DEVELOPMENT OF A BRAIN ORGANOID-ON-CHIP PLATFORM FOR NEUROTOXICITY TESTING

BOOTH #317

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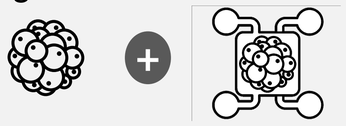
BACKGROUND

Cerebral organoid + **Microfluidic device**



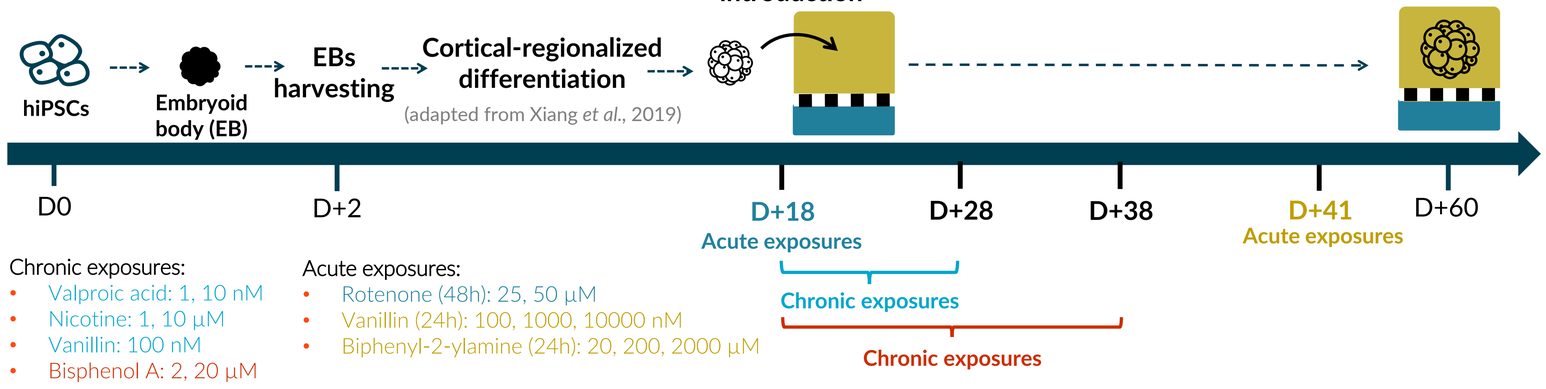
Cerebral organoids have emerged as relevant *in vitro* models of human brain development. To facilitate their use for **large-scale compound screening and testing**, they require **a gain of reproducibility and scalability** (Castiglione *et al.*, 2022). To address this challenge, we have:

- Developed a **Brain Organoid-on-Chip platform**, combining **cerebral organoid** culture in a **NETRI microfluidic device**;
- Optimized an on-chip culture protocol for cerebral organoids, with **improved reproducibility** compared with conventional culture supports;
- Established a **Quality Control** for cerebral organoids based on a **scoring system** (Castiglione *et al.*, 2025, under review), along with the development of a **Prediction Algorithm** for **toxicological evaluation of cortical organoids exposed to chemical compounds.**

Using this platform, we have initiated **neurodevelopmental toxicity evaluations** of several chemicals under different exposure modes.

MATERIALS & METHODS

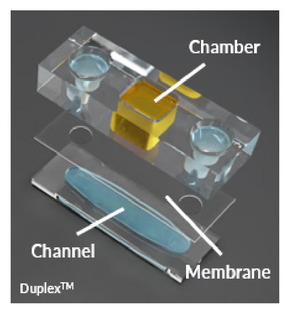
On-chip culture conditions & compound exposures



Chronic exposures:
- Valproic acid: 1, 10 nM
- Nicotine: 1, 10 µM
- Vanillin: 100 nM
- Bisphenol A: 2, 20 µM

Acute exposures:
- Rotenone (48h): 25, 50 µM
- Vanillin (24h): 100, 1000, 10000 nM
- Biphenyl-2-ylamine (24h): 20, 200, 2000 µM

NETRI's microfluidic device: Duplex



Adapted to 3D cell culture:
Two **compartments** separated by a porous **membrane**:
- Open well for 3D culture
- Perfusion channel

Anti-adherence protocol

Adapted to industrial transfer

Pumpless

Standardized on-chip protocol for cortical organoid culture

- Expected morphology, cell types, cytoarchitectures & RNA expression levels up to D+120
- **Improved reproducibility:** sizes & cytoarchitectures at D+60 & D+120

Quality scoring (Castiglione *et al.*, 2025, under review)

- Cortical organoid characterization
- Scoring scale: 5 to 0 *(from most to least optimal)*

