Magnetofection Accelerates the Loading of SPIO Nanoparticles for MRI Cell Tracking

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Introduction: Superparamagnetic iron oxide (SPIO) particles are widely used for MRI-based cell tracking because they can be loaded into cells using simple protocols and provide a high degree of image contrast due to the large magnetic moment which they impart to labeled cells [1]. Previous research has indicated that using FDA-approved protamine sulfate as a "transfection" agent in combination with approved SPIO contrast agents such as Feridex enables efficient labeling for a variety of cell types [2]. However, this labeling method requires exposing cells to coated SPIO particles for long periods of time (~ 12-14 hours), often in low-serum or no-serum media. Recently, it was shown that SPIO-labeled cells may exhibit signs of oxidative stress and that this is primarily a result of the labeling process rather than the mere presence of SPIO particles within the cell [3]. Consequently, it is highly desirable to accelerate the process of loading cells with SPIO's. *Magnetofection* is a process in which the transfection of DNA, RNA or viral fragments into cells is enhanced by conjugation of these species to superparamagnetic particles and the application of magnetic force [4]. In practice, this is accomplished simply by incubating cells in dishes placed on top of an array of strong permanent magnets. In this work, we hypothesized that magnetofection could increase the speed and quantity with which SPIO's could be loaded into cells, and thereby shorten the time required to label cells with sufficient iron to be detectable in vivo by MRI.

Methods: HeLa cells were grown in DMEM media supplemented with 10% fetal bovine serum (Hyclone, Provo, UT), penicillin-streptomycin reagent, L-glutamine, and fungizone (Invitrogen, Carlsbad, CA). 35 mm tissue culture plates containing ca. 2 million cells each were incubated at 37 °C in a 95% air: 5% CO_2 v/v atmosphere. In preparation for cell labeling, Feridex suspension and protamine sulfate solution were mixed in serum-free OptiMEM media (Invitrogen) and pre-incubated for 10 minutes. The media in each dish of plated cells was replaced with 1.5 ml of the Feridex-Protamine complex in OptiMEM. This labeling media contained a total of 50 µg Fe and 4 µg protamine per ml. Control experiments were performed on separate dishes of cells incubated with OptiMEM only or with OptiMEM containing Feridex at a concentration of 50 µg Fe/ml but without protamine. Six dishes were incubated on top of a magnetofection plate magnet (OZ Biosciences, Marseille, France) while a separate set of dishes was incubated under the same conditions without a magnet (see Fig. 1). At regular intervals of 0.5, 2 and 14 hours, dishes from both groups were removed from the incubator for analysis. Cells were released by trypsinization, washed 3 times with DPBS and counted. The cells from each dish were then digested by adding 2 ml of 6M HCl and 50 µL of 3% H_2O_2 to prepare for iron quantification using atomic absorption spectrometry (AAS) [3] After overnight digestion, filtration and addition of 4 ml H_20, each sample was then analyzed for iron content using AAS. Iron uptake per cell was calculated by multiplying the observed iron concentration in each digest by the total digest volume and dividing by the measured cell count.

Results: After HeLa cells were incubated for 14 hours on top of the magnetofection magnet in OptiMEM media containing Feridex at a concentration of 50 µg/ml but no protamine, cellular iron content did not differ significantly from that of cells incubated with neither Feridex, protamine or magnetofection (0.3 ± 0.2 pg Fe/cell vs 0.3 ± 0.3 pg Fe/cell, N=2 each). When cells were incubated with both Feridex and protamine for 0.5 or 2 hours, significantly higher iron uptake was observed with magnetofection than without magnetofection (Fig. 2). After overnight incubation, magnetofection resulted in somewhat higher iron uptake, but this trend was not statistically significant due to the limited sample size. Comparing the two sample groups, we found that magnetofection increased mean cellular iron uptake by 283.2%, 73.7% and 49.5% after 0.5, 2, and 14 hours incubation, respectively.

Discussion: We have found that the use of magnetofection does accelerate the process of loading cells with iron and increases uptake at every time point, though the effect was not significant during a 14 hour loading period due to a limited number of samples. Magnetofection has its greatest effectiveness during shorter loading times and offers diminished advantage over standard loading techniques for overnight incubation. Despite this, magnetofection does not obviate the need for transfection agents such as protamine sulfate. This result is not surprising since while magnetic force may increase net contact of SPIO particles with cell membranes, these particles must be coated with a suitable cationic agent to be effectively internalized by the cells. In conjunction with protamine sulfate, 2 hours of incubation with magnetofection results in mean iron uptake roughly equal to that achieved by overnight incubation without magnetofection. While we have demonstrated the ability of magnetofection to accelerate and enhance SPIO uptake into HeLa cells, its real utility lies in labeling less robust cells such as primary cardiac myocytes. These more delicate cells would benefit from the decreased loading times made possible by magnetofection. Moreover, in protocols where cells must be labeled in low-serum or serum-free media due to competitive binding of serum proteins to SPIO's and/or transfection agents [5], magnetofection would decrease the amount of time cells must spend in a serum-deprived environment. Finally, the greater iron uptake obtained at each time point via magnetofection suggests that this technique could be useful in improving iron loading and therefore image contrast for cells which are difficult to label using transfection agents alone.

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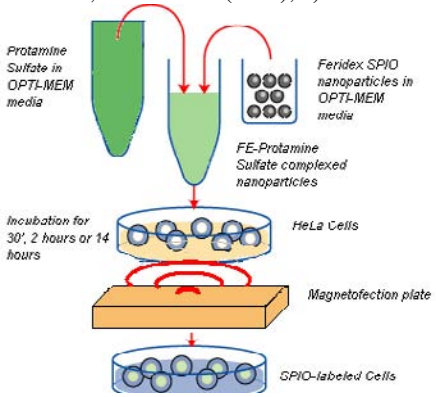
References: 1) Bulte JW et al, *NMR Biomed*, **17**:484-499 (2004); 2) Arbab A et al, *Blood,* **104:**1217-1223 (2004); 3) Stroh A et al, *Free Radical Biol Med*, **36**:976-984 (2004); 4) Plank C et al, *Expert Opin Biol Ther*, **3:**745-758 (2003); 5) Arbab A et al, *Transplantation*, **76**:1123-1130 (2003)



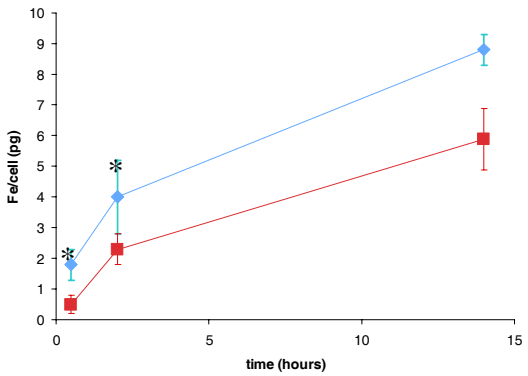


Figure 2: Comparing the uptake of protamine-coated Feridex SPIO nanoparticles into plated HeLa cells with and without magnetofection. Samples were labeled for 0.5 hours, 2 hours and 14 hours. Error bars represent standard deviations at each time point. A T-test was performed comparing the magnetofection and non-magnetofection groups at each time:

0.5 h: p = 0.003, N= 6 (mag), 6 (no mag)
2 h: p = 0.031, N = 5 (mag), 4 (no mag)
14 h: p = 0.107, N = 2 (mag), 2 (no mag)