Microfluidic high-throughput screening platform to screen pre-clinical stage compound effects on neurite outgrowth of human Motor Neurons post-injury

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PROVIDING ORGANS-ON-CHIP FOR THE INDUSTRY

Background

Pharmaceutical industry **needs relevant** *in vitro* **models of Traumatic Spinal Cord Inury** (SCI) (Omelchenko *et al*., 2020), capable of:

- inducing **localized axonal injury** with a **robust and reproducible protocol**
- **not affecting cell viability** to allow **quantification of axonal regeneration post-injury**

thanks to an appropriate **method of quantification**.

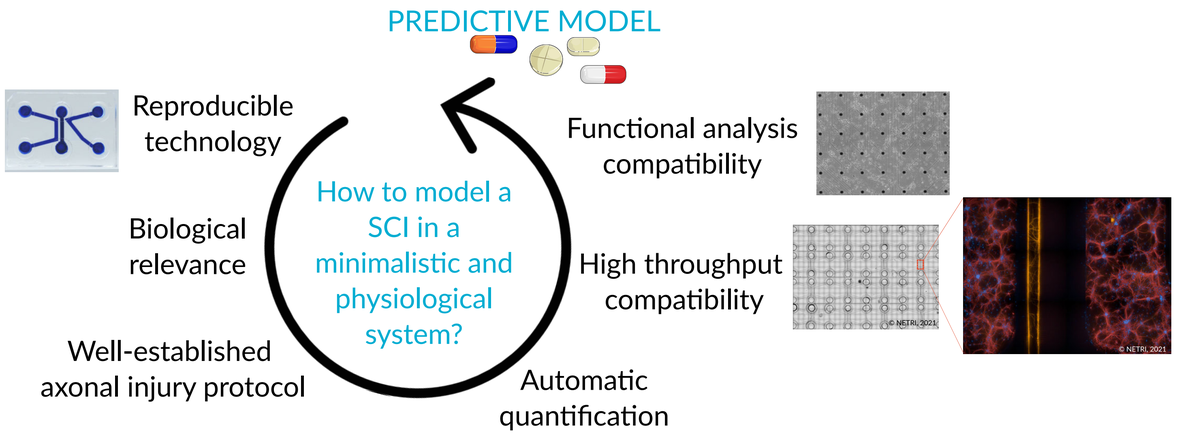


Fig. 1: Resolution fluorescence microscopy compatibility acquired with Operetta, Perkin Elmer (Tau-Orange, MAP2-Red, DAPI-Blue).

Results

1 Reproducible technology

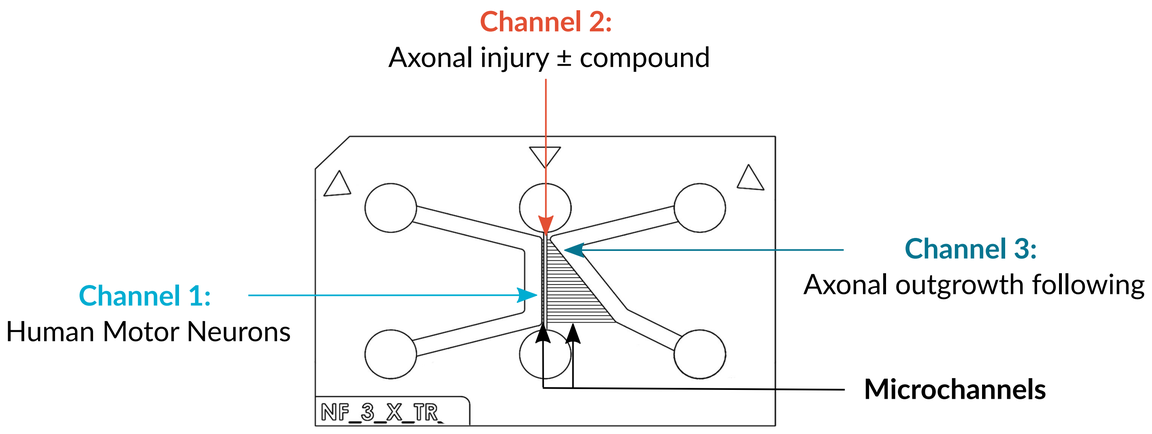


Fig. 2: Three-compartimentalized triangle shaped microfluidic device.

