Robust & scalable process enables the transplantation of hPSC-derived functional microtissues composed of dopaminergic neurons with best-in-class results in preclinical model of Parkinson's disease

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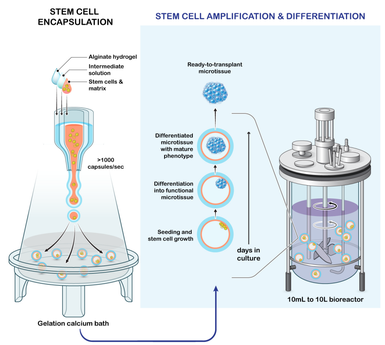
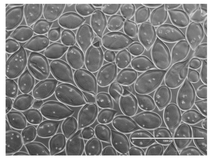
Introduction

Despite the development of efficient protocols to differentiate human pluripotent stem cells (hPSC) into dopaminergic neurons, the transplantation of mature dopaminergic neurons to treat Parkinson's disease is generally considered as a non-viable option. In mature neurons cultured *in vitro*, neurite outgrowth indeed greatly complexifies cell harvest and transplantation. To circumvent this issue, most cell replacement therapies in development for Parkinson's disease use dopaminergic progenitors. However, the clinical use of these multipotent cells remains challenging with regards to : i) the *in situ* control over the graft's cellular identity and ii) the management of cell proliferation risks. In addition, such progenitor-based cell therapies rely on differentiation protocols which do not meet industrial requirements for scalable manufacturing in bioreactors.
Since 2018, we have been addressing some of these challenges with the C-Stem™ technology . Using high-speed cell encapsulation microfluidics, hiPSC were grown in alginate shells and differentiated into functional microtissues composed of dopaminergic neurons.

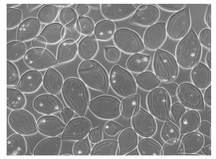
iPS exponential growth and differentiation into 3D neural microtissues in bioreactors

Using high-speed cell encapsulation microfluidics[1], the C-Stem™ technology enables the growth of hiPSC in alginate shells and their differentiation into functional neural microtissues.

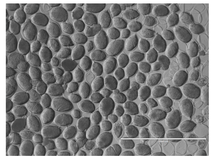




SEEDING
hiPSC are seeded in the capsules as a single cell suspension, each capsule containing 2 to 10 cells.



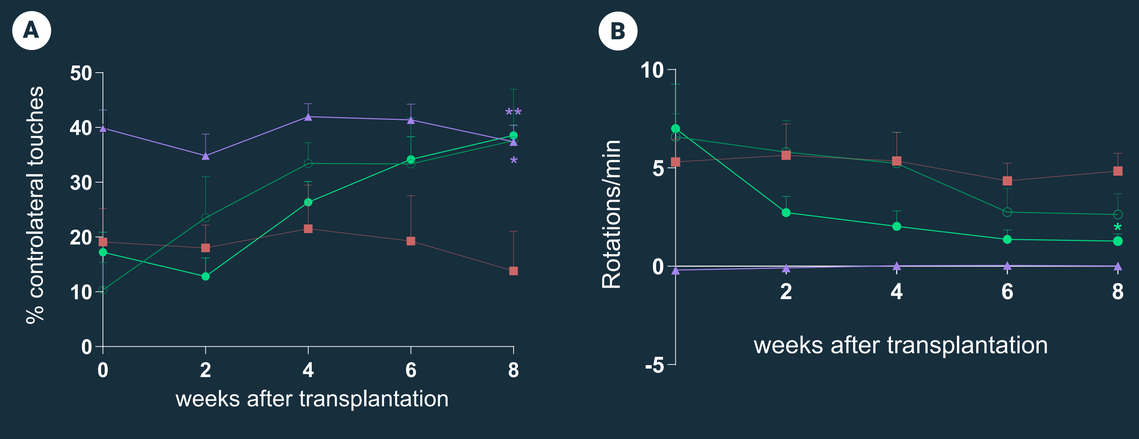
AMPLIFICATION
hiPSCs self-organize and expand within the capsules.



NEURO-DIFFERENTIATION
Upon neurodifferentiation, functional neural microtissues with dopaminergic neuronal content are harvested.

