Short-term compatibility assessment of cryogel microcarriers for improving the engraftment of human iPSC-derived dopaminergic progenitors in immunosuppressed Sprague-Dawley rats

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Introduction

Cellular based brain repair is a therapeutic option to treat Parkinson's disease (PD) but engraftment remains limited with poor survival and differentiation of Induced Pluripotent Stem cells (iPSCs) *in situ* in the brain[1]. Cryogel microcarriers can potentially aid with engraftment of iPSCs. The microcarriers bind GDNF and BDNF, releasing them in a controlled manner to aid the growth of cells[2].

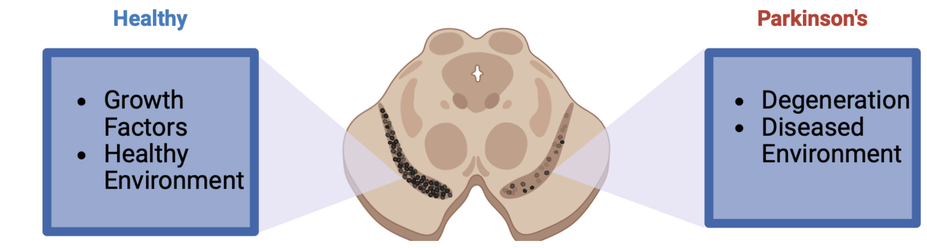


Figure 1 – iPSCs struggle to survive in the Parkinsonian brain

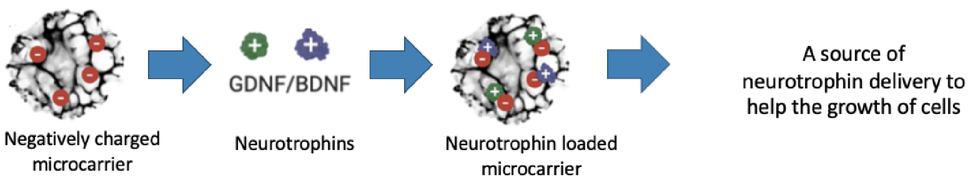


Figure 2 – Microcarriers can bind and release neurotrophins

Hypothesis and Objectives

Aim: To improve the engraftment of iPSC-derived dopaminergic progenitors[3].
Hypothesis: Microcarriers are cytocompatible with iPSC-DAPs and therefore can improve the survival of iPSC-DAPs *in situ* in the brain

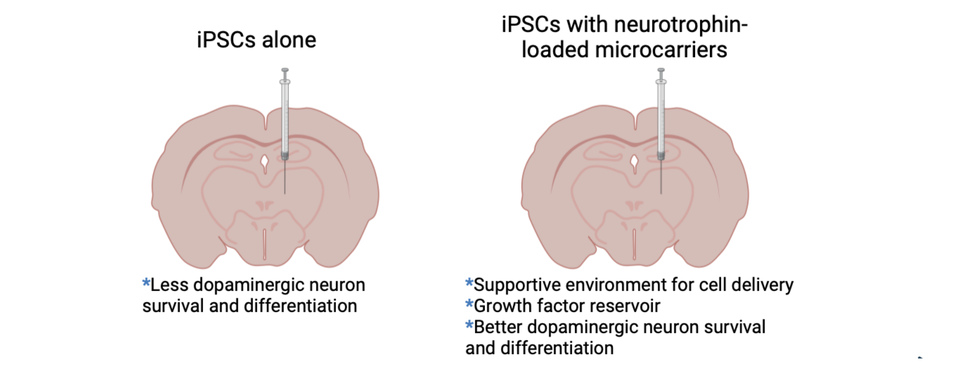


Figure 3 – Transplanting microcarriers with iPSCs provides growth factors to aid the growth of cells

