Development of a Magnetosonoporation-Enhanced Stem Cell Labeling Technique

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PURPOSE: Monitoring of stem cells migrating or homing to the targets is essential for the success of stem cell-based therapies. For non-invasive magnetic resonance imaging (MRI) to serially track cell migration, recent efforts have focused on labeling cells with MR contrast agents, such as superparamagnetic iron oxide (SPIO) particles[1]. The currently-available MR cell labeling techniques include simple incubation and magnetoelectroporation[1,2]. We attempted to develop an alternative instant cell labeling technique, using ultrasound to facilitate MR-labeling of cells, called magneto-sonoporation (MSP), which is based on the fact that ultrasound can increase cell membrane permeability to external molecules [3].

METHODS: We tested the MSP approach using mouse LacZ-neural stem cells, C17.2. For in vitro study, the cells were suspended in PBS at a density of 1-5x10^6 cells/mL and mixed with Feridex (Berlex Imaging, Wayne, NY) at 2mg Fe/mL. Then, the Feridex-cell mixture was transferred to the sterilized cell labeling container and subjected to 1-MHz ultrasound at 0.3W/cm^2 intensity, 50% duty cycle, and 5-mins exposure time. After the MSP treatment, we performed Prussian blue stain and immnocytochemistry to confirm the success of Feridex-labeling. Then, we evaluated the cell viabilities and metabolic assimilation rate using trypan blue exclusion and MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay (CellTiter 96 AQueous, Promega). For in vivo MR imaging, the left brain hemispheres of nude mice were locally implanted with approximate 8x10^4 labeled or unlabeled C17.2 cells through stereotaxic injections. Ten-days after the cell implantation, the mice were imaged with T2-weighted imaging (FSE: 2000/40ms TR/TE) and T2*-weighted imaging (FFE:100/9ms TR/TE) in a 3T MRI scanner (Philips, Cleveland, OH) using a solenoid animal coil. The brains of mice were harvested and sectioned for subsequent hisotological confirmation of successful cell implantations.

RESULTS: Of the in vitro study, both Prussian blue staining and immnocytochemistry demonstrated successful labeling of cells with Feridex- or dextran-positive cells (Figure 1). The viability of MSP-labeled cells was 0.938±0.03, which was close to the metabolic assimilation rate, 0.935±0.045. Both in vivo T2-weighted and T2*-weighted MR imaging showed MR signal void at the brains injected with Feridex-labeled cells, which was not seen at the control brains injected with control cells. The Prussian blue staining and anti-dextran/β-gal double-staining confirmed that the cells were successfully localized in the target brains and consistent with the MR signal changes of MR imaging (Figure 2).

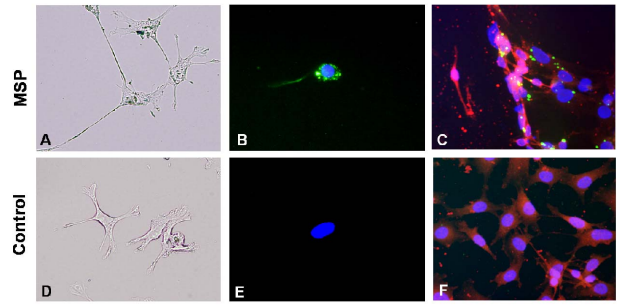

Fig.1. MSP-enhanced C17.2 stem cell labeling. (A) Prussian-blue staining of Feridex-labeled cells, showing successful cell labeling (blue dots) following MSP treatment. (B) Immunocytochemistry to detect dextran, a component of the coat of Feridex particles, showing successful cell labeling (green dots) following MSP treatment. (C) Double staining with β–gal for LacZ and anti-dextran for Feridex, demonstrating pink-colored LacZ- and green-dotted dextran-positive cells. These dotted findings are absent in the control groups without MSP treatment (D-F).

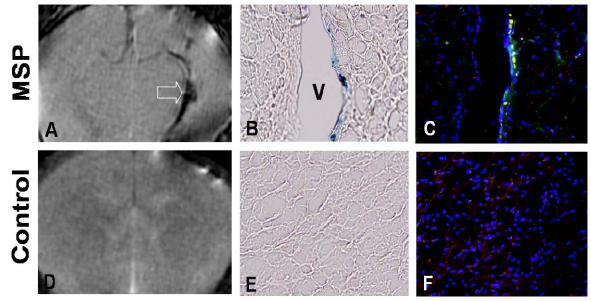

Fig.2. In vivo MRI and histological correlation of a mouse brains implanted with MSP/Feridex-treated cells. (A) T2*WI shows the hypointense signal area (arrow) at the injection site nearby the left ventricle, which is absent in the control brain (D). (B) Histological Prussian blue staining confirms the presence of injected MSP/Feridex-treated cells (blue) nearby the left ventricle (V); (C) immuno-histochemical staining for beta-galactosidase (pink) and dextran (green) confirms the presence of the MSP-enhanced, Feridex-labeled neural stem cells. These findings are not seen in the control brain (E-F).

Conclusion: This technical development demonstrates the potential of using magnetosonoporation to facilitate MR labeling of stem cells, which may become a safe and efficient MR labeling approach to instantly label large amounts of stem cells for preclinical and clinical applications.

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Reference:

1. Bulte, J. W. et al. Magnetodendrimers allow endosomal magnetic labeling and in vivo tracking of stem cells. *Nat Biotechnol* **19**, 1141-7 (2001).
2. Walczak, P., Kedziorek, D. A., Gilad, A. A., Lin, S. & Bulte, J. W. Instant MR labeling of stem cells using magnetoelectroporation. *Magn Reson Med* **54**, 769-74 (2005).
3. Liu, J., Lewis, T. N. & Prausnitz, M. R. Non-invasive assessment and control of ultrasound-mediated membrane permeabilization. *Pharm Res* **15**, 918-24 (1998).