# Magnetoelectroporation: Ultrafast, One-Step Magnetic Labeling of non-Phagocytic Cells Without the Need For Transfection Agents

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**Introduction:** Magnetic resonance (MR) tracking of stem cells and hematopoietic cells is a new emerging application that aims to obtain a better understanding of the dynamics of cell-tissue interactions, as well as to aid in guiding the development of effective cell therapies. During the past few years, much effort has been directed toward developing suitable procedures that can be applied to achieve an efficient *intracellular* magnetic labeling of *non-phagocytic* cells (phagocytic cells, such as macrophages and certain hematopoietic cells, readily take up unmodified iron oxide particles). The current most widely used method is based on coating the iron oxides with polycations, facilitating particle binding to the anionic cell membrane, followed by internalization. Initial studies introduced arginine/lysine-rich cationic (TAT) peptides [1] or dendrimers [2] as transfection agents. Subsequently, other transfection agents [3] and polycations, such as poly-L-lysine (PLL) [4], have been applied to achieve this "magnetofection." Most recently, the use of an arginine-rich low molecular weight transfection agent, which is FDA-approved, has been shown to be suitable as well [5].

One of the current magnetofection limitations is the need to incubate cells with the iron oxide/transfection agent complexes for prolonged time periods (1-48hrs, depending on the cell type) in cell suspensions or cell culture (Petri dishes). It is well known that placing freshly isolated cells in culture, as a result of exposure to charged plastics and cell adherence, may alter the activation state as well as affect the pluripotency potential of early stem cells. It would, therefore, be desirable if a rapid, simple one-step procedure could be developed that labels cells instantly. We introduce "magnetoelectroporation" (MEP) as a new method that fulfills these requirements. It is based on the use of electroporation, which has long been used to shuttle negatively charged DNA complexes into cells. We modified this "classic" transfection procedure for negatively charged Feridex particles, and show here that neural stem cells can be labeled as efficiently as with the use of transfection agents but with much greater speed and without the need for incubation longer than 1 minute.

**Materials and Methods:** The immortalized mouse neural stem cell line C17.2 was used as a prototype cell line throughout the studies. For MEP, cells were trypsinized, washed, and resuspended in PBS at $10^7$ cells/ml. The dextran-coated FDA-approved iron oxide formulation Feridex (Berlex Imaging, Wayne, NY) was added at 224 μg Fe/ml (i.e., 20 μl from the stock solution of 11.2 mg Fe/ml) and then mixed with the cell suspension. Cells were loaded into sterile 0.4 mm gap electroporation cuvettes (Bio-Rad, Hercules, CA), placed in a cuvette holder of a BTX electroporation system (Harvard Apparatus, Holliston, MA), and pulsed using a variety of conditions (75-400 V; 0.3-30 ms). A square wave pulse of 130V and 17ms was determined to be optimal. After electroporation, cells were left in the cuvette holder for 1 min to allow membrane recovery, transferred on ice for 5 min and then washed 2 times with PBS. Labeling of cells using PLL-Feridex was included for comparison. The iron uptake was assessed both qualitatively by diaminobenzidine (DAB)-enhanced Prussian blue staining and quantatively by a Ferrozine-based spectrophotometric assay [2]. Feridex uptake was also evaluated immunohistochemically using anti-dextran monoclonal antibody (StemCell Technologies, Vancouver, CA). The metabolic mitochondrial rate following magnetofection was measured using an MTS assay (Promega, Wisonsin, WI). MEP-treated cells were compared to control-treated cells, i.e., Feridex-incubated cells that were not electroporated.

**Results and Discussion:** Prussian blue staining of MEP-treated cells showed an intracellular, endosomal distribution of iron particles without localization in the nucleus (Fig 1A). The pattern and intensity of staining was identical to that obtained with Feridex-PLL run in parallel. When cells were incubated with the same dose of Feridex, but without electroporation, very little or no uptake could be observed (Fig. 1B). These results were confirmed by anti-dextran immunofluorescent FITC staining (Fig 1C - electroporated; Fig 1D - non-electroporated), with this technique being more sensitive, as few Feridex particles could be observed in non-electroporated cells that were undetectable by DAB-enhanced Prussian blue staining (compare Fig 1B with 1D). The amount of intracellular iron determined by the Ferrozine-based colorimetric assay ranged between 1-5 pg Fe/cell, depending on the electroporation pulse conditions. No detrimental cellular effects were observed after MEP, as these cells proliferated normally following their return to culture, showed no drop in trypan blue vital dye exclusion, and exhibited a metabolic rate similar to that of non-electroporated cells (Fig. 2).

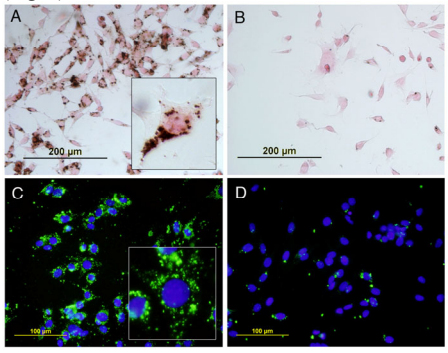


Fig. 1

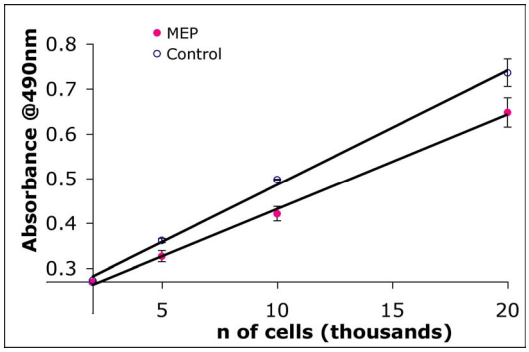


Fig. 2

**Conclusions:** The present results indicate that magnetoelectroporation may be applied as an alternative magnetic labeling procedure to magnetofection. The method is very rapid, straightforward, non-specific and should be widely applicable as electroporation equipment is already available in most molecular biology labs. Compared to the use of transfection agents, MEP has the advantage that no prolonged incubation/cell culture is required, and that it can be used to label hard-to-transfect cell lines, such as embryonic stem cells. The method should also be clinically translatable as Feridex is FDA-approved and no second compound is needed.

**References:** 1) Josephson, L., et al., Bioconjug Chem, 1999. **10**(2): p. 186-91; 2) Hoehn, M., et al., Proc Natl Acad Sci U S A, 2002. **99**(25): p. 16267-72; 3) Bulte, J.W., et al., Nat Biotechnol, 2001. **19**(12): p. 1141-7; 4) Frank, J.A., et al., Radiology, 2003. **228**(2): p. 480-7; 5) Arbab, A.S., et al., Blood, 2004. **104**(4): p. 1217-23.