Advancing Neural Microtissues (TFG-001) Toward a Clinically Viable Cell Therapy for Parkinson's Disease

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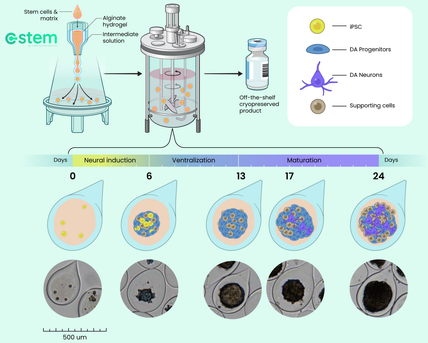
Introduction

- Multiple preclinical studies[1-6] and ongoing clinical trials[7-10] support the rationale of **pluripotent stem cell-derived cell replacement therapies** to alleviate motor symptoms in Parkinson's Disease.
- The target for replacement is the major dysfunctional cell population in the disease: ventral mesencephalic A9 dopaminergic **(DA) neurons**, which are particularly vulnerable to the *in vitro* manipulations entailed by monolayer cell culture, harvest, and intracerebral administration.[11]
- Alternative **3D formats** have been explored to reduce anchorage-dependent programmed cell death, known as anoikis. However, these formats introduce **additional challenges**, such as much faster sedimentation, which hinders homogenization, a critical factor for accurate dose delivery.
- Multiple non-clinical studies demonstrated that the **extent of graft-derived reinnervation** is a critical determinant of functional recovery in Parkinson's disease animal models[12-14], supporting reinnervation as a key mechanistic correlate of efficacy.
- Here, we present **extensive graft-derived projections** of neural microtissues (**TFG-001**) both *in vitro* after replating and *in vivo* in the 6-OHDA hemiparkinsonian rat model. The development of an **appropriate delivery strategy for clinical translation** was further validated by demonstrating graft survival following transplantation in a healthy non-human primate (NHP).

Scalable bioproduction of neural microtissues in stirred tank bioreactors



1. **Encapsulation** of human induced pluripotent stem cells (hiPSCs) into hollow alginate capsules using a microfluidic chip (C-Stem®).[15-17]
2. **Neurodifferentiation** in 1L stirred tank bioreactors (24 days).[6]
3. **Harvest & cryopreservation** of neural microtissues (~250 µm) after removal of the alginate capsule.

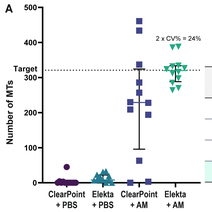
Extensive TFG-001 graft-derived projections *in vitro* and *in vivo*

Cannula and medium testing

Two cannulas (ClearPoint **(A, C)** and Elekta **(B, D)**) were tested by loading 200 µL of 30% (v/v) microtissues (MTs) resuspended in PBS **(A, B)** or administration medium (AM) **(C, D)** and performing 10 consequent injections of 18 µL at 2 µL/min (3 technical replicates).

In AM conditions (ClearPoint/Elekta): The drag was sufficient to displace the MTs.
- **ClearPoint (C):** The MTs accumulated because the inner diameter (0.5 µm) was too small relative to their average size (242 µm), resulting in heterogeneous delivery.
- **Elekta (D):** For the first 4 injections, the number of MTs injected was constant and approached the desired target value → Usable volume = 72 µL

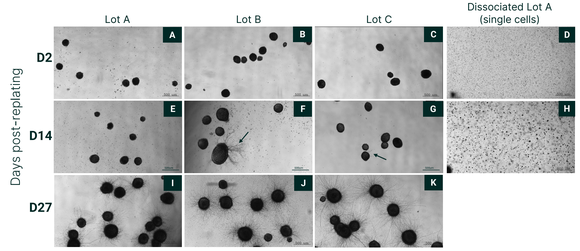
Precision and accuracy for a usable volume of 72 µL:



Combination	Precision (SD/ average)	Accuracy (median/ target dose)
ClearPoint + PBS	N/A	N/A
Elekta + PBS	N/A	N/A
ClearPoint + AM	66.2%	0.71
Elekta + AM	**12.2%**	**0.99**

