Superparamagnetic iron oxide labelling for in vivo follow-up of hRPE cell implants in non-human primate

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Introduction

Recently, intrastriatal implantation of human retinal pigment epithelial (hRPE) cells attached to gelatin microcarriers (GM) has been shown to produce long-term motor improvement in Parkinson's disease (PD) patients as well as in rodent and monkey models of PD. Studying the mechanism and efficacy of this therapy requires in vivo identification and the follow-up of survival of the implanted hRPE cell over extended periods of time. Recently, cell labelling methods using superparamagnetic iron oxide (SPIO) nanoparticles have been implemented to allow the in vivo tracking of transplanted cells through MRI [1]. We present here the results of a preliminary study to assess the safety and efficacy of SPIO labelling RPE cells for non invasive imaging of the labelled hRPE cells using MRI.

Methods

RPE19 cells were labelled using the SPIO nanoparticle suspension Feridex (FE) complexed to varying concentrations of the transfection agent protamine sulfate (Pro) [2]. For in vitro studies, 3 groups of hRPE cells (12,500-25,000 cells each) were transfected with 50 µg/ml FE using varying concentrations of Pro (0-4.5 µl/ml) and sandwiched between 6% gelatine layers. Live cells of the highest Pro content (4.5 µl/ml) were assessed for short term changes in cell viability and doubling time.

For in vivo experiments, FE-Pro labelled RPE19 cells were attached to GM using previously described methods [3]. One normal monkey was implanted with 3 injections of non-labelled RPE19-GM cells and 3 injections of FE-Pro labelled RPE19-GM cells in opposite hemispheres. The monkey was imaged at 3 and 7 weeks post implant, and subsequently sacrificed for post-mortem immunohistochemistry. MRI experiments were carried on a 3T clinical scanner (Philips, The Nethrlands) using Fast Field Echo sequence (FOV = 10 cm, 320x320 matrix, TR/TE = 10/400 ms, slice thickness = 1 mm).

Results and Discussion

Live-cell viability assessment demonstrated no differences in cell viability or cell doubling time between non-labelled and FE-Pro labelled RPE19 cells. MR images of in vitro RPE19 cell samples (Fig.1) clearly showed differences in image intensity depending on both cell and Pro concentrations. Figures 2 shows gradient echo cross-sections of the implant site 7 weeks post-implantation. FE-Pro labelled cells are identified as hypo-intense regions in the right striatum surrounded by a characteristic "blooming" artifact. Note there are minimal hypo-intense regions in the contralateral non-labelled hemisphere likely due to the formation of scar tissue. Figure 3 demonstrates post-mortem identification of intracellular iron inclusions through Prussian blue histochemistry, confirming the presence of FE-Pro labeled RPE19 cells at the implant site.

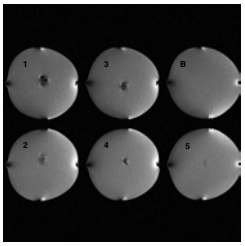


Figure 1. In vitro MRI image of FE-Pro labelled RPE19 cells in 6 wells. FE:Pro and cell concentrations are as follows:
1. 50:4.5 µg/ml; 25,000 cells.
2. 50:4.5 µg/ml; 12,500 cells.
3. 50:3 µg/ml; 25,000 cells.
4. 50:3 µg/ml; 12,500 cells.
5. 50:1.5 µg/ml; 12,500 cells.
B. Blank

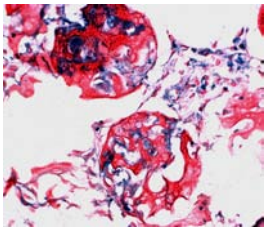
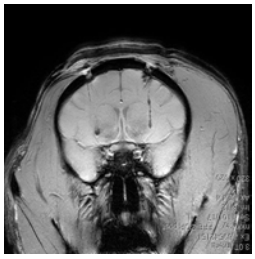
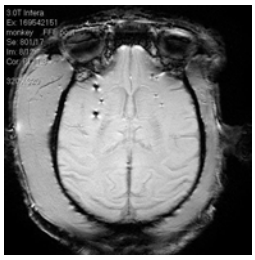


Figure 3. 40x micrograph showing post-mortem PB staining of FE-Pro RPE19 – GM 7 weeks post implant. Numerous blue intracellular iron inclusions can be identified





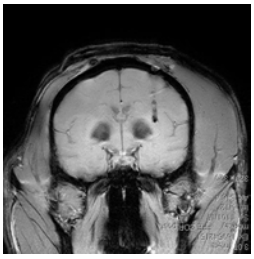


Figure 2. MRI showing the appearance of FE-Pro labelled and non-labelled RPE19 cells in the monkey 7 weeks post implant. Panels show coronal and axial cross sections. Note dark hypo-intense regions in the caudate-putamen at the level of the implant sites.

Conclusions

These preliminary studies show both the safety and efficacy of SPIO labelling for non invasive MR imaging. This technique may prove to be a valuable method to follow the in vivo survival of RPE cells in a new cell therapy technique for PD.

Acknowledgements

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References: [1] Frank JA, et al. *Radiology*, 2003: **228**, 480–487; [2] Arbab AS, et al. *Blood* 2004: **104**, 1217-1223; [3] Doudet DJ, et al. *Exp Neurol*, 2004: **189**, 361-368;