TRANSLATIONAL PLATFORMS OF INJURY & PAIN-ON-CHIP

BOOTH #926

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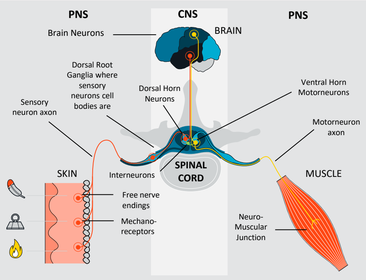
BACKGROUND



Peripheral nerves are made of motor and sensory nerves, two very distinct types of neurons that are linked but each have their specific function. **Organs-on-chip** (OoC) offer the advantage to **isolate** neuron **somas** from their **axons**, thus reproducing the human anatomical architecture and enabling injury or treatment paradigms aligned with real life situations. To tease apart each cell type and allow their study separately, we adapted the culture of **motorneurons** and **sensory neurons** onto our OoC platform. To bridge the gap between *in vivo* models and first-in-human studies, as well as increase relevance, we developed our models using **hiPSC**-derived neurons. We took advantage of the versatility of our architectures to develop a traumatic pain model (**Nerve Injury**) and a Chemotherapy-Induced Peripheral Neuropathy (**CIPN**) model.

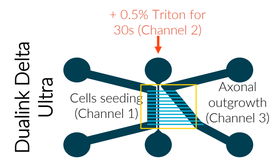
RESULTS

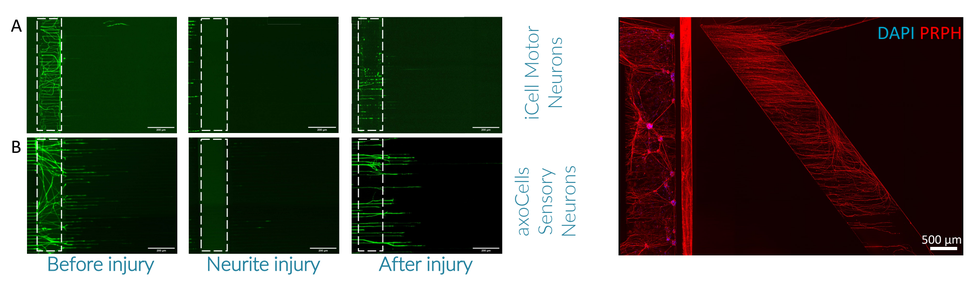
NERVE INJURY-ON-CHIP

FUJIFILM Cellular Dynamics · AXOL

Neurite injury method in DuaLink Delta Ultra

- **Localized neurite injury** with a reproducible protocol
- **Neurite regeneration** post-injury



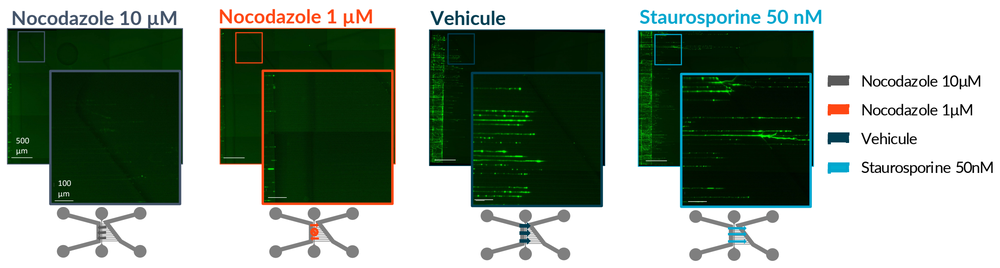


Human iPSCs-derived neurons cultured in DuaLink Delta Ultra.
A) Neurite visualization of iCell Motor Neurons (01279, FCDI) ICC anti-βIII Tubulin, 3 days post-injury. B) Dynamic neurite outgrowth of axoCells™ Sensory Neurons (ax0555, AxolBioscience), before to 2 days post-injury with calcein live staining (0.1 µg/mL).

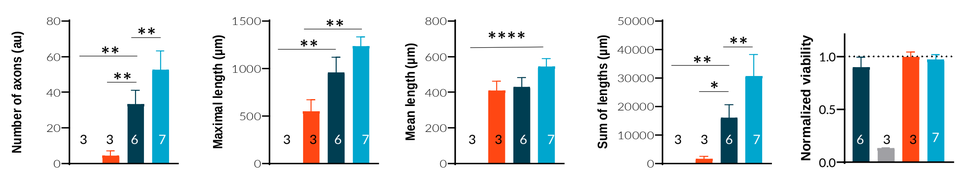
axoCells™ Sensory Neurons cultured 3-weeks in DuaLink Delta Ultra.
Peripherin, a marker of mature peripheral neurons.

