Compatibility of Iron Nanoparticle-based MRI Cell Tracking and ^{31}P MRS Bioenergetic Measurements

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Introduction: The MRI visualization and tracking of cells by means of in vitro labeling with superparamagnetic iron oxide (SPIO) nanoparticles has been a subject of intense interest in recent years. In particular, MRI has been used to locate labeled therapeutic cells in vivo, following direct injection into myocardium[1] or during homing toward a stroke lesion following remote injection[2]. Despite these advances, no non-invasive evaluation of the bioenergetic state of these labeled cells has been reported thus far. Moreover, there is a need to evaluate the bioenergetic status of damaged tissue undergoing repair by therapeutic cells over time. ^{31}P MRS offers a highly attractive means for obtaining these physiological data, but the acquisition of usable ^{31}P NMR data is expected to be hampered by the broadening effect induced by the presence of intracellular SPIO particles in close vicinity to the metabolites of interest. Indeed, the same paramagnetic effect that enables relatively small clusters of iron-labeled cells to be detected in a T_2*-weighted ^{1}H MRI scan might render important species such as ATP and PCr totally invisible in a ^{31}P NMR spectrum of the same subject. It is the goal of the present work to evaluate the feasibility of obtaining bioenergetic data via ^{31}P MRS for a population of SPIO-labeled cells growing in a MR-compatible perfusion chamber.

Methods: Two cell lines were used in these experiments: HeLa cells and C2C12 mouse myoblasts, a potential therapeutic line that has demonstrated myotube formation in vitro in response to reduced serum concentration[3]. SPIO nanoparticles (Feridex, Berlex) were initially coated with the transfection agent Lipofectamine 2000 (Invitrogen, 20µl/mg Fe). Cells were then incubated overnight in OptiMEM I media (Invitrogen) containing a coated SPIO suspension at a concentration of 20 µg Fe/ml and 3×10^4 cells/cm^2. Unincorporated iron particles were removed by repeated centrifugation and washing. Samples of the labeled cells were set aside for evaluation of viability by Trypan blue exclusion, apoptosis by flow cytometry with annexin-V/PI staining, iron particle uptake by Prussian blue staining and myotube formation by light microscopy. The remaining cells (ca. 20×10^6 per experiment) were suspended in a 1.2% solution of sodium alginate (Sigma). This suspension was added dropwise to 100 mM $CaCl_2$ to form beads of diameter ca. 2 mm. Beads were washed, suspended in DMEM media containing 20% (C2C12) or 5% (HeLa) fetal bovine serum (FBS) and packed into a MR-compatible perfusion system consisting of a 10 mm screw-top NMR tube fitted with porous Teflon plates (Small Parts, Inc.) threaded onto a central PEEK media inlet tube. A 1 mm diameter glass capillary containing 0.4 M MDP (Sigma) was inserted into the central tube as a ^{31}P NMR standard. Media was circulated through this system via a peristaltic pump using a loop of gas-permeable silicone tubing enclosed in a 95% air: 5% CO_2 v/v atmosphere. The temperature of the media reservoir and NMR tube were maintained at 37.0 +/- 0.1°C via a water bath and the NMR probe heater system, respectively. ^{1}H MRI and ^{31}P MRS data were acquired for the perfused cells beads using a Bruker DMX400 spectrometer equipped with a three-axis shielded gradient set and 30 mm ^{1}H birdcage resonator or 10 mm ^{1}H/X broadband probe, respectively. Prior to the acquisition of each image or spectrum, global shimming was performed on the ^{1}H signal from H_2O. ^{1}H gradient echo images were acquired with a single, 0.5 mm sagittal slice, 3.0×1.2 cm FOV (V×H), 256^2 MTX, TR=3s, TE=1.5 ms and NEX=1. Unlocalized ^{31}P NMR spectra were acquired using a one-pulse sequence with TR=3s, FA=90°, SW=10 kHz, and 27K averages (60 blocks X 450 scans). ^{31}P data were zero-filled from 4K to 8K complex points and exponentially multiplied with LB=12 Hz prior to Fourier transformation. Following spline baseline correction and chemical shift calibration with MDP=0 ppm, Lorentzian deconvolution was performed to obtain widths and areas for each of the major spectral peaks. No corrections for saturation effects were performed. Quantitative data are presented as mean ± std. dev. Comparisons between unlabeled (N=4) and labeled (N=3) C2C12 preparations were made using a two-tailed unpaired Student's T-test. After MR scanning, cells were released from beads by exposure to 25 mM EDTA for 10 min. The Trypan blue and flow cytometry assays were then repeated. Iron loading histograms were calculated by counting dark pixels per cell ROI in Prussian blue images using MetaMorph™.

