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

H. Castiglione[1,2,3], J. Rontard[3], L. Madrange[1,2], C. Baquerre[3], T. Honegger[3], F. Yates[1,2], P.-A. Vigneron[1,2]

[1] SupBiotech/CEA-IBFJ-SEPIA, 92260 Fontenay-aux-Roses, France, [2] SupBiotech, Ecole d'ingénieurs en Biotechnologies, 94800 Villejuif, France, [3] NETRI, 69007 Lyon, France ; pierre-antoine.vigneron@supbiotech.fr

Cerebral organoids

- *In vitro* models of **human developing brain**
- **3D self-organized** cell structures
- **Lack of reproducibility & transferability to industrial scale** [1]

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NETRI microfluidic devices

- Controlled **fluid flows**
- **Cellular microenvironment** modeling
- Compatible with **industrial readouts** & **automation processes**

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Brain-Organoid-on-Chip

- Promising technology to facilitate cerebral organoid **scalability** for **preclinical applications** [1]
- Establishment of an **on-chip culture protocol** for cerebral organoids, with improved **reproducibility**

MATERIALS AND METHODS

NETRI's DUPLEX WELL MICROFLUIDIC DEVICE

Adapted to 3D cell culture:

Two compartments separated by a porous membrane:

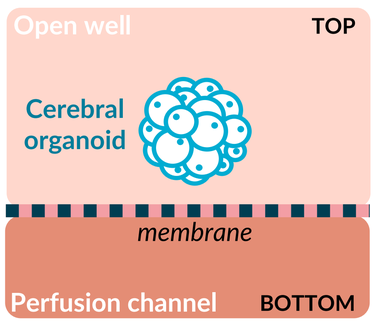
- Open well for 3D culture (5x6x4.6 mm)
- Perfusion channel

Adapted to industrial transfer:

- Standardized fabrication procedures
- Compatible with automation processes
- Adapted to pharmaceutical readouts (microscopy, MEA, HTS)

Membrane anti-adherence method:

- To limit organoid adhesion
- Based on fluid mechanics



OPTIMIZATION OF ON-CHIP CULTURE CONDITIONS

Regionalized differentiation into cortical organoids and assayed conditions:

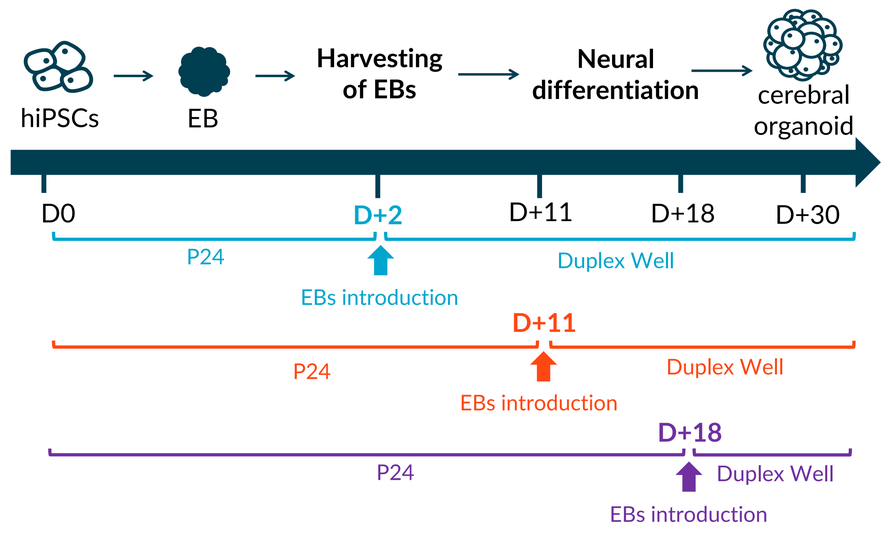


Fig. 1. Main steps of guided differentiation protocol into cortical organoids (adapted from [2]) and three assayed timepoints of EBs introduction into Duplex Well (*hiPSCs: human induced pluripotent stem cells, EB: embryoid body). hiPSCs were reprogrammed from BJ fibroblasts (ATCC, CRL-2522).*