2 Standardization of Human motor neurons characterization

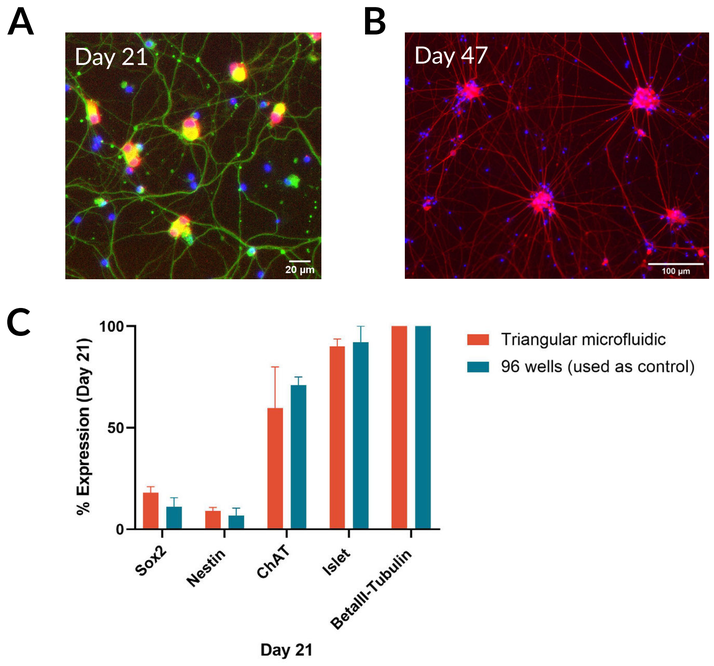


Fig. 3: Characterization of Human iCell Motor Neurons from Fujifilm Cellular Dynamics (#01279) in NETRI's microfluidic devices. (A) Immunofluorescence pictures of βIII-Tubulin (Green) and Islet-1 (Red) at Day 21 and (B) MAP-2 (Red) at Day 47 counterstained with DAPI (Blue). (C) Quantifications performed with semi-automatic proprietary software in Fiji in the entire active zone of the device after Day 21.

FUJIFILM Value from Innovation — Cellular Dynamics

- Standard Operating Procedure in microfluidic devices (available upon request)
- Classical morphology with clustering
- Long-term viability up to Day 50
- Fully-differentiation process
- Expression of markers characteristic: Islet-1, ChAT, βIII-Tubulin, MAP2

