MULTIMODAL ASSESSMENT OF PESTICIDE-INDUCED NEUROTOXICITY USING A HUMAN iPSC-BASED ORGAN-ON-CHIP MODEL

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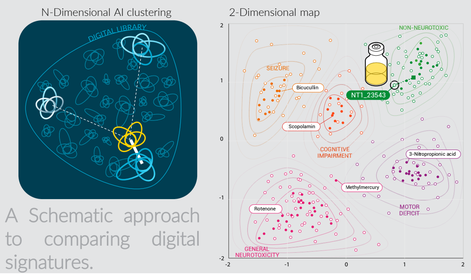
BACKGROUND



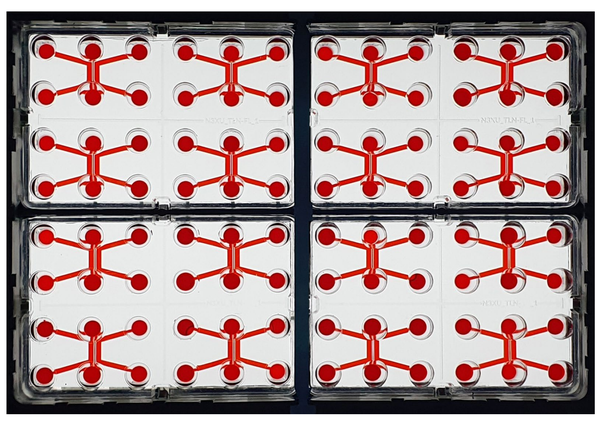
A Schematic approach to comparing digital signatures.

We described here a collaborative project between NETRI and ANSES to develop a human-relevant *in vitro* platform for pesticide-induced neurotoxicity assessment. This study combines:

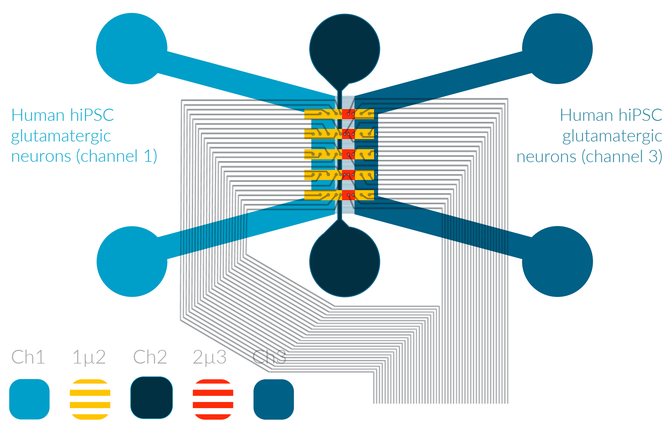
- Human induced pluripotent stem cell (hiPSC)-derived neurons integrated within NETRI's Dualink organ-on-chip system,
- Exposure to Rotenone as a reference neurotoxic compound inducing Parkinsonian-like mechanisms,
- Microelectrode array (MEA) recordings to monitor neuronal network activity and,
- Enzyme-linked immunosorbent assays (ELISA) to quantify molecular biomarkers associated with neuronal stress and toxicity.

The integration of functional and biochemical endpoints enables the generation of compound-specific "digital neurotoxicity signatures" supporting the discrimination of neurotoxic and non-neurotoxic compounds within a scalable, predictive, and human-relevant screening framework.

METHODS



NeoBento™ contains 16 microfluidic devices arranged in an SBS-standard international format, compatible with automated processing systems. Its 96-well plate footprint ensures compatibility with standard industry handling, imaging, and a wide range of readout platforms. The system integrates multiple user-friendly features and operates without external pumping equipment.



Dualink™ Shift MEA microfluidic device. The device comprises three interconnected compartments with an asymmetric design: glutamatergic neurons are seeded in channels 1 and 3, while channel 2 is positioned closer to channel 1 and farther from channel 3 for synaptic isolation while maintaining fluidic isolation. Neurons are cultured on integrated MEAs with 48 × 50 µm electrodes per chip.

