Standardization criteria of hiPSC-derived neurons for Brain-on-Chip applications

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PROVIDING ORGANS-ON-CHIP FOR THE INDUSTRY

Abstract

Brain Organ-on Chips (OoC) is the creation of humanized neural networks culture in relevant microfluidic compartmentalization architecture with the possibility to analyze specific readouts such as functional activity recording. In order to further explore the potential of Brain-on-Chips for preclinical trials and to trigger the adoption by the scientific community, cell culture protocols need to be standardized to achieve a high reproducibility. Here, we present a methodology to control the microfluidic system variability, to optimize culture protocol adapted to specifics of microfluidic technology and to establish analytical methods including validation criteria to quantify axonal outgrowth/ functional activity. We designed a microfluidic system with compartments connected by microchannels. We established specific protocols to maintain differentiated neurons derived from hiPSCs (glutamatergic, GABAergic, dopaminergic, motor and sensory neurons). For each cell type, we evaluated the (i) cell morphology and long-term viability, (ii) expression of pluripotency and biological markers by immunofluorescence approach, (iii) growth kinetics and (iv) electrophysiological recordings using multielectrode array (MEA). Reproducibility of cell culture was assessed using semi-automatic image analysis using several cell providers and operators. To conclude, we have applied our methodology to characterize five different neural types, for which Standard Operating Procedures (SOPs) have been developed. We determined and validated specific cell culture renewal media in our devices. Our data highlight that the markers of differentiation are more expressed in microfluidic devices than in conventional cell culture devices. We suggest that this validation methodology should be an essential point when using Organ-on-Chip and to facilitate the regulatory acceptance during medicinal product development. Our hope is to open the route to standardize neural cultures for Brain-on-Chip applications.

Introduction

Adoption by the pharmaceutical industry of Organ-on-chip (OoC) models requires the establishment of normalized cell culture conditions and validation criteria before utilization.

① Standardization of microfluidic quality control

② Human cell type characterization and establishment of Standard Operating Procedure (SOP)

③ Standardization in quality control procedures of OoC

Results

1 Normalization of physiological microsystems and quality control

For each NETRI's technology, we **control the technical variability** in order for each steps of fabrication of the microfluidic system to be reproducible and not affecting the cells viability.

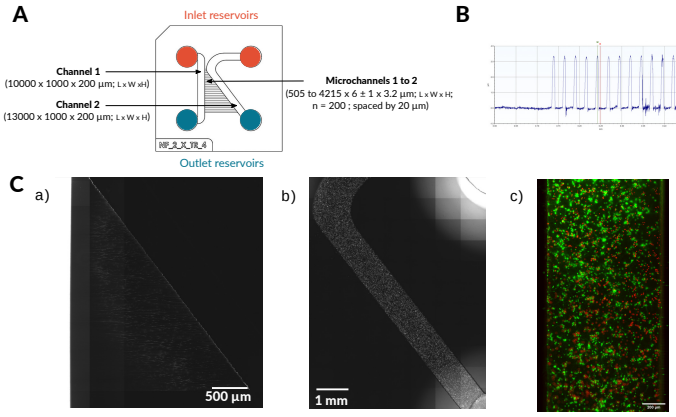


Fig. 1: (A) Triangular shape chip characteristics. (B) Dimensions are characterized by Dektak profilometry and respecting microfluidic equations. (C) Every microfluidic device design is fluidically controled and characterized with (a) fluorescence for microchannels vizualisation, (b) colloides and (c) Live (Green)-Dead (Red) Assay for cell viability. (D) Liquid replacement tests are made to determine the protocol of cell culture media renewal at 50%, 75% or 100%.

2 Normalization of human cell cultures and characterization

For each human cell type, establishment of **SOPs optimized and adapted** for microfluidic devices:

- **Reproducibility** (2 vials and 2 devices) with conventional cell culture used as control
- **Robust and controlled** cell seeding density
- Evaluation of **long-term viability** (up to 4 weeks)
- **Characteristic markers quantification** using proprietary software
- **Compatibility**, functionality assessment in Multi-Electrode Array (MEA)

