Supporting Information for:

Immobilization of Iron Oxide Magnetic Nanoparticles for Enhancement of Vessel Wall Magnetic Resonance Imaging – An *Ex Vivo* Feasibility Study

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METHODS

Acquisition of vein specimens and vein conduit labeling: Fresh discarded human vein segments (n=3) from bypass surgeries and major amputations were collected by following approved institutional protocols and used for labeling experiments. The sample size used in this proof of principle experiment (n=3 total) is small, and has largely been limited by the scarcity of adequately sized fresh discarded human vein specimens.

Fe-NPs that have carboxylic groups on the surface (~ 30 nm) were purchased from Ocean Nanotech LLC USA. The surface carboxylic groups have been activated through *N*-hydroxysuccinamide ester reaction as follows: 50 µL of Fe-NPs were (5 mg/mL water) diluted with 150 µL of double distilled water (ddH_2O). The particle solution was centrifuged at 13,000 rpm for 6 min. The supernatant was removed and 200 µL of dimethyl formamide (DMF) was added, then the solution was vortexed and centrifuged for 6 min. The supernatant was again removed, and the pellet re-dispersed in 200 µL of DMF containing *N, N'*-diisopropylcarbodiimide (DIC, 1 mmol/L) and *N*- hydroxysuccinamide (1 mmol/L). The reaction continued at room temperature overnight. The activated particles were then spun down and washed with DMF (200 µL). The particles were finally re-dispersed in phosphate buffer saline (PBS) and used immediately for coating the vein graft adventitia. With the luminal side protected via ligatures on either end, the test human vein sample (n=1) was placed in a PBS filled Petri-dish. The activated Fe-NPs (200 µL in PBS) were added and incubated for 30 minutes at room temperature. The procedure was repeated for the unlabeled control vein segment (n=1), but incubated with plain PBS without the addition of Fe-NPs. Finally, all vein segments were thoroughly rinsed thrice with PBS to remove nonspecifically bound nanoparticles. Different sets of instruments were employed for each study group to avoid cross contamination.

Dose-responsive samples: To non-covalently fixate different concentrations of Fe-NPs on the vein, four concentrations of Fe-NPs (50, 25, 12.5 and 6 μg) were each mixed with a drop of Cyanoacrylate adhesive and placed on the adventitia of a segment of unlabeled vein (n=1) prior to imaging. This method resulted in non-covalent localization of Fe-NPs on the vein.

Evaluation of contrast localization—SEM and EDAX: Scanning Electron Microscopy (SEM) with energy dispersive spectroscopy (EDAX) was used to map the presence of Fe-NPs on the surface of the vein following MR imaging. The Fe-NP-coated vein segments were mounted on an aluminum stub with carbon tape, and directly examined using Environmental SEM (FEI/Phillips XL30 FEG-ESEM) operated at 10 kV. EDAX and elemental mapping analysis data was also collected from the same samples at 10 kV using X-ray detector (Torr Scientific Ltd., UK) coupled with FEI/Phillips XL30 FEG-ESEM.

MR Imaging: T1-weighted (T1W), T2*-weighted (T2*W), and proton density-weighted (PDW) high resolution MR Imaging of labeled, control, and dose-response vein specimens was performed on a clinical 3 T MRI scanner (HDx, General Electric, Milwaukee, WI) equipped with 40 mT/m gradients (150 T/m/s slew rate). T1-weighted contrast images were obtained using a standard 3D spoiled gradient recalled echo sequence using the following parameters: 30° flip angle, 12.8 ms TR, 3.5 ms TE, ±32 kHz receiver bandwidth, 224×224×84 image matrix, 0.5 mm slice thickness and 6.8 cm field-of-view. T2*-weighted contrast images were obtained using the same sequence except with 5 ms TE, 100 ms TR, 160×160×68 image matrix and 4.8 cm field-of-view. Proton density-weighted images were obtained using a high sampling efficiency 3D fast

spin echo (FSE) sequence with 1800 ms TR, 9 ms TE, ±16 kHz receiver bandwidth, 150×150×92 image matrix, 0.5 mm slice thickness and 4.5 cm field-of-view. Spatial resolution was 0.3×0.3×0.5 mm^3 for all imaging experiments.

