#116 - Modeling the human Brain-on-Chip with human iPSC-derived Glutamatergic neurons

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Background

- Brain-on-Chip is the **creation of humanized neural networks culture** in **relevant microfluidic architectures** that mimics the **human brain function** with the possibility to analyze specific readouts.
- Adoption by the pharmaceutical industries of OoC models and Regulatory Bodies requires the **establishment of standardized and normalized cell culture conditions and validation criteria.**
- Here, we present **(i) data characterizing NETRI's Brain-on Chip** with **(ii) BrainXell's hiPSC-derived neurons** as a relevant minimalist model of the human brain recapitulating morphological and functional characteristics of human brain tissue.

Methods

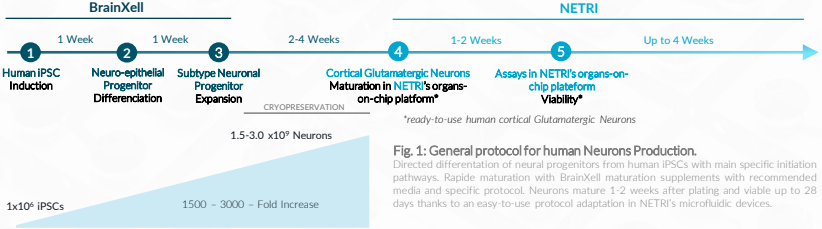

Fig. 1: General protocol for human Neurons Production.
Directed differentiation of neural progenitors from human iPSCs with main specific initiation pathways. Rapide maturation with BrainXell maturation supplements with recommended media and specific protocol. Neurons mature 1-2 weeks after plating and viable up to 28 days thanks to an easy-to-use protocol adaptation in NETRI's microfluidic devices.

Results

1 Use of characterized human Glutamatergic Neurons to mimic human brain tissue

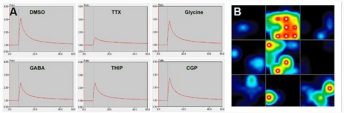

Fig. 2: Functional activity of human cortical Glutamatergic neurons.
A. Calcium changes after electrical stimulation at Day 10 in the presence of DMSO (vehicle condition), TTX (sodium channel blocker), glycine, GABA, THIP (selective GABA-A receptor agnoist), and CGP64626 (selective GABA-B antagonist). **B.** Spontaneous activity, including spikes, burst and synchronous network activity at Day 12 recorded by MultiElectrode Array (MEA).

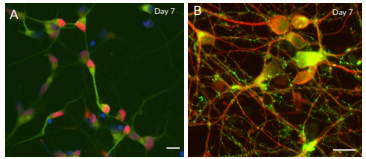

Fig. 3: Synaptic specific markers in human cortical Glutamatergic Neurons.
Images of ICC stained Glutamatergic neurons for **A.** MAP2 (Green), FOXP1 (Red) and nuclei (Blue) and **B.** Tuj1 (Red) and synaptophysin (Green) at Day 7.

2 Standardized morphological characterization of human cortical Glutamatergic Neurons in NETRI's microfluidic devices

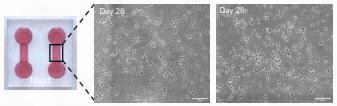

Fig. 4: Validation of long-term viability of human cortical Glutamatergic Neurons in microfluidic devices.
Cellular morphology and viability up to Day 28 with reproducticible and adapted cell culture protocol in NETRI's microfluidic devices (Honegger *et al.*, 2022). Images © NETRI 2022.

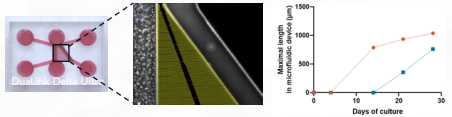

Fig. 5: Measurement of axonal outgrowth kinetics of human cortical Glutamatergic Neurons in microfluidic devices. Architecture of device allow a soma and neurites compartimentalization (Maisonneuve *et al.*, 2021) and semi-quantification of axonal growth with and without pharmacological compound. Images © NETRI 2022.

3 Fully-differentiation process and standardized functional characterisation of human cortical Glutamatergic Neurons in NETRI's microfluidic devices

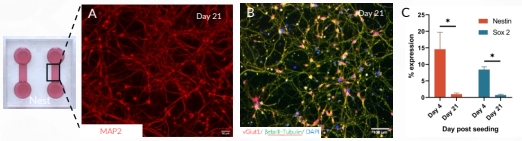

Fig. 6: Characterization of human cortical Glutamatergic Neurons in microfluidic devices. A. B. Immunofluorescence pictures with specific markers staining. C. Quantification using NETRI's proprietary software in the entire active microfluidic area. Images © NETRI 2022.

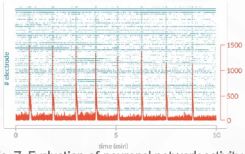

Fig. 7: Evaluation of neuronal network activity and synchronicity of human cortical Glutamatergic Neurons in microfluidic device.
Cumul of all the spikes (Red) and by each electrode (Blue) according to the time at Day 21 . Images © NETRI 2022.

Conclusions and perspectives

Thanks to characterized and ready-to-use neurons from BrainXell's and NETRI's microfluidic devices **(Booth #110)**, there are Brain-on-Chips:

- ✓ Using **hiPSCs-neurons derived in relevant architectures with a reproducible SOP;**
- ✓ **Compatible with specific equipment** (MEA, high resolution microscopy, etc.) including pharmaceutical readouts;
- ✓ **Aligned to 96-well plate format** and **easy-to-use** (pump free);
- ✓ **User friendly** (technical support and training available).

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