Human Glutamatergic neurons derived from induced Pluripotency Stem cells in NETRI's microfluidic devices

Introduction

Glutamate is the most abundant and the major excitatory neurotransmitter in the mammalian central nervous system. It is involved in different aspects of development (neurogenesis, cell migration, synaptogenesis) and participates in many brain functions (memorization, learning). Glutamate can still be neurotoxic and plays a role in seizure generation and neurodegenerative disorders. Glutamate acts post-synaptically on three families of ionotropic receptor (NMDA, AMPA and kainite receptors). NETRI has characterized BrainXell's Glutamatergic neurons derived from induced Pluripotency Stem Cells (iPSCs, Cortical Glutamatergic Neurons, BX-0300) in microfluidic devices thanks to an adapted and reproducible protocol. The key results of this characterization are presented in this Data Sheet (cf. Fig1.).

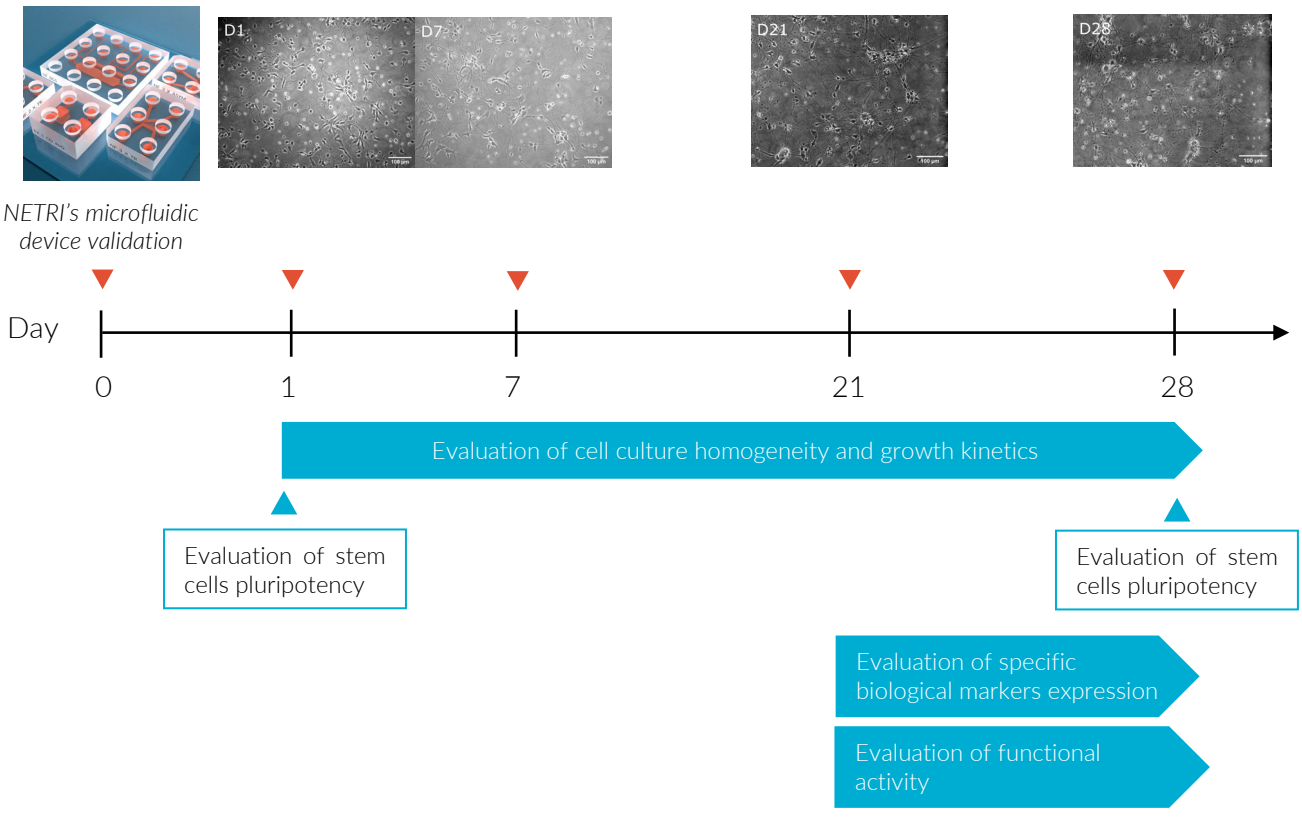

Figure 1. The main steps of BrainXell's iPSCs Glutamatergic neurons characterization. Brightfield pictures of human Glutamatergic neurons from day 1 up to day 28 in NETRI's microfluidic devices.

