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

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Background

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

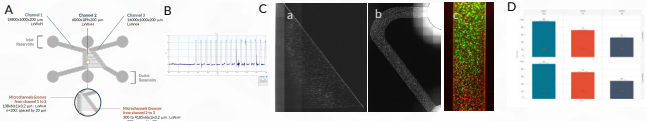


Fig. 1: (A) Triangular shape chip, DuaLink™ Delta Ultra, characteristics. (B) Dimensions are characterized by Dektak profilometry and respecting microfluidic equations. (C) Every microfluidic device design is fluidically controled and characterized with (a) fluorescence for microchannels vizualisation, (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

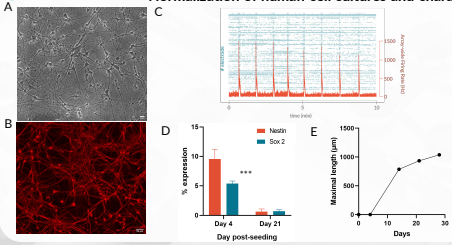
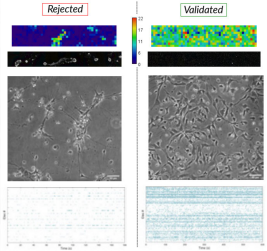


Fig. 2: Validation of human Cortical Glutamatergic Neurons derived from induced pluripotent stem cells (iPSc) in microfluidic device. (A) Cell viability until Day 28. (B) MAP2 staining at Day 21. (C) Electrophysiological recording with MEA at Day 21: raster plot, cumul of all the spikes (red) and by each electrode (blue). (D) Quantification of pluripotency markers (unpaired sample t-test, pvalue = 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.*

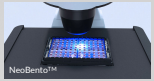
Conclusion & Perspectives

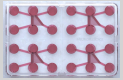
This process opens new route to standardization of neural cultures for Brain-on-Chip applications:

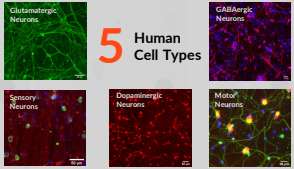
- ✓ 5 validated and characterized human neurons
- ✓ Facilitate regulatory acceptance during drug development

All NETRI's microfluidic devices are in NeoBento™ format:

- ✓ Standard SBS alignment on 96-well microplates format
- ✓ Compatible with HTS equipments (liquid handlers, MEA and microscopes)
- ✓ No expensive equipment needed
- ✓ Optimized design to ensure the maturation of cell cultures







- ✓ Up to 28 Days *in vitro*
- ✓ Expected morphology
- ✓ Expression of specific markers and functional activity
- ✓ Reproducible and adapted protocols

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