Imaging the Distribution of Iron Oxide Nanoparticles in Hypothermic Perfused Tissues

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Supporting Information:

Detailed Tissue Perfusion Protocol:

Hindlimb:

Hindlimb tissue preparation and perfusion was performed at the Medical University of South Carolina and Tissue Testing Technologies, LLC (North Charleston, NC, USA). Both left and right back legs were harvested from male Lewis rats, anesthetized with Ketamine/Xylazine IP, shaved and heparinized with 300 µL via tail vein. Anesthesia was maintained with 1.5% isofurane in O_2 by nose cone. Femoral arteries were cannulated using a custom carotid artery catheter and the limbs were flushed with Lactated Ringers, containing 120 µg/ml papavarine, and 40 mg/ml heparin, using a Masterflex peristaltic pump at 0.35 – 45 ml/min rate and 10 – 15 mmHg pressure for a total of 20 mL. The femoral vein was opened on each leg to promote blood evacuation. The cannulas were sewn in place and secured to the skin on the back of each leg. The legs were harvested

with the femurs intact at the hip with preservation of as much muscle tissue as possible. The legs transported into the lab in Lactated Ringers solution on ice. The legs were then loaded, and in some cases unloaded, with ice cold vitrification solutions by perfusion using the same pump, catheter and settings as above in several steps of increasing concentration (30 - 40 mL total) or decreasing concentration during removal (30 - 40 mL total), respectively, with addition of IONPs (10 mg Fe/mL) in the final addition step. The limbs were also immersed in solutions at each step of addition and removal during perfusion, for at least 15 minutes per step, similar to the process previously described (1,2). After loading with the final concentration of vitrification solution the limbs in 50 mL bags filled with the perfusate were rapidly cooled to below -130°C and stored in a mechanical storage freezer as described previously for blood vessels (1,2). These samples were shipped to UMN on dry ice. All animal procedures and care were approved by the Institutional Animal Care and Use Committee of Medical University of South Carolina.

Liver:

Liver isolation and perfusion were performed at UNC Charlotte as previously described (3). Briefly, male Sprague-Dawley rats 200-250 g were placed under isoflurane anesthesia. The isolated perfused liver (IPL) operation was performed with a transverse abdominal incision. The bowel and the duodenum were retracted to the left using moist gauze to expose the portal vein (PV), the common bile duct (CBD) and the inferior vena cava (IVC). One mL of saline containing 200 units of heparin was injected through the penile vein. After ligation of the left diaphragmatic vein, several liver ligaments were dissected. A 16 gauge cannula (Terumo, Somerset, NJ) attached to a syringe was inserted into the lumen of the PV and the liver was flushed with cold William's E media. The IHVC was tied with 3-O silk, the diaphragm was opened, and a 14 gauge cannula was inserted into the Suprahepatic Vena Cava. Additional William's E media was flushed through the liver to remove any residual blood. The liver was removed and placed on the isolated perfusion system. The liver was perfused with the Euro-Collins solution at a flow rate of 0.3 mL/min/g liver. A separate syringe pump containing the IONPs in either Euro-Collins or VS55 was set at the same flow rate as the perfusion system. A 3-way stopcock controlled the introduction of the IONPs. For the VS55 group, the liver is perfused at 15

min step intervals of VS55 concentrations varying between 1-6 M. At the end of the VS55 introduction, the IONPs (642.9 mM_{Fe}; 36 mg_{Fe}/mL) are introduced via the syringe pump. All animal procedures and care were approved by the Institutional Animal Care and Use Committee of University of North Carolina Charlotte.

Kidney:

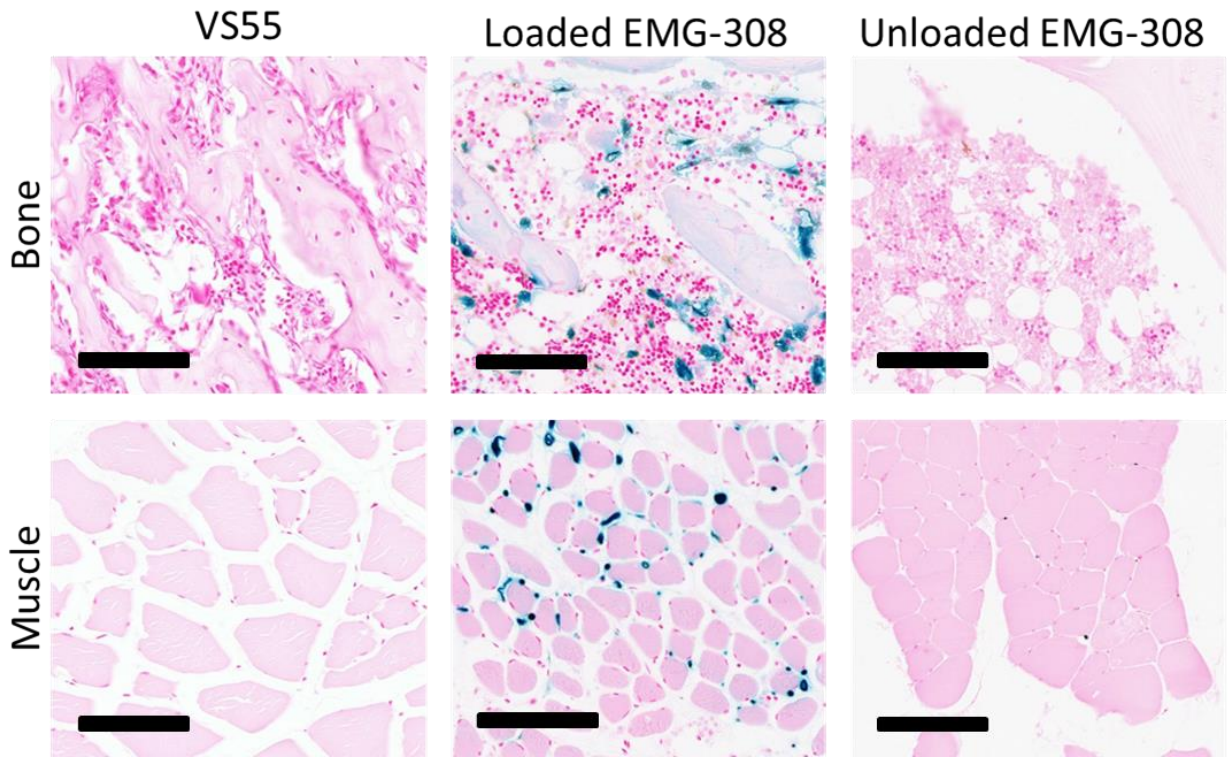
Rabbit kidneys were prepared and perfused at 21st Century Medicine, Inc. (Fontana, CA). Male New Zealand White rabbits were induced with IM ketamine/xylazine and then transferred to isoflurane anesthesia with 1.5-2 L/min oxygen by face mask and hydrated with 60-80 ml of lactated Ringer's solution containing 4.2 mg/ml of sodium bicarbonate and given dropwise via a 22-24 gauge needle inserted into the marginal ear vein. Arterial blood pressure was maintained at ≥45 mmHg as monitoring via a 20-22 gauge needle introduced into the central ear artery. A mid-ventral laparotomy was performed, and the right kidney was mobilized, flushed with room temperature Renasol-14 and then with 4°C Renasol-14, and the kidney was transferred to a perfusion machine. M22 was introduced essentially as described before (4-7), except that, following 20 min of M22/LM5 perfusion at -22°C, the kidney was perfused for an additional 10 min with an M22 solution in which all of the water was replaced with the stock nanoparticle solution (resulting in a final nanoparticle concentration 38% of the stock concentration, or about 14 mg/ml). After perfusion, the kidneys were immersed in a 5-fold dilution of the M22/nanoparticle solution (final nanoparticle concentration ~2.8 mg/ml). However, the M22/nanoparticle solution was filtered through a 1.2 micron filter prior to use, which may have removed an unknown number of nanoparticles prior to use. All procedures involving animal use were done according to USDA standards with IACUC approval.

Ovary:

Procurement – Whole reproductive tract from female sows (ovaries and uterus) were procured en bloc with vasculature in-tact from deceased donors (up to ~250 lb) provided by the local abattoir. Briefly, following euthanasia, the animals were exsanguinated and hung, the abdominal viscera were removed en bloc, and the entire reproductive tract was then dissected by the scientific staff on a back-table. The procured reproductive tracts were quickly submerged in ice-cold buffered saline solution, and stored on-ice for

