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

Introduction

Gamma-aminobutyric acid (**GABA**) is one of the main inhibitory neurotransmitter. The balance between inhibitory/excitatory neuronal transmission (GABA *vs* glutamate) is essential for **physiological neurologic function** in developing and adult brain. Various diseases have been associated with low levels of GABA such as psychiatric illnesses and epilepsy. The role of GABA receptors is also particularly important in chronic pain and in « the gut-vagus-brain » pathway. **NETRI** has **characterized** FUJIFILM Cellular Dynamics International's (FCDI) **GABAergic neurons** derived from induced Pluripotency Stem Cells (iPSCs, iCell GABANeurons, C1008) in **microfluidic devices**. The key results of this characterization are presented in this Data Sheet (cf. Fig1.).

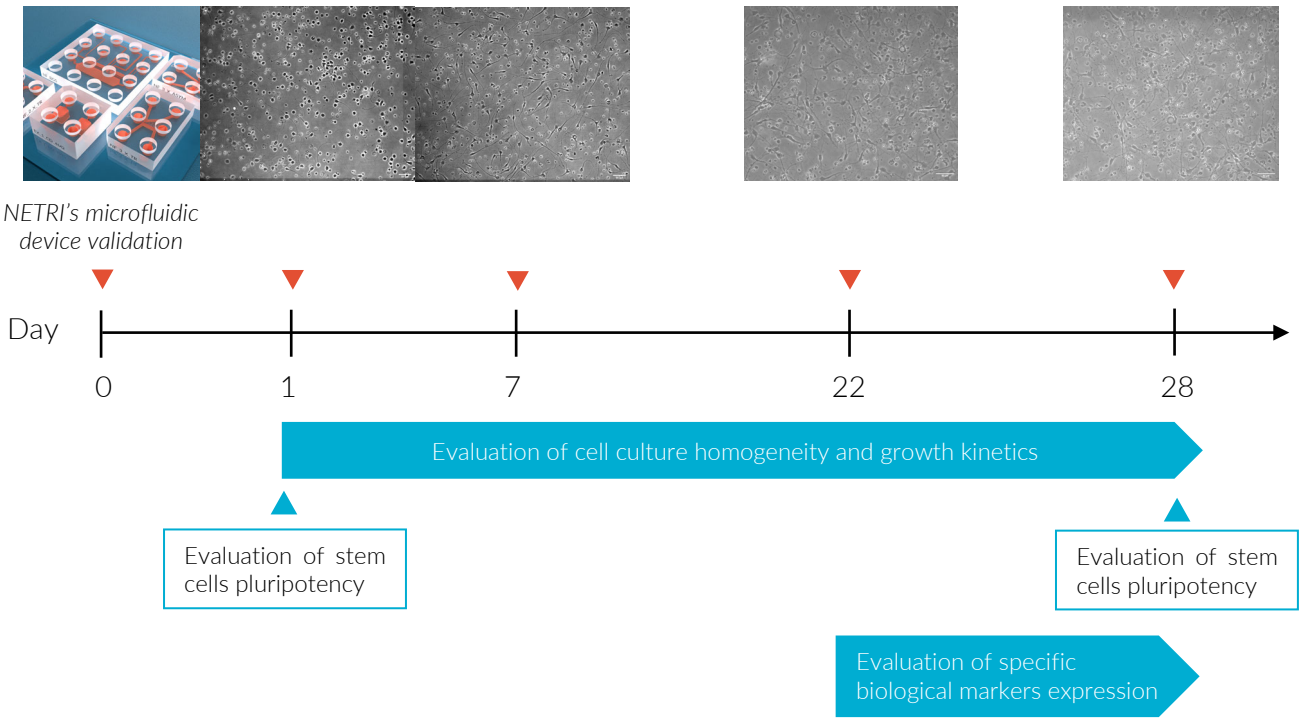

Figure 1. The main steps of FCDI's iPS Cells-derived GABAergic neurons characterization. Brightfield pictures of human GABAergic neurons from day 1 up to day 28 in NETRI's microfluidic devices.

Keys Datas

To specifically characterize FCDI's human GABAergic 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**.

Cell culture homogeneity

Human GABAergic neurons were seeded in NETRI's 3D-Deposition Chamber microfluidic device allowing long-term viability and cell homogeneity (c.f. Operating Protocol – FCDI GABA Neurons). It has been possible to evaluate this neuronal homogeneity thanks to a proprietary software, from DAPI fluorescent pictures, labelling cell nucleus to a heat map representing number of neurons in each square (Fig. 2).

Human neurons were maintained 100% of media change the first 3 days of culture then half media change up to 28 days twice a week.

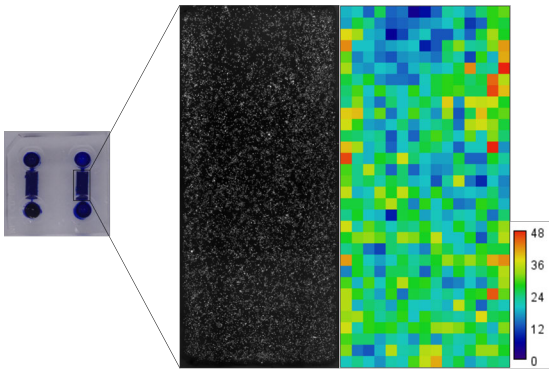

Figure 2. Cell culture homogeneity analysis. (A) Fluorescent pictures of DAPI staining. **(B)** Heat Map of neuronal homogeneity in a 3D-Deposition Chamber microfluidic device.