Methodology

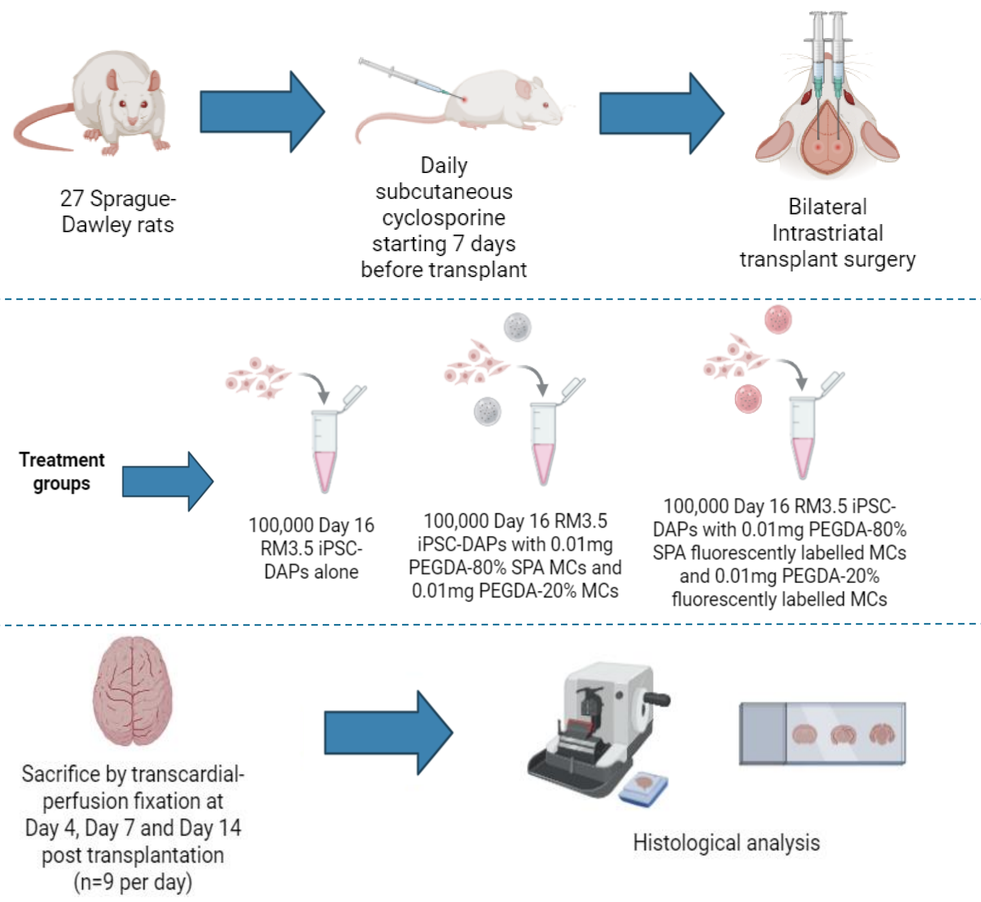


Figure 4 - The microcarriers were evaluated *in vivo* using female cyclosporine-immunosuppressed Sprague Dawley rats[4]

Results

Immunocytochemistry

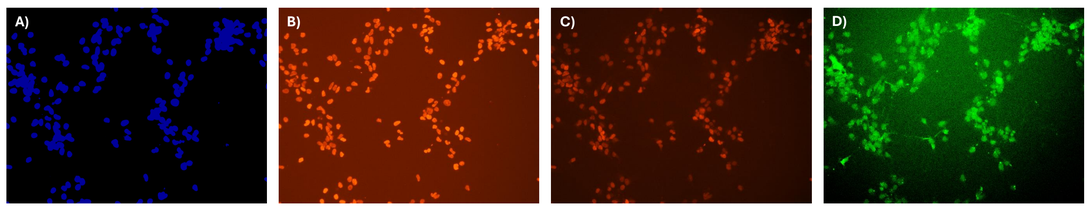
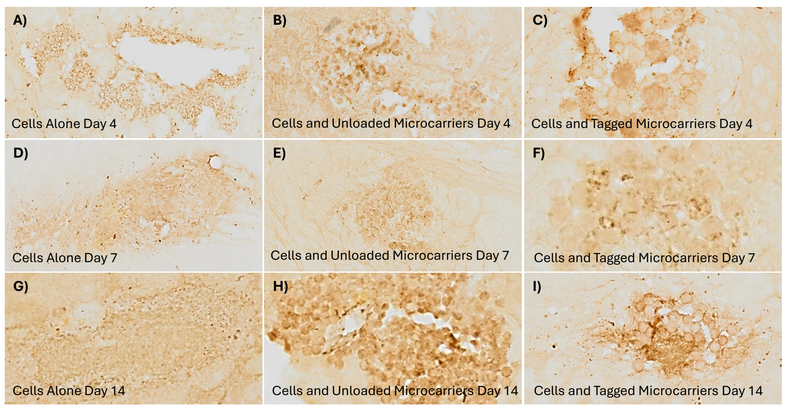


Figure 5 - The differentiation of the iPSC-DAPs (Day 23) was evaluated *in vitro.* The cells were stained with A) DAPI , B) FOXA2, C) OTX2 and D) TH.

