Instant Magnetic Labeling of Stem Cells Using Magnetosonoporation (MSP)

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Introduction: Stem cells are currently explored for tissue/organ repair, revascularization, and other therapeutic interventions [1]. Monitoring of the delivery, migration and homing of stem cells to their targets is essential for obtaining clinical success. Using non-invasive magnetic resonance imaging (MRI) to track cell migration, recent efforts have focused on labeling cells with MR contrast agents, in particular superparamagnetic iron oxide (SPIO) particles [2]. The most commonly used method for magnetic labeling of cells is prolonged incubation of cells with Feridex and polycationic transfection agents such as lipofectamine [3] or poly-L-lysine. However, since prolonged (24-48hrs) cell incubation may change the cellular properties, and a secondary (transfection) agent is required, it is not an ideal MR labeling approach for clinical applications. To overcome these problems, magnetoelectroporation (MEP) was developed as an instant magnetic labeling method without the need for secondary agents[5]. In this study, we attempted to develop an alternative instant magnetic labeling method, magnetosonoporation (MSP), based on the membrane permeabilization properties of ultrasound [6].

Materials and Methods: We tested the MSP approach using two types of cells: mouse C17.2 neural stem cells and human 293T tumor cells. Using a 10-cm diameter cell culture dish, the cells were first suspended in phosphate-buffered saline (PBS) at an approximate density of $1x10^5$ cells/mL. Feridex (Berlex Imaging, Wayne, NY, USA) was then added to the cell suspension at 2 mg Fe/mL. After placement of the ultrasound probe (Richmar 3.5, Rich-Mar Co., Inola, OK) into the cell-Feridex suspension, 1-MHz ultrasound energy was applied at 100% duty cycle and an intensity of $1w/cm^2$. Four MR-labeling experiments were performed using different ultrasound exposure times of 1, 5, and 10 minutes, as well as no ultrasound exposure (control). After ultrasound treatment, the cells were transferred into a 15 ml tube that was placed on ice for 5 min, followed by washing to remove unbound Feridex. Feridex uptake was assessed by Prussian Blue staining and anti-dextran immunohistochemistry. For in vivo MR imaging, approximate $1x10^5$ MSP-labeled and unlabeled 293T cells were injected into the left and right back muscles of a nude mouse. Four-days after injection, the mouse was imaged at 4.7 T using with T2-weighted imaging (2000/40ms TR/TE).

Results: Of all three cell groups with ultrasound exposure, both Prussian blue staining and immunohistochemistry demonstrated successful intracellular Feridex-labeling of cells (Figure.1). Very few iron- or dextran-positive cells were detected in the control cell group. The *in vivo* T2-weighted MR images revealed MR signal voids at the site injected with Feridex-labeled cells, but not at the control site injected with unlabeled cells (Figure. 2).

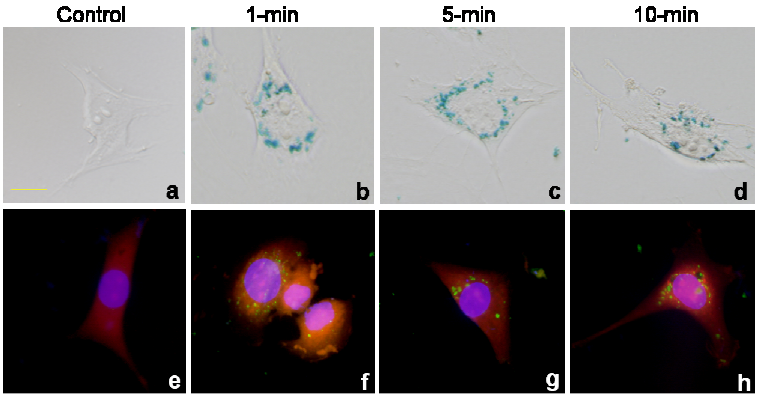

Figure 1. MSP-labeling of C17.2 neural stem cells. (Upper row) Prussian blue staining for Feridex and (lower row) immnohistochemistry for dextran. Successful intracellular localization of Feridex (blue spots in b-d) and dextran (green spots in f-h) is achieved for all three MSP exposure times (1 min- b&f), (5 min-c&g) and (10 min-d&h), while no labeling occurred without MSP treatment (a&e). e-h: blue=nuclei; and red=cytoplasm.

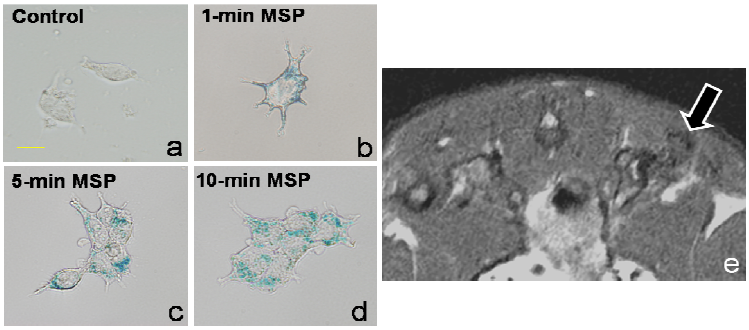

Figure 2. (a-d) Prussian blue staining of 293 T cells with MSP-mediated Feridex labeling, showing successful intracellular localization of Feridex (blue spots on b-d) in all labeled cell groups treated with MSP exposure times of 1 min (b), 5 min (c), and 10 min (d). No labeling occurred without MSP treatment (a). (e) In-vivo MR imaging of a mouse with percutaneous local injection of MSP-labeled 293T cells into the left back muscle and unlabeled 293T cells into the right back muscle. MSP-labeled cells can be detected as a region with signal void (arrow).

Conclusion: Our results demonstrate the potential of using magnetosonoporation to label cells using a single FDA-approved agent. While the MSP optimization and effect on the viability and proliferation as well as differentiation of labeled cells need to be investigated further, we anticipate that this simple and straightforward magnetic labeling technique may be widely applicable for MRI cell tracking studies.

Reference:

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