# Noninvasive monitoring of stem cell delivery and muscle regeneration

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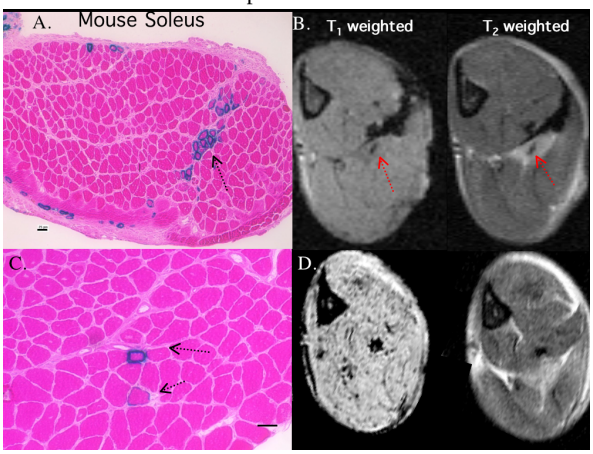
**INTRODUCTION:** Cast immobilization causes skeletal muscle disuse atrophy and an increased susceptibility to muscle damage. The underlying muscle atrophy occurs due to apoptosis and the silencing of endogenous muscle stem cells.[1] Whereas, subsequent reambulation promotes extensive muscle regeneration through the recruitment of muscle stem cells.[2] Stem cell transfer for enhanced muscle regeneration has tremendous theraputic potential especially in the musuclar dystrophies and during sacropeana. Previous analysis of stem cell delivery has relied on traditional invasive techniques to monitor the effectiveness of delivery. The purpose of this study was to evaluate the ability of MR imaging to noninvasively monitor muscle stem cell transplantation, homing to damaged tissue, and regeneration.

**METHODS:** Ten female C57BL6 mice underwent hindlimb immobilization for 2 weeks using a bilateral casting procedure that produces a well defined model of soleus muscle damage and regeneration during reloading.[3] Multipotent muscle derived stem cells ($0.5x10^6$) constituently expressing a histological marker gene (β-galactosidase) were delivered either via direct intra-muscular injection into the mouse hindlimb or arterial injection into the abdominal aorta following 48 hours of muscle reloading. At this time point, muscle damage and $T_2$ contrast in the reambulated mouse soleus is maximal.[3] Unilateral occlusion of the iliac artery ensured that the arterial injected cells were delivered to one hindlimb. Muscle stem cells were labeled with a supraparamagnetic iron-oxide contrast agent (ferumoxide-poly-l-lysine) to allow for the detection of stem cell transfer using $T_2$ and $T_2$* weighted MR images.[4] MR measurements were performed on a 4.7 Tesla Bruker Avance Imaging Spectrometer with a single tuned 1.6 cm solenoid coil immediately post-injection and at 1, 3, and 7 days post-injection. $T_2$ weighted images were acquired immediately post injection and at 3 days post injection using a spin echo sequence (TE=14, 40ms, TR=2000ms, FOV=1.6cm, 256x128, 2nex, thk=1.0mm) and $T_1$ weighted images were acquired using a 3D gradient echo sequence (TE=4.5,7ms, TR=17ms). $T_2$ maps were created by using the decay in pixel signal intensity as a function of TE on the spin-echo sequences. $T_2$ was used as an indicator of muscle damage. Cells were visualized on all scans as the absence of signal. Histological analyses (Hematoxylin & Eosin; X-gal; Prussian Blue) were performed to confirm muscle damage and stem cell incorporation.

**RESULTS:** Reambulation following cast immobilization induced specific muscle damage to the soleus, an important postural muscle. Examination of the MR images acquired during reambulation showed an increase in $T_2$ in the soleus muscle consistent with muscle damage (Figs B&D). Regions of increased $T_2$ in the soleus muscle on MR scans were confirmed using hematoxylin and eosin stains. As previously reported muscle derived stem cells were efficiently labeled *in vitro* by transfection with ferumoxide-poly-L-lysine complexes.[4] Labeled stem cells underwent normal myogenesis *in vitro* and *in vivo*. Transplantation of labeled stem cells resulted in regions of hypo-intensity on $T_1$, $T_2$, and $T_2$* weighted MR scans. Following the direct intra-muscular injection of labeled stem cells, regions of hypo-intensity could be detected in the regenerating soleus musculature as well as other posterior hindlimb muscles (FIG B). Engrafted cells were detected by analysis of β-galactosidase (LacZ) activity, and iron content. LacZ expressing fibers were identified in regions corresponding to the MR images (FIG A). Additionally, Prussian blue staining of the corresponding serial section revealed the presence of iron accumulation in the LacZ positive fibers. However, a small number of non-muscle cells contained iron. Due to the number of macrophages seen in regenerating muscle fibers, we hypothesize these to be scavenger cells removing the remains of labeled cells that fail to engraft. Additionally, MRI allowed for the non-invasive analysis of mc13 cell engraftment following arterial delivery. Small, punctuate areas of decreased signal intensity were seen in the musculature of the leg that received the labeled cell infusion (FIG D). The contra-lateral limb and control limbs injected with unlabeled cells did not contain these characteristic areas. Histological analyses of the leg musculature revealed cells within the vasculature distributed in patterns corresponding to the MR images. Importantly, in direct contrast to the local injection of labeled cells, label from non-engrafted cells was efficiently and rapidly cleared from the musculature. Following arterial delivery of c2c12 cells, a cell type that results in extremely low engraftment rates, labeled cells were removed by 3 days post injection. Following the arterial delivery of mc13 cells to the hindlimbs, the majority of cells remained trapped in the capillary lumens for up to 2 weeks post-injection. X-gal staining confirmed the presence of stem cell integration in the soleus following vascular delivery (FIG C).




**CONCLUSION:** MRI can be utilized to monitor not only muscle damage and regeneration following hindlimb immobilization but also therapeutic muscle stem cell transfer. Although the rate of stem cell incorporation was significantly lower following vascular delivery compared to the direct intra-muscular injection, stem cell derived myotubes could still be identified in the regenerating soleus.

**REFERENCES:**

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