- 24-well plate (P24): control organoids cultured in conventional support (+ control)
- 96-well plate (P96): control organoids cultured in 150µL (volume in Duplex) (- control)
- Duplex Well:

→Three timepoints of EBs introduction (Fig. 1): D+2, D+11, D+18

→Three conditions of medium renewal (Fig. 2.): 1, 2, 3

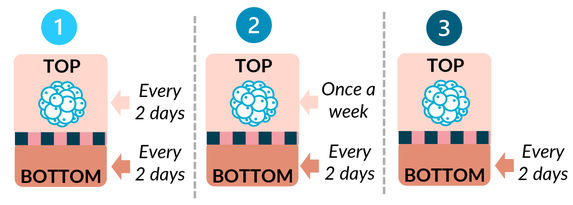


Fig. 2. Three conditions of medium renewal in Duplex Well.

RESULTS

EXPECTED MORPHOLOGIES UP TO D+60

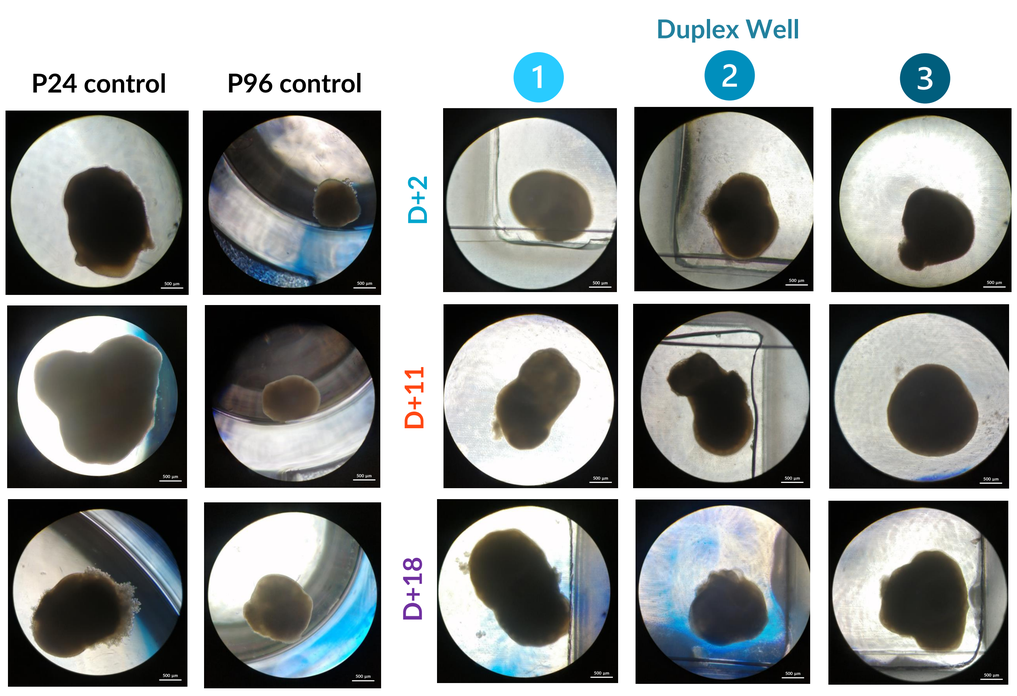


Fig. 3: Morphology of cerebral organoids at D+60 in control and on-chip conditions, for different EBs introduction timepoints and methods of medium renewal (brightfield, 5X).

- Some organoids adhered on the membrane for Timepoint D+2 and for Condition 3 of medium renewal

