

Feasibility of imaging mesenchymal stem cells *in vitro* using standard MRI sequences

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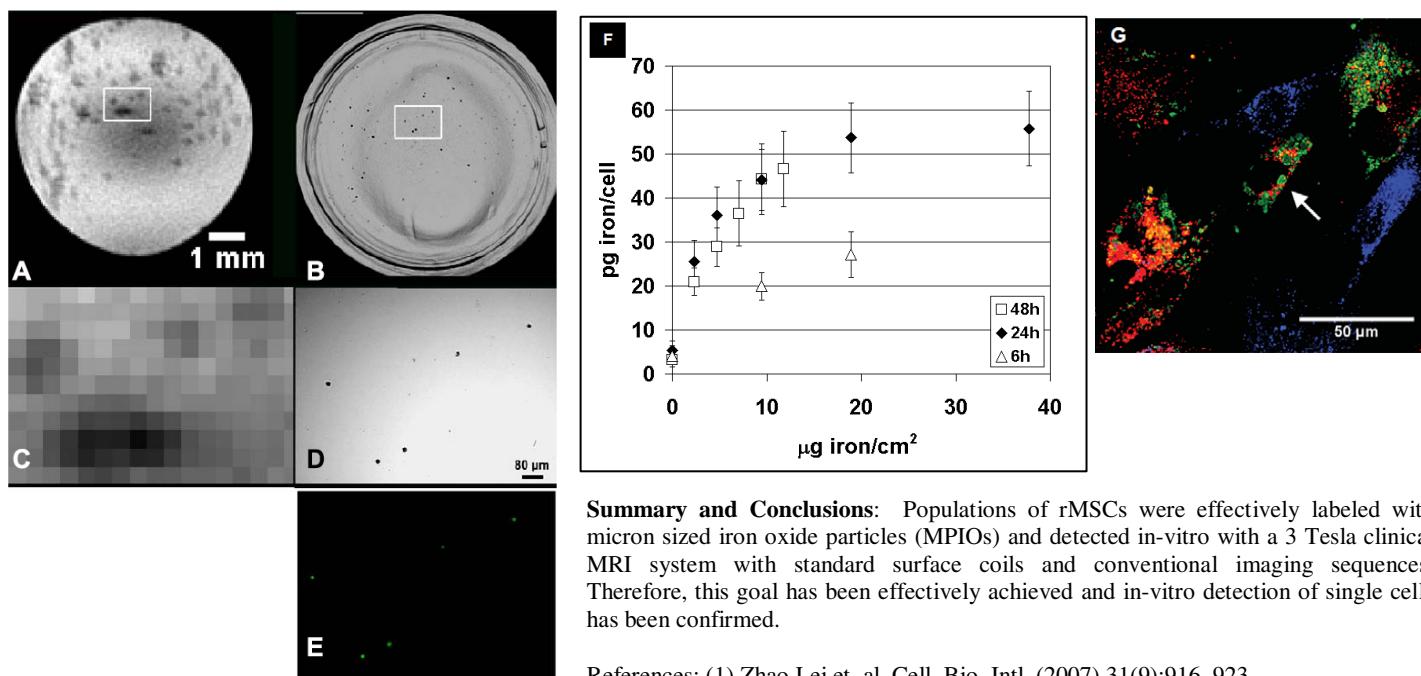
Introduction. Treatment of neurodegenerative diseases such as stroke continues to be an elusive goal for the medical community. While stem cells hold great promise for neuroregeneration, their migration and mechanism of repair are yet to be clearly defined. Literature suggests that mesenchymal stem cells (MSC) can be influenced to differentiate into neural cells *in-vitro* [1]. Several strategies have been developed for understanding their regenerative mechanism; this investigation makes use of micron-sized superparamagnetic iron-oxide (MPIO) particles for tracking MSCs in MRI. We demonstrate the successful labeling and imaging of rMSC-MPIOs.

Methods. Derivation: Rat-derived mesenchymal stem cells (rMSCs) were harvested from bone marrow aspirates obtained from the tibias and femurs of male Long Evans rats 60 days post gestation.

Labeling: Cells were subsequently grown and labeled with MPIO particles to achieve \approx 54 pg iron/cell. The MPIOs are composed of a divinyl benzene polymer with a mean diameter of $0.9\mu\text{m}$ immersed with 62% magnetite (Fe_3O_4) by weight and a Dragon Green fluorophore with 480 nm peak absorption and 520 nm peak emission spectra (Bangs Labs, MC05F). To allow fluorescent visualization of the intracellular space MPIO-rMSCs and rMSCs were labeled with carbocyanine dyes [DiI (540 nm, 580 nm) or DiD (640 nm, 680 nm) cell labeling solutions (Molecular Probes, Invitrogen, V-22889)]. DiI was used to label MPIO-rMSCs (DiI-MPIO-rMSCs) and DiD was used to label control rMSCs (DiD-rMSCs) such that the cell populations could be differentiated when imaged together.

MRI: Agar phantoms with various rMSC-MPIO concentrations were scanned at 3T (clinical GE Signa) using a custom-designed surface coil. 2D-SPGR acquisition of the cellular plane with TR/TE: 57/24 ms, 70 NEX, 30 \times 30 mm FOV, 1 mm slice thickness and 512 \times 512 matrix resulting in voxel dimensions of 60 \times 60 μm in plane.

Results. (A) MRI from a single microwell showing signal voids in the presence of MPIO-rMSCs on a single plane. (B) Phase contrast microscopy of the same microwell from A showing distribution of cells. (C) Magnification of the region of interest from the MRI in A showing five signal voids in a distinct pattern; when the cells are close the signal void 'overlaps' voxels. (D) Phase contrast microscopy of the region of interest showing five cells in the same distinctive pattern. (E) Epifluorescence microscopy showing five distinct regions of MPIO particles fluorescing green which co-localize to the same distinctive pattern from both the cells in D and the signal voids from C. (F) Labeling efficiency. Average iron uptake per cell is shown as a function of iron incubation concentration for three different incubation times. Error bars are representative of the standard deviation of 3 samples per data point. After 6, 24 and 48 hours of incubation with MPIO particles cells were washed, counted and loaded into a 96 well microplate. Treatment with hydrochloric acid and potassium ferrocyanide produces a blue pigment proportional to the amount of ferric iron quantified by absorbance at 650 nm on a microplate reader. (G) Confocal microscopy performed on DiI-MPIO-rMSCs and DiD-rMSCs. Multi-channel co-localization of endosomal DiI (red) and MPIO particles (green) revealing a cytoplasmic, granular distribution of MPIO particles within the MPIO treated cells whereas untreated control rMSCs (blue) show no green fluorescence. The MPIO loading was found to be highly efficient and without serious adverse effects on rMSCs based on assessment of viability, proliferation, metabolism and plating efficiency (data not shown).



Summary and Conclusions: Populations of rMSCs were effectively labeled with micron sized iron oxide particles (MPIOs) and detected *in-vitro* with a 3 Tesla clinical MRI system with standard surface coils and conventional imaging sequences. Therefore, this goal has been effectively achieved and *in-vitro* detection of single cells has been confirmed.

References: (1) Zhao Lei et. al. Cell. Bio. Int'l. (2007) 31(9):916–923