda t)$, where $C(t)$ is SPIO concentration in blood as a function of time t and C_0 is initial concentration.

^b The distribution volume, V_d [L/kg], is equal to $\frac{\text{total injected iron [kgFe]}}{C_0 \left[\frac{\text{kgFe}}{\text{L}} \right] * \text{Body weight [kg]}}$

^c injected dose was 6 mgFe/kg