Results: Trypan blue staining indicated at least 93% cell viability before and after each experiment. At most 12% of cells were apoptotic in each preparation, and this percentage was unaffected by labeling or overnight perfusion in alginate beads. Iron-labeled and unlabeled C2C12 cells showed similar myotube formation following culture in media with 5% FBS. No significant difference was observed in water ^{1}H linewidth (48±7 vs 53±16 Hz). Gradient echo images (Fig. 1) clearly showed excellent contrast for beads containing SPIO-labeled cells despite the short TE used. In Fig. 2, representative ^{31}P spectra of labeled (left) and unlabeled (right) C2C12 cell populations are shown. Iron-labeled cells exhibited broader metabolite peaks than unlabeled cells, but all major peaks are still visible and adequately-resolved. Significant differences between labeled and unlabeled samples were observed for γ, α and β-NTP linewidths (51±3 vs 147±71 Hz, p=0.038; 52±5 vs 112±14 Hz, p<0.001; 71±13 vs 127±35 Hz, p=0.029). Peak height ratios relative to MDP for all three NTP peaks showed a trend toward smaller values in labeled samples, but this difference was not significant (p=0.059; 0.061; 0.068). A similar, though less pronounced trend was observed for NTP/MDP peak area ratios. No significant differences were observed in Pi, PCr or NTP chemical shifts. For HeLa cells (Fig. 3), however, linewidths did not increase appreciably with labeling yet peak height and area ratios decreased markedly. Light micrographs of Prussian blue-stained labeled HeLa cells (Fig. 4 top, 300X) demonstrated a very inhomogeneous distribution of SPIO particles among cells, as shown in the histogram below.

Discussion: We have demonstrated that usable ^{31}P NMR spectra can be obtained from immobilized, perfused cells despite the presence of intracellular SPIO particles at a concentration commonly used for visualization and tracking by ^{1}H MRI. The fact that metabolite peaks are not broadened excessively suggests that at least some subpopulation of cells is only lightly labeled. This seems particularly true for the HeLa cells, where the lack of appreciable broadening and decrease in peak area ratios suggests that only sparsely-labeled cells may be visible, while more heavily-labeled cells do not contribute to the observed ^{31}P spectrum. We conclude that the interpretation of ^{31}P NMR spectra of iron-labeled cells will probably require assessment of the iron loading distribution or new labeling methods which ensure homogeneous loading.

Fig. 1

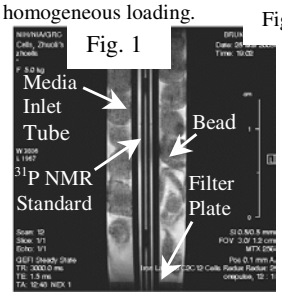
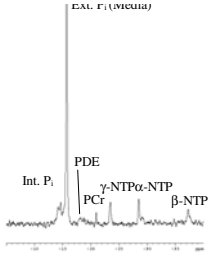


Fig. 2: C2C12 Unlabeled



C2C12 Labeled

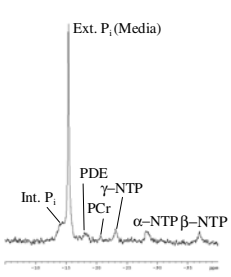
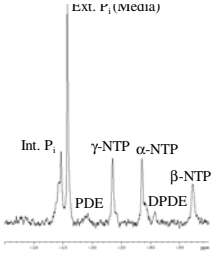
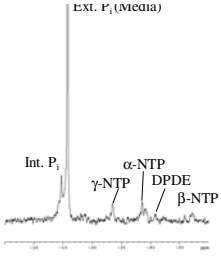
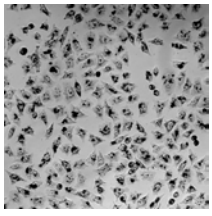


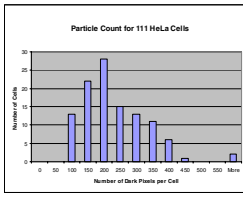
Fig. 3: HeLa Unlabeled



HeLa Labeled







References: 1. Jendelová P et al, J Neurosci Res 76:232-243 (2004); 2. Himes N et al, Magn Reson Med 52:1214-1219 (2004).
3. Yaffe D, Saxel O, Nature 270:725-727 (1977).