Developing Cellular MPI: Initial Experience

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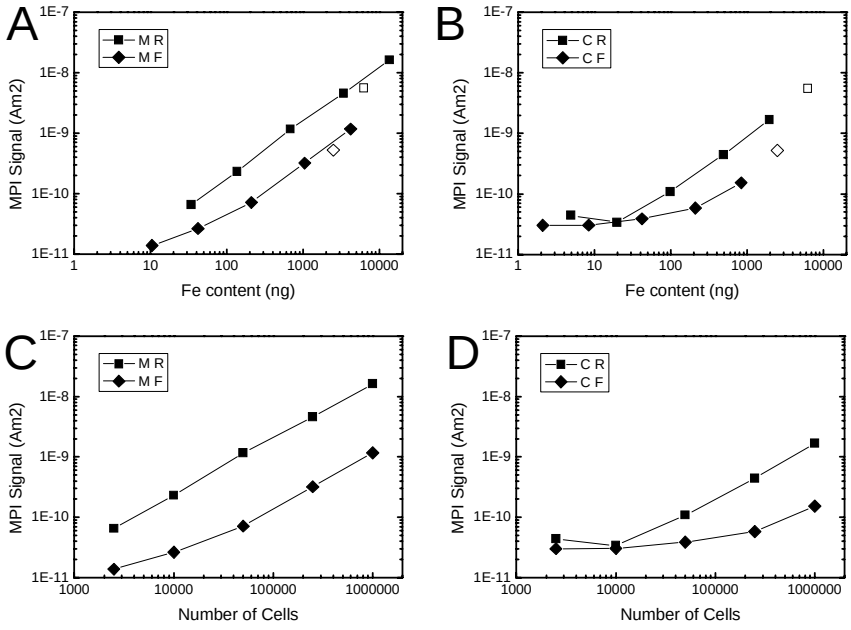
Introduction: MRI cell tracking using superparamagnetic iron oxide particles (SPIO) has found many applications in understanding cell biology and developing cell therapy. However, due to its indirect detection of cells through the SPIO effect on proton relaxation, there are several limitations that prevent its full exploitation. These include 1) the difficulty to absolutely quantify cell concentration and iron content - part of the difficulty relies in the existence of different relaxation regimes (dependent on the agglomeration state and size of SPIO cluster); 2) the difficulty of discriminating SPIO-labeled cells in areas of hemorrhage and traumatic injury (which are often present in targets of cell therapy), as caused by the proton dephasing effects of methemoglobin, ferritin, and hemosiderin (especially at higher fields); 3) the occasional misinterpretation of isolated "black spots" due to differences in magnetic susceptibility effects around blood vessels and air-tissue interfaces (i.e. stomach and GI tract); and 4) the inability to track cells in areas devoid of proton signal (i.e., the lungs). ^{19}F MRI cell tracking can overcome several of these limitations[1] but suffers from inherent lack of sensitivity. In the present study, we have determined the feasibility of developing cellular magnetic particle imaging (MPI). MPI relies on the non-linear response of magnetic material as a direct manner for detecting the presence of an iron oxide nanoparticle agent in an oscillating magnetic field[2]. Spatial encoding can be realized by a static, inhomogeneous magnetic field, saturating the magnetic material almost everywhere except in the vicinity of a special point, the field free point.

Methods: C17.2 neural stem cells (NSCs) and rat mesenchymal stem cells (MSCs) were labeled with the clinical SPIO formulations Resovist (R) or Feridex (F) combined with poly-L-lysine (48 hr incubation at 62.5 - 25 µg Fe/ml culture medium). These two cell types were chosen in order to assess the MPI dependence on cell size and cytoplasmic iron content for smaller cells (NSCs, ~10 µm) and larger cells (MSCs, ~25 µm). Cells were washed, counted, and 50 µl gelatin samples were prepared containing between $2.5x10^3$ and $1x10^6$ cells. The mean cellular iron content was determined using a Ferrozin-based spectrophotometric assay. For MPI analysis, the non-linear magnetic response of labeled and reference (free iron oxide particles in solution) samples was measured in a spectrometer using an oscillating magnetic field (10 mT amplitude, 25 kHz frequency, 30 sec sampling time). After Fourier transformation the intensity of the harmonic signals, i.e. 3rd and 11th, was monitored.

Results: The mean iron content was determined as 13.67 pg Fe/cell for MSCs+R, 4.21 pg Fe/cell for MSCs+F, 1.97 pg Fe/cell for NSCs+R, and 0.84 pg Fe/cell for NSCs+F. Figure 1 shows the MPI signal plots as function of iron content (A,B) and corresponding cell number (C,D). Reference gelatin samples are included for reference (A,B). Except for the lowest amount of the smaller NSCs, which had < 1pg Fe/cell, there was a linear relation between the MPI signal and iron content. Slope values for the 3rd harmonic were $1.2x10^{-3}$ Am^2/g Fe for MSCs+R, which is equivalent to $1.7x10^{-14}$ Am^2 per cell; $2.8x10^{-4}$ Am^2/g Fe for MSCs+F, or $1.2x10^{-15}$ Am^2 per cell; $8.6x10^{-4}$ Am^2/g Fe for NSCs+R; and $1.7x10^{-4}$ Am^2/g Fe for NSCs+F. Note that for both R and F, the reference samples have the same MPI value vs. unit of iron as for the cell samples. For all samples, as compared to Feridex, Resovist had a 4-fold higher MPI efficacy (on 3rd harmonic) per unit of iron. This large difference was observed for both NSCs and MSCs. With the current equipment the efficiency for Fe oxide with infinite susceptibility is $3x10^{-2}$ Am^2/g Fe so that, for MSCs+R, the detection limit for imaging is below 100 cells.

Figure 1: MPI signal measurements of magnetically labeled stem cells. Shown are the signal amplitudes as a function of Fe content (A,B) and the corresponding number of cells (C,D) obtained for the 3rd harmonic. Data are shown for MSCs (A,C) labeled with Resovist (MR) and Feridex (MF), and NSCs (B,D) labeled with Resovist (CR) and Feridex (CF). Reference (free, non-cell bound particles) gelatin samples are included in A,B (open symbols).



Conclusions: Stem cells can be readily detected with MPI at biologically relevant concentrations. MPI enables a linear quantification of both cell number and iron content over a wide range of concentrations, regardless of the state of SPIO as free or intracellular entity. Unlike its use as MRI contrast agent, we found a large difference (4-fold) in MPI efficacy between Feridex and Resovist. While the underlying mechanism is not fully understood, it opens up a new rationale for synthesis and testing of novel magnetic nanoparticles. There appear to be no physical constraints towards developing a whole body human scanner[3], which should encourage further development of cellular MPI given that SPIO formulations can be used that are already in use as clinical MRI cell tracking agents.

References: 1. Ahrens, E.T. et al. *Nature Biotechnol.* **23**, 983-987 (2005); 2. Gleich, B. et al. *Nature* **435**, 1214-1217 (2005); 3. Weizenecker, J. et al. *Phys Med Biol* **52**, 6363-6374 (2007).