Evaluating the effect of a neurotrophin-loaded collagen hydrogel on engraftment of RM3.5 iPSC-derived dopaminergic progenitors in immunosuppressed hemi-parkinsonian rats

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THE MICHAEL J. FOX FOUNDATION FOR PARKINSON'S RESEARCH

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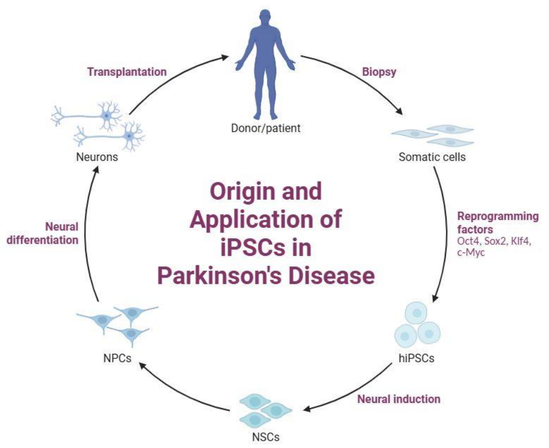
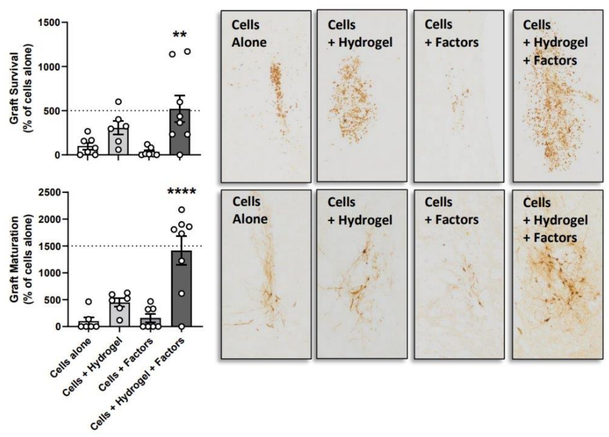
Background

Current treatments for Parkinson's disease (PD) are not curative and solely target symptoms[1]. Cell-based brain repair, particularly using induced pluripotent stem cells (iPSCs), is a promising avenue for the treatment of PD.

iPSCs are useful in the context of PD as they are taken from an adult human, bypassing ethical concerns associated with their embryonic counterparts [2,3].

The adult human cells are then reprogrammed to a pluripotent state using transcription factors and can therefore be differentiated to different lineages, for example, neuronal lineages [3,4]. Once differentiated they are suitable for transplantation. In this particular study the iPSCs were differentiated to dopaminergic neuronal progenitors (iPSC-DAPs).





Engraftment of these cells in the neurotrophin-depleted environment of Parkinsonian brains remains a hurdle [4]. However, a previous study by the Dowd group have shown that a collagen hydrogel enriched with glial-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) significantly improves the survival and maturation of NAS2 iPSC-DAPs in athymic nude rats[5]. These results are yet to be replicated in immunocompetent animals. Due to the NAS2 cell line displaying poor survival, the RM3.5 cell line is being investigated instead, which has more favourable survival across studies[6]. This is the first time the RM3.5 cell line has been transplanted with the collagen hydrogel.

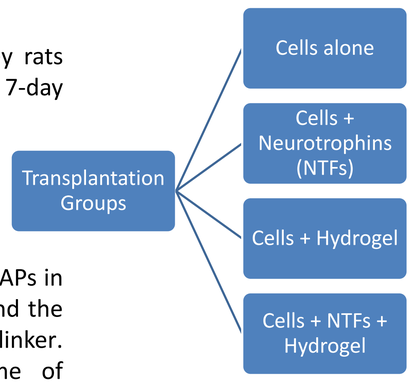
Aims

The aim of this study was to determine the effect of the neurotrophin-loaded hydrogel on engraftment of RM3.5 iPSC-DAPs in cyclosporine preconditioned rats.

