Imaging of Grafted Mesenchymal Stem Cells in Bone Tissue

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Introduction. Mesenchymal stem cells (MSC's) have high potential for a treatment of bone loss disease such as osteoporosis and bone fracture. Progress in this promising area of research will require an *in vivo* technique to observe the grafted cells and engraftment pattern overtime. Our previous studies indicate that iron labeled cells can be successfully imaged *in vivo* with gradient echo MR imaging. However iron labeled cells and bones appeared dark on gradient echo images, which make interpretation of data very difficult or impossible. In this project, we propose to implement SWIFT pulse sequence, which can produce a positive contrast from structures with short T2, such as bone and iron labeled cells, and develop an *in vivo* imaging protocol to monitor MSC engraftment in the bone tissue.

Methods. *Cell culture*: Mouse MSCs were maintained on uncoated flasks in media with DMEM and 10% FBS. *Phantoms*: 250 µl tubes were filled 0, 50, 100, 200, 300, 600, 1200, 3000 µg/ml of Fe. *Intra-tibia injection:* 50 µl of iron-oxide (100 µg/ml of Fe) were injected into high limbs of rat carcass. *Imaging:* Three-dimensional (3D) radial SWIFT images were acquired with 2.6 µsec duration of segmented RF pulse with excitation bandwidth of 100 kHz. Data were acquired with TR = 2.4 ms and TE = 3.9 µsec. Images were reconstructed with 66 µm resolution.

Results. Fig.1A depicts SWIFT MR image of a phantom with different concentrations of iron oxide particles acquired with 2- (A), 10-(B) at 20-degree (C) excitation flip angles. Images of the same phantom acquired with gradient or spin echo sequences allowed signal detection only from first two iron concentrations (image not shown). SWIFT acquisition with low flip angle provides weak but detectable signal from pure water. An increase of the excitation flip angle leads to suppression of pure water signal due to saturation and an increase of signal intensity from iron containing solutions. Fig.1D depicts a graph of MRI signal intensity as a function of iron concentration. MRI signal increases initially, reaches a maximum and decreases again with increasing the iron concentration. It is important to mention that even SWIFT sequence was not able to recover MRI signal from vial with very high concentration of iron (30000 µg/ml). Fig.1E shows dependence of MRI signal intensity as a function of the flip angle. In experiments with different iron concentrations we observed maximum signal intensity at a different flip angle (panel E)(water (red)- too small to measure, 50 µg/ml (green) – 8^0, 200 µg/ml (cyan) – 15^0, 600 µg/ml (purple) – too big to measure (hardware limitation)). These flip angles are in the excellent agreement with theoretical predictions (Ernst angle) and correlate with T1 relaxation times for these solutions.

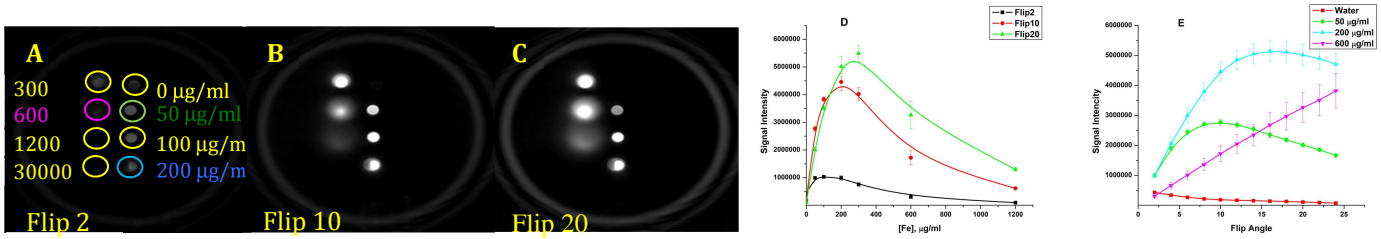

Fig.1 ***SWIFT pulse sequence allows to generate positive MRI signal from iron oxide particles.*** *SWIFT MR images of phantoms with different concentrations of iron oxide particles. A, B and C – images of phantom acquired at 2 (A), 10 (B) and 20 (C) degree flip angles. D is a graph of signal intensity vs. iron concentration at different flip angles. E - Signal intensity as a function of flip angle of different concentrations of iron oxide particles. Each iron concentration curve reaches a maximum at definite flip angles*

Fig.2 shows *ex vivo* SWIFT MR images of a mouse hind limb after injection of ~50 µl of 100 µg/ml solution of iron oxide particles into tibia. Imaging at different flip angles revealed that maximum contrast between tissue and iron solution can be achieved at 6-degree flip angle.

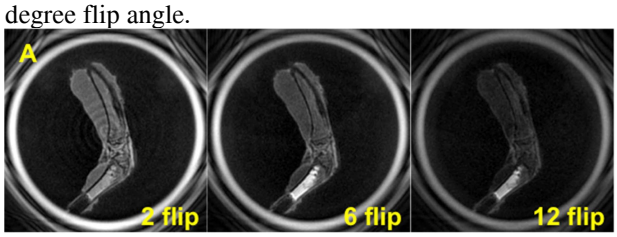

Fig.2. ***Positive MRI signal from high concentration of iron oxide particles in mouse tibia.*** *A –Ex vivo SWIFT MR images of mouse tibia after intra-trabecular injection of ~50 µL of iron oxide particles (100 µg/ml). 2, 6, 12 degree flip angles. Images have 66 µm isotropic resolution. B – Signal intensity from iron oxide particles (red) and muscular tissue (green) as a function of flip angle.*

Result and discussion. The goal of this project is to develop a robust MRI protocol for detection and monitoring of grafted (MSCs) in bone tissue. In our preliminary experiments we have shown that SWIFT pulse sequence allowed to generate a positive MR signal from iron labeled cells, which was not conceivable with traditional MR imaging. Phantom experiments indicate that an optimization of excitation flip angle of SWIFT sequence will be very beneficial to maximize signal intensity and produce maximum contrast between tissue and iron labeled cells. Proposed method will be very useful in all areas of stem cell therapy and have a profound impact on stem cell treatment of bone degenerative diseases and fracture repair.