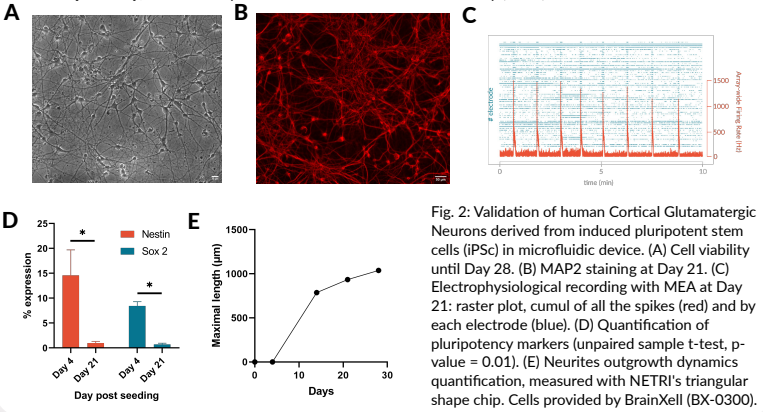
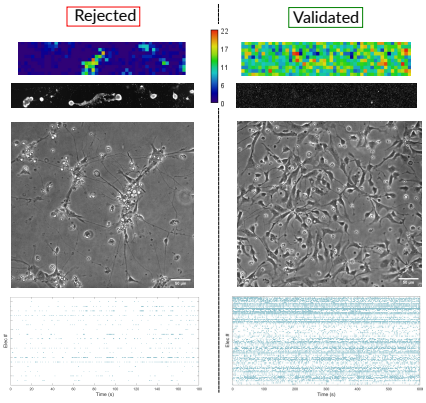


Fig. 2: Validation of human Cortical Glutamatergic Neurons derived from induced pluripotent stem cells (iPSc) in microfluidic device. (A) Cell viability until Day 28. (B) MAP2 staining at Day 21. (C) Electrophysiological recording with MEA at Day 21: raster plot, cumul of all the spikes (red) and by each electrode (blue). (D) Quantification of pluripotency markers (unpaired sample t-test, p-value = 0.01). (E) Neurites outgrowth dynamics quantification, measured with NETRI's triangular shape chip. Cells provided by BrainXell (BX-0300).

3 Standardization of validation criteria

By standardization we mean a process making validation criteria (microfluidic quality control and human cell types characterization) conform to a norm. Example of not acceptable versus validated criteria for human cell characterization in NETRI's technology.



Homogeneous seeding criteria:
Seeding validation of human Spinal Motor Neurons (BrainXell, BX-0100) in the entire active microfluidic device (proprietary software) at Day 1.

Observation criteria:
Morphological evaluation of human Midbrain Dopaminergic Neurons (BrainXell, BX-0200).

Number of active electrodes criteria:
Functionality analysis of the neural network of human Cortical GABAergic Neurons (BrainXell, BX-0400) recorded using MEA at Day 21.

Conclusions and Perspectives

This process opens new route to standardization of neural cultures for Brain-on-Chip applications:

- 5 validated and characterized human neurons
- Facilitate regulatory acceptance during drug development.

All NETRI's microfluidic devices are in NeuroBentoTM format:

- Standard SBS alignment on 96-well microplates format
- Compatible with HTS & all 96-well microscope jigs
- Optical transparency
- Compatible with all transmissive imaging methods

or any microscope, direct or inverted

- No pump or mechanical stirrer needed
- Optimized design to ensure the maturation of cell cultures

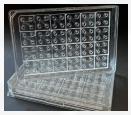
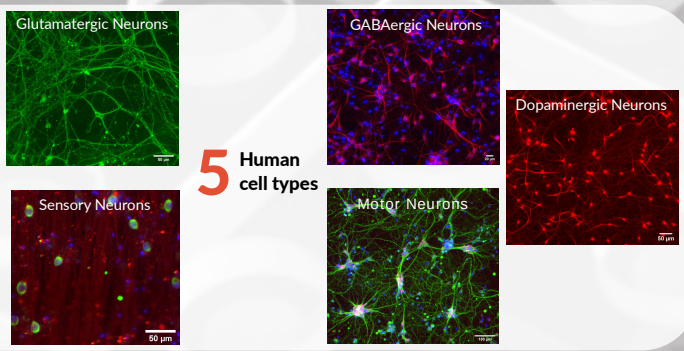


Fig. 3: NeuroBento™ Pharma.

- Up to 28 Days in vitro
- Expected morphology
- Expression of specific markers and functional activity
- Reproducible Operating Protocol



5 Human cell types

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We thank BrainXell team for scientific advices and providing human cell. BrainXell

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