CO-CULTURE MODEL OF GLUTAMATERGIC NEURONS AND PEDIATRIC HIGH-GRADE GLIOMA CELL LINES IN MICROFLUIDIC DEVICES TO EVALUATE ELECTROPHYSIOLOGICAL IMPACT

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Introduction

Pedriatic high-grade gliomas (pHGG) are the most lethal brain cancers in children and adolescents and 80 % of the cases lead to death within 2 years (Ostrom *et al.*, 2015; Mackay *et al.*, 2017). Lack of therapeutic options underlies the need of *in vitro* models to rapidly test new drugs and therapeutical approaches.

pHGG have characteristics **dependent of cellular environment.** They are closely **intricated into neural networks** and induce **neuronal hyperexcitability** (Krishna *et al.*, 2012).

The aim of this study was to **develop a functional *in vitro* model of pHGG** reproducing their microenvironment and their electrical effects.

To mimic physiological conditions, we establish a robust *in vitro* protocol to co-culture glutamatergic cells derived from **human induced pluripotent stem cells (hiPSC)** and pHGG cell lines, into **microfluidic devices** (NF_1_CD_100). To monitor electrical shift of neurons activity induced by pHGG cell lines, devices are bounded onto **microelectrode arrays (MEA)** (Fuchs *et al.*, in prep).

Methodology

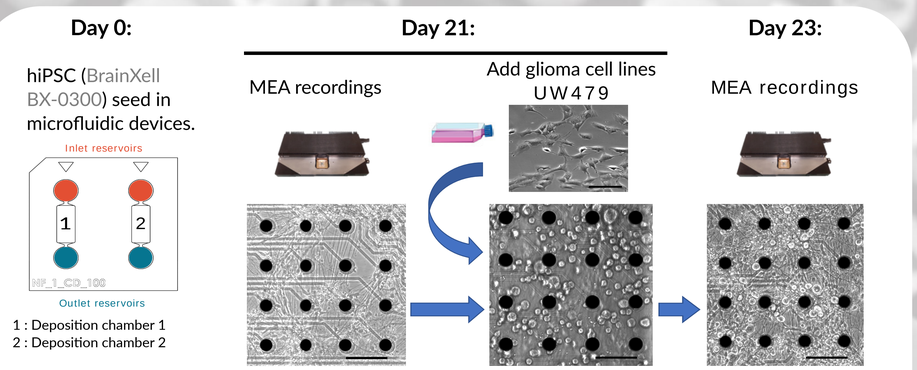

Fig. 1: The NF_1_CD_100 microfluidic devices allow two cultures and is combined with MEA. The deposition chamber contains the electrodes and the cultured cells (light transmission photographs). At Day 21, a first MEA recording is performed, then pHGG cell lines (UW479) are added to the culture. At Day 23, a second MEA recording is performed. Scale bar 100 µm.

Conclusions

- We developed an accurate functional *in vitro* model to evaluate the interactions between hiPSC-derived cortical glutamatergic neurons and brain tumoral cells in microfluidic devices.
- We opened a new approach to explore electrical impact of tumoral cells.
- Many applications arise from this methodology:
  - functional studies,
  - mechanistic studies,
  - drug testing.
- New pharmacological agents blocking interaction of the pHGG cells could be studied.
- Moreover, patient iPS-derived neurons for the development of personal medicine could be used.

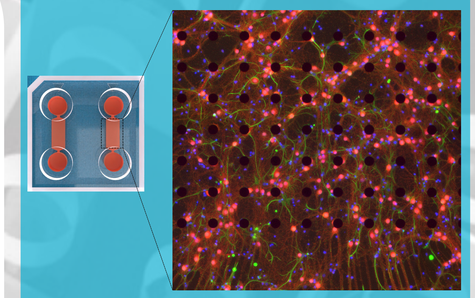

Fig. 4: Immunostaining of neurons using beta III tubulin (green), GFAP (red) and Dapi (blue) into NF_1_CD_100 microfluidic device combined with MEA which electrodes are visible (black).