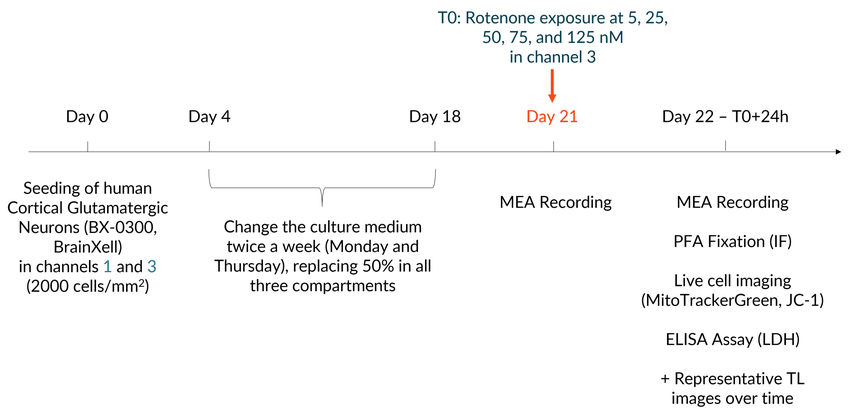
Human iPSC-derived glutamatergic neurons on chip with multimodal Readouts:

- Electrophysiology (MEA): non-invasive neuronal activity monitoring in channels 1 and 3
- Imaging: JC-1 mitochondrial integrity assay and immunostaining for pan neuronal and synaptic markers (β3-tubulin, PSD95, vGlut1).
- Biochemical assays: LDH release (ELISA) for cytotoxicity assessment.
- Data integration: digital signatures combining functional, structural, and molecular data using multivariate analysis and AI-based compound classification.

RESULTS

ESTABLISHMENT AND CHARACTERIZATION OF A PHYSIOLOGICAL ON-CHIP MODEL

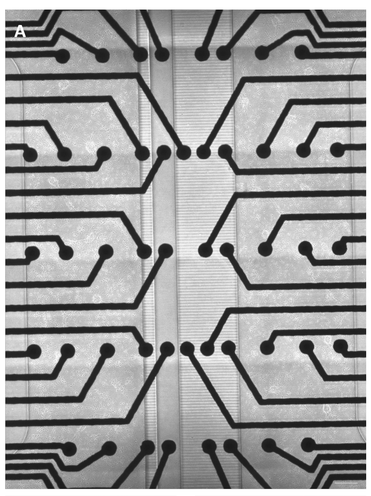
Human iPSC-derived glutamatergic neurons were cultured in the Dualink Shift MEA microfluidic platform to establish a physiologically relevant on-chip neuronal network. Structural and functional characterization was performed using immunostaining and electrophysiological recordings.



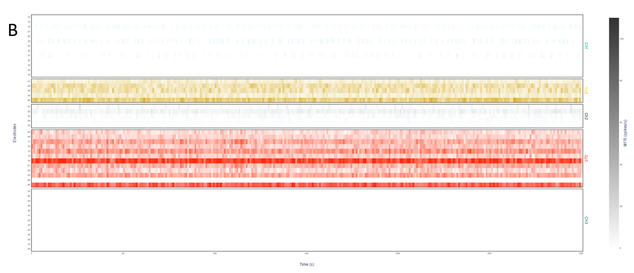
Experimental workflow for human iPSC-derived neuronal (BrainXell BX-0300) co-culture on Dualink Shift MEA devices and multimodal neurotoxicity assessment.

