Team NETRI / SupBiotech-CEA: Prediction Algorithm for Neurotoxicity Evaluation based on Brain Organoid-on-Chip

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Cerebral organoid + **Microfluidic device (NETRI)**

Brain-Organoid-on-Chip

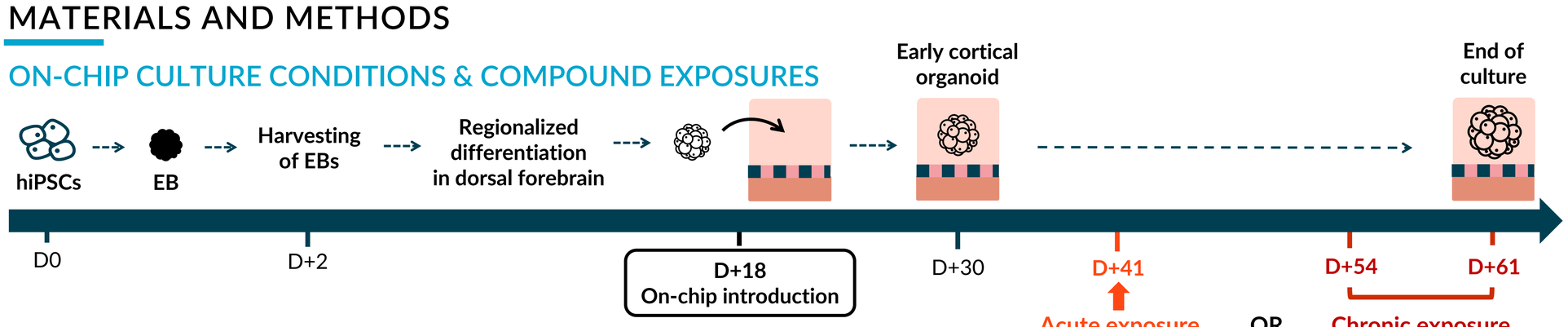
- Enhanced cerebral organoid **reproducibility**
- Promising technology to facilitate organoid **scalability** & to **overcome current limitations** due to high organoid heterogeneity [1]

Neurotoxicity studies: NAM Designathon

- Participation to **EPAA NAM Designathon Challenge**: proposition of a NAM-based classification system for predicting **compound neurotoxicity**
- Evaluated compounds: **vanillin** & **biphenyl-2-ylamine**

MATERIALS AND METHODS

ON-CHIP CULTURE CONDITIONS & COMPOUND EXPOSURES



Vanillin *(CAS n°121-33-5)*: 100, 1 000, 10 000 nM
Biphenyl-2-ylamine *(CAS n°90-41-5)*: 20, 200, 2 000 µM *(meOH)*

Biphenyl-2-ylamine: 200 µM *(meOH)*

Controls: non-exposed and vehicle-exposed organoids

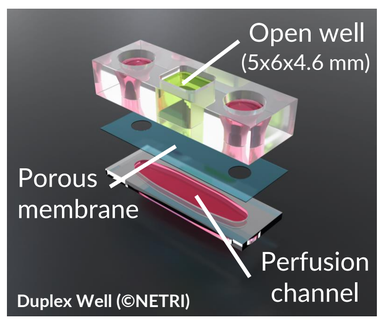
NETRI's MICROFLUIDIC DEVICE

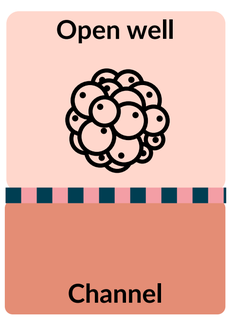
Adapted to 3D cell culture:

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

Adapted to industrial transfer

Pumpless





QUALITY SCORING

- Cortical organoid characterization (D+60)
- Scoring scale: 5 to 0 *(from most to least optimal)*

EXPOSURE SCORING

- For compound-exposed cortical organoids (acute & chronic)
- Compared to controls

PREDICTION ALGORITHM

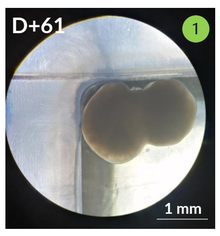
- For compound classification into 3 neurotoxicological categories

Timeline of cortical organoid generation and culture protocol (adapted from [2]), on-chip culture conditions, compound exposures, and Duplex Well schematic representations *(hiPSCs: human induced pluripotent stem cells, EB: embryoid body).*