Keys Datas

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

Human Glutamatergic neurons were seeded in NETRI's 3D-Deposition Chamber microfluidic device allowing long-term viability and cell homogeneity and were maintained 50% of media change up to 28 days twice a week (c.f. Operating Protocol-BrainXell Gluta Neurons).

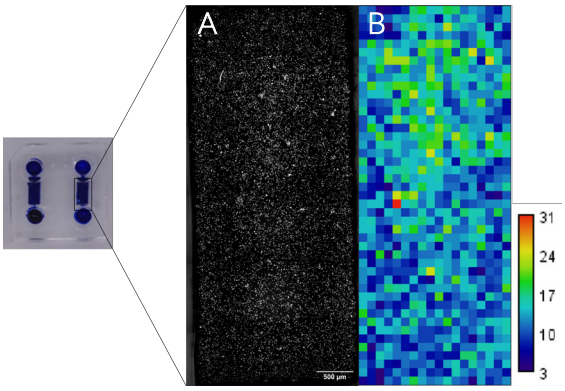

Figure 2. Cell culture homogeneity analysis. (A) Fluorescent pictures of DAPI staining. **(B)** Heat Map of neuronal homogeneity in a 3D-Deposition Chamber microfluidic device.

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 from DAPI fluorescent pictures, labelling cell nucleus.

Growth kinetics

The outgrowth kinetic of human Glutamatergic Neurons from iPSCs was performed in our triangular shaped microfluidic device. White arrows show neurites coming out of microchannels into channel 2 (Fig. 3 A).

Thanks to its architecture and proprietary software drug assay on outgrowth kinetic with different concentrations and conditions could be performed precisely, from day 0 to day 28 here (c.f. Application Protocol - Neurite Length Measurement). It has been possible to precisely evaluate Glutamatergic neurites maximal length up to 1120 µm at Day 28 in NETRI's microfluidic device with two different compounds (condition 1 and 2) (Fig. 3 B) (c.f. Maisonneuve *et al.*, 2021).

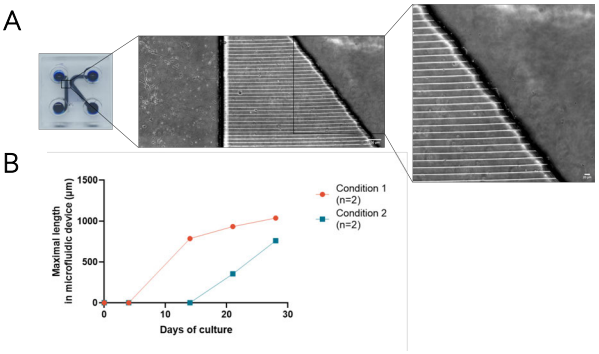

Figure 3. Growth kinetics measurement. (A) Brightfield picture of a triangular shape microfluidic device seeded with Glutamatergic neurons. **(B)** Graph of neurite outgrowth kinetic of Glutamatergic neurons seeded in NETRI's microfluidic device with two compounds.

Evaluation of pluripotent stem cells expression

Human Glutamatergic 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 (Day 4), these markers

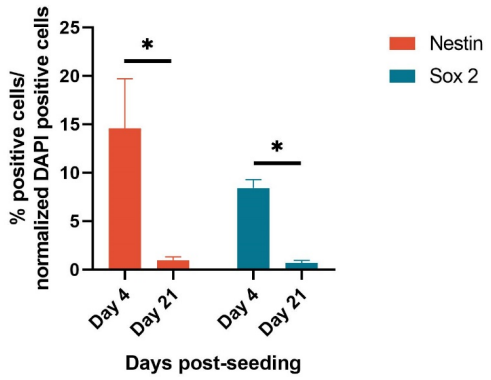

Figure 4. Evaluation of pluripotent stem cells expression (Sox 2 and Nestin markers) at Day 4 and Day 21.

represent 8% for Sox2 and 14% for Nestin of number of total cells and decreased to 3% and 4% respectively at late days (Day 21) (Fig. 4). As expected, pluripotency markers expression decreased at late days showing a fully differentiation of Glutamatergic neurons seeded in NETRI's microfluidic devices.

Evaluation of specific biological markers expression

NETRI has developed several robust immunofluorescence staining protocols allowing a specific characterization of human Glutamatergic neurons at Day 28 (c.f. Operating Protocol - ImmunoStaining).

Human Glutamatergic Neurons could be stained with specific markers such as vGlut1 and GABA (Fig. 5 A-B). Expression percentage of theses specific Glutamatergic markers normalized by β-III-tubulin was quantified in the entire active microfluidic area, using proprietary software.

