Human Dopaminergic Neurons derived from induced Pluripotency Stem cells in NETRI's microfluidic devices

Introduction

Dopaminergic neurons are found particularly in the substantia nigra. The loss of Dopamine is associated with Parkinson's disease. **NETRI** has **characterized** FUJIFILM Cellular Dynamics International's (FCDI) **Dopaminergic neurons** derived from induced Pluripotency Stem Cells (iPSCs, iCell DopaNeurons, C1087) in **microfluidic devices** thanks to an adapted and reproducible protocol. The key results of this characterization are presented in this Data Sheet (cf. Fig1.).

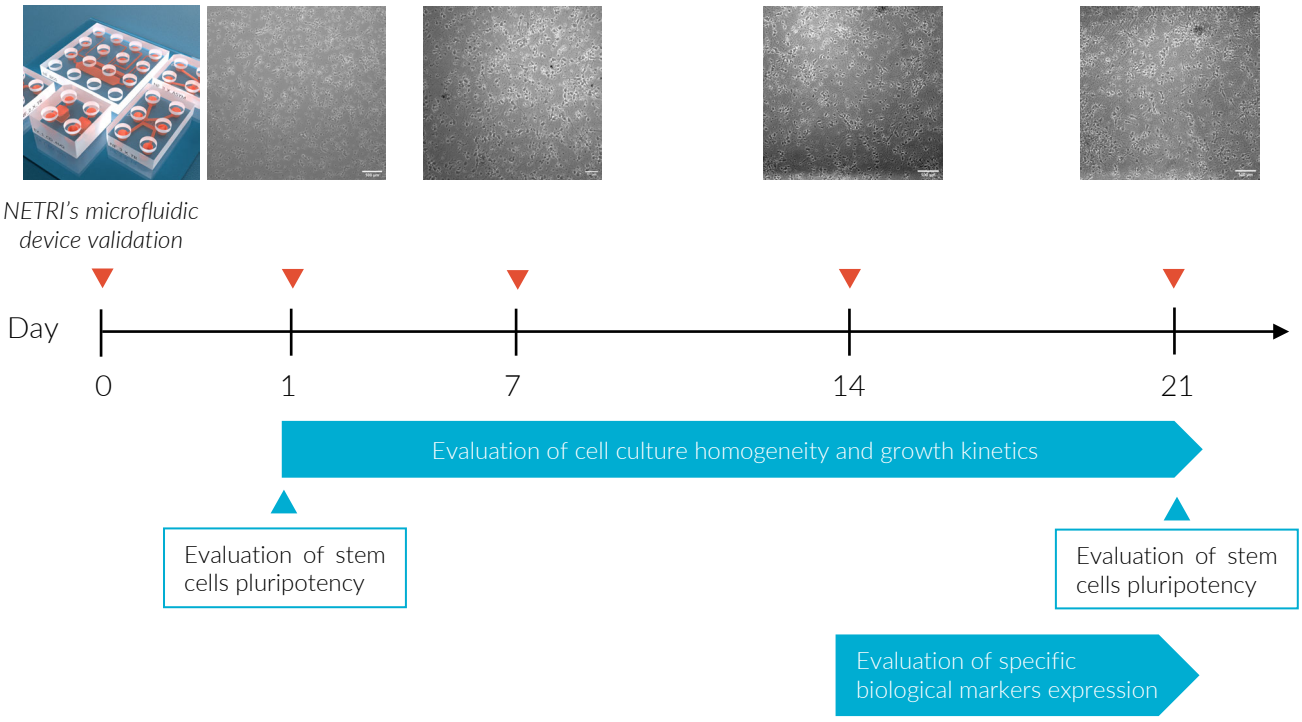

Figure 1. The main steps of FCDI's iPS cells-derived human Dopaminergic neurons characterization in NETRI's microfluidic devices. Brightfield pictures of human Dopaminergic neurons from day 1 up to day 21 in NETRI's microfluidic devices.

(Differentiated in protocols licensed and adapted from the Lorenz Studer lab (Memorial Sloan Kettering) and industrialized for scale at FCDI)

Keys Datas

To specifically characterize FCDI's human Dopaminergic neurons, different steps were performed:

- validation of **cell culture homogeneity**,
- measure of axonal **growth kinetic**,
- evaluation of **stem cells pluripotency**,
- evaluation of the expression of **specific biological markers**,
- and analysis of the **neuronal functional activity**.

NETRI was validated several seeding and homogeneity protocols in microfluidic devices allowing reproductible media change for long term viability of neurons seeded. Several experiments were performed in 3D-Deposition Chamber microfluidic devices to have the best human neurons culture protocol (Maisonneuve *et al.*, 2021).