3 Performance of the axonal injury method

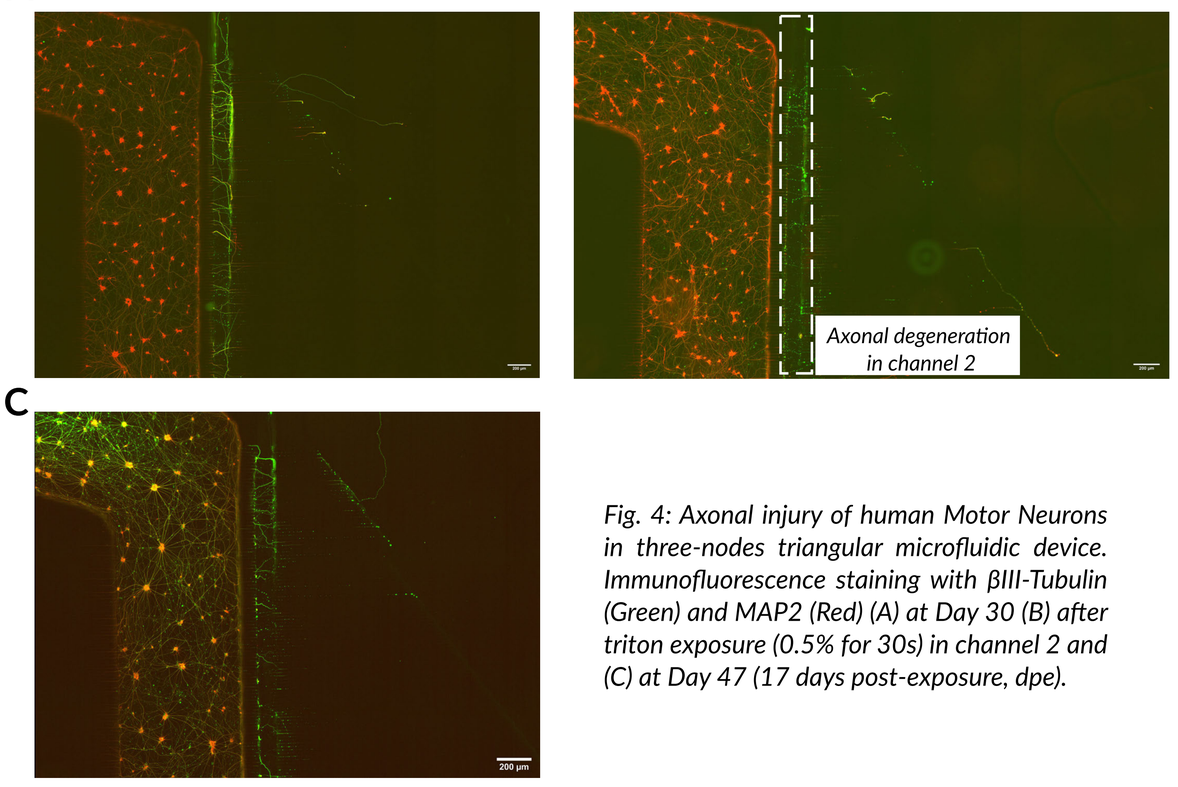


Fig. 4: Axonal injury of human Motor Neurons in three-nodes triangular microfluidic device. Immunofluorescence staining with βIII-Tubulin (Green) and MAP2 (Red) (A) at Day 30 (B) after triton exposure (0.5% for 30s) in channel 2 and (C) at Day 47 (17 days post-exposure, dpe).

- Neurite degenerescence after triton application
- Neurite regeneration in 2-3 days post-injury
- Cell viability (with/ without) axonal injury in microfluidic device up to day 47

4 Quantification of neurites elongation

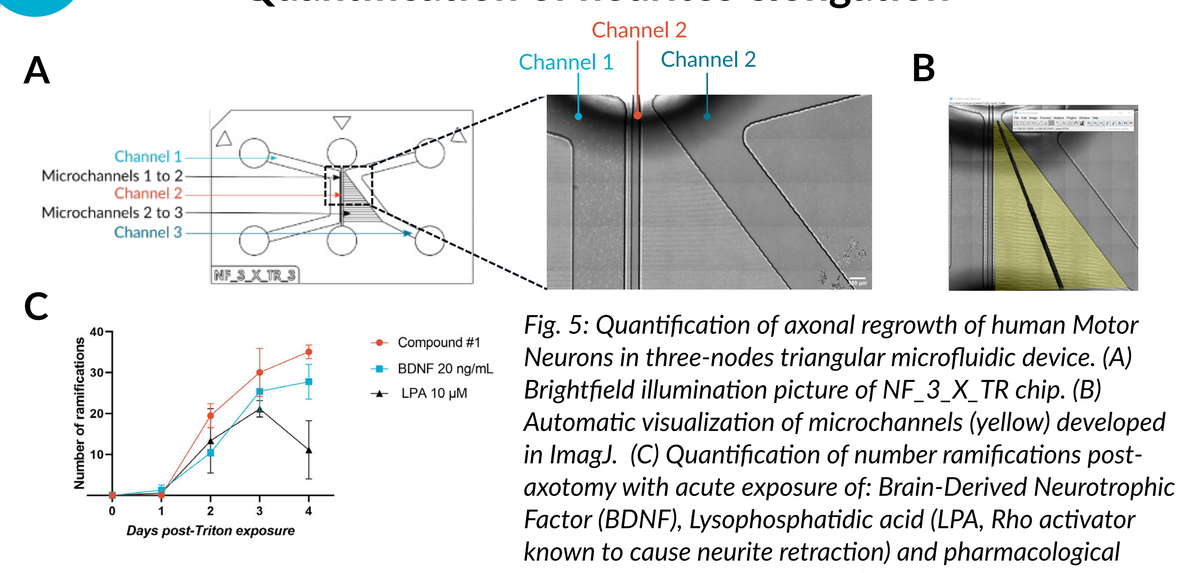


Fig. 5: Quantification of axonal regrowth of human Motor Neurons in three-nodes triangular microfluidic device. (A) Brightfield illumination picture of NF_3_X_TR chip. (B) Automatic visualization of microchannels (yellow) developed in ImagJ. (C) Quantification of number ramifications post-axotomy with acute exposure of: Brain-Derived Neurotrophic Factor (BDNF), Lysophosphatidic acid (LPA, Rho activator known to cause neurite retraction) and pharmacological compound (called compound #1).