HuNu



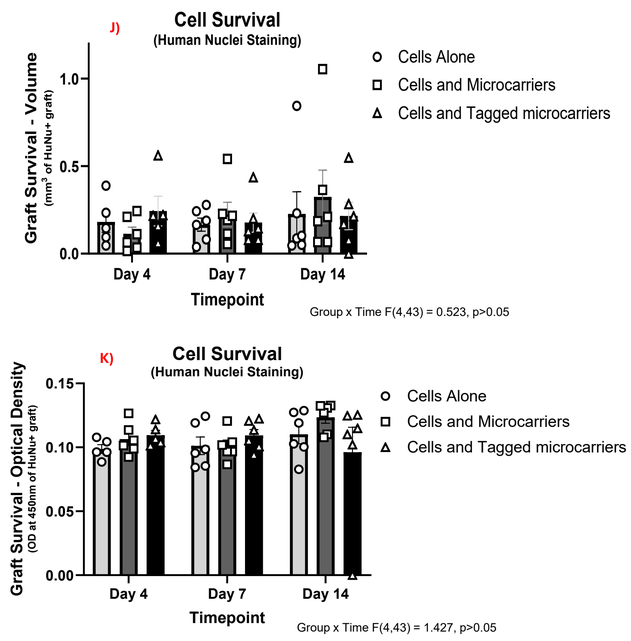
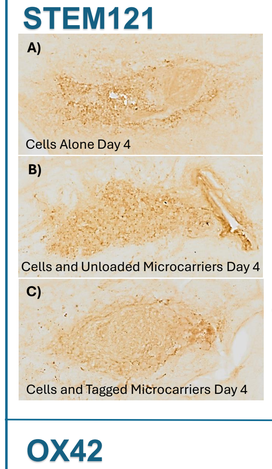


Figure 6 – Cell survival was assessed via Human Nuclei staining. Representative images are shown for each treatment group and timepoint (A-I). There were no significant differences for J) Volume of the HuNu graft or K) Optical Density of the graft. Data is expressed as mean ± SEM and was analysed via a two-way ANOVA with multiple comparisons.

STEM121



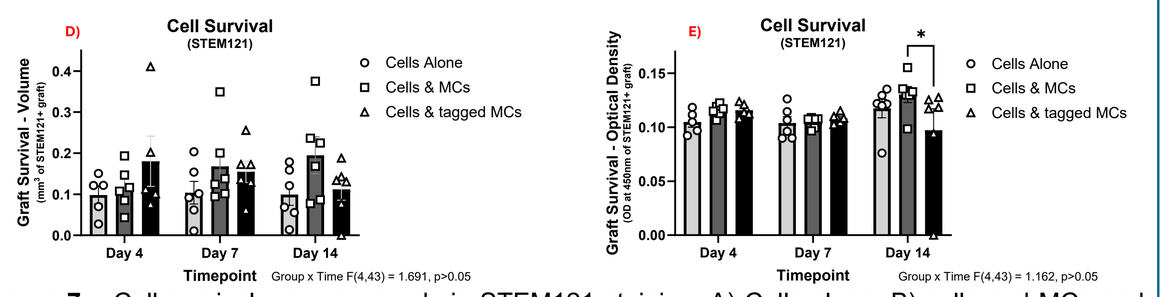
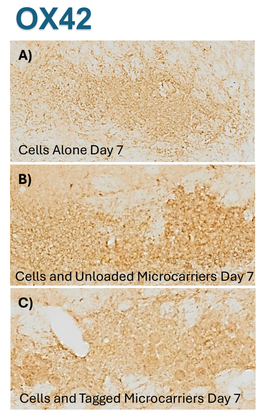


Figure 7 – Cell survival was assessed via STEM121 staining, A) Cells alone, B) cells and MCs and C) cells and tagged MCs. There was no significant differences for volume (D). Cells and MCs had a higher density than cells and tagged MCs at day 14 (E). Data is expressed as mean ± SEM and was analysed via a two-way ANOVA with multiple comparisons.

OX42



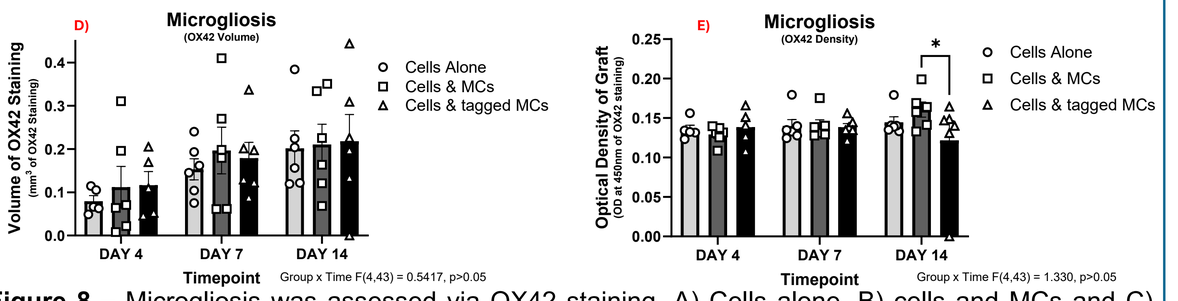
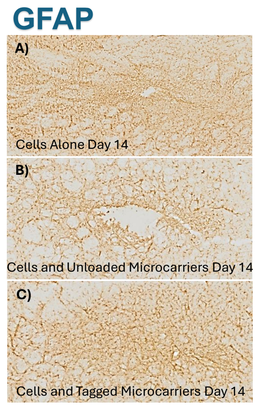


Figure 8 – Microgliosis was assessed via OX42 staining, A) Cells alone, B) cells and MCs and C) cells and tagged MCs. There was no significant differences for volume (D). Cells and MCs had a higher density than cells and tagged MCs at day 14 (E). Data is expressed as mean ± SEM and was analysed via a two-way ANOVA with multiple comparisons.