Exposure scoring

- For **compound-exposed** cortical organoids
- Compared to controls

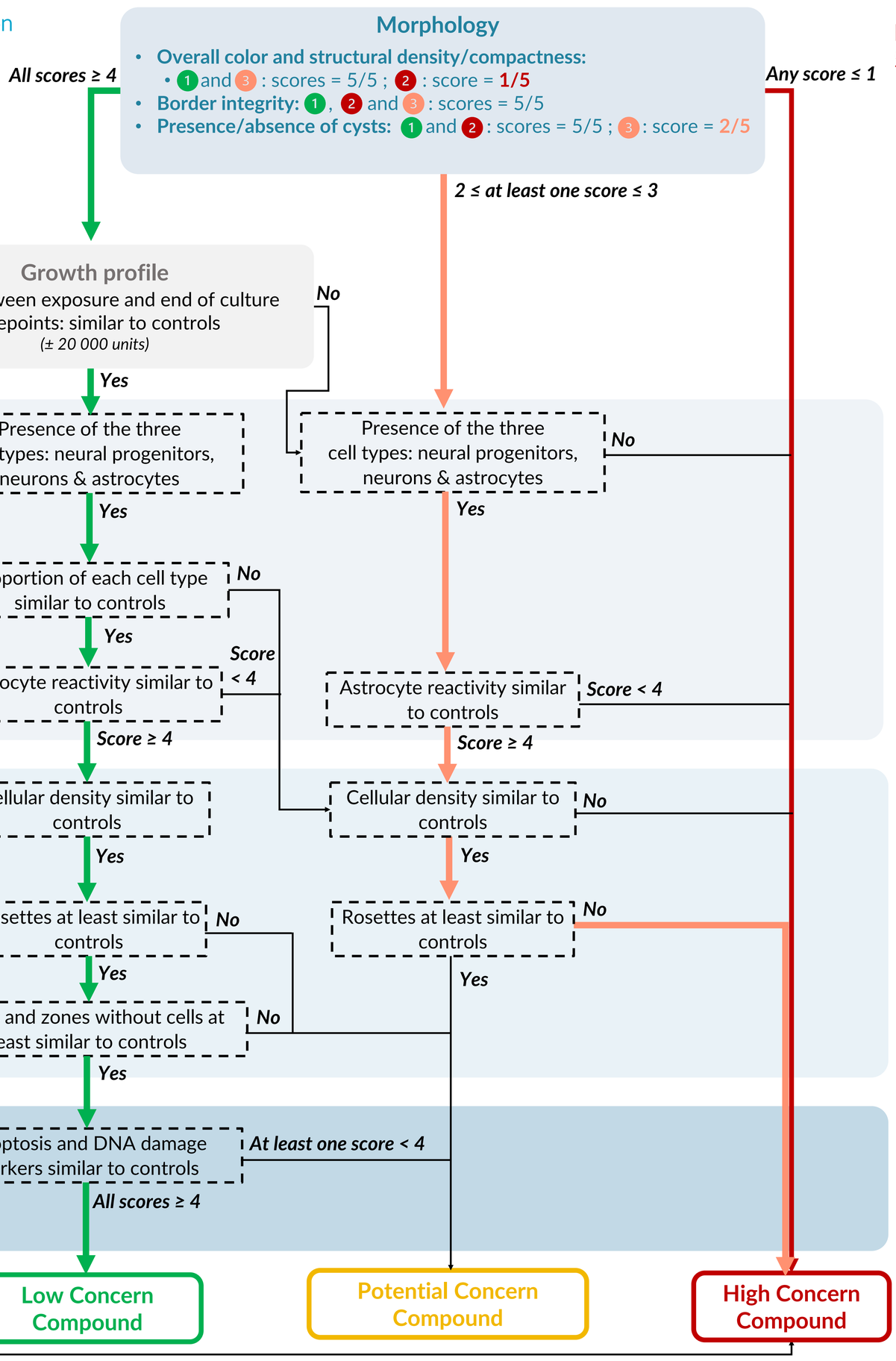
Prediction algorithm

For compound classification into **3 neurotoxicological risk categories**

Timeline of cortical organoid generation and culture protocol, on-chip culture conditions, compound exposures, and schematic representation of the Duplex device (*hiPSCs: human induced pluripotent stem cells).*

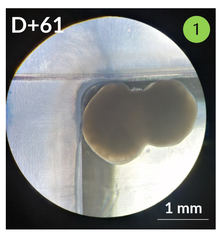
RESULTS

Prediction Algorithm for compound classification



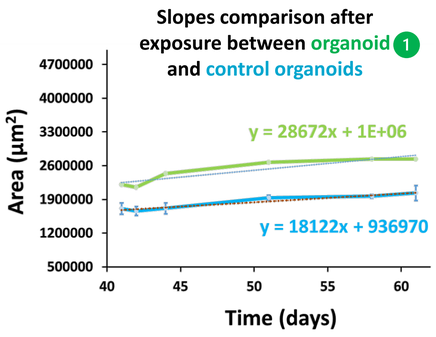
Example 1 : acute exposure with 10 000 nM vanillin