TENDENCY TO DISPLAY MORE REPRODUCIBLE SIZES AND GROWTH PROFILES COMPARED TO CONTROLS

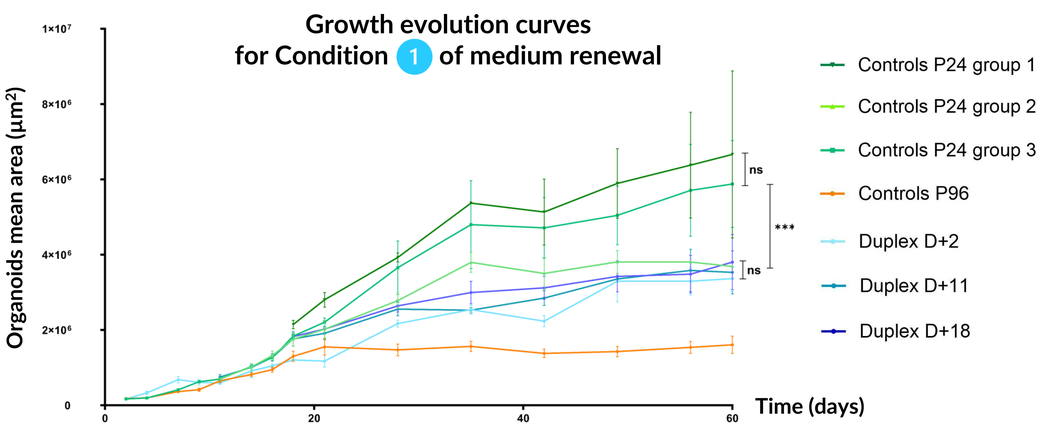
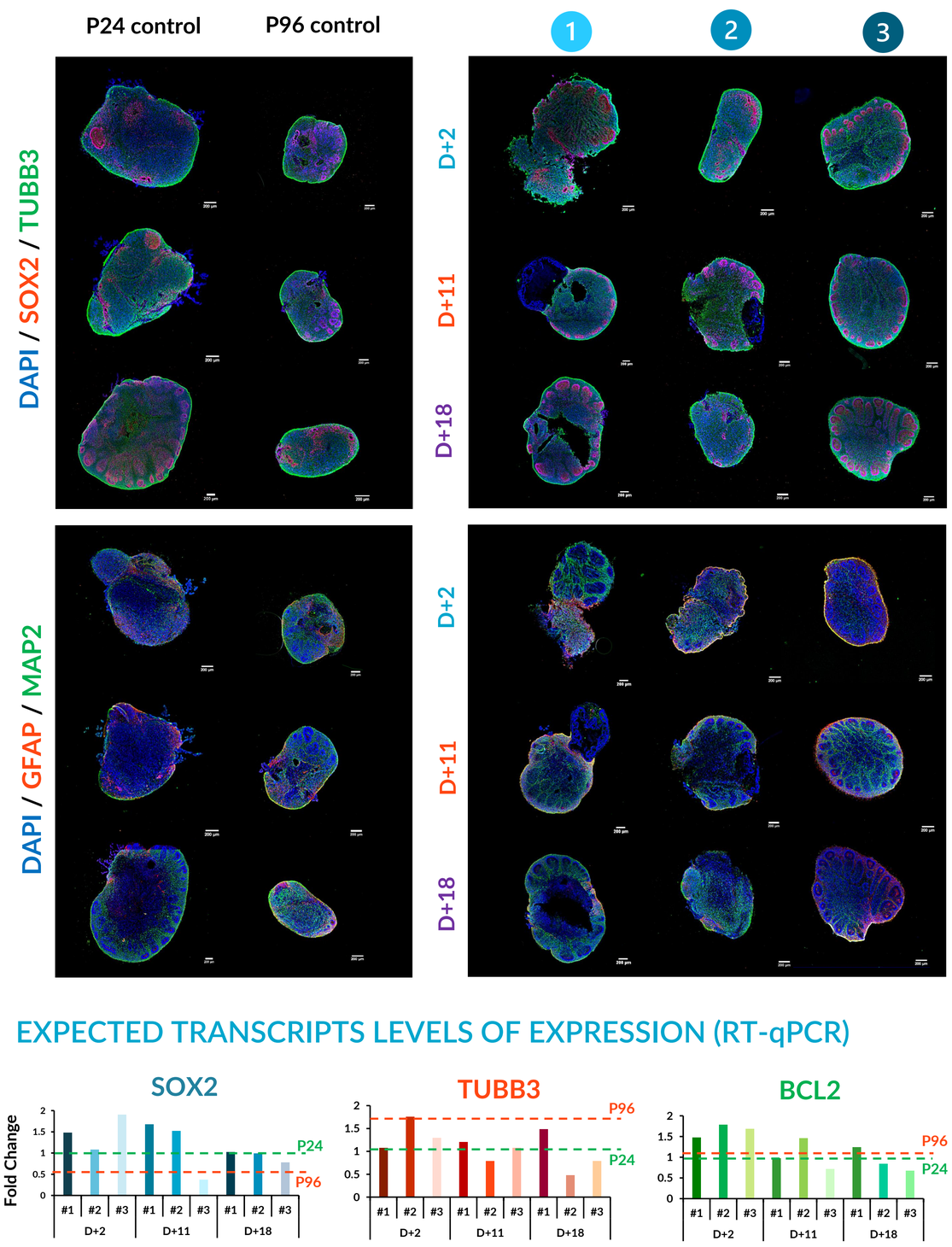


