Ferumoxytol labeling of human neural progenitor cells for tracking with MRI in the porcine spinal cord

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Purpose/Target Audience: Multiple clinical investigations of cell-based therapies transplanted in to the spinal cord are underway for a range of neurological diseases. Accurate assessment of clinical outcomes and therapeutic efficacy is complicated by the unknown fate of transplanted cell grafts, secondary to limited evidence confirming graft delivery and survival. We propose a straightforward, clinically relevant method to pre-label transplanted cell grafts with ferumoxytol, a Superparamagnetic Iron Oxide Nanoparticle (SPION), for *in vivo* graft tracking in the porcine spinal cord. The purpose of this study is to track labeled cell grafts directly injected in to the large animal spinal cord using a clinical MR scanner and correlate these findings with post-mortem histology.

Methods: Human neural progenitor cells (Klein et al. 2005 *Hum Gene Ther*) cultured as non-adherent neurospheres were incubated with increasing concentrations of ferumoxytol [0, 100, 200, 400, and 1000 ug/mL] for seven days following mechanical passage. The neurospheres were dissociated to single cells, washed, and then assessed for viability with trypan blue, *in vitro* MR contrast, cellular internalization of ferumoxytol with Transmission Electron Microscopy (TEM), and differentiation capacity with immunocytochemistry. Ferumoxtyol-labeled ([200] and [400]) and unlabeled [0] cell grafts of 2.5e5 cells/graft were transplanted in to the thoracolumbar spinal cord of Gottingen minipigs with non-traumatic intraspinal microinjection following laminectomy. The spinal cord was imaged with whole body 3T MRI pre-operatively and on post-operative day (POD) 14 (n = 9) and POD 28 (n = 6) using a transverse T2*-weighted gradient echo sequence. Images were reformatted in to coronal sections using 3D slicer (Fedorov et al. 2012 *Magn Reson Imaging*). Three (3) pigs were sacrificed at POD14 and spinal cords were harvested, sectioned and stained with an antibody specific to the human nuclear antigen to confirm graft survival.

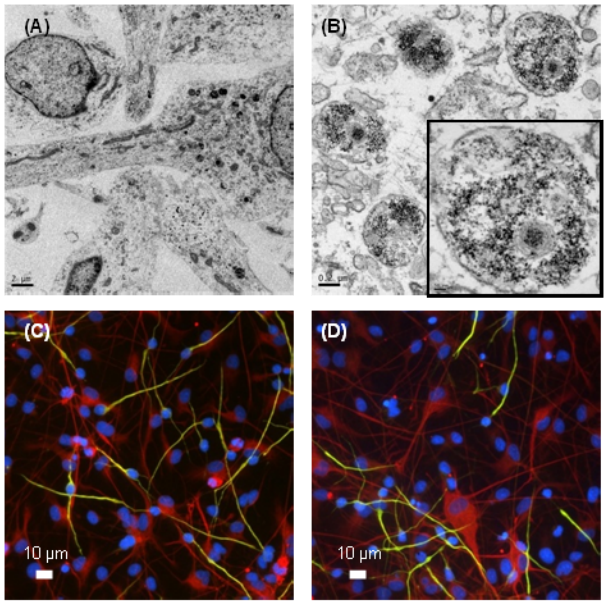

Figure 1: In vitro analysis of ferumoxytol-labeled cells. TEM demonstrates labeled cells [200] and [400] contain numerous lysosomes **(A)** with ferumoxytol nanoparticles **(B + Insert)**. Immunocytochemistry of differentiated cells demonstrates numerous GFAP positive astrocytes (red) and β-tubulin III positive neurons (green) in unlabeled **(C)** and labeled **(D)** cells.