N/A: not applicable

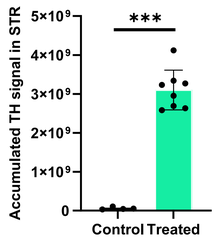
In vitro agarose embedding and projection capacity



- Embedded TFG-001 microtissues show **extensive projection capacity**, with axonal outgrowth observed as early as day 14 (up to day 27)
- In contrast, dissociated TFG-001 (single cells) show no projections
- The **3D microtissue format enhances projection capacity**, supporting strong reinnervation potential

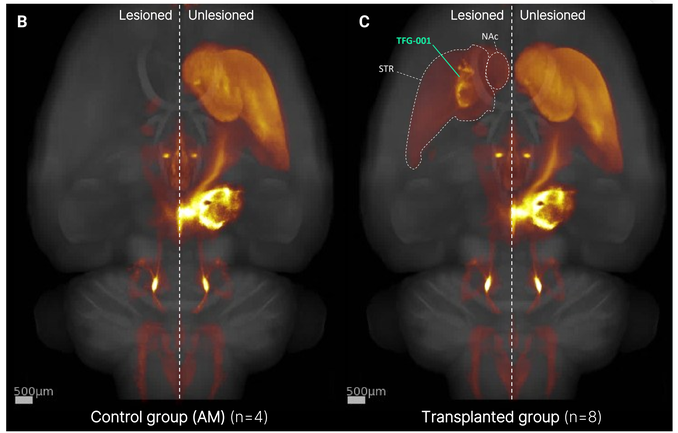
Quantification of graft-derived reinnervation using 3D whole-brain imaging in the 6-OHDA rat model

- Quantitative analysis shows **widespread graft-derived innervation across the entire striatum (STR)**, extending beyond the injection site (C, D)



Voxel-wise quantification of total accumulated signal across groups (A). Dunnett's test negative binomial generalized linear model. ***: P < 0,001 compared to Control

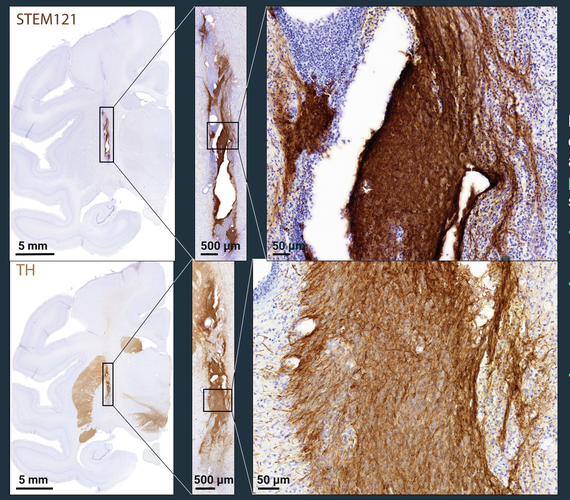
The signal is visualized in a perceptually uniform color map, with brighter color indicating higher signal intensity (B, C). NAc: nucleus accumbens



TFG-001 enables **homogeneous** and **consistent dose control**

Successful & fast engraftment of neural microtissues in a Non-Human Primate (NHP)

- **Delivery strategy:** Elekta cannula + 25.5% microtissues (v/v) in custom-made administration medium.
- The selected delivery strategy was validated in a non-clinical study in a healthy cynomolgus macaques (*Macaca fascicularis*).



Post-mortem histological characterization at 1 month post-transplantation showed the presence of:

- **surviving human cells** (STEM121)
- **surviving human DA neurons** (TH+ cells co-localizing with the STEM121 staining)
- **TH+ fibers reinnervating the host brain**

Conclusion & Perspectives

- Unprecedented 3D whole-brain imaging in the 6-OHDA hemiparkinsonian rat model demonstrates **extensive whole-striatal graft-derived reinnervation** with the 3D TFG-001 format, a potential surrogate of functional efficacy.
- A **delivery strategy adapted to the 3D neural microtissue format (TFG-001)** was developed and validated by confirming graft survival 1-month post-transplantation in a healthy non-human primate.
- These data support entry into a first-in-human study; the in-life phase of the GLP toxicological study is completed and data analysis is ongoing to finalize the regulatory package required for clinical readiness.

References

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