Supplementary File

Ferumoxytol-based Dual-modality Imaging Probe for Detection of Stem Cell Transplant Rejection

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Methods:

Measurement of the number of peptides conjugated to ferumoxytol. To evaluate the conjugation efficiency of peptide on Feru-AFC NPs, we synthesized gadolinium(III) functionalized ferumoxytol (Feru-Gd) NPs through a similar approach to facilitate the quantification of Gd number on NP surface by inductively coupled plasma mass spectrometry (ICP-MS), indicating that ~150 Gd(III) were successfully chelated to each NP, suggesting that ~150 peptides have been conjugated to each nanoparticle.

The detailed approach is described as following: KKKKDEVDC was conjugated on the ferumoxytol NPs through the same approach of EDAC-coupling reaction used in Feru-AFC synthesis. Maleimido-mono-amide-DOTA was further immobilized through thiol-maleimide coupling reaction, followed by chelation of Gd(III) from gadolinium(III) chloride to afford Feru-Gd(III) NPs. After harsh washing steps using PBS buffer and DI water to eliminate excess Gd(III), the obtained NPs were sent for ICP-MS analysis to determine the contents of Fe and Gd and their ratio for estimation of the number of Gd(III) on each NP. The calculation was based on the formulation of Feraheme: $Fe_{5874}O_{8752}$-$C_{11719}H_{18682}O_{9933}Na_{414}$ (https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/022180lbl.pdf).

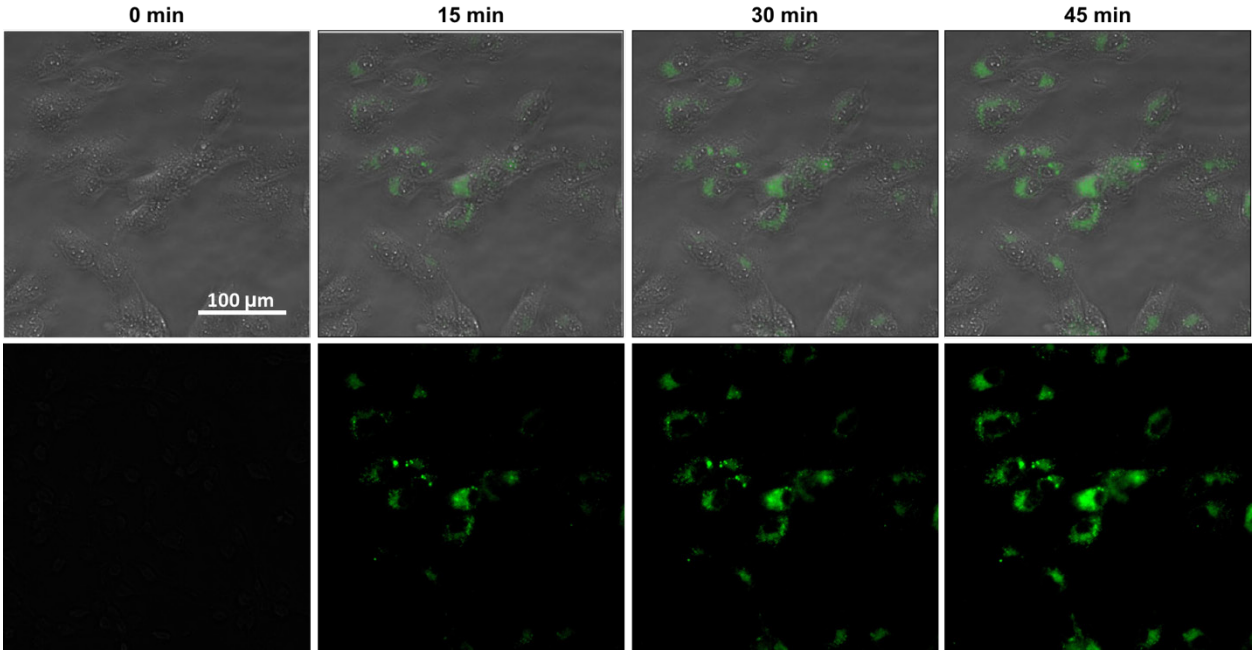

Figure S1. Real-time fluorescence imaging of apoptosis. Live cell imaging shows that at the starting point (0 min), the cells labeled with Feru-AFC NPs showed no green fluorescence because there was not enough activated intracellular caspase-3 in viable MSCs. 15 min after addition of staurosporine (1 µM) in the cell culture well, the cells underwent apoptosis and the green fluorescence from the cell cytoplasm increased over time in the FITC channel (30 and 45 min).

