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IN VIVO TRACKING OF HUMAN NEURAL STEM CELLS IN THE MOUSE BRAIN WITH 19F MRI

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Introduction: MRI is a promising tool for monitoring stem cell based therapy. Conventionally, cells are loaded *in vitro* with iron oxide particles, which appear hypointense on ^{1}H MR images. However, the cell signal is not specific due to ambiguous background and not quantitative. An emerging field to overcome these drawbacks is ^{19}F MRI of cells labeled with perfluorocarbon (PFC) emulsions [1]. Fluorine is virtually absent in the body, i.e. only labeled cells generate contrast, and quantification is possible as the signal is proportional to the concentration of ^{19}F. Here it is shown, for the first time, that human neural stem cells (hNSCs), which have shown high therapeutic potential for brain pathology [2], can be labeled with PFCs, detected and quantified in vitro and after brain implantation.

Methods: hNSCs were incubated with a PFC emulsion (CELSENSE 1000, Celsense, USA), viability was assessed with trypan blue exclusion, and immunocytochemistry was carried out to evaluate the influence of the label on cell phenotypes. For *in vitro* MRI, hNSCs were suspended in gelatin at varying densities. For *in vivo* experiments, 150.000-300.000 labeled hNSCs or non-labeled controls were implanted into the striatum of mice (n=4). Animals were scanned with ^{1}H MRI and ^{19}F MRI (total time<1.5 h, 400x400x1000 μm^3 ^{19}F image resolution) on an 11.7 T scanner (Bruker BioSpec, Ettlingen, Germany) and transcardially perfused after the last MRI session.

Results: hNSCs were labeled most efficiently at a concentration of 80 µl emulsion/ml medium. The labeling lead to a decrease of cell viability compared to controls directly after incubation (83±4 % vs. 93±7 % living cells, $p<0.05$), which renormalized after 7 days in culture of the replated cells. We did not detect label-related changes in the numbers of Ki67, nestin, GFAP, or ßIII-tubulin+ cells during both, proliferation and differentiation. The dilution series indicated that 1.000 hNSCs needed to accumulate in one image voxel to generate significant signal to noise ratio (SNR) in vitro. We detected grafts in vivo with ^{19}F MRI over several days but not the controls. From SNR analysis we estimate a detection limit of a few 10.000 cells/voxel. The location and density of human cells (hunu+) on histological sections correlated well with observations in the ^{19}F MR images.

Conclusion: Our results show that hNSCs can be labeled with ^{19}F with little effects on viability or proliferation and differentiation capacity. Our optimized settings allowed higher resolution and a lower detection limit than in other reports on MRI of ^{19}F labeled cells. This proves for the first time that ^{19}F MRI can be utilized for cell tracking in brain implantation studies, in which the graft may be initially small or diluted through cell migration induced by the damage.

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