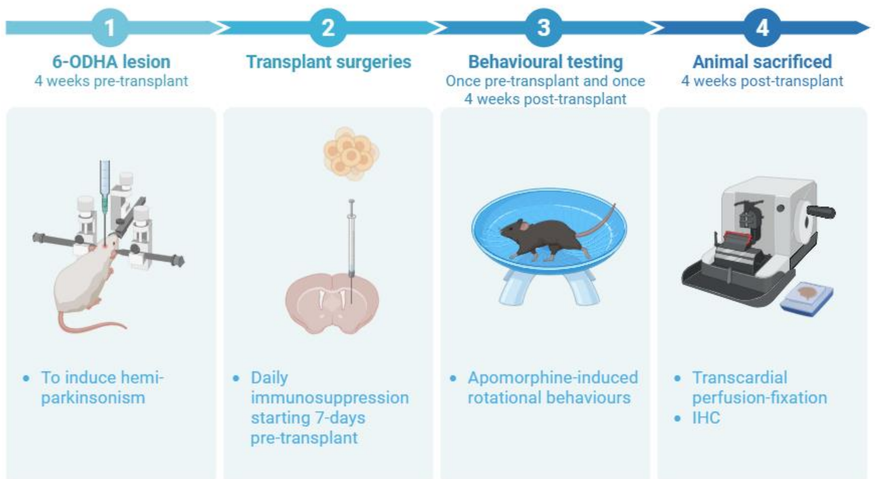
Methods

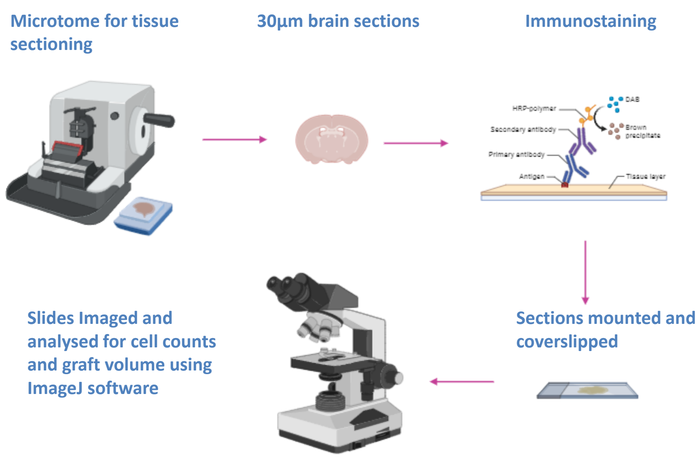
This 4-week transplantation study included 24 Sprague-Dawley rats undergoing daily cyclosporine immunosuppression including a 7-day pre-conditioning period prior to cell transplantation.

There were 4 transplant groups (n=6/group)



Each intra-striatal transplant consisted of 100,000 RM3.5 iPSC-DAPs in transplantation media. The NTFs used were BDNF and GDNF and the Hydrogel consisted of Bovine Type I Collagen and starPEG crosslinker. iPSC-DAPs were at day 16 of differentiation at the time of transplantation.





Histological analysis was performed on the brains post-mortem using free-floating immunohistochemistry (IHC) for:

- **Cell survival** - Human Nuclear (HuNu+) staining
- **Differentiation to Dopaminergic neurons** – Tyrosine Hydroxylase (TH+) staining
- **Graft Volume** – Human cell cytoplasm (STEM121+) staining

Results – *In Vitro*

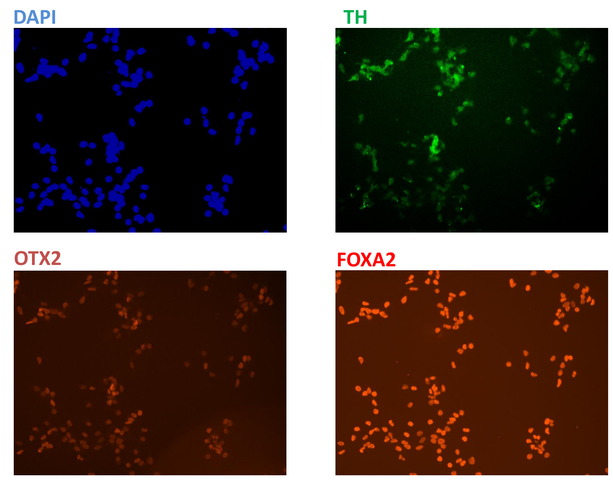


Figure 1: Immunocytochemistry

Leftover iPSC-DAPs from transplants were plated and brought to day 23 of differentiation. Cells were stained for DAPI, TH, OTX2 and FOXA2 using fluorescent immunocytochemistry. Cells were 93% double positive for OTX2 and FOXA2 (markers of differentiation) and 39% positive for TH (dopaminergic neuronal marker) indicating the cells were correctly differentiating down the dopaminergic neuronal lineage.

Results - Behavioural

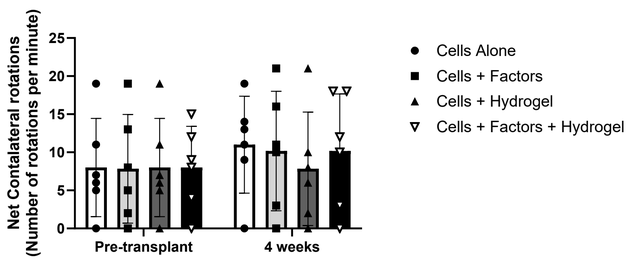


Figure 2: Apomorphine-induced rotational behaviours

Contralateral rotations are indicative of the lesion causing motor defects, a characteristic of the 6-ODHA Parkinson's model. Neither the iPSC-DAPs nor the Hydrogel treatment improved rotational behaviours at 4 weeks – the cells had likely not differentiated fully to mature dopamine neurons by this time.