Results/Discussion: A dose-dependent decrease in cell viability was observed at [1000] ($p < 0.05$). A significant increase of *in vitro* MR contrast was observed at concentrations of ferumoxytol [200] and above ($p < 0.05$). Based on this data, [0], [200], and [400] incubation conditions were chosen for further evaluation and transplantation. Ferumoxytol nanoparticles were observed in labeled [200] and [400], but not unlabeled [0] cells with TEM (Figure 1). Differentiation to astrocytes and neurons for labeled and unlabeled cells was confirmed (Figure 1). Ferumoxytol-labeled cell grafts of both [200] and [400] were identified *in vivo* with MRI as hypointense foci on POD14 and POD28 (Figure 2). No signal changes were observed in the areas of unlabeled cell grafts. Unlabeled and labeled [400] and [200] cell engraftment and survival at POD14 was confirmed with immunohistochemistry (Figure 2).

Conclusion: We demonstrated a clinically relevant, rapidly translatable approach for tracking intraspinal cell grafts in a large animal model. This approach could allow clinicians to assess the delivery of cell-based therapeutics *in vivo* for upcoming clinical trials in the spinal cord. Future work will include long-term tracking, quantitative imaging, and quantitative post-mortem staining including Iron.

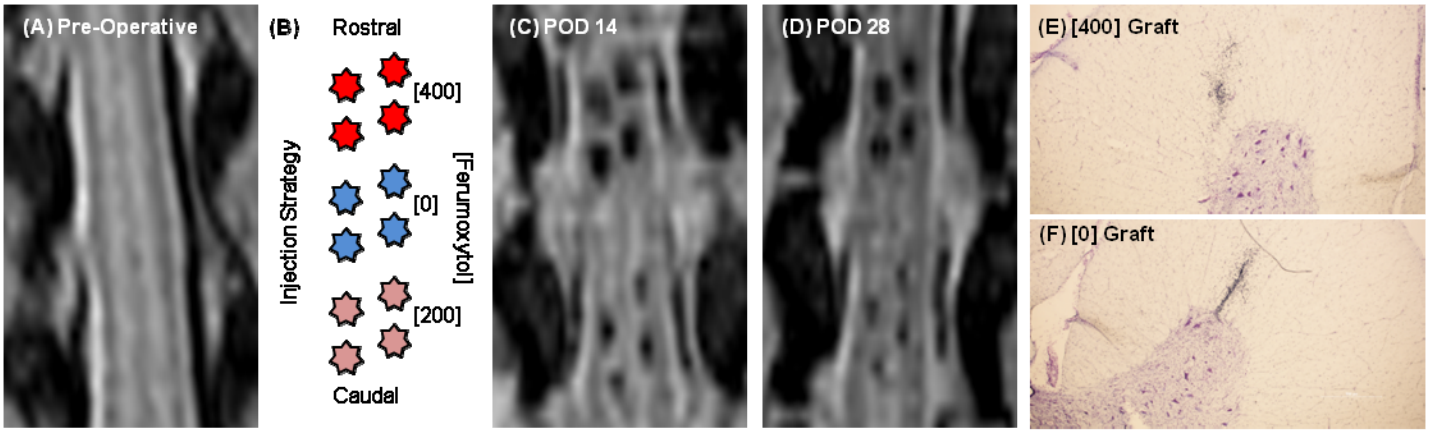

Figure 2: In vivo MR-tracking and post-mortem identification of ferumoxytol-labeled grafts in the spinal cord. Coronal T2*-weighted gradient echo image before transplantation demonstrates normal spinal cord anatomy **(A)**. Unlabeled [0] and ferumoxytol-labeled [200] and [400] 2.5e5 cell grafts were transplanted in to the pig spinal cord bilaterally **(B)**. Hypointense foci, representing grafted labeled cells, are observed on post-operative day (POD)14 **(C)** and 28 **(D)**. Unlabeled cell grafts were not visualized. Post-mortem immunohistochemistry at POD14 shows a transplanted labeled [400] **(E)** and unlabeled [0] **(F)** human cell graft (black - human nucleus) integrated in to the pig tissue (purple - cresyl violet).