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

Introduction

Motor neurons (MTN) control the contraction of a muscle (volitional, automatic, reflex movement). Spinal Motor neurons regulate effector muscles in the periphery and have been particularly studied for the neurite regeneration following traumatic injury and pathogenesis of Motor Neuron diseases. **NETRI** has **characterized** BrainXell's **Motor neurons** derived from induced Pluripotency Stem Cells (iPSCs, Spinal Motor Neurons, BX-0100) in **microfluidic devices** thanks to an adapted and reproducible protocol. The key results of this characterization are presented in this Data Sheet (cf. Fig1.).

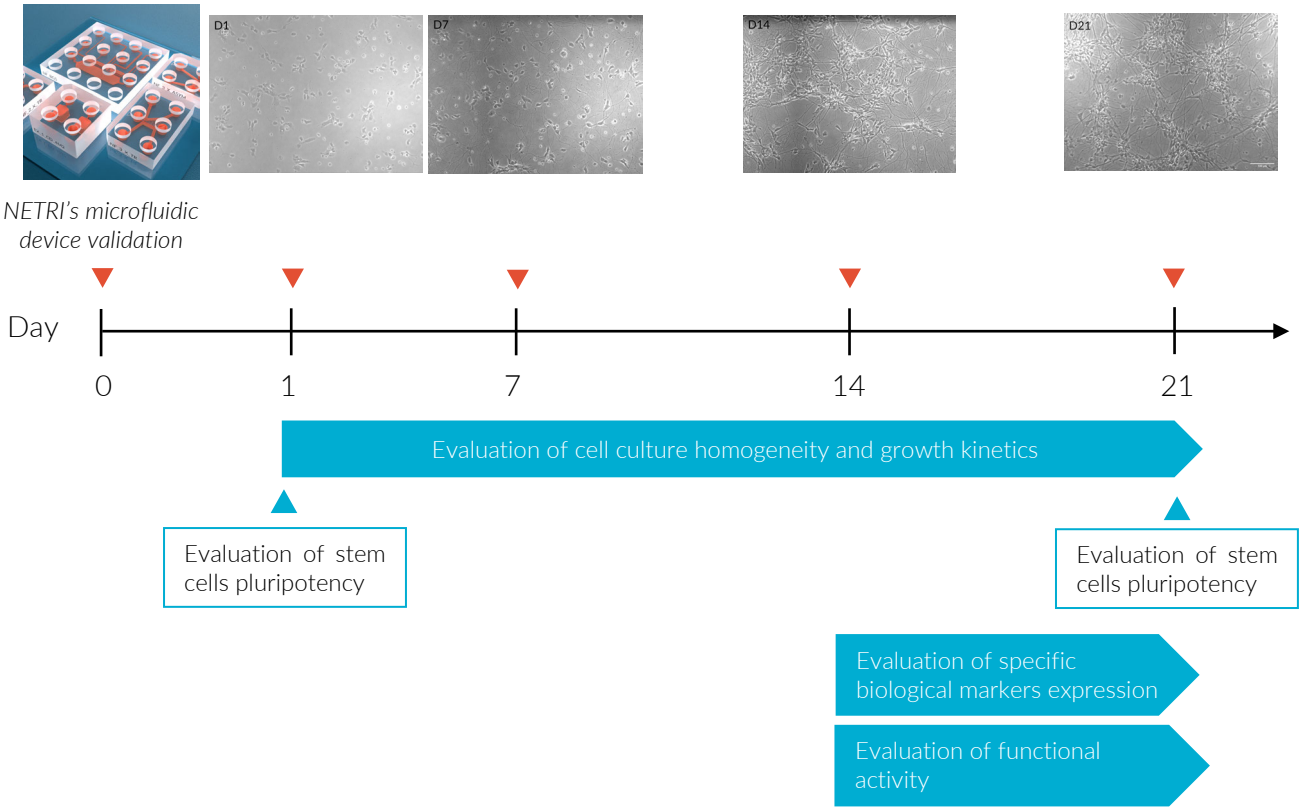

Figure 1. The main steps of BrainXell's iPSCs Motor neurons characterization. Brightfield pictures of human motor neurons from day 1 up to day 21 in NETRI's microfluidic devices.

Keys Datas

To specifically characterize BrainXell's human Motor 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 Motor Neurons attachment and long-term viability, microfluidic devices were previously coated with Poly-D-Lysine (PDL).

Human motor neurons were seeded in NETRI's 3D-Deposition Chamber microfluidic device allowing long-term viability and cell homogeneity and were maintained 100% of media change the first 4 days of culture then half media change up to 28 days twice a week (c.f. Operating Protocol BrainXell Motor Neurons).