Twice faster motor function recovery

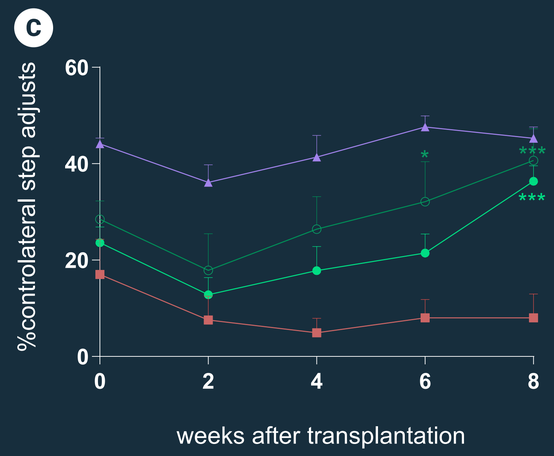
with 3D dopaminergic neuron transplants



Motor function recovery: 8 weeks (vs 16 weeks with progenitors[2])

Asymetry and akinesia were measured on hemiparkinsonian rats before and 2, 4, 6 and 8 weeks after the transplantation compared with their control. (A-C) Asymetry was evaluated in the cylinder test (A) and the apomorphine-induced rotations test (B) measuring the percentage of controleral touches in five minutes in a cylinder and the number of rotations per minute after apomorphine injection in a rotometer, respectively. Akinesia was evaluated by the stepping test (C) measuring the percentage of controlateral step adjusts.

Motor functions of hemiparkinsonian rats transplanted with neural microtissues (NM) derived from two different hiPSC lines (6-OHDA / NM002, n= 4-8, dark green; 6-OHDA / NM003, n= 5-10, light green) were compared with hemiparkinsonian rats without neuron transplantation (6-OHDA, n= 4, red). Sham group was used as control (n= 8, purple). Comparisons were made using Two-Way ANOVA and Tuckey's test for multiple comparison (*p<0.05, ***p< 0.001). Data are presented as mean ± SEM.



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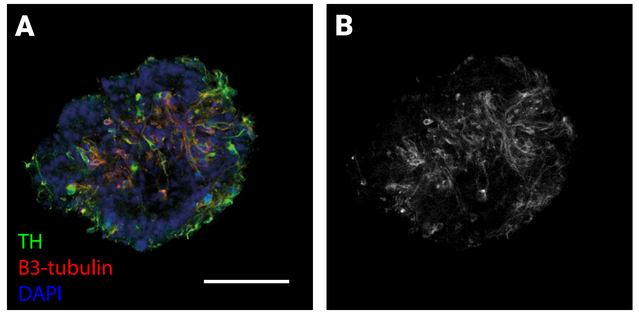

Confocal image of a 3D neural microtissue. (A) Confocal image showing expression of neuron specific marker (red: Beta-3-tub) and dopaminergic neuron specific marker (green: Tyrosine hydroxylase). (B) Beta-3-tubulin. (C) Tyrosine hydroxylase (D) DAPI. Scale bar: 100 µm

Conclusion

In 2018, the tranplantation of functional neural microtissues with dopaminergic neuronal content into hemiparkinsonian rats led to full motor recovery within 8 weeks (vs 16 weeks with progenitors[2]). Here, we replicated our results with two supplementary cell lines differentiated "*in capsulo*", demonstrating the robustness of the process. We believe the reduction of time-to-effect is partly linked with the transplantation of mature neurons, which eliminates the time lag required for progenitor *in situ* differentiation.

Perspectives

To further investigate the mechanism of action and the safety benefits of our cell therapy product, we are currently 1) characterizing neurospheres at the single cell level, and 2) launching long-term in vivo non-clinical studies, with the aim of a first-in-human trial in 2024.

C-Stem™ has been further developed to reach cGMP compliance by Q2 2022.
It is now a closed, automated, and single-use technology allowing for the large-scale expansion and differentiation of hiPSCs in bioreactors.

In April 2021, TreeFrog announced a world's first, with the production of a single batch of 15 billion hiPSCs in a 10L bioreactor with an amplification factor of 276x in 6.59 days.

References:
[1] Alessandri, K., et al. Cellular capsules as a tool for multicellular spheroid production and for investigating the mechanics of tumor progression in vitro. Proc Natl Acad Sci 110(37):14843-8 (2013).
[2] Doi, D., Magotani, H., Kikuchi, T. et al. Pre-clinical study of induced pluripotent stem cell-derived dopaminergic progenitor cells for Parkinson's disease. Nat Commun 11, 3369 (2020)

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