The graph shows 94% of vGlut1, 50% of GABA and 85% of MAP2 expression in human Glutamatergic neurons seeded in NETRI's microfluidic devices. These datas show that Glutamatergic neurons are perfectly differentiated in NETRI's microfluidic devices. Same results were obtained in 96-well plates (data not shown but used as control).

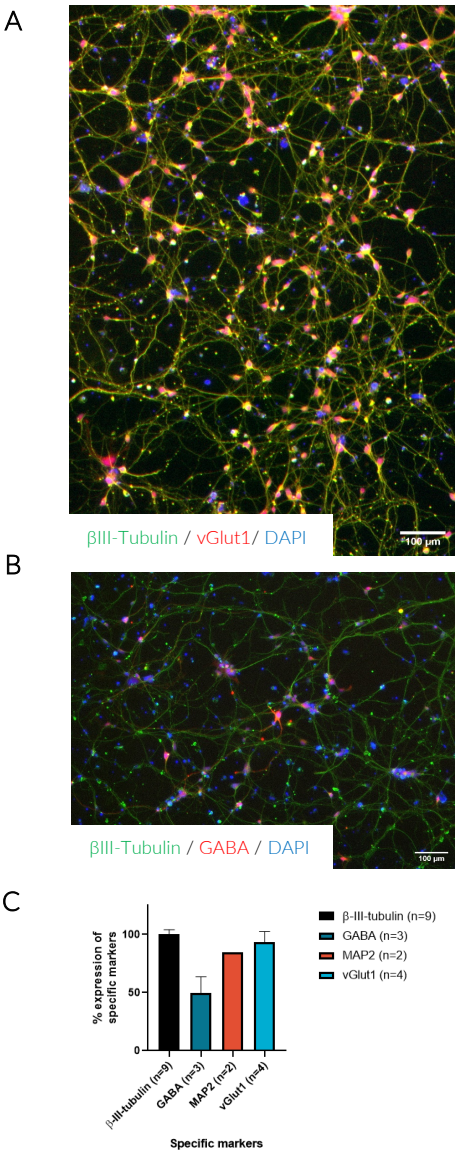

Figure 5. Evaluation of specific biological markers expression. (A), (B) Immunofluorescence at Day 28 in NETRI's microfluidic device **(C)** Percentage expression of Glutamatergic neurons specific markers at Day 28 in NETRI's microfluidic device.

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

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

At 21 days, human Glutamatergic neurons derived from iPSC were matured with 0.5 spikes/s recorded, corresponding to mean firing rate (MFR). Post-recording the cell viability is observed by immunofluorescence approach.
Glutamatergic neurons have an excitatory action, as expected, they are roughly synchronous in time (Fig. 6).

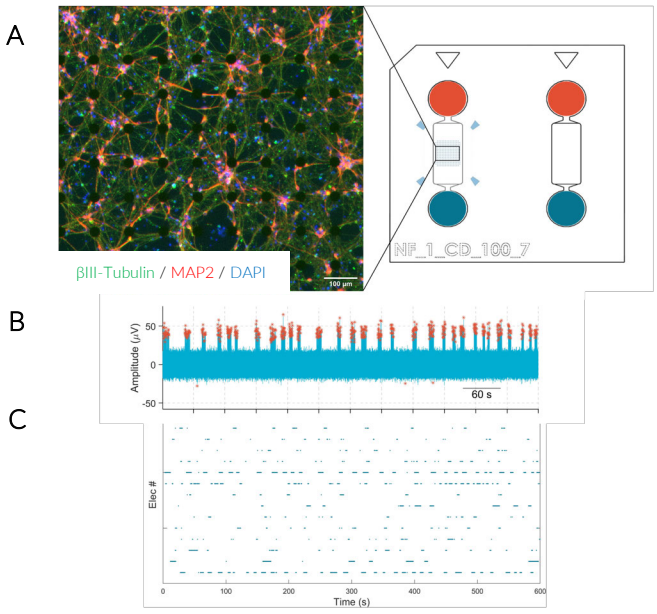

Figure 6. Functional activity analysis. (A) Immunofluorescence picture of Glutamatergic neurons seeded in a 3D-Deposition Chamber with MEA coupled. **(B)** Functional activity plot of one electrode during 10 min recording. Each red spot showing one action potential. **(C)** Raster plot of active electrodes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

Conclusion

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

This process allows different potential human Glutamatergic Neurons applications like – co-culture protocol.

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 – BrainXell Gluta Neurons
- Operating Protocol - ImmunoStaining