Poster #1219

Off-the-shelf Bioreactor Produced, iPSC-derived Neural Microtissues Containing Dopaminergic Neurons Innervate the Striatum and Normalize Behavior in a Parkinson Rat Model

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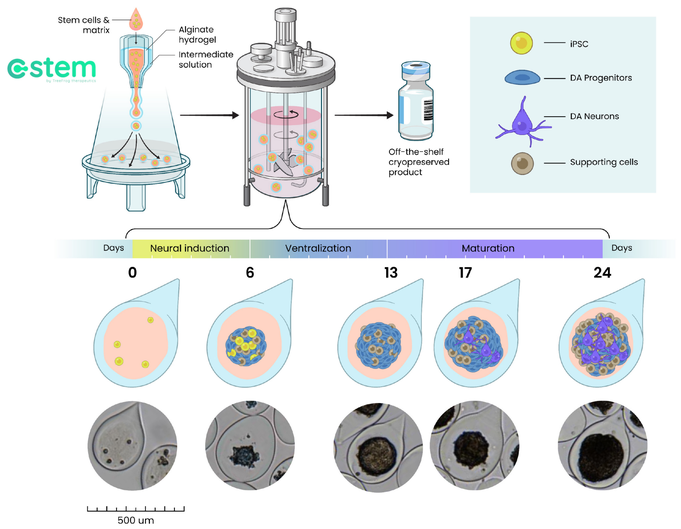
Introduction

- Multiple preclinical studies and ongoing clinical trials support the rationale of pluripotent stem cell-derived cell replacement therapies to alleviate motor symptoms in Parkinson's Disease.[1-6]
- 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 that monolayer cell culture, harvest and intracerebral administration entail.[7]
- The manufacturing of these cells needs to address major pharmaceutical hurdles including scalability for commercial phases and off-the-shelf availability that does not compromise cell function.
- **Here, we propose a shift in bioproduction strategy: from single cell suspensions to 3D microtissues.**
- Using a high-throughput cell encapsulation technology (**C-Stem™**) and standard bioreactors, **we demonstrate a scaled-up process yielding cryopreservable 3D ventral midbrain microtissues that restore motor function in a pre-clinical Parkinsonian model.**

Scalable bioproduction using C-Stem™ and stirred tank bioreactors

- **Encapsulation:** Human induced pluripotent stem cells (hiPSCs) were encapsulated into hollow alginate capsules using a microfluidic chip[8-10]
- **Differentiation:** Cells were cultured in 500 mL stirred tank bioreactors and neuro-differentiated for 24 days:
  - Neural induction: dual SMAD inhibition
  - Ventral midbrain patterning: CHIR99021, SHH, Purmorphamine, FGF-8b
  - Dopaminergic maturation: BDNF, GDNF, TGF-Beta3, FGF-20, dbcAMP, DAPT, Compound E and Trichostatin A
- **Harvest & cryopreservation:** At d24, the neural microtissues (~200µm) were harvested and cryopreserved after removal of the alginate capsule



References

1. Doi et al., Nat Commun. 2020
2. Piao et al., Cell Stem Cell 2021
3. Hiller et al., Npj Regen Med 2022
4. Kirkeby et al., Cell Stem Cell 2023
5. Park et al., Cell Stem Cell 2024
6. NCT04802733, NCT05635409, UMIN000033564, NCT05887466
7. Marchionini et al., J Comp Neurol 2023
8. **Cohen et al. Biomaterials 2023**
9. Alessandri et al., PNAS 2013
10. Alessandri et al., Lab Chip 2016

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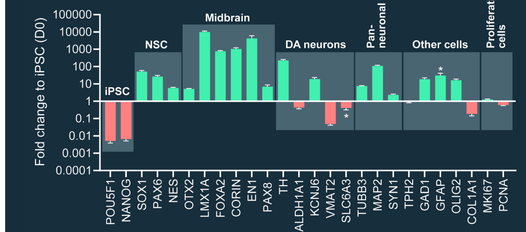
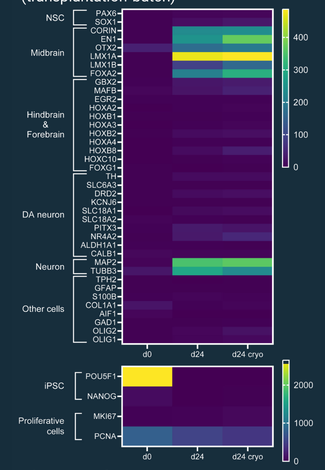
Characterization of the ventral midbrain DA microtissues

RTqPCR analysis
(13 production batches)