Motor nerve injury model

- We normed our platform to allow molecules screening and comparisons with validated mechanistic controls.
- Nocodazole destabilized microtubules and can be used as internal **neurodegenerative** control[1].
- Staurosporine, a wide spectrum protein kinases inhibitor enhancing neurite outgrowth, is used as internal **neurotrophic** control[2].



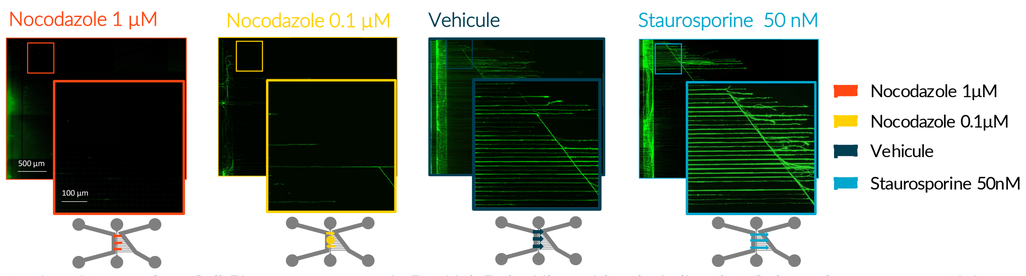
Illustrative pictures of iCell Motor Neurons in DuaLink Delta Ultra stained with calcein live dye, 3 days of exposure post-injury.



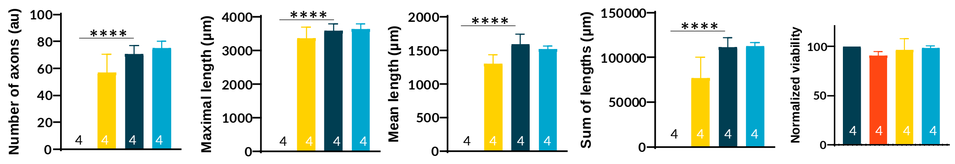
iCell Motor Neurons regrowth quantification post-injury. *Quantifications were performed using ImageJ..Graphs and statistical analysis were generated using GraphPad Prism.* Independent t-test with Welch's correction (* p-value ≤ 0.05, ** ≤ 0.01).

Sensory nerve injury model

- Nocodazole is more potent on axoCells™ sensory neurons compared to iCell Motor Neurons. Our platform can detect differences of efficacy depending on the cell model used.
- At the same concentration, staurosporine is less effective on iCell Motor Neurons. Dose should be adjusted to use it as internal neurotrophic control.



Illustrative pictures of axoCells™ sensory neurons in DuaLink Delta Ultra with calcein live dye, 2 days of exposure post-injury.

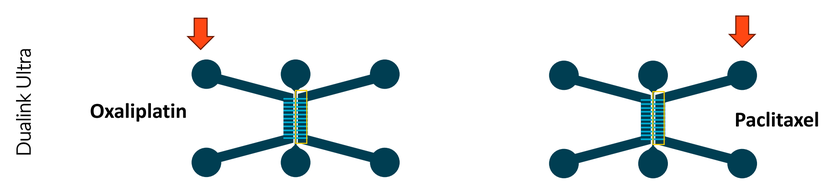


axoCells™ Sensory Neurons regrowth quantification post-injury. *Quantifications were performed using ImageJ and a homemade pre-processing algorithm. Graphs and statistical analysis were generated using GraphPad Prism.* Independent t-test with Welch's correction.

CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY-ON-CHIP

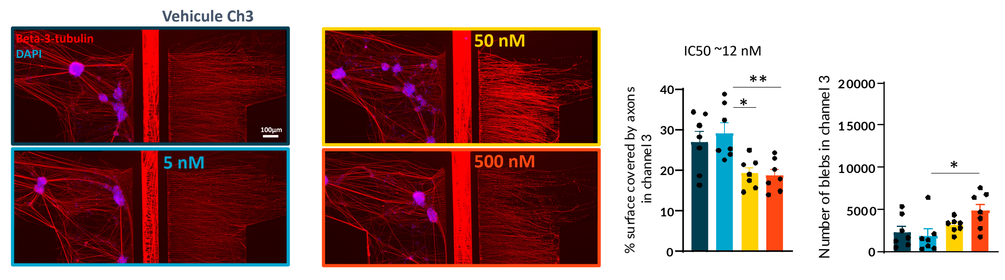
Compartimentalization to study mode of action

- The major hallmark of **CIPN neuropathology** is a "**dying back**" axon degeneration that proceeds in a distal-to-proximal fashion.
- Our compartmentalized architecture offers the advantage to i) **isolate** distal axons from soma and proximal axons, and (ii) **segregate the modes of action** of each chemotherapy agent onto each cell compartment.
- Mature axoCells™ sensory neurons were treated on the **soma only** (channel 1) for oxaliplatin and on the **distal axons only** (channel 3) for paclitaxel. After 72h of exposure, cells were fixed and stained for β3-Tubulin, a specific marker of neuronal cytoskeleton used to assess neuronal health.