Cell culture homogeneity

To promote human Dopaminergic Neurons attachment and long-term viability, microfluidic devices were previously coated with Poly-L-ornithine (PLO) and laminin.

Human Dopaminergic neurons were seeded in NETRI's 3D-Deposition Chamber microfluidic device allowing long-term viability and cell homogeneity and were maintained 100% up to day 5 and then 50% of media change up to 28 days twice a week.

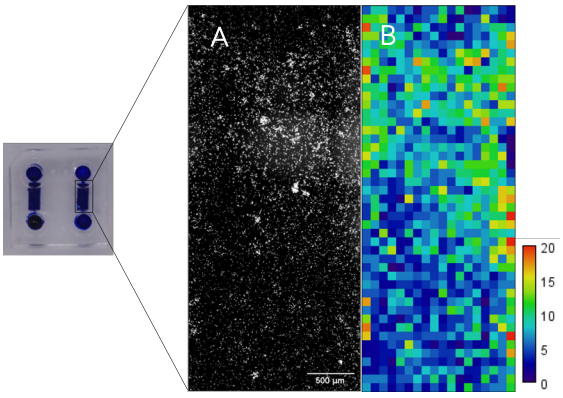

Figure 2. Cell culture homogeneity analysis. (A) Fluorescent pictures of DAPI staining. **(B)** Heat Map of neuronal homogeneity in a 3D-Deposition Chamber microfluidic device.

It has been possible to evaluate this neuronal homogeneity, thanks to a proprietary software, to a heat map representing number of neurons in each square (Fig. 2). This tool is based on DAPI fluorescent pictures, labelling cell nucleus.

Growth kinetics

The outgrowth kinetic of human Dopaminergic neurons from iPSCs was performed in our triangular shaped microfluidic device (Fig. 3 A).

Thanks to its architecture and proprietary software outgrowth kinetic could be performed precisely, from day 1 to day 28 here (c.f. Application Protocol - Neurite Length Measurement). It has been possible to measure Dopaminergic neurites maximal length up to 987 µm at Day 28 in NETRI's microfluidic device (Fig. 3 B) (c.f. Maisonneuve *et al.*, 2021). It's also possible to perform drug assay on outgrowth kinetic with different concentration.

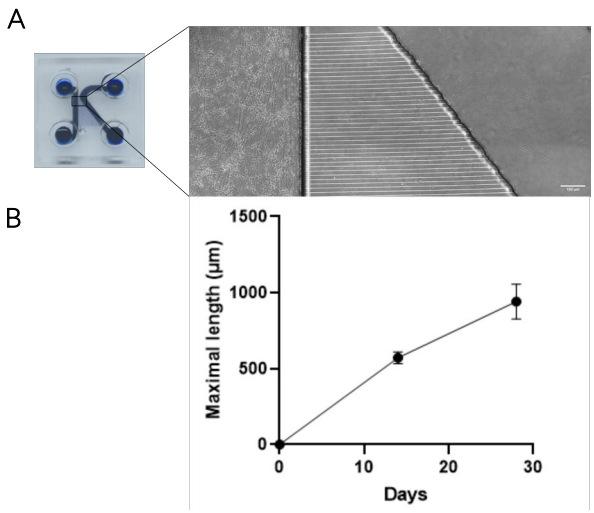

Figure 3. Growth kinetics measurement. (A) Brightfield pictures of a triangular shape microfluidic device seeded with Dopaminergic neurons. **(B)** Graph of neurite outgrowth kinetic of Dopaminergic neurons seeded in NETRI's microfluidic device.

Evaluation of pluripotent stem cells expression

Human Dopaminergic Neurons are derived from pluripotency stem cells. To validate the fully differentiation process, the expression level of usual potency markers such as Nestin was tested by an immunofluorescence approach at early and late days. Percentages of expression of this marker is quantified by a proprietary software (c.f. Operating Protocol - ImmunoStaining).

At Day 1, this marker represents 5% for Nestin of number of total cells and decreased to 1% at late days (Day 21) (Fig. 4). As expected, pluripotency markers expression decreased at late days showing a fully differentiation of Dopaminergic neurons seeded in NETRI's microfluidic devices.

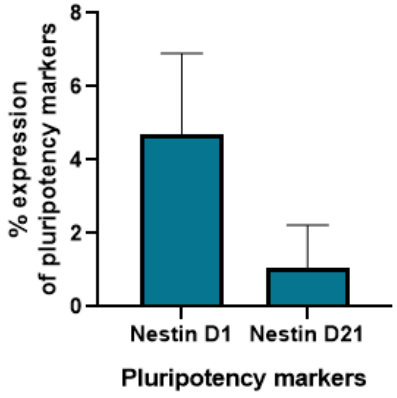

Figure 4. Evaluation of pluripotent stem cells expression (Nestin markers) at Day 1 and Day 21.