RESULTS COMPOUND CLASSIFICATION USING THE PREDICTION ALGORITHM

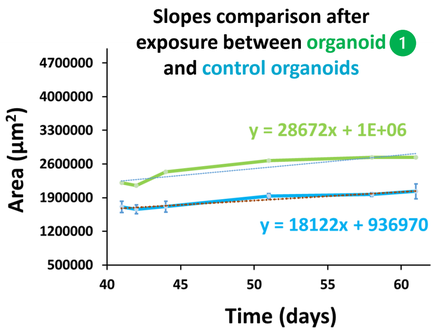
Example 1: acute exposure with 10 000 nM vanillin

Morphology: optimal



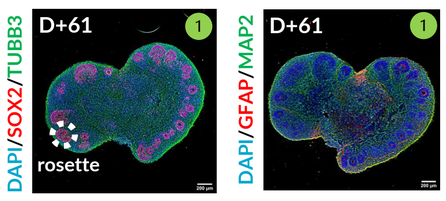
Morphology of cortical organoid #1 at 61 days of culture *(brightfield, 5X).*

Growth profile: similar compared to controls



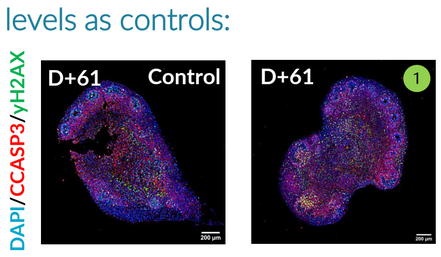
Cortical organoids growth curves and slopes between exposure (D+41) and end of culture (D+61) *(for controls: mean ±SEM, n=4).*

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 (CCASP3) and DNA damage (yH2AX) *(Thunder microscope, Leica, objective 20X).*

Morphology
- Overall color and structural density/compactness: 1 and 2 = score 4
- Border integrity: 1 and 2 = score 4
- Presence/absence of cysts: 1 = score 4; 2 = score 2

All scores ≥ 4 → **Growth profile**: Slope between exposure and end of culture timepoints: similar to controls *(± 20 000 units)*

No → *Any score = 0*

Yes → *1 ≤ at least one score ≤ 3*

Cellular populations

Presence of the three cell types: neural progenitors, neurons & astrocytes — *No* / *Yes*

Proportion of each cell type similar to controls — *No* / *Yes*

Astrocyte reactivity similar to controls — *Score < 4* / *Score ≥ 4*

Cytoarchitectures

Cellular density similar to controls — *No* / *Yes*

Rosettes at least similar to controls — *No* / *Yes*

Cysts and zones without cells at least similar to controls — *No* / *Yes*

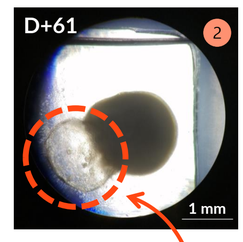
Cytotoxicity

Apoptosis and DNA damage markers similar to controls — *At least one score < 4* / *All scores ≥ 4*

Low Concern Compound | **Potential Concern Compound** | **High Concern Compound**

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

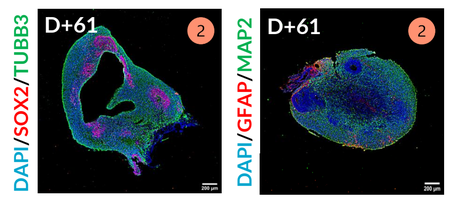
Morphology: altered



Morphology of cortical organoid #2 at 61 days of culture *(brightfield, 5X, circle: cyst).*

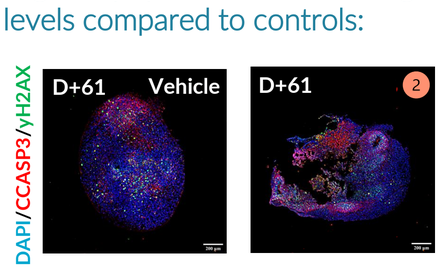
Presence of a large cyst
(> 25% of total surface area)

Expected cell types, but disorganized cytoarchitectures:
- Altered pattern of neurogenic areas (rosettes)
- Presence of a cyst and a large zone without cells (necrotic core)



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

Higher apoptosis & DNA damage levels compared to controls:



Immunofluorescence staining of apoptosis (CCASP3) and DNA damage (yH2AX) *(Thunder microscope, Leica, objective 20X).*

CONCLUSION

- Brain Organoid-on-Chip platform + Scorings + Prediction Algorithm: adapted to neurotoxicity evaluations
- Vanillin exposures: no discernable impact on morphology, cytoarchitectures & viability → low concern
- Biphenyl-2-ylamine exposures: altered morphology & disorganized cytoarchitectures in a dose-response manner → high concern

PERSPECTIVES

- Implementation of additional criteria for organoid cytotoxicity characterization
- *Paves the way for neurotoxicological studies & drug screening*

[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., B.G.C.M. and T.H. are employees of NETRI. T.H. is Chief Executive Officer and Chief Scientific Officer at NETRI.*

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