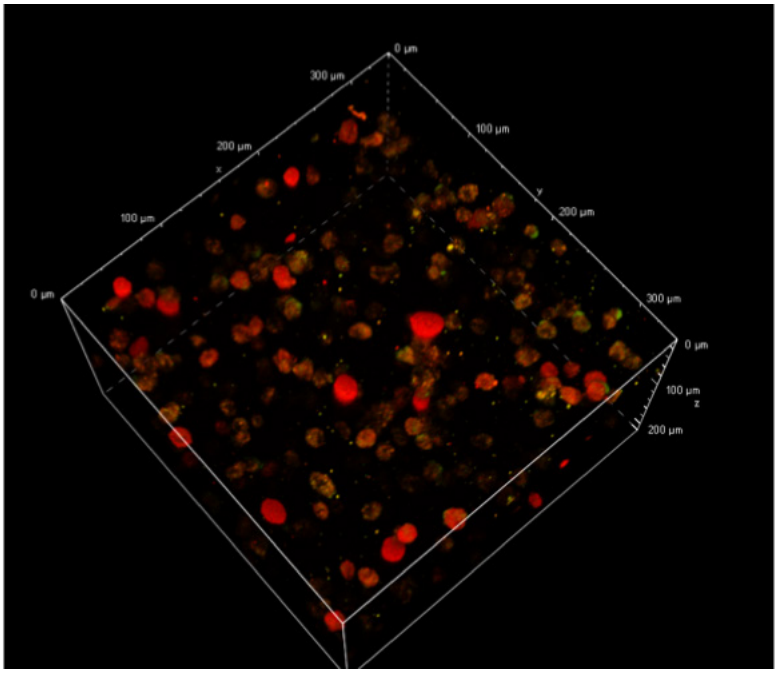

Figure S2. Intravital imaging (IVM) of pig MSCs prior to implantation. To confirm that the pig MSCs that were transplanted into the calvarial defect were non-apoptotic, td-tomato-labeled pig MSCs were incubated with the Feru-AFC NPs prior to embedment in the scaffold. IVM was performed as described in Figure 5a. 3-D volumetric images showed that no apoptotic cells were detected.

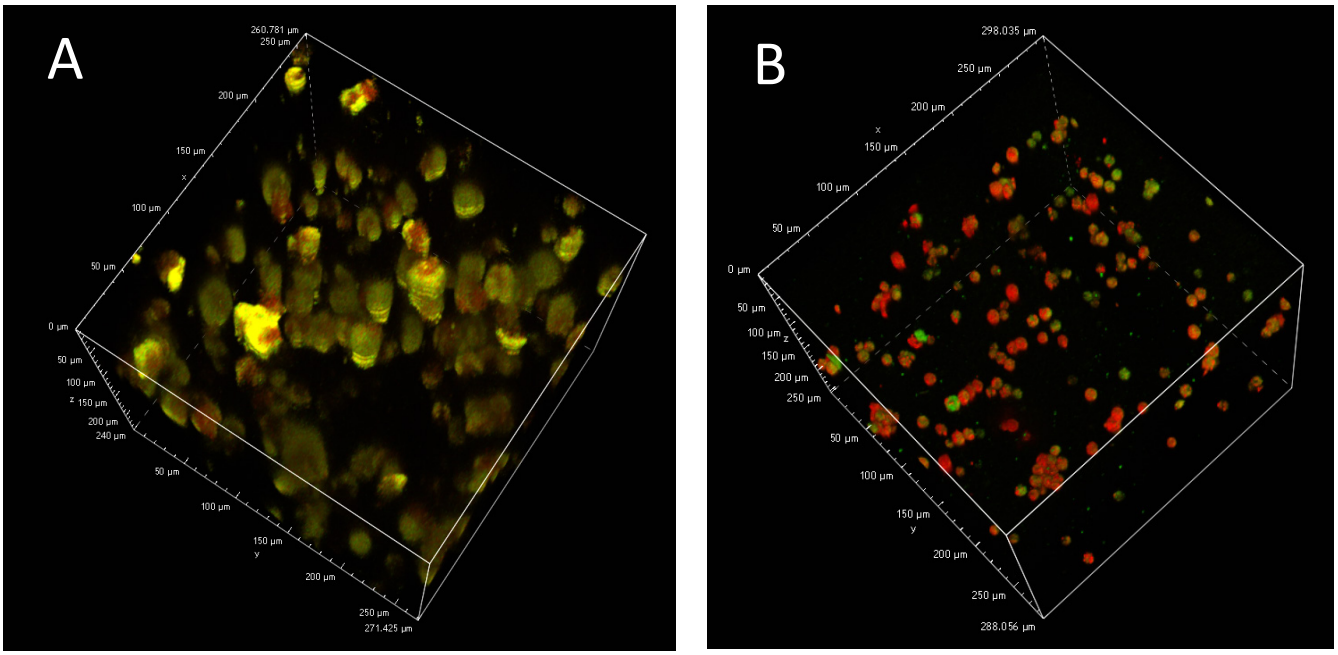

Figure S3. Intravital imaging (IVM) of apoptotic pig MSCs Day 1 post implantation. Higher magnification of the 3-D volumetric image from Figures 5A and B, in which individual apoptotic and non-apoptotic pig MSCs (Panel A) and mouse MSCs (Panel B) can be observed.