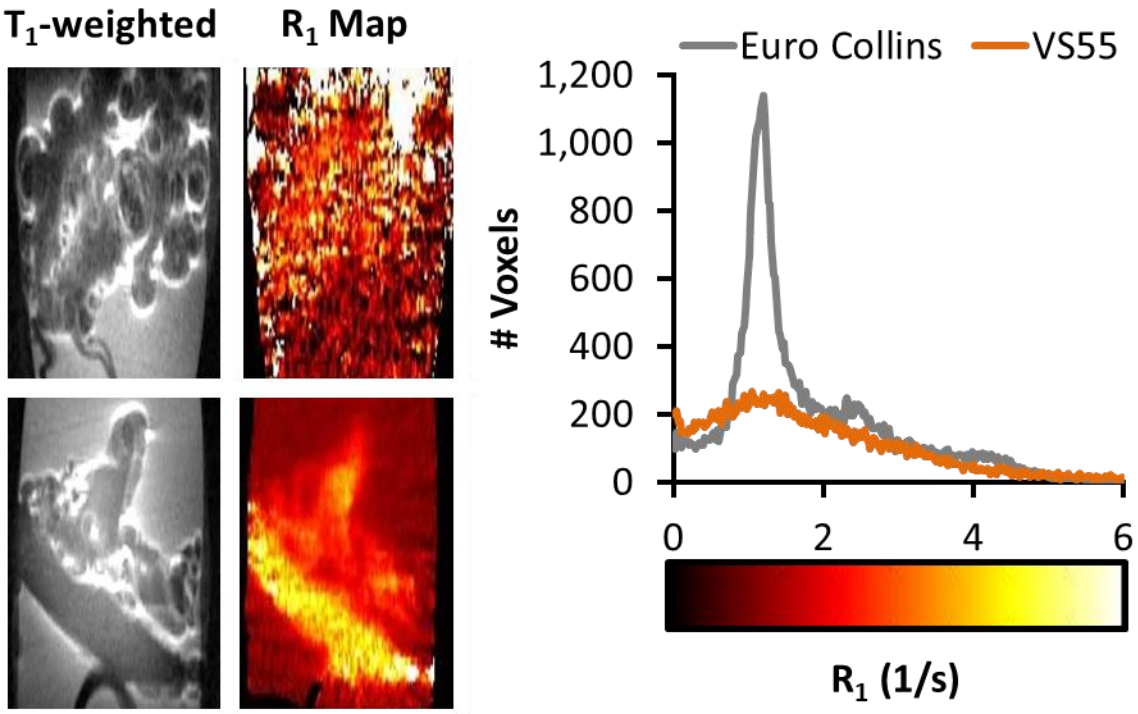
transport to the laboratory. Cannulation and Exsanguination – Upon arrival at the lab the recovered tissues were prioritized based on visual inspection of tissue quality (blood content, standard morphology and access to primary artery). Each ovary-uterus was then placed on a chilled surgical tray and the ovary was carefully dissected from the surrounding uterine tissues to expose the primary ovarian artery. The artery was quickly identified and cannulated using an appropriately sized polyurethane cannula (1-3 fg. size) by experienced laboratory staff. Following cannulation, the ovarian artery was further exsanguinated and manually perfused with 10 ml of ice-cold preservation solution (Unisol UHK with 1000 U/L of Heparin) at a flow rate of approximately 1 ml/minute (chosen based on literature and our experience). The perfusion-flush procedure was then repeated as needed until all of the blood was flushed from the tissue as determined by visual observation of the effluent fluid (when it runs clear). The flushed ovary was then trimmed of excess uterine tissue, catalogued and stored at 4°C in UHK until being designated for an experimental treatment (20-30 minutes). CPA and Nanoparticle loading - The cannulated porcine ovaries were then machine perfused with 18.75%, 25%, 50%, 75%, 100% VS55 for 15 min in each loading step, at 0.1 mL/min·g, and then for ovaries loaded with IONPs, a final step of 2 mL of 24 mg Fe/mL PBG300 nanoparticles (a PEGylated IONP, Ferrotec) suspended in VS55 solution were perfused into ovaries at the same flow rate. For each ovary, the ovarian artery and adjacent ovarian veins were then ligated at the cannula position and at the nexus of the ovarian vascular pedicle and the ovary itself, to prevent any leakage of nanoparticles from the ovarian vasculature during transport. Shipment and Transport - The cannula was then removed, and the whole ovaries (with attached vascular pedicle) were placed in individual 50ml conical tubes, which were then filled to the top with fresh VS55 solution, and capped. The tubes were sealed with copious amounts of laboratory film (Parafilm M), and then further wrapped in absorbent pads and placed into plastic bags for shipment to the University of Minnesota. The plastic bags were then shipped overnight, in insulated packaging filled with frozen ice-packs, to ensure a constant shipment temperature of approximately 0°C. Upon arrival at the University of Minnesota, the ovaries were stored at 4°C in a refrigerator until prepared for scanning procedures as described in the main methods.

