#389.06 - A NEW HUMAN BRAIN ORGANOID-ON-CHIP MODEL MEETING BIOLOGICAL AND INDUSTRIAL REQUIREMENTS OF NEUROLOGICAL PRECLINICAL STUDIES

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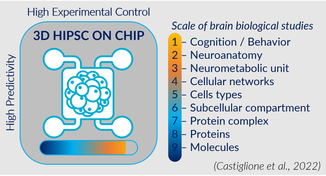
BACKGROUND



Cerebral organoids appear as relevant alternative solutions to model human cerebral cellular organization and development in 3D. Nevertheless, implementing organoids for **large-scale drug screening** requires **reproducibility and scalability**. To address this challenge, we propose to generate a **Brain Organoid-on-Chip platform** with:

- A microfluidic technology for recreating cellular microenvironment
- An on-chip cell culture protocol to improve **reproducibility** of cerebral organoids
- Compatibility with a full process of **cerebral organoid characterization**

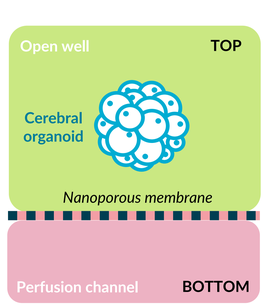
EXPERIMENTAL DESIGN

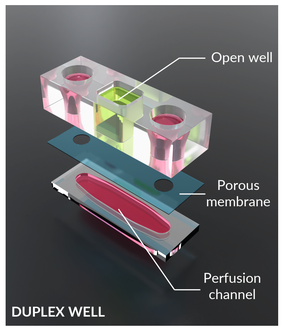
NETRI's DUPLEX WELL MICROFLUIDIC DEVICE.

Adapted to 3D cell culture.

Two compartments separated by a porous membrane:
- Open well for 3D culture (5x5x5mm; LxWxH)
- Perfusion channel to enhance nutrient diffusion

Adapted to industrial transfer.
- Standardized fabrication procedures
- Compatible with automation processes and adapted to pharmaceutical readouts (microscopy, MEA, HTS)





OPTIMIZATION OF CULTURE CONDITIONS ON-CHIP.

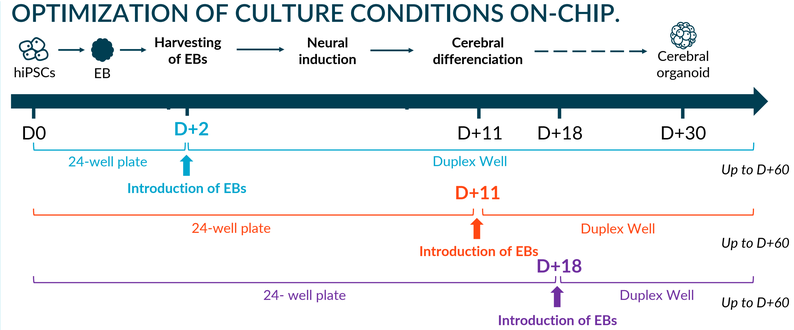


Fig. 1. Main steps of guided differentiation protocol into cortical organoids (adapted from Xiang *et al.*, 2019) and three timepoints of EBs introduction into Duplex assayed (*hiPSCs: human induced pluripotent stem cells, EB: embryoid body*).

- Three timepoints of EBs introduction into Duplex tested (Fig. 1): D+2, D+11, D+18
- Three media renewal conditions tested per timepoint (Fig. 2.): 1, 2, 3

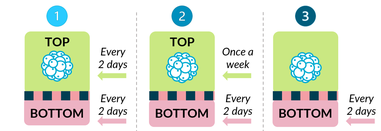


Fig. 2. Three conditions of medium renewal in Duplex Well.

Controls: - 24-well plate (P24)
- 96-well plate (P96)

RESULTS

ESTABLISHMENT OF A STANDARD PROTOCOL FOR ORGANOIDS ON-CHIP CULTURE.

Viability and expected morphology up to D+60

Anti-adherence protocol established to limit organoid adhesion
- Timepoint D+2: partial adherence
- Medium renewal #3: partial adherence

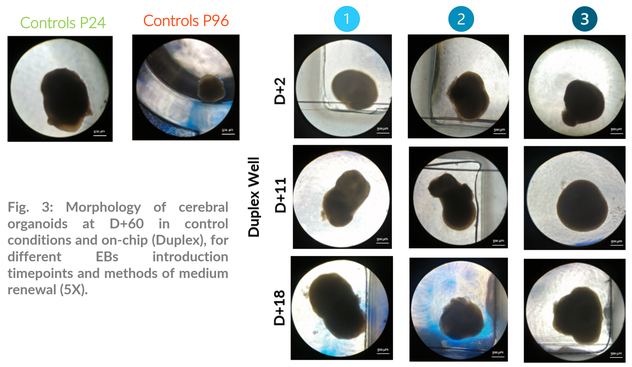


Fig. 3: Morphology of cerebral organoids at D+60 in control conditions and on-chip (Duplex), for different EBs introduction timepoints and methods of medium renewal (5X).