Results

1 Characterization of hiPSC into microfluidic devices

The number of cells expressing **Sox2 and Nestin decrease** from Day 4 to Day 21

A majority of cells are neurons with a total of 45% express **mGluR2 marker at Day 21** in microfluidic device

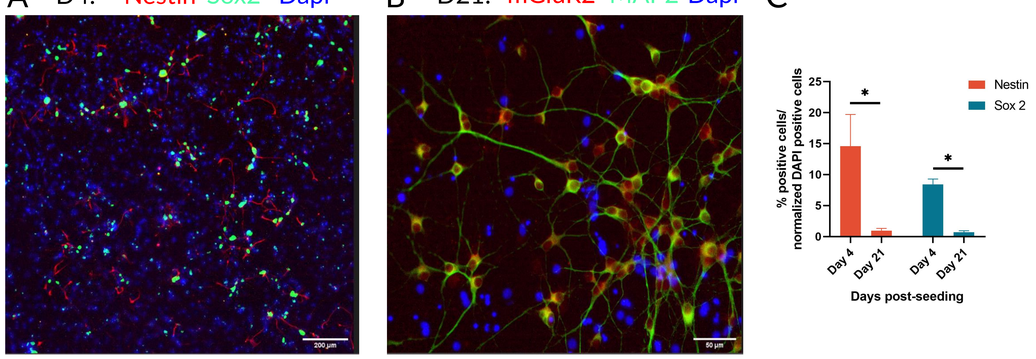

Fig. 2: Immunofluorecences characterisation of glutamatergic neurons. At Day 4 using Nestin (A) and Sox2 (B) and at Day 21 using mGluR2. Quantification of positive cells (expressing Nestin or Sox2) versus total cells number (Dapi counter stained) at Day 4 and Day 21 (C).

2 pHGG cells modify the activity of neural networks

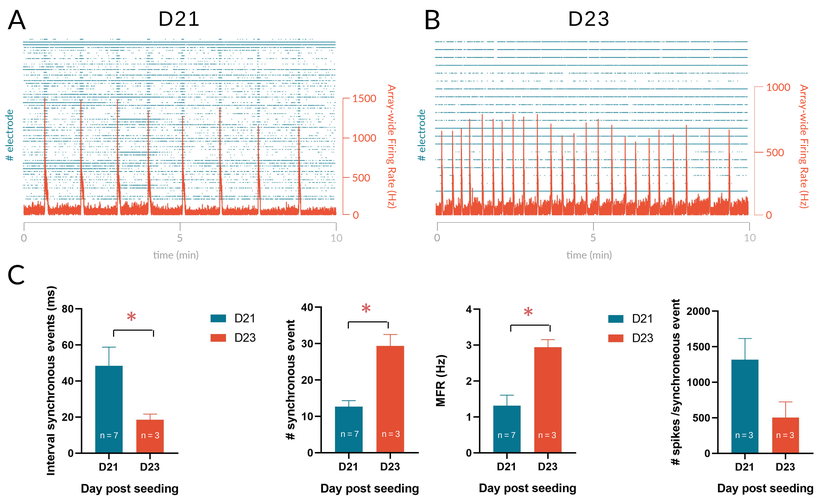

Fig. 3: (A) Each Raster plot's line (blue) reflects all the spikes detected by one electrode. Cumul of instantaneous firing rate of each electrode (red) underlies the presence of synchroneous events. (B) Two days after addition of pHGG cells, hiPSC-derived glutamatergic neurons activity shifts. (C) Mean firing rate (MFR) and number of synchroneous events increase. Conversely, the delay between events and the number of spikes per synchroneous event tends to decrease.

The pHGG cells induce an **hyperexcitability** and alter the **neuronal connectivity** without disturbing cell viability (data not shown)

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