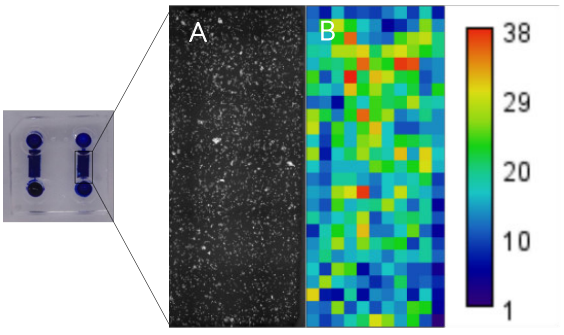

Figure 2. Cell culture homogeneity analysis. (A) Fluorescent pictures of DAPI staining. **(B)** Heat Map of neuronal homogeneity in a 3D-Deposition Chamber microfluidic device.

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 Motor 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 15 here (c.f. Simple quantitative method of neurite length measurements in triangular microfluidic device). It has been possible to evaluate Motor neurites maximal length up to 1764 µm at Day 15 in NETRI's microfluidic device (Fig. 3 B) (c.f. Maisonneuve *et al.*, 2021).

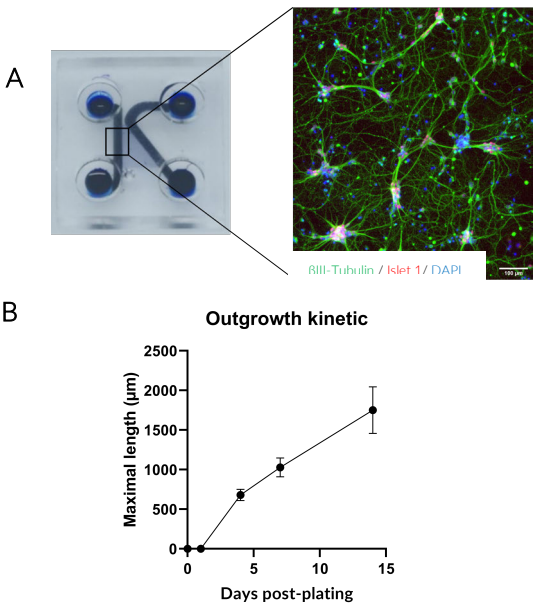

Figure 3. Growth kinetics measurement. (A) Immunofluorescence pictures of a triangular shape microfluidic device seeded with motor neurons. **(B)** Graph of neurite outgrowth kinetic of motor neurons seeded in NETRI's microfluidic device.

Evaluation of pluripotent stem cells expression

Human Motor Neurons are derived from pluripotency stem cells. To validate the fully differentiation process, the expression level of two usual potency markers such as Nestin and Sox2 was tested by an immunofluorescence approach at early and late days. Percentages of expression of theses markers are quantified by a proprietary software (c.f. Operating Protocol - ImmunoStaining).

In early days, these markers represent 80% for Sox2 and 60% for Nestin of number of total cells and decreased to 25% and 5% respectively at late days (Fig. 4). As expected, pluripotency markers expression decreased at late days showing a fully differentiation of motor neurons seeded in NETRI's microfluidic devices.

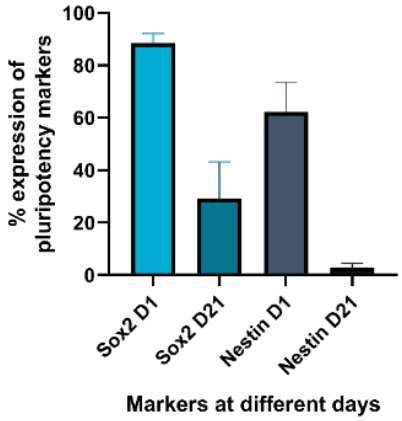

Figure 4. Evaluation of pluripotent stem cells expression (Sox 2 and Nestin markers) at Day 1 and Day 21.

Evaluation of specific biological markers expression

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

Human Motor Neurons could be stained with specific markers such as Islet 1 and ChAT antibodies (Fig. 5 A-B). Expression percentage of theses specific motor neurons markers normalized by β-III-tubulin was quantified in the entire active microfluidic area, using proprietary software.

The graph shows 100% of ChAT and 50% of Islet 1 expression in human motor neurons seeded in NETRI's microfluidic devices. These datas show that motor neurons are perfectly differentiated in NETRI's microfluidic devices. Same results were obtained in 96-well plates (data not shown but used as control).

