NETRI's APP NOTE

CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY-ON-CHIP MODEL: UTILIZING THE STRENGTH OF COMPARTMENTALIZATION

- Human sensory neurons-on-chip as a relevant model for the study of toxic peripheral neuropathy.
- Discriminate mode of action and mechanism of neurotoxicity, thanks to compartmentalization and associated fluid isolation: soma versus neurites.
- Segregate therapeutic modality: topical versus systemic.
- Platform compatible with High-Throughput Assays and MicroElectrode Array (MEA).

OVERVIEW

Pains have multiple etiologies and can be classified as nociceptive (damage to the tissue) or neuropathic (damage to the somatosensory nervous system). Peripheral neuropathic pain resulting from chemotherapy affects 60 to 80% of patients during the course of treatment, and for 20 to 40%, the pain will become chronic after treatment with a high impact on the patient's quality of life.

Chemotherapy agents fall into six main categories which are (ordered by the incidence of neuropathies): platinum compounds (such as oxaliplatin), taxanes (such as paclitaxel), immunomodulatory agents, epothilones, proteasomes inhibitors and vinca alkaloids [1,2]. Symptoms of sensory peripheral neuropathies include paresthesias («pin and needles»), dysesthesias and numbness in the hands and feet. Pain management options are limited and not fully effective [2,3] which can partly be explained by the lack of relevant research models available. Indeed, current animal-based *in vivo* models lack translationability and can only be based on evoked pain, while current *in vitro* assays lack anatomical relevance and complexity to understand modes of action and toxicity [4,5].

Pain signals are primarily detected by free nerve endings of sensory neurons and transmitted via their axons to the cell body in the dorsal root ganglia then to the dorsal horn neurons. Local integration and modulation of the signal is performed by spinal cord circuits before reaching the brain, area of consciousness of the pain and central modulation (Figure 1).

This highly segregated circuit can easily be reproduced with Organs-on-Chip (OoC) technology. OoC offer the advantage to isolate neuron somas from their axons, thus reproducing the human neuroanatomical architecture and enabling injury or treatment paradigms aligned with real-life situations [6].

To bridge the gap between *in vivo* models and first-in-human studies and increase relevance, we developed our pain models using human induced pluripotent stem cell (hiPSC)-derived sensory neurons and adapted the culture onto relevant microfluidic devices (Figure 2).

We chose the DuaLink™ from the NeuroFluidics™ line to develop two Chemotherapy-Induced Peripheral Neuropathy (CIPN) models. We use the strength of DuaLink's compartmentalization to explore chemotherapy agents with distinct modes of action and by selectively adding them to either the cell body or distal axonal compartment. The data highlights degenerescence in the axonal compartment as described in previous studies but for the first time performed on a high-throughput format.

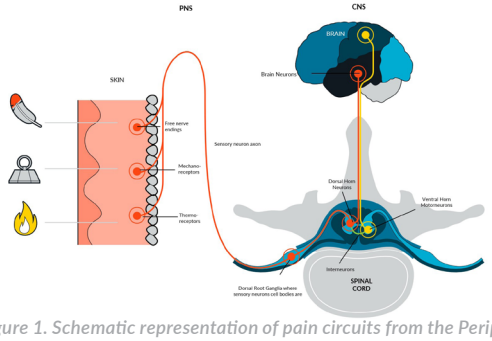


Figure 1. Schematic representation of pain circuits from the Peripheral Nervous System (PNS) to the Central Nervous System (CNS). Distinct sensory neurons with specific receptors are present in the skin (and all organs). Their cell bodies are located in the Dorsal Root Ganglia (DRG), and they transmit their signals to dorsal horn neurons in the spinal cord. The message will be modulated via interneurons before reaching the brain for consciousness. Brain neurons can modulate and control spinal cord circuits.

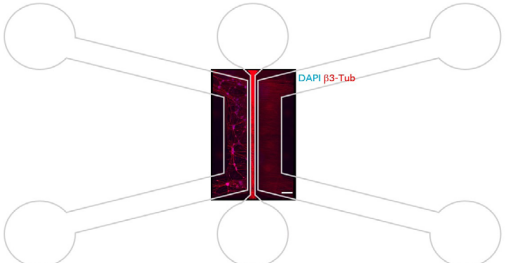


Figure 2. Axol Bioscience human iPSC-derived sensory neurons cultured 21 days in DuaLink Ultra™ according to our Operating Protocol Axol Sensory Neurons. Neurons were fixed then stained for β3-Tubulin. For more details, see our Operating Protocol - Immunostaining. Scale bar is 500 µm.

RESULTS

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 [8,9].

This feature is also critical to determine if the mode of action of a pain relief molecule is compatible with its therapeutic modality. Furthermore, our models can be used to assess potential risk of neuropathy from emerging classes of therapeutics from which toxicological data are scarce.

Paclitaxel CIPN Model

Paclitaxel stabilizes microtubules and thus interferes with axonal transport [8]. 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 [8,10]. The doses were chosen to be physiologically relevant by being in the range of measured plasma concentrations seen in patients (80-280 nM) [11].