Growth kinetics

The growth kinetics of human GABAergic neurons from iPSC was performed in our triangular shaped microfluidic device (data not shown).

Thanks to its architecture and proprietary software the neurite growth could be measured precisely, from day 0 to day 28 (c.f. Application Protocol - Neurite Length Measurement). It has been possible to evaluate GABAergic neurites maximal length up to 860 µm at Day 28 in NETRI's microfluidic device. (c.f. Maisonneuve *et al.*, 2021).

Evaluation of pluripotent stem cells expression

Human GABAergic 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.
Pluripotency markers were stained at early and late days. Percentages of expression of theses markers are quantified by a proprietary software.

At Day 1, these markers represent 29% for Sox2 and 21% for Nestin of number of total cells and decreased to 6.5 and 0.4% respectively at late days (Day 21) (Fig.3). As expected, pluripotency markers expression decreased at late days showing a fully differentiation of GABAergic neurons seeded in NETRI's microfluidic devices.

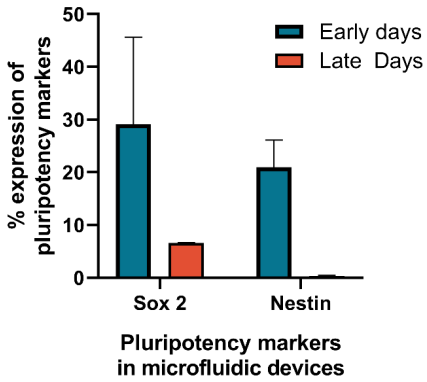

Figure 3. Evaluation of pluripotent stem cells expression (Sox 2 and Nestin markers) at Days 1 and 21.

Evaluation of specific biological markers expression

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

Human GABAergic neurons could be stained with specific markers as GABA and vGAT antibodies (Fig. 4 A-B). Expression percentage of theses specific GABAergic markers normalized by β-III-tubulin was quantified using proprietary software. The graph shows 68% of GABA, almost 100% of vGAT expression in human GABAergic neurons seeded in microfluidic device (Fig. 4 C), showing they were perfectly differentiated in NETRI's microfluidic devices. Same results were obtained in 96-well plates (*data not shown but used as control*).

Conclusion

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

This process allows different potential human GABAergic neurons applications like motor neuron disease models as amyotrophic lateral sclerosis or even neurodevelopmental disorders. Insofar as these are the main inhibitory neurotransmitter in the human brain, it can be used to mimic different aspects of the human brain and thus be compatible with a wide range of applications. It is also possible to study the *in vitro* communication between neuronal cells and glioma cells (Fuchs *et al.*, 2021).

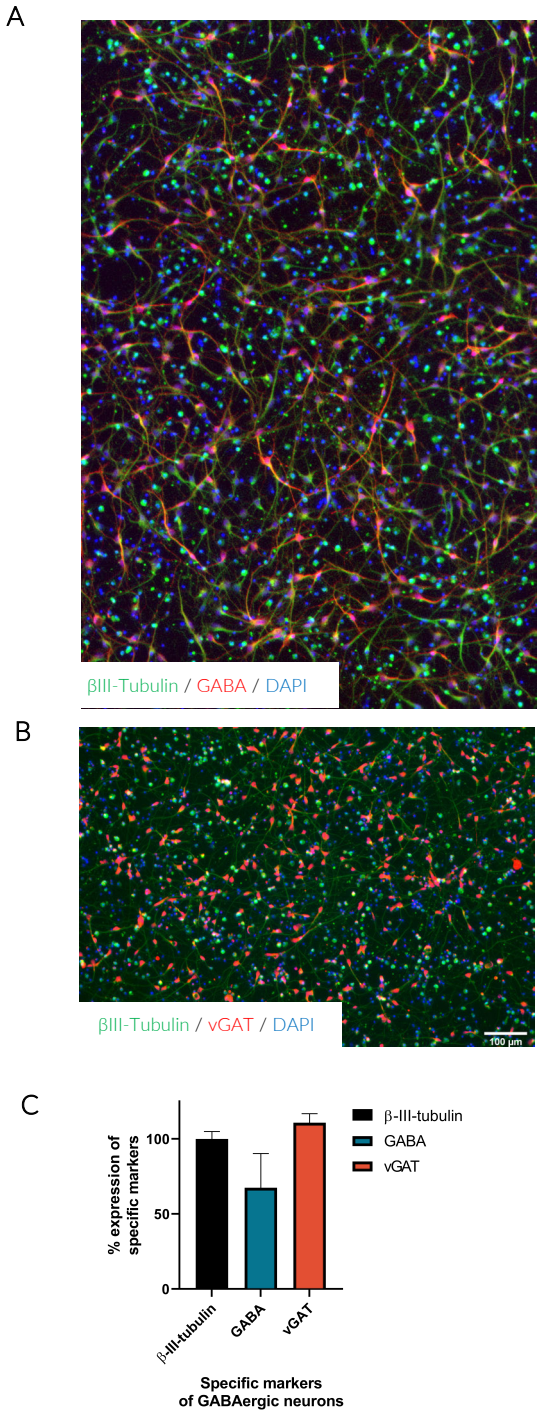

Figure 4. Evaluation of specific biological markers expression. (A), (B) Immunofluorescence at Day 28 in NETRI's microfluidic device **(C)** Percentage expression of GABAergic neurons specific markers at Day 28 in NETRI's microfluidic device.

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

- Operating Protocol – FCDI GABA Neurons
- Operating Protocol - ImmunoStaining
- Application Protocol - Neurite Length Measurement