Fig. 4: Cerebral organoids growth from D+2 until D+60 of culture, for on-chip and control organoids. **On-chip organoids exhibit more reproducible intra- and inter-batch sizes and growth evolutions.** (Mean ±SEM, n=3 per group). Statistical differences between the groups were determined by simple linear regression (****p<0.001), with Duplex 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: ****).

EXPECTED CYTOARCHITECTURES AT D+60 AND TENDENCY TO EXHIBIT MORE REPRODUCIBLE STRUCTURES COMPARED TO CONTROLS



Structural organizations:

- D+11 and D+18 more optimal than D+2
- 1 and 2 similar
- 3 display most optimal patterns of neurogenic areas (rosettes)

Improved reproducibility for on-chip conditions compared to controls:

- Rosettes with expected morphologies (oval shapes) & well-organized patterns (present along organoid borders)

Fig. 5: Immunofluorescence staining of neural progenitors (SOX2), neurons (TUBB3, MAP2), and astrocytes (GFAP) in cerebral organoids cultured at D+60. (Thunder microscope, Leica, objective 10X).

EXPECTED TRANSCRIPTS LEVELS OF EXPRESSION (RT-qPCR)

Fig. 6: Fold gene expression values in control and on-chip cerebral organoids for neural progenitors (SOX2), neurons (TUBB3), anti-apoptotic (BCL2), and pro-apoptotic (BAX) markers.

CONCLUSION

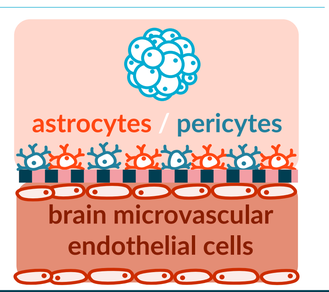
- On-chip cerebral organoids: expected morphology, cell types, cytoarchitectures & RNA expression levels
- Improved reproducibility: size, growth profile & cytoarchitectures *(also demonstrated with another hiPSCs line)*
- Several possible timepoints of EBs introduction & methods of medium renewal:

	1	2	3
D+2	✗	✗	✓
D+11	✓	✓	✓
D+18	✓	✓	✓

PERSPECTIVES

- Further organoid viability characterization
- Blood-brain barrier modelling

→ *Paves the way for drug screening & toxicological assessments*



[1] Castiglione, H.; Vigneron, P.-A.; Baquerre, C.; Yates, F.; Rontard, J.; Honegger, T. Human Brain Organoids-on-Chip: Advances, Challenges, and Perspectives for Preclinical Applications. Pharmaceutics 2022, 14, 2301.
[2] Xiang, Y.; Tanaka, Y.; Cakir, B.; Patterson, B.; Kim, K.; Sun, P.; Kang, Y.; Zhong, M.; Liu, X.; Patra, P.; Lee, S.; Weissman, S. M.; Park, I. hESC-derived thalamic organoids form reciprocal projections when fused with cortical organoids. Cell Stem Cell 2019, 24(3), 487–497.

Conflicts of Interest: *The authors declare no conflict of interest. H.C., J.R., C.B. and T.H. are employees of NETRI. T.H. is Chief Executive Officer and Chief Scientific Officer at NETRI.*

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- www.netri.com
- Phone: +33 4 78 23 08 66
- Email: contact@netri.com