GFAP



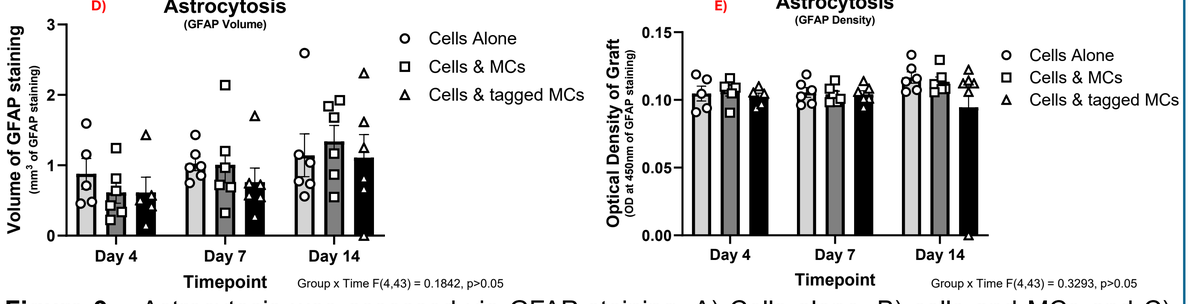


Figure 9 – Astrocytosis was assessed via GFAP staining, A) Cells alone, B) cells and MCs and C) cells and tagged MCs. There were no significant differences for volume or optical density (D&E). Data is expressed as mean ± SEM and was analysed via a two-way ANOVA with multiple comparisons.

Immunofluorescence

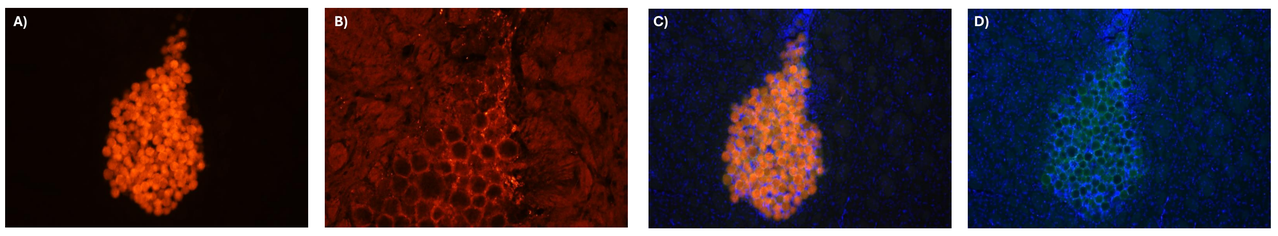


Figure 10 - Brain sections were stained for HuNu and DAPI to view the iPSC-DAPs and fluorescently labelled microcarriers. The microcarriers were visible in every channel but fluorescent in the far-red channel. A) The microcarriers in the far-red channel, B) HuNu cell staining, C) Microcarriers with DAPI, D) Microcarriers in green channel with DAPI.

References

[1](Comini G et al 2025, Neural Regeneration Vol 20 p. 2315-2316), [2](Hakami A et al 2024, J Control Release Vol 369 p. 404-419), [3](Narasimhan K et al 2024, J Neural Engineering Vol 21), [4](Moriarty N et al 2017, Scientific Reports Vol 7)

Acknowledgements

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Conclusion

- Based on the results of this *in vivo* short-term compatibility study, the microcarriers did not affect cell survival (Human Nuclei and Stem121 staining), microgliosis (OX42 staining) or astrocytosis (GFAP staining). The fluorescently tagged microcarriers remained successfully labelled *in vivo* and the RM3.5 iPSC-DAPs showed promising differentiation patterns *in vitro.*
- Based on the preferential results seen in this short-term study, further research should be carried out investigating the efficacy of the microcarriers.
- Further studies should focus on testing the neurotrophin-loaded microcarriers with RM3.5 iPSC-DAPs to evaluate their potential and investigate if the microcarriers can enhance cell survival.
- Microcarriers can potentially aid the engraftment of iPSC-DAPs, therefore improving cellular based brain repair for Parkinson's Disease.

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