High Resolution Imaging of Arterially Delivered Mesoangioblasts

M. Liu[1], Q. Yang[1], B. Thattaliyath[2], G. Cossu[3], S. Germain[1], and G. A. Walter[1]

[1]Physiology, University of Florida, Gainesville, FL, United States, [2]Powell Gene Therapy Center, University of Florida, Gainesville, Fl, United States, [3]Stem Cell Research Institute, Milan, Italy

INTRODUCTION:

With new potential therapies on the horizon for muscular dystrophy, there is an urgent need for noninvasive imaging to study muscle and cardiac function. Gene and/or stem cell transfer, two therapeutic strategies that have tremendous potential, currently rely heavily on invasive techniques to evaluate the effectiveness of the intervention. This is problematic for pre-clinical or clinical studies evaluating the efficacy of these interventions in patients with extensive muscle damage or cardiac involvement. Therefore, it is imperative that we develop noninvasive techniques capable of providing high-resolution images of muscles at the cellular level. It is now well established that widespread gene expression can be achieved in adult cardiac and skeletal muscle using recombinant viruses and that MRI/MRS can be used to monitor foreign gene expression as well as the incorporation of therapeutic genes in muscle. Cell based therapies; on the other hand, represent a greater challenge for noninvasive monitoring due to the variability in stem cell incorporation that occurs in the presence of massive cell death(1). In this work MR based strategies have been developed to track the migration/integration of stem cells into skeletal muscles and the bone marrow of dystrophic mice.

METHODS:

Cell labeling and in vitro migration assays: SPIO labeling (Feridex™) was optimized on muscle progenitor cells (C2C12;ATCC and primary human myoblasts (Bioheart) using electroporation, cationic transfection agents (poly-L-lysine or protamine sulfate(PS)), or passive endocytosis. The use of 5µg/mL PS and Feridex™ at 50 µg/ml in MEMS for 12hr followed by a heparin wash (10U/ml) was found to result in optimal labeling based on cell viability, labeling consistency and the absence of cell surface aggregates and was used in all the subsequent studies using muscle progenitor and stem cells (mesoangioblasts;(2,3)). Labeled cells were further enriched by magnetic cell sorting (invitrogen dynal). To determine if SPIO labeling altered the ability of muscle cells to migrate, we tested the migration of SPIO labeled and unlabeled C2C12 cells in the presence of recombinant SDF-1(R&D,10ng/ml), IGF-1(PeproTech Inc., 100ng/ml), or 10% Fetal Bovine Serum(invitrogen,) for 24 hrs using migration chambers (Millipore).

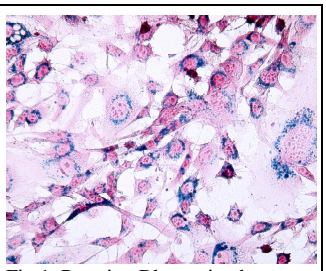

Fig 1. Prussian Blue stained mesogangioblast cells after 12 hrs of SPIO-PS labeling.

Animals and *in vivo* migration assays: Ten, 4-8wk old female *mdx* mice received femoral artery injections, as previously described(4). 100,000-500,000 SPIO labeled mesoangioblasts were injected and MR imaged between 1hr to 30 days post injection. After the final imaging session, hindlimb muscles were extracted and processed for high-resolution MRI and histological verification of iron using Prussian Blue staining. In addition, immunofluorescent staining for dystrophin expression identified regenerated muscle fibers.

MRI: Mice were imaged at 4.7 and 11.1T using Paravision® software (PV3.02;Bruker Medical). The animals were anesthetized using 2% Isofluorane in oxygen. The hindlimbs were placed inside a 1cm transmit-receive solenoid (4.7T) or loop-gap coil (11.1T) and imaged with a 3D-FLASH scan sequence at 4.7 (TR/TE=100/7.5&9,BW=100kHz,NEX=1,flip=30º, FOV=0.8×0.8×2.0cm^3, matrix=384×192×128) and at 11.1T(TR/TE=100/2.9&9,BW=100kHz,NEX=1,flip=30º, FOV=0.8×0.8×2.0cm^3, matrix=384×192×128). Mice hindlimb were also imaged using 3D-RARE at 4.7T (TR/TE=2000/45, RARE factor=8, BW=75kHz, FOV=0.8x0.8x1.8cm^3, Matrix=128x96x256,NEX=1) and at 11.1T (TR/TE=1000/22, RARE factor=4, BW=75kHz, FOV=0.8x0.8x1.8cm^3, Matrix=128x96x256,NEX=1). Isolated muscles were imaged at 17.6T in perfluorocarbon at 12°C with a 5mm i.d coil (PV4;Bruker Medical) using 3D-FLASH(TR/TE=150/3.4, BW=100 kHz, Flip=30º, FOV=1.6×0.4×0.2cm^3 ,matrix=512×128×64, NEX=4). Images were analyzed with OsiriX and IDL (ITT) software.