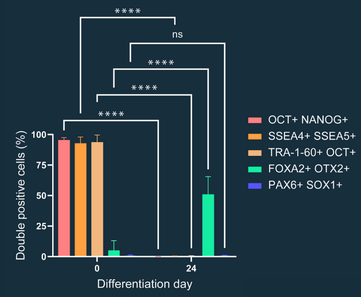
- **Enrichment in midbrain genes** (*OTX2, LMX1A, FOXA2, CORIN, EN1, PAX8*)
- **Decrease of pluripotent stem cell genes** (*POU5F1* and *NANOG*)
- **Enrichment of neuronal genes** (*MAP2, TUBB3, SYN1*), including A9 DA neurons (*TH, KCNJ6*)
- No enrichment in serotoninergic marker (*TPH2*), VLMC marker (*COL1A1*), and astrocyte marker (*GFAP**)
  ** raw value below the detection threshold ($2^{\Delta Ct} < 10^{-4}$)*

BulkRNAseq analysis
(transplantation batch)



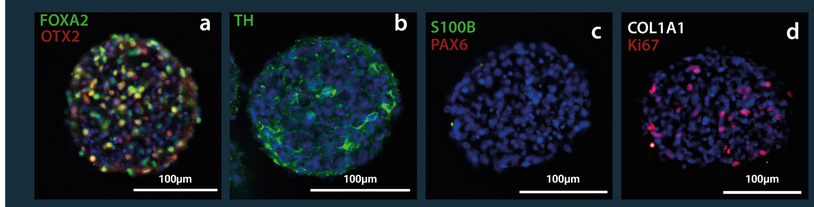
Absence of RNA transcript enrichment from contaminants:
- Forebrain (*FOXG1*)
- Hindbrain (*GBX2, MAFB, EGR2, HOXA2, HOXB1, HOXA3, HOXB2, HOXA4*)
- Spinal cord (*HOXB8, HOXC10*)

Flow cytometry analysis
(13 production batches)



At day 24:
- Negligible hiPSCs (0.07% ± 0.06 % OCT+NANOG+, 0.28% ± 0.35% SSEA4+SSEA5+ and 0.50% ± 0.39% TRA-1-60+OCT+)
- <1% neural stem cells (NSC) (0.72% ± 0.42 % PAX6+SOX1+)
- **50.8% ± 14.8% FOXA2+OTX2+** midbrain dopaminergic progenitors

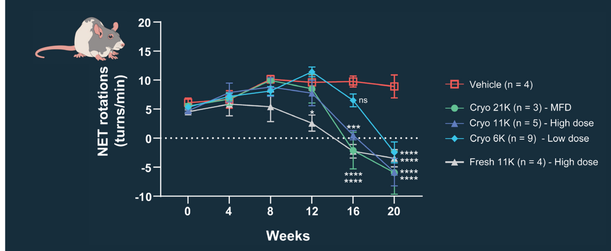
Immunofluorescence analysis



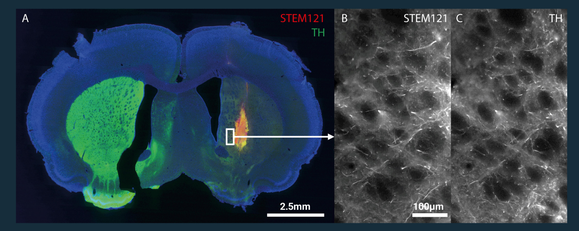
Presence of:
- Midbrain dopaminergic progenitors (FOXA2+OTX2+ cells)
- Neurons (MAP2+ cells)
- DA neurons (TH+ cells)
- Proliferative cells (KI67+ cells)

Absence of:
- Astrocytes (S100B+, GFAP+, SOX9+ cells), NSCs or forebrain progenitors (PAX6+ cells), and VLMCs (COL1A1+ cells)
- iPSCs (NANOG+ cells)
- Oligodendrocytes (OLIG2+ cells)

Transplanted microtissues dose-dependently normalize rotational bias and re-innervate the host striatum



- Hemiparkinsonian rats (6-OHDA-lesioned) were transplanted with cryopreserved product at Low Dose (n = 9), cryopreserved or fresh product at High Dose (n = 5 and n = 4, respectively) and cryopreserved product at the Maximum Feasible Dose (MFD, n = 3)
- **Functional recovery was observed at 16 weeks post-transplantation for the cryopreserved and fresh High Dose, and the cryopreserved MFD**
- The cryopreserved Low Dose group achieved recovery at 20 weeks



- Post-mortem histological characterization at 5 months post-transplantation showed that **surviving TH+ human dopaminergic neurons were present in the rat brain**
- The majority of TH+ neurons localise to the periphery of the graft
- TH+ fibers are seen to **innervate the striatum**

Conclusion & Perspectives

- We report the first bioproduction of a cryopreservable & efficacious 3D cell therapy for Parkinson's disease in a scalable bioreactor.
- Ongoing studies include formal demonstration of process reproducibility and toxicology assessment.
- This represents a cornerstone in our ultimate goal to establish cost-effective, large-scale & reproducible processes for manufacturing allogeneic iPSC-based cell therapies.