Neuronal network of human iPSC-derived glutamatergic neurons within the Dualink Shift microfluidic platform

- Neuronal viability was maintained up to day 22 with expected morphology on MEA substrate
- Recording of spontaneous electrophysiological activity across the neuronal network (channels 1, 2, and 3)



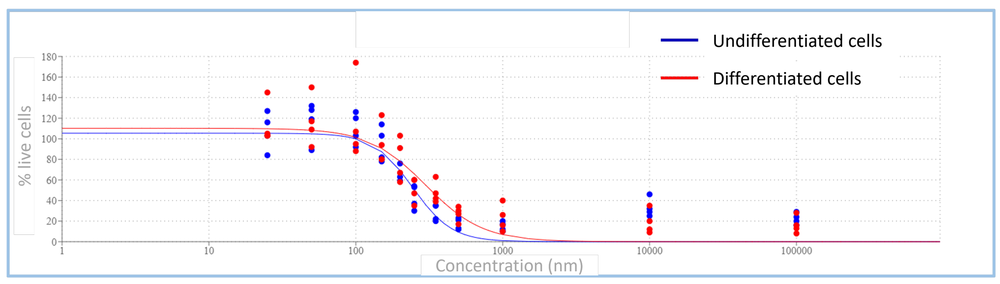
A) Immunofluorescence images of human glutamatergic neurons at Day 22, cultured in Dualink Shift MEA devices. Neurons are labeled in red with βIII-tubulin, a neuronal lineage marker and major constituent of neuronal microtubules (1:100). PSD-95 immunostaining is shown in green and was used as a postsynaptic marker, a membrane-associated protein enriched in the postsynaptic density of forebrain neurons (1:200). Scale bar: 200µm. Images were acquired using a Zeiss LSM980 confocal microscope (x20).



B) Representative raster plot of spontaneous electrophysiological activity recorded from neuronal networks cultured on a Dualink Shift MEA device. The detected spikes are binned in 1s bins; the color intensity corresponds to the MFR in each bin for each compartment (channels 1, 2, 3, and in the microchannels connecting the compartments). In this example, the majority of the detected activity is localized in the microchannels and in compartment 2 (synaptic compartment).

PRELIMINARY NEUROTOXICITY ASSEMENT USING ROTENONE

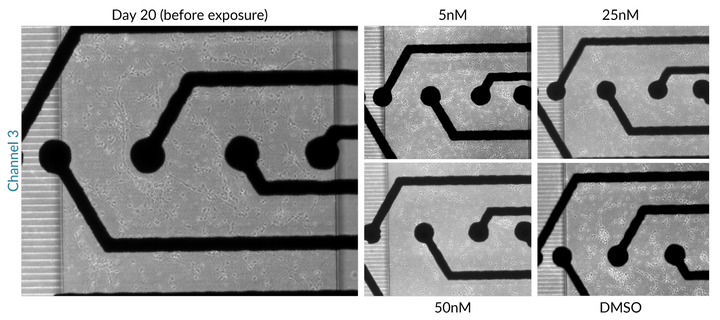
Human iPSC-derived glutamatergic neuronal networks cultured in the Dualink Shift MEA platform were exposed to Rotenone, a reference neurotoxic compound associated with Parkinsonian-like mechanisms. Morphological and functional alterations were assessed using transmitted-light imaging and MEA electrophysiological recordings.



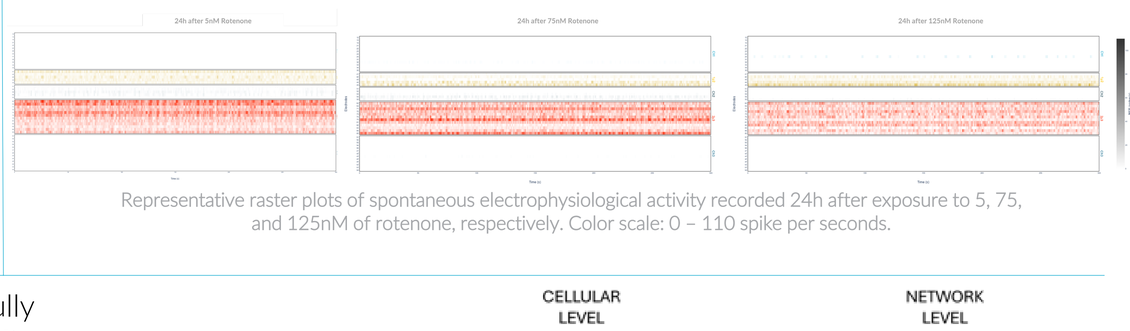
Viability of SH-SY5Y cells exposed to rotenone. Dose–response analysis was performed following Rotenone exposure to determine the IC50 value in SH-SY5Y neuroblastoma cells.