Evaluation of specific biological markers expression

NETRI has developed several robust immunofluorescence staining protocols allowing a specific characterization of human Dopaminergic neurons (c.f. Operating Protocol - ImmunoStaining).

Human Dopaminergic Neurons could be stained with specific markers such as β-III-tubulin and TH antibodies (Fig. 5 A-B).

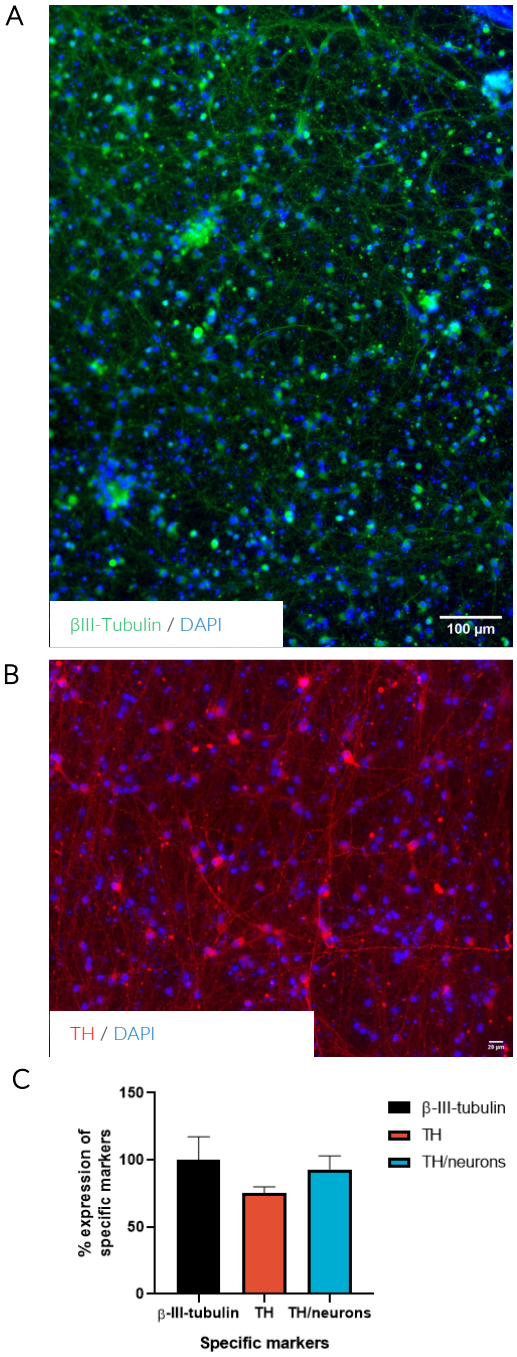

Figure 5. Evaluation of specific biological markers expression. (A), (B) Immunofluorescence at Day 21 in NETRI's microfluidic device (C) Percentage expression of Dopaminergic neurons specific markers at Day 21 in NETRI's microfluidic device.

The graph shows around 75% of TH and 93% of TH on neurons in human Dopaminergic neurons seeded in NETRI's microfluidic devices. In addition, Dopaminergic neurons have been stained with vGAT, the main inhibitory neurotransmitter to evaluate neuronal composition (*data not shown*). These datas show that d/Dopaminergic neurons are perfectly differentiated in NETRI's microfluidic devices. Same results were obtained in 96-well plates (*data not shown but used as control*).

Conclusion

Human Dopaminergic neurons derived from iPSC are reproducibly characterized in NETRI's microfluidic device which are compatible with high throughput screening. Human Dopaminergic neurons were structurally and functionally fully differentiated in NETRI's microfluidic devices.

This process allows different potential human Dopaminergic neurons applications like Dopaminergic maturation is a challenge in microfluidic device culture or axonal projections in Substantia Nigra involved in Parkinon's disease.

Resources

- Maisonneuve B. G. C., Libralesso L., Miny L., Batut A., Rontard J., Gleyzes M., Boudra B., Vieira J., Debis D., Larramendy F., Jost V. and Honegger T. Deposition chamber technology as building blocks for a standardized brain-on-chip framework. bioRxiv (2021) doi : 10.1101/2021.06.21.449231.
- Maisonneuve B. G. C., Batut A., Varela C., Vieira J., Gleyzes M., Rontard J., Larramendy F., and Honegger T. Neurite growth kinetics regulation through hydrostatic pressure in a novel triangle-shaped neurofluidic system. bioRxiv 2021.03.23.436675 (2021). doi : 10.1101/2021.03.23.436675
- Application Protocol - Neurite Length Measurement
- Operating Protocol - ImmunoStaining