- Few neurites in channel 3
- Neurite regeneration quantification using number of ramifications
- Testing pharmacological compounds

5 New technical feature: microgrooves technology

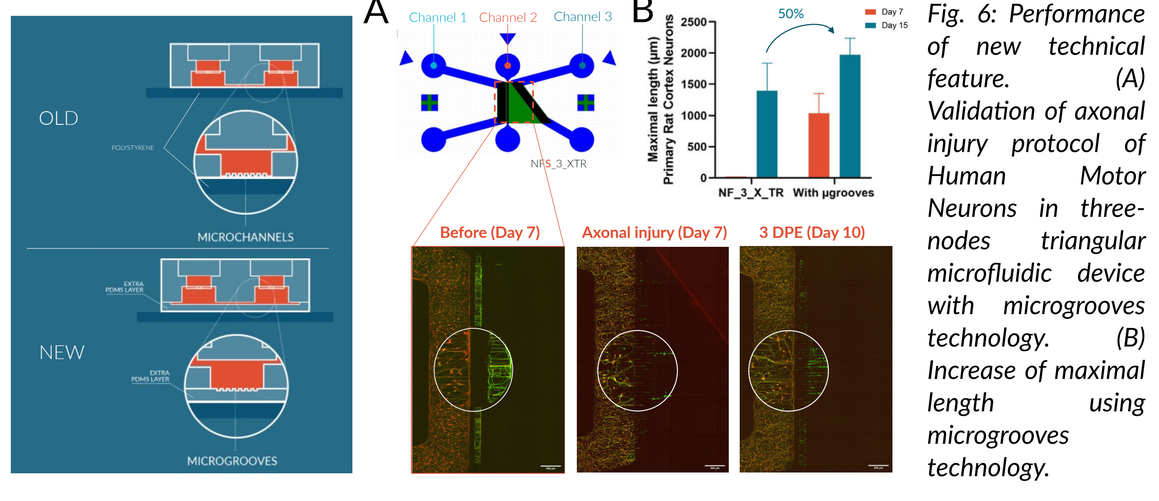
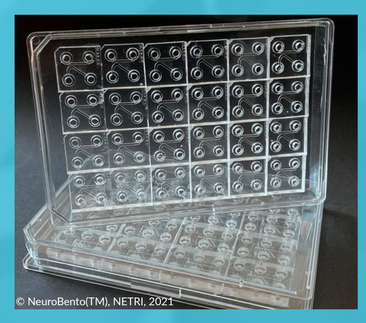


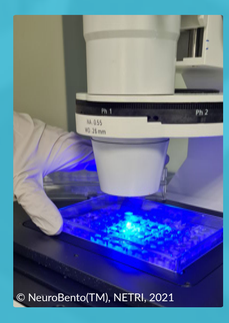
Fig. 6: Performance of new technical feature. (A) Validation of axonal injury protocol of Human Motor Neurons in three-nodes triangular microfluidic device with microgrooves technology. (B) Increase of maximal length using microgrooves technology.

- Characterization of three-compartment-triangle-shaped microfluidic device with microgrooves.
- Addition of a PDMS layer in which the microgrooves are hollowed out.
- Increase the rate of axon projections in channel 3.
- Axonal injury protocol validation following axonal regeneration.

Conclusions and Perspectives

- **Organ-on-Chip technology** combined with human cells, opens **new route to the quantification of neurite outgrowth** dynamics post-axonal injury, avoiding animal experiments and **open new field of therapeutic application**.
- This model can be used to **record functional activity** using Multi-Electrode Arrays (MEA) and access the recovery process thus providing relevant insights on the mode of action of pharmacological compounds.
- Our data suggest that this model can be used for **high-throughput drug-induced axonal regeneration screening** for preclinical stages pharmaceutical compounds.





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