Quantitative MR Image Analysis: Three regions-of-interest (ROI) were drawn using direct planimetry for each of the control and labeled samples in T1W images for 17 consecutive image sections (8.5 mm longitudinal extent) sufficiently away from the Petri dish mounting setup. First, a central ROI was used to delineate the lumen. A second ROI was used to delineate the outer extent of the vessel wall tissue. Finally, the third ROI was used to delineate the region surrounding the vein. For the labeled vein, this ROI was used to delineate the outer extent of the Fe-NP susceptibility-induced signal void. For the control vein segment, this ROI was used to delineate a similar sized region containing the saline surrounding the vein (Figure 6). These ROIs were used to perform two analyses; the first analysis was aimed at testing the hypothesis that the vessel wall thickness could be accurately measured despite the presence of the Fe-NPs. For this analysis, the two inner-most ROIs (lumen and vessel wall ROIs) were assumed to represent ideal disks in order to obtain average radii as $r = \sqrt{\text{ROI area}/\pi}$. The average vessel wall thickness was then calculated by subtracting the radius corresponding to the lumen ROI from the radius corresponding to the vessel wall ROI. The unpaired t-test was used to compare the average vessel wall thickness of the labeled versus the unlabeled vein segments. This comparison was based on the assumption that the wall thickness of the two short (appx. 1.5 cm extent) vein segments did not vary significantly in thickness given that they were harvested from consecutive portions of the same donor vein.

The second analysis was aimed at assessing the ability of the Fe-NP label to effectively enhance the delineation of the vessel wall boundary in comparison to surrounding tissues. First, we measured the average thickness of the iron oxide label, as this places a limit on the image resolution required to detect it. This analysis was similar to that described for the wall thickness, except using the wall and outer region ROIs. Second, we measured the contrast-to-noise-ratio (CNR) achieved using the label. This analysis was performed by comparing the average signal in the vessel wall ROI to the average signal in the ROI surrounding the vessel wall. Average signal in each ROI was calculated by subtracting the total signal in the inner ROI from the total signal in the enclosing ROI, divided by the number of pixels in the enclosing ROI minus that in the enclosed ROI. For example, the average vessel wall signal was obtained as the total signal in the vessel wall ROI minus the total signal in the lumen ROI, divided by the number of pixels in vessel wall ROI minus the number of pixels in the lumen ROI. CNR was then computed as $CNR = \frac{(\text{average vessel wall signal} - \text{ average surrounding signal})}{\sigma_{\text{noise}}}$, where σ_{noise} the noise standard deviation measured in an ROI placed in empty space.

A final quantitative analysis was performed for the dose-response experiment in order to determine the correlation between tissue Fe-NP concentration, and label width. For this analysis we measured the length of the susceptibility-induced signal void orthogonal to the vessel wall for each axial image containing one of the drops of the Fe-NP-loaded adhesive (Figure 6). The lengths of the signal voids were additionally measured on a single sagittal reformat of the 3D volume in order to ensure that axial slice measurements were not significantly biased by the angle of the vein segment relative to the image plane. Linear regression was used to correlate the measured widths of the Fe-NP label to the label concentration.

Evaluation of contrast localization—Iron histochemistry: Perl's Prussian Blue protocol was used to detect the presence of iron bound to the vein adventitia. Sections from the Fe-NPs labeled vein conduit were incubated with 2% aqueous potassium ferrocyanide-hydrochloic acid incubating solution (composed of equal volumes of 4% aqueous potassium ferrocyanide and 4% aqueous hydrochloric acid) for 15 minutes. The sections were washed twice for 2 minutes with ddH_2O, and then counterstained for 2 minutes with 1% Neutral Red. Subsequently, these slides were rinsed with ddH_2O (2 washes for 2 minutes each) and then dehydrated rapidly using increasing concentrations of ethanol (70%, 95%, 100%; 10 seconds each). They were then examined under polarized microscopy.