Morphology: optimal



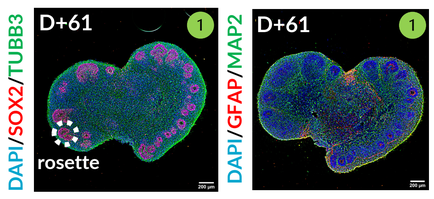
Morphology of cortical organoid #1 at 61 days of culture, exposed to 10 000 nM vanillin at D+41 (brightfield, 5X).

Growth profile: similar compared with controls



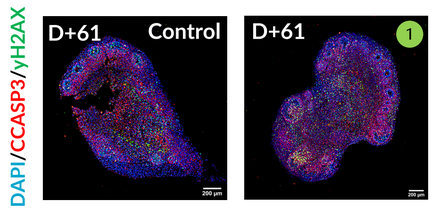
Cortical organoids growth curves and slopes between exposure (D+41) and end of culture (D+61) (for controls: mean ±SEM, n=4).

Expected cell types and optimal cytoarchitectural organization:



Immunofluorescence staining of neural progenitors (SOX2), neurons (TUBB3, MAP2), and astrocytes (GFAP) (Thunder microscope, Leica, objective 20X, circle: rosette).

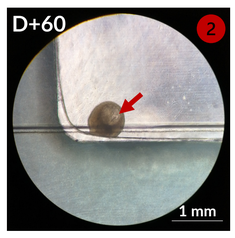
Similar apoptosis & DNA damage levels as controls:



Immunofluorescence staining of apoptosis (Cleaved Caspase-3, CCASP3) and DNA damage (γH2AX) (Thunder microscope, Leica, objective 20X).

Example 2 : acute exposure with 50 µM rotenone

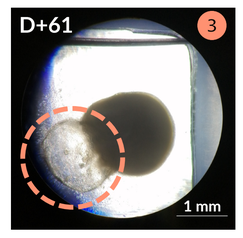
Morphology: not optimal
- Poor density/compactness (visible cell-less area, red arrow)



Morphology of cortical organoid #2 at 60 days of culture, exposed to 50 µM rotenone at D+18 (brightfield, 5X, arrow: poorly-dense area).

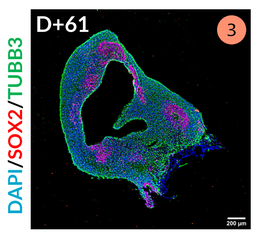
Example 3 : acute exposure with 2 000 µM biphenyl-2-ylamine

Morphology: altered
- Presence of a **large cyst** (> 30% of total surface area, orange circle)



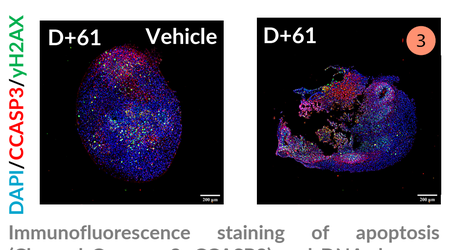
Morphology of cortical organoid #3 at 61 days of culture, exposed to 2 000 µM biphenyl-2-ylamine at D+41 (brightfield, 5X, circle: cyst).

Expected cell types, with disorganized cytoarchitectures:
- Altered pattern of neurogenic areas (rosettes)



Immunofluorescence staining of neural progenitors (SOX2) and neurons (TUBB3) (Thunder microscope, Leica, objective 20X).

Higher apoptosis & DNA damage levels compared to controls:



Immunofluorescence staining of apoptosis (Cleaved Caspase-3, CCASP3) and DNA damage (γH2AX) (Thunder microscope, Leica, objective 20X).

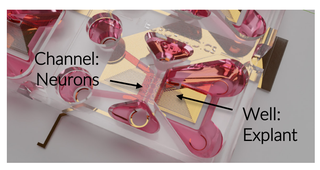
CONCLUSION & PERPSECTIVES

Brain Organoid-on-Chip platform & Prediction Algorithm:

- Adapted to **neurotoxicity testing**
- Open the way towards **compound screening for neurotoxicity evaluations** in drug development, chemical risk assessment, food safety, ecotoxicology, ...

Model complexifications:

- Functional readout: **electrophysiological activity recording** on-chip
- Blood-Brain Barrier modeling



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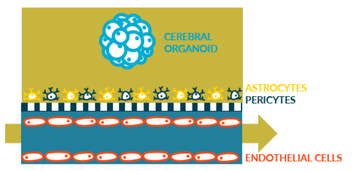

Blood Brain Barrier-on-chip

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