Supporting Information Figure S1: Hindlimb Histology



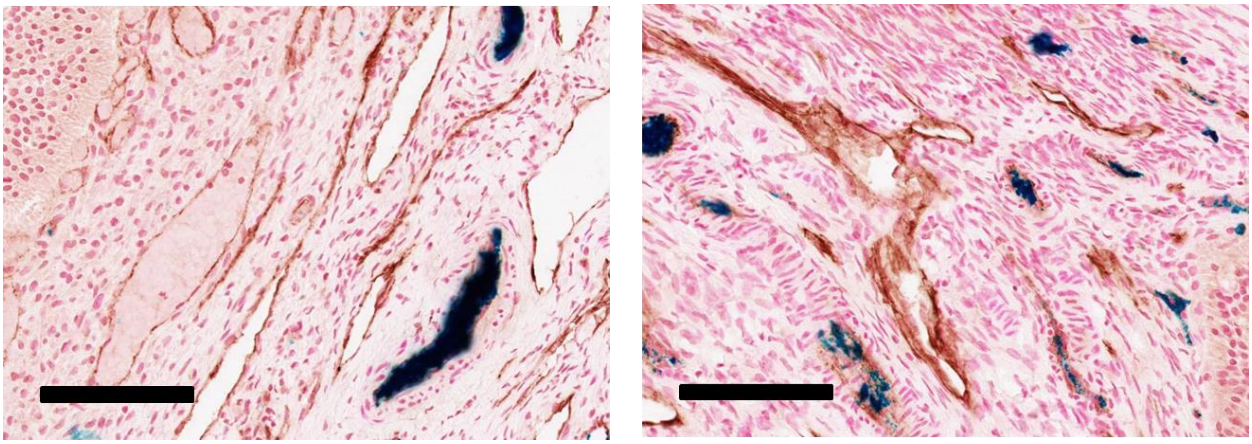
Supporting Information Figure S1: Hindlimb histology demonstrating that IONPs are present within the hindlimb loaded with EMG-308. In contrast, the unloaded EMG-308 appears to be similar to the negative control. The histological observations of the unloaded EMG-308 hindlimb are in conflict with the MRI results which indicate that there is much more iron present in the tissue after washout. Black bars indicate 100 µm.

Supporting Information Figure S2: Liver R_1 Maps



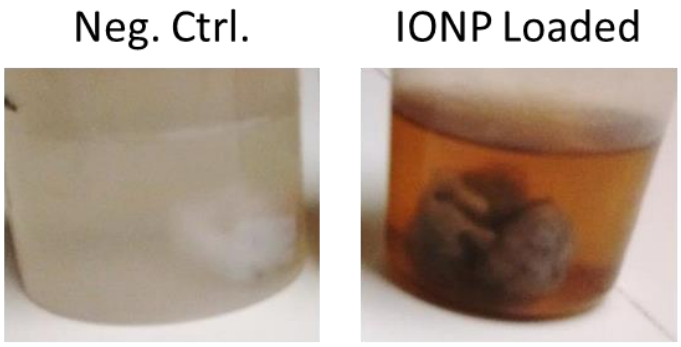
Supporting Information Figure S2: The difference in distribution from rat livers perfused with IONPs (EMG-308) using VS55 in EC and or EC as perfusate (a & c) with their corresponding R_1 Maps (b & d). The pile-up artifacts observed in the T_1-weighted image (a) of the rat liver perfused with VS55 and 357 mM_{Fe} (20 mg_{Fe}/mL), prevent the measurement of a robust R_1 map (b) where the lobes of the liver can be distinguished. In contrast the rat liver perfused with EC and 643 mM_{Fe} (36 mg_{Fe}/mL) is observed to have distinguishable lobes in the T_1-weighted image (c) and the R_1 map (d). A histogram across the entire image (e) demonstrates the broad range of values in the R_1 map for the VS55 perfused liver compared to the peaks at R_1 = 1.2, 2.5, and 4.1 1/s, which correspond to the VS55 surrounding the liver and two different lobes within the image.

Supporting Information Figure S3: Ovaries 48 h Fixation



Supporting Information Figure S3: The histology demonstrates the loss of IONPs within the fixation process for the ovary. Within this images, IONPs are not visibly consistent throughout the vasculature of the tissue. Both µCT and MRI indicate that there is not a loss of IONPs throughout the tissue. Black bars are 100 µm.

Supporting Information Figure S4: Ovaries 48 h Fixation



Supporting Information Figure S4: Ovary histology preparation shows a loss of IONPs from the ovary after 48 h of 10% formalin fixation.

Perfusion References:

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