GFP-Transfected Autologous Schwann Cells are Rejected after Transplantation in the SCI Minipig Model

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Introduction - Schwann cells have shown substantial evidence of salutatory effects after implantation into damaged central nervous tissues [1-2]. Transplantation of autologous peripheral nerve derived Schwann cells (aSC) into spinal cord injuries (SCI) are currently being evaluated in a human clinical trial (www.clinicaltrials.gov NCT01739023). The Yucatan minipig model provided a pivotal pre-clinical platform for human studies as a closer approximation to human dimensions [3-5], particularly regarding the study of cell survival. In this work, we test if *ex vivo* expanded aSC are immunogenic after implantation and/or if the green fluorescent protein (GFP) viral vector commonly used in our studies caused an immune response towards the transplanted aSC. The spinal cord (SC) tissues were evaluated with high resolution high field MRI and histological studies.

Experimental - Five female Yucatan minipigs underwent 3 left hemi-contusion SCIs at T6, T9 and T12. A nerve was extracted for cell preparations which were divided into GFP transfected (GFP-aSC) and non-transfected (aSC) groups. 52 days after SCI, cells were injected at the injury sites (100,000 cell/μL) to fill the entire cavities. 70 days after transplantation, animals were euthanized, perfused and the SCs dissected. For *ex vivo* MRI, tissues were washed in 1X PBS following immersion in Fluorinert. Using the 11.75-T magnet at the FSU-FAMU College of Engineering, T_2*-weighted, 3D gradient recalled echo (GRE) sequences were acquired at 80-μm isotropic resolution. The tissues then were prepared for immunohistochemistry analysis.

Results and Discussion - In Fig 1, *ex vivo* MRI defines the left-sided hemi-contusion boundaries using a 3D GRE sequence (TE/TR = 15/ 250 ms) at 11.75 T; comparative H&E and Luxol staining displays outstanding correlation with the MRI. The hypointense signal in MRI corresponds to the macrophage filled cavity created from the contusion; although the effect from the GFP-aSC is not obvious, the hyperintense signals clearly define the transplant site (arrows). In Fig 2, sections have been immunostained for GFP, astrocytes (GFAP), Schwann cells (p75) and a nuclear label (Hoechst). GFP-aSC show an irregular pattern likely related to monocyte engulfment, lack of p75 cells and massive cellular infiltration separating the graft from the astroglial margin; aSC without GFP, appear healthy, spread through the cavity and confluent with the glial margins.

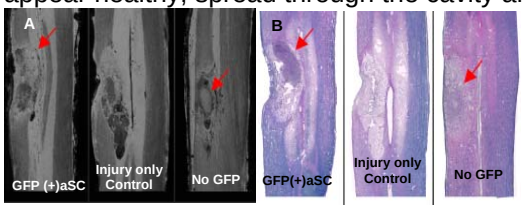

Fig 1A: Ex vivo MRI of the SC showing the SCI.
B: corresponding images stained for H&E/Luxol.

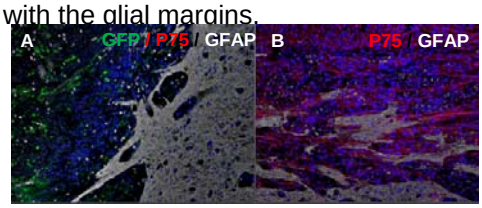

Fig 2: Transplant organization of the aSC in the astrocytic margin showing low survival of GFP-aSC compared to aSC without GFP. Green-GFP, Red-P75

Conclusions

This study demonstrates that high resolution, high field MRI of excised Yucatan minipig SC with SCI showed excellent correlation with histological analysis of the injury. Histological results demonstrated a lymphocytic reaction against GFP(+)aSC compared to a lesser or no response to GFP(-)aSC, which also had superior survival and integration. Future work involves acquiring diffusion and tractograhy (DTI) sequences as well as labeling aSC with an MRI contrast agent to better evaluate the transplants (the only feasible tool for human application).

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