Paclitaxel CIPN Model

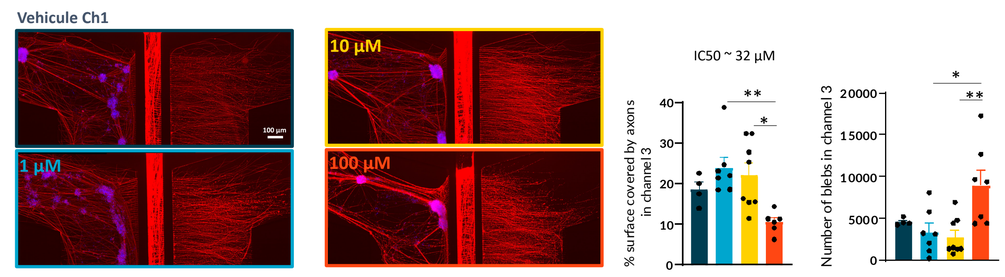
- **Paclitaxel** stabilizes microtubules and thus interferes with axonal transport. The modality of administration was based on previous literature data showing that **only distal axons** are affected by paclitaxel, and not soma and mid-axons[3,4]. The doses were chosen to be physiologically relevant by being in the range of measured plasma concentrations seen in patients (80-280 nM)[5].
- **Paclitaxel induces a dose-dependent degenerescence** of the axons. Those results, obtained with a high throughput platform, are consistent with previous reports on individual chips showing no effect of 10 nM paclitaxel on the axon area covered but a marked decrease at 50 nM[3].



Paclitaxel-Induced Peripheral Neuropathy model. *Vehicle and Paclitaxel were applied in channel 3. The whole chip was imaged, but quantification only performed on distal axons, in channel 3. One-way ANOVA with Tukey's multiple comparison test. * p-value<0.05. ** p-value<0.01.*

Oxaliplatin CIPN Model

- Oxaliplatin is a third-generation platinum agent and one of the most used in clinic. It binds to DNA, creating crosslinking that will interfere with DNA replication and transcription[6].
- At 100 µM, **oxaliplatin** applied onto the **soma** reduces the surface covered by the distal axons present in channel 3 and increases the number of axonal debris drastically. These results confirm the **neurotoxicity of oxaliplatin** in our model[6,7].

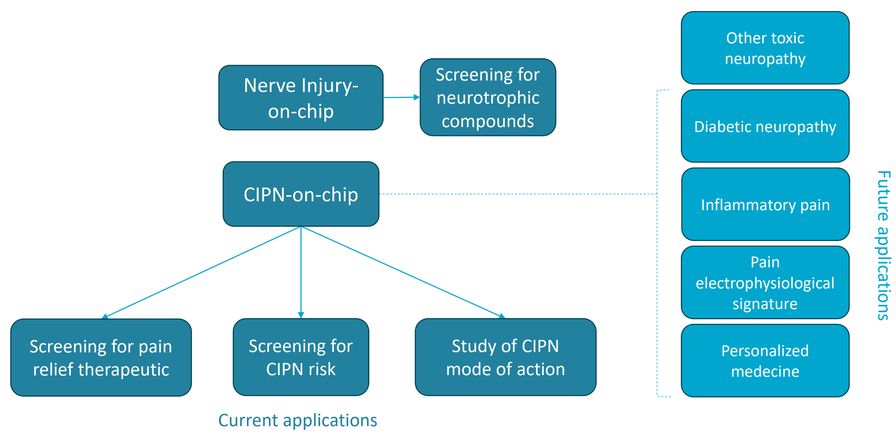


Oxaliplatin-Induced Peripheral Neuropathy model. *Vehicle and oxaliplatin were applied in channel 1. The whole chip was imaged, but quantification only performed on distal axons, in channel 3. One-way ANOVA with Tukey's multiple comparison test. * p-value<0.05. ** p-value<0.01.*

CONCLUSION & PERSPECTIVES

By **combining NETRI's engineering, biological & digital expertise**, we **validated our nerve injury platform** by comparing axonal regeneration following treatment with a neurotrophic molecule or a drug inhibiting neurite outgrowth and **our CIPN-on-chip platform** by recreating two modalities of toxic neuropathies. NETRI's **Injury & Pain-on-chip** platforms offers pharmaceutical companies and researchers a **new translational platform** including digital analysis tools to study the efficacy and mode of action of novel therapeutic modalities.

This methodology paves the way to model different types of pain such as diabetic neuropathy or inflammatory pain. It will also be enhanced by resolving the **electrophysiological digital signature of pain** with NETRI's **NeuroFluidics™ MEA** Line.



Ask at our booth to learn more about screening models for neuroprotective molecules.

Visit our website netri.com for the related information.

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