Human iPSC-derived glutamatergic neuronal networks cultured on the Dualink Shift MEA platform were exposed to Rotenone, a reference neurotoxic compound

- Human Glutamatergic cell damage was observed from 50nM rotenone after 24h exposure. No evident morphological changes were detected by transmitted-light imaging at 24h at lower concentrations, including in channel 1 conditions. Needs to be confirmed with LDH release and JC-1 assays.
- MEA recordings revealed functional modifications in spontaneous neuronal network activity with exposure doses as low as 5nM (analysis ongoing).



Representative transmitted-light images of human neuronal cultures following rotenone exposure (channel 3). Cell damage was observed from 50nM rotenone after 24 h.
No evident morphological alterations were detected at lower concentrations at 24 h post-exposure, including in channel 1. Scale bar: 30 µm.



Representative raster plots of spontaneous electrophysiological activity recorded 24h after exposure to 5, 75, and 125nM of rotenone, respectively. Color scale: 0 – 110 spike per seconds.

CONCLUSION & PERSPECTIVES

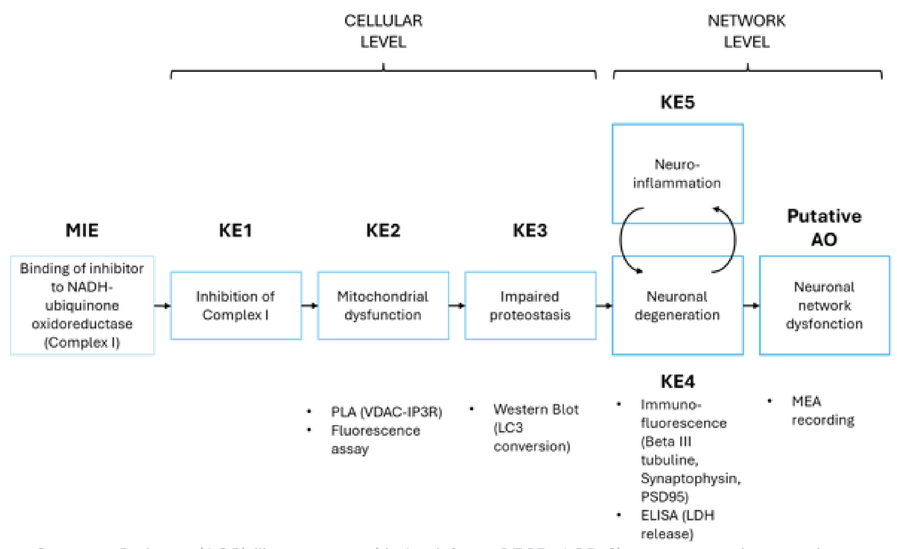
This collaborative project between NETRI and ANSES aims to develop a fully humanized organ-on-chip (OoC) platform for pesticide-induced neurotoxicity assessment. The model uses human iPSC-derived glutamatergic neurons cultured on NETRI's Dualink Shift microfluidic platform and combines multimodal functional, morphological, and biochemical readouts.

Preliminary results show :

- Successful establishment of a functional human neuronal network within the Dualink Shift OoC platform
- MEA-based detection of Rotenone-induced functional alterations.
- Definition of an effective Rotenone concentration range for neurotoxicity testing.

Ongoing studies include:

- Optimization of rotenone exposure conditions (5–125nM), including DMSO concentration, as well as the timing and duration of exposure
- Mitochondrial integrity assessment using MitoTracker staining
- Cytotoxicity quantification via LDH release



Adverse Outcome Pathway (AOP)-like structure (derived from OECD AOP 3) to ensure coherence between the validated readouts and the anticipated biological endpoints.

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