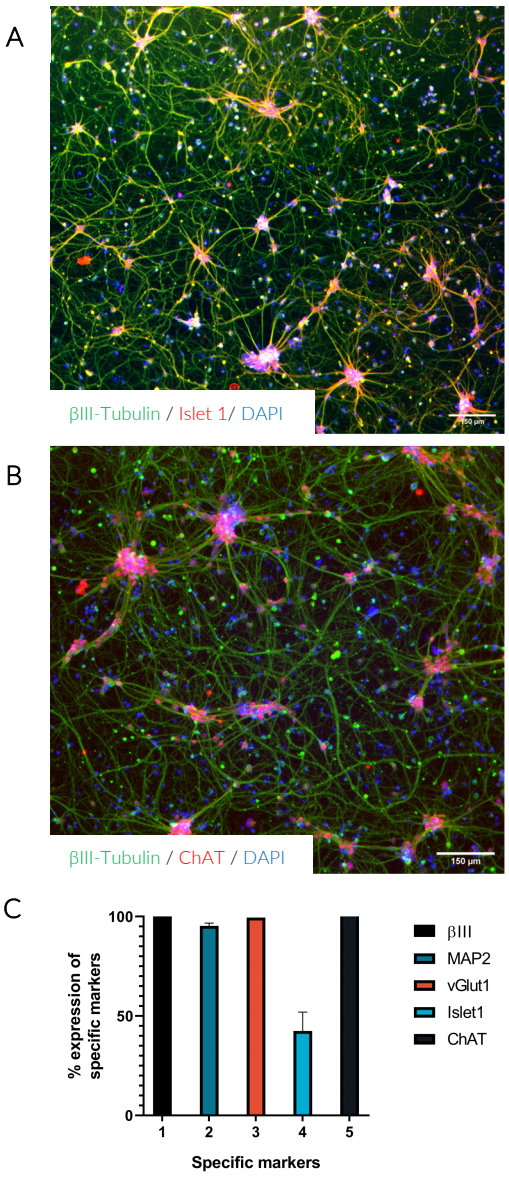

Figure 5. Evaluation of specific biological markers expression. (A), (B) Immunofluorescence at Day 21 in NETRI's microfluidic device (C) Percentage expression of Motor neurons specific markers at Day 21 in NETRI's microfluidic device.

Analysis of electrophysiological recordings using Micro-Electrode Array (MEA)

The functional activity of human motor neurons was recorded in a 3D-Deposition Chamber microfluidic device on a microelectrode array (MEA) plate.

At 21 days, human motor neurons derived from iPSC were matured with 1.5 spikes/s recorded, corresponding to mean firing rate (MFR). Post-recording the cell viability is observed by immunofluorescence approach (Fig. 6).

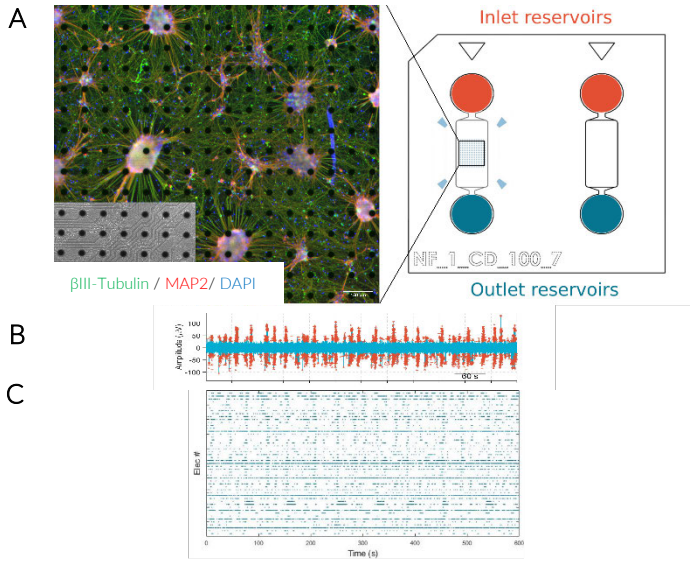

Figure 6. Functional activity analysis. (A) Immunofluorescence picture of motor neurons seeded in a 3D-Deposition Chamber with MEA coupled. **(B)** Functional activity plot of one electrode during 10 min recording. Each red spot showing one action potential. **(C)** Raster plot of active electrodes during 10 min recording.

Conclusion

Human Motor Neurons derived from iPSC are reproducibly characterized in our microfluidic device which are compatible high throughput screening. Human MTN were structurally and functionally fully differentiated in NETRI's microfluidic devices.

This process allows different potential human MTN applications like motor neuron disease models as amyotrophic lateral sclerosis or pain or even neurodevelopmental disorders. It will be possible to perform neurites axotomy (motor neurons sections) to investigate neuronal regeneration in a particular triangular shaped microfluidic device).

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
- Simple quantitative method of neurite length measurements in triangular microfluidic device
- Operating Protocol BrainXell Motor Neurons
- Operating Protocol - ImmunoStaining