Tendency to obtain more reproducible sizes on-chip compared to 24-well controls

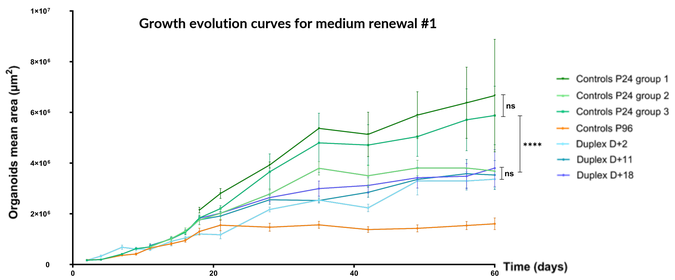


Fig. 4: **Cerebral organoids growth from D+2 until D+60 of culture, for control organoids and organoids-on-chip with method #1 of medium renewal** (Mean ±SEM, n=3 per group). Statistical differences between the groups were determined by simple linear regression (****p<0.001): D+2 vs D+11, D+2 vs D+18, D+11 vs D+18: ns, P24 grp1 vs grp3: ns; P24 grp1 vs grp2, P24 grp3 vs grp2: ****).

CHARACTERIZATION OF BRAIN ORGANOID-ON-CHIP.

Transcript level of expression (RT-qPCR)

Condition (D+11 & renewal #1) on-chip most similar to Controls P24 (Fig. 5)

Expected cytoarchitectures at D+60 with characteristic neurogenic areas (rosettes)

Tend to be more reproducible in terms of structural organization compared to Controls P24 (Fig. 6)

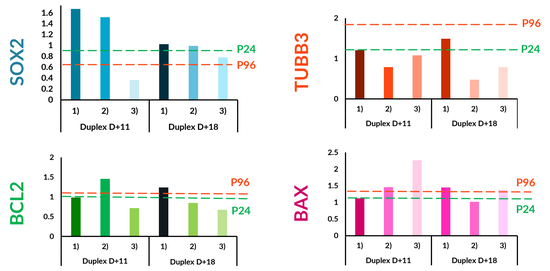


Fig. 5: Fold gene expression values in cerebral organoids on-chip and controls for neural progenitors (SOX2), neurons (TUBB3), anti-apoptotic (BCL2), and pro-apoptotic (BAX) markers.

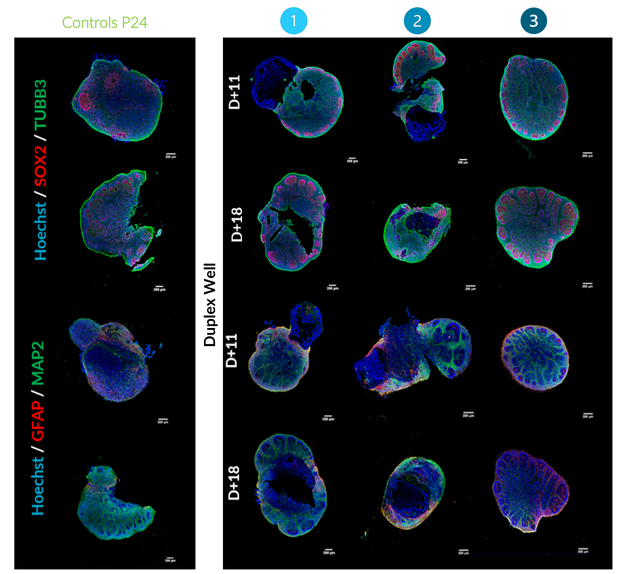


Fig. 6: Immunofluorescence staining of neural progenitors (SOX2), neurons (TUBB3), astrocytes (GFAP), and mature neurons (MAP2) in cerebral organoids cultured at D+60. (Thunder microscope, Leica, objective 10x).

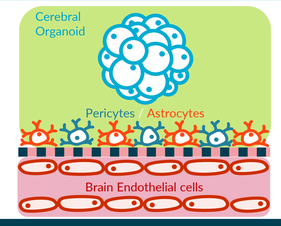
CONCLUSION & PERSPECTIVES

By combining **NETRI's microfluidic device** and **cerebral organoids**, we have set up a **Brain Organoid-on-Chip platform** meeting the needs **of preclinical applications** with organoids exhibiting **expected characteristic morphology**, **cell types**, **cytoarchitectures**, and **transcript levels of expression at D+60**.

Our cerebral organoids on-chip protocol allows:
- Several possible **timepoints of EBs introduction** and **methods of medium renewal** on-chip, depending on the experimental design

Future perspectives include further **organoid viability characterization**, **on-chip analyzes** (microscopy, MEA) and complexification of the model by adding cell types for **blood-brain barrier** (BBB) modelling.

Our Brain Organoid-on-Chip platform paves the way for **HTS drug screening** and **toxicological assessments.**



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