F (1.654, 16.54) = 0.3264, P>0.05

Results - Histology

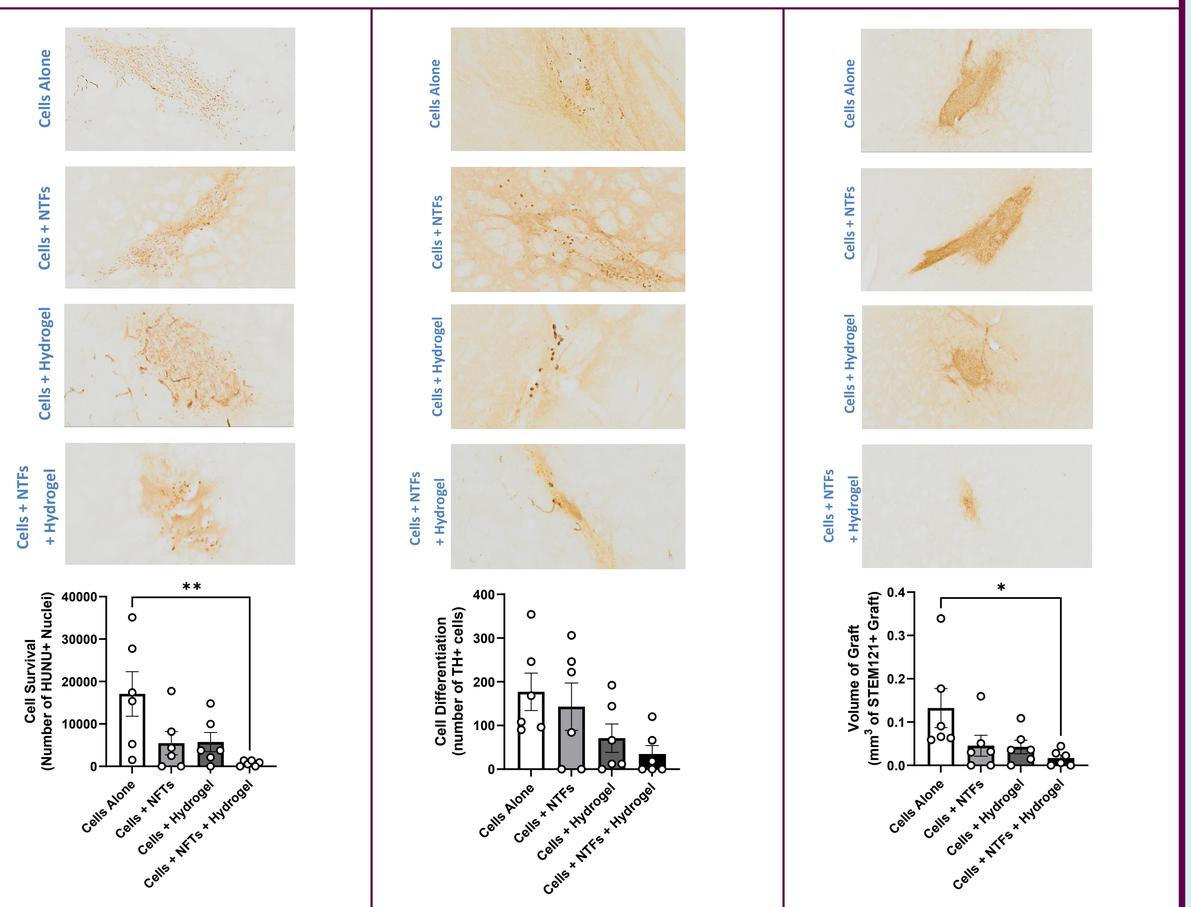


Figure 3: Cell Survival

HUNU+ staining revealed good survival when the cells were transplanted alone but the NTFs and Hydrogel unfortunately did not improve this survival. Survival in the Cells + NTFs + Hydrogel group was poor. Representative images show examples of HUNU+ grafts in each group

F (3, 20) = 4.795, P<0.05

Figure 4: Cell Differentiation

TH+ staining showed low levels of differentiation to dopaminergic neurons overall, with no significant effect of hydrogel treatment in this 4 week study. Representative images show low amounts of TH+ cells in each group.

F (3, 20) = 2.742, P>0.05

Figure 5: Volume of Graft Survival

Stem121+ staining for human cytoplasm revealed the volume of the grafts in each group. Reflecting the results of the HUNU+ staining, the survival is seen to be poor in the Cells + NTFs + Hydrogel group in comparison to Cells Alone. Representative images show the volume of the Grafts in each group.

F (3, 20) = 3.478, P<0.05

Conclusions and Future Directions

The 6-ODHA model successfully induced motor defects in the animals which is a hallmark of Parkinson's disease. These motor defects were not improved by either the RM3.5 cells or the NTF-enriched hydrogel. However, 4 weeks post-transplant is a very early timepoint and it would be unexpected to see behavioural improvements that early with iPSC-DAPs. The overall cell survival of the study was satisfactory with the exception of the Cells + NTFs + Hydrogel group. The results did not reflect that of the athymic rat study[5]. There was some evidence of cell differentiation to dopaminergic neurons across all groups, but this was low, as would be expected at this early post-transplantation timepoint. A longer study would better ascertain the differentiation capacity of these cells *in vivo*.

This study warrants further investigation to see if the results of the athymic study can be replicated with the RM3.5 iPSC-DAPs. The best way to asses this is to repeat the above study in athymic rats or to alter the immunosuppression regime in immunocompetent animals. Short-term studies will be the best way to assess if changing factors such as the immunosuppressant drug improves cell survival with the NTF-enriched hydrogel.

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