RESULTS:

Cell labeling and in vitro migration assays: We found that labeling with PS was efficient at 5µg/mL and Feridex™ at 50 µg/ml and did not hinder differentiation or characteristics of myoblasts as evident by the efficient expression of desmin in the differentiated human myotubes. Labeling with PS has the advantage over PLL in that surface SPIO aggregates could be competed off using heparin washes and resulted in consistent endosomal labeling from batch to batch. We found that PS-Feridex labeling did not alter the ability of the muscles cells to grow or migrate *in vitro*. Moreover, PS-Feridex labeling did not diminish the cell's response to SDF-1a or IGF-1 treatment. PS-Feridex labeling in muscle progenitor cells was extremely efficiently resulting in ~99% labeling, but was reduced to ~50% in mesoangioblasts. Despite this, cells used for transplantation studies were readily enriched to 100% labeling by the use of a magnetic cell-sorting column prior to injection (Fig 1).

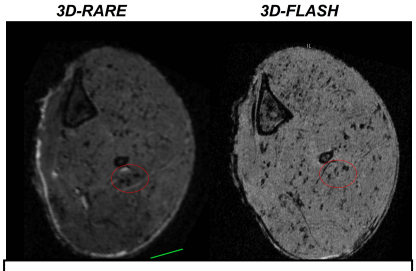

Fig 2. Axial images from a dystrophic mouse hindlimb 12hr after the femoral artery injection of 5x10^5 SPIO labeled mesioangioblasts. Cells appear as hyperintense regions on both T2 (3D-RARE) and T2* images. Red circle indicates areas in which a single muscle (soleus) was extracted for high-resolution imaging. Note the accumulation of cells throughout the muscle and bone marrow.

Animals and *in vivo* migration assays: At both 4.7 and 11.1T, areas devoid of signal on both FLASH and RARE images could be readily detected throughout the leg muscles and bone marrow following femoral artery delivery of as few cells as 100,000 between one and 24 hrs post cell delivery. The majority of the cells could be visualized in muscles surrounding the injection site in upper limb muscles (quadriceps/hamstrings), but cells were also visualized along the entire length of the lower limb muscles and bone marrow (Fig 2). Prussian blue staining revealed cells with iron within the vasculature and within the muscles (Fig 3C). Unlike intramuscular delivery, there was a dramatic loss of muscle MR contrast as early as 12 hr post delivery. Whereas the 3D-FLASH images resulted in higher signal to noise and better contrast and cell detection at 11T compared to 4.7T, the 3D-RARE images at 4.7T highlighted areas of tissue damage to a greater extent than 11.1T. Immediately after 11T imaging the soleus from the mouse in Fig 2 (red circle) was isolated and placed in perfluorocarbon to be imaged at 17.6T (Fig 3). High-resolution (31x31x31um^3) images clearly showed the presence of SPIO throughout the isolated soleus (Fig 3).

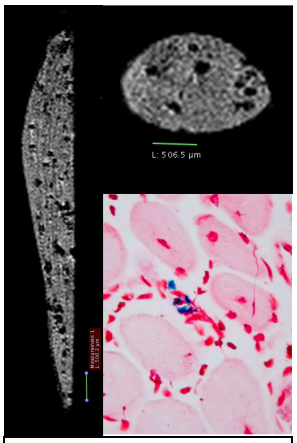

Fig 3. Images from isolated soleus muscle 12hr after cell transplantation at 17.6T. Prussian blue staining revealed cells with iron within the muscles

CONCLUSIONS:

The initial tracking of mesoangioblasts through muscle can be followed *in vivo* following the arterial delivery of SPIO labeled cells. It is this initial binding and transmigration of cells during the first 6-24hrs that is thought to be the most therapeutically relevant for vascular cell delivery therapies and MRI is ideally suited to follow these initial events. Noninvasive and longitudinal tracking will provide valuable feedback to cell biologist for enhancing cell delivery and tissue regeneration.

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