Cellular MR Imaging of Single Human Mesenchymal Stem Cells with Extreme High Resolution at 14.1 Tesla

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Introduction

Magnetically labeled mammalian and stem cells has been a crucial corner of *in vivo* molecular MR imaging. Detection of intravenously injected, implanted, transplanted, or possibly metastasized magnetically labeled cells has another significance for *in vivo* and *in vitro* or *ex vivo* MR trafficking and validation. Superparamagnetic iron oxide (SPIO) particles appear in MR images larger than their actual size, which benefits for sensitive detection of labeled cells. On the other hand, this might interfere accurate characterization of the target. The purpose of this study is to evaluate a limit of cellular MR imaging at the present and the feasibility of MR trafficking at the cellular level.

Materials and Methods

Cells: Fresh human mesenchymal stem cells (HMSC) from bone marrow isolated and cultured until 90% confluence of the surface area of the culture flask. before co-culture with SPIO. Size and number of the cells were measured on hemacytomer (Brihgt-Line; Hausser Scientific, Horsham, PA).

Preparation of Feridex-PLL complex: Dextran coated superparamagnetic iron oxide (Feridex IV; Berlex Laboratories, Inc., Wayne, NJ) was used labeling agent and Poly-$_L$-lysine (PLL; Sigma, St. Louis, MO) was used for transfection agent. Feridex at a concentration of 50 μg/mL was put into a conical tube containing 16mL serum free media and then PLL was added to the solution at a concentration of 1 μg/mL. The solution was slowly mixed in a rotator for 60 minutes at 4°C. After 60 minutes, old media was discarded and replaced with Feridex-PLL complex solution, add FBS in a concentration of 10 % 2 hours later, and then incubated for 22. hours.

Preparation of cells: Cells were washed and centrifuged three times by phosphate buffered saline for removing excess Feridex-PLL complex. Cells were fixed with 4 % glutaraldehyde for 5 minutes, washed, incubated for 20 minutes 2% potassium ferrocyanide (Perl's reagent for Prussian blue staining) in 3.7% hydrochloric acid, washed again and counterstained with nuclear fast red. Labeling efficiency was determined by manual counting of Prussian blue stained and unstained cells using microscope. Labeled cells are prepared for MR imaging by mixing with 4 % gelatin and loaded at the micro capillary tube (internal diameter = 800 μm). Final concentration of labeled cells was 1.5 x 10^5 / mL.

Cellular MR Imaging: High resolution cellular MR imaging was done using 14.1 Tesla system based on a BioSpin DMX 600 (Bruker Instruments, Inc., Billerica, MA). Custom made Micro RF coil and NMR microscopy probe was used for imaging. The imaging protocol consisted of gradient echo sequences with TR/TE = 1000/5.04 ms, flip angle = 30°, acquisition = 340, matrix size = 128 x 128, slice thickness 25 μm, in-plane resolution 10 μm x 10 μm, echo position = 40%, and an experimental imaging time of 12 hours 5 minutes 20 seconds. Experiment was done at 5 °C to prevent cellular movement during the long imaging time.

Results and Discussion

Mean size of the cells was 18.7μm (Figure A). It was supposed that 4 pixel would be occupied by the HMSCs on cellular MR imaging in this study. Intracellular SPIO-labeling efficiency was 100 %. MR imaging of magnetically labeled HMSC with high resolution was successfully acquired. Signal void lesion occupies 4 to 5 pixels. Maximum signal void lesion was 1 to 2 pixels at the center. Control study with non labeled cells and gelatin without cells did not produce significant signal intensity.

Conclusion

Blooming artifact did not extend over the estimated cell boundary on 10 μm pixel high resolution MR imaging. Cellular MR imaging was possible when stem cells were magnetically labeled and our results may support that individual stem cells trafficking would be possible.

References

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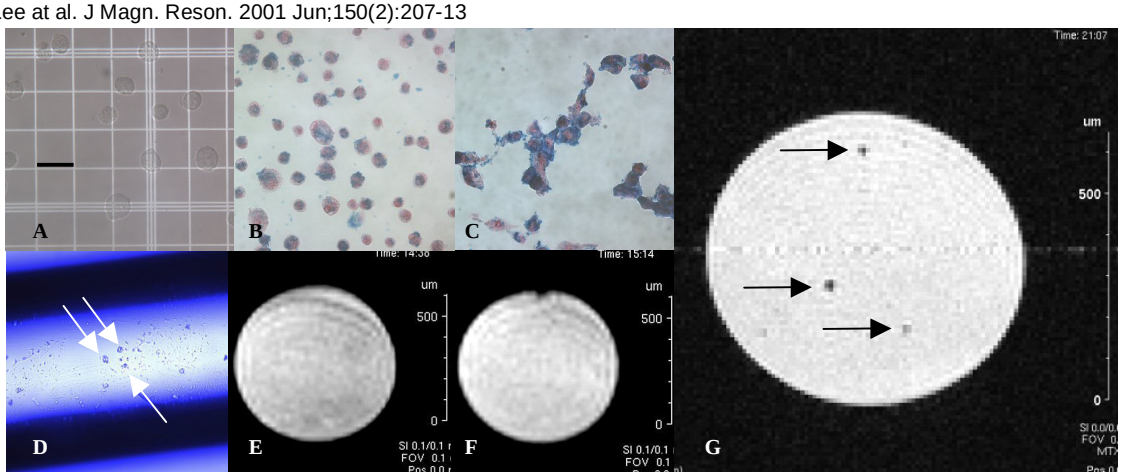

Figure A: Fresh HMSCs counted and measured by hemacytomer. Black transverse line represents 40 μm (LM x 400).
Figure B: Prussian blue staining shows intracytoplasmic vacuoles containing SPIO (LM x 400).
Figure C: Fixed cells with glutaraldehyde before transferred to cytospin slide show decreased cytoplasmic volume (LM 400)
Figure D: Photomicrograph of labeled cell loaded at the 800 μm (internal diameter) micro capillary tube. Relatively even distributed labeled HMSCs are shown (arrows) (LM x 100).
Figure E: Control study with non labeled cells in a concentration of 4 x 10^4 / mL. There is no significant signal difference indicates cells.
Figure F: Control study with 4 % gelatin not containing cells.
Figure G: One pixel indicates 10 μm x 10 μm. Signal void lesions are identified at 3 point (black arrows) and 4 to 5 pixels are occupied.