Mature hiPSC-derived sensory neurons were treated on the distal axons only (channel 3) by applying three paclitaxel doses (5 nM, 50 nM and 500 nM). After 72 h of exposure, cells were fixed and stained for β3-Tubulin, a specific marker of neuronal cytoskeleton. The surface covered by the axons was reduced at the highest doses tested and the number of axonal debris increased indicating that paclitaxel induces a dose-dependent degenerescence of the axons (Figure 3). 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 [8].

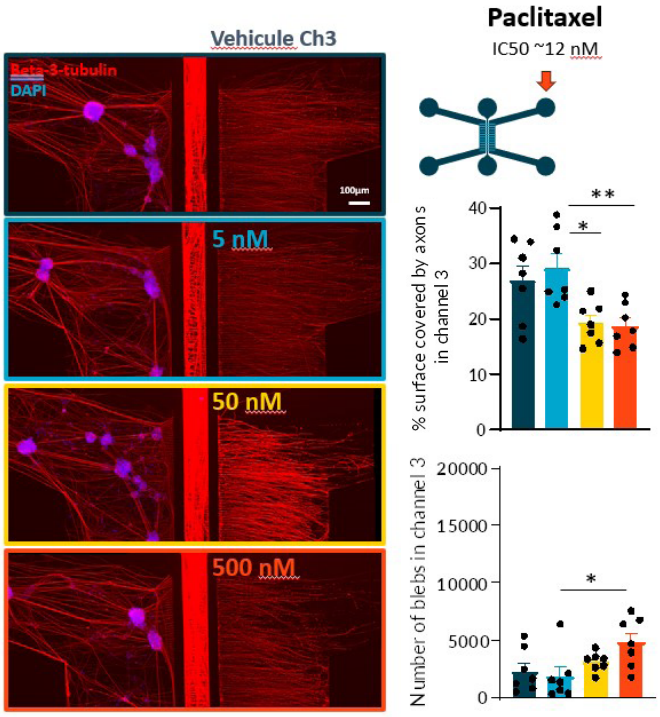


*Figure 3. Paclitaxel-Induced Peripheral Neuropathy model. Vehicle and Paclitaxel were applied in channel 3. The whole chip was imaged, but quantification only performed on distal axons, in channel 3. One-way ANOVA with Tukey's multiple comparison test. * p-value<0.05. ** p-value<0.01.*

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 [12]. As the main target of oxaliplatin is in the nucleus, we applied the three doses (1 µM, 10 µM and 100 µM) on the cell bodies only (channel 1, Figure 4).

The methodology used for the paclitaxel model, was applied to the oxaliplatin model: after 72 h of exposure, cells were fixed and stained for β3-Tubulin, used as a marker for neuronal health. Previous *in vitro* studies show that axonal retraction can be observed for 100 µM oxaliplatin, whereas effects of lower doses can only be detected by electrophysiological recordings [13]. Here, at 100 µM, oxaliplatin 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 [2,12].

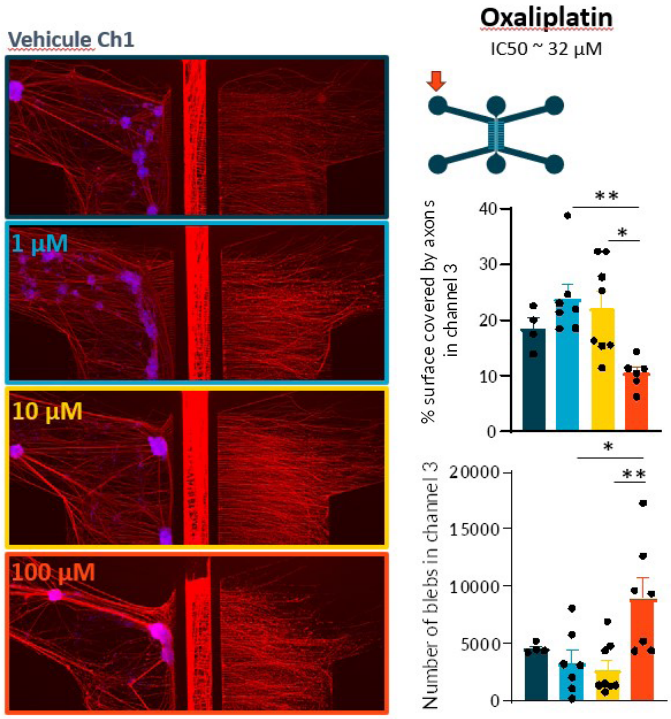


*Figure 4. Oxaliplatin-Induced Peripheral Neuropathy model. Vehicle and Oxaliplatin were applied in channel 1. The whole chip was imaged, but quantification only performed on distal axons, in channel 3. One-way ANOVA with Tukey's multiple comparison test. * p-value<0.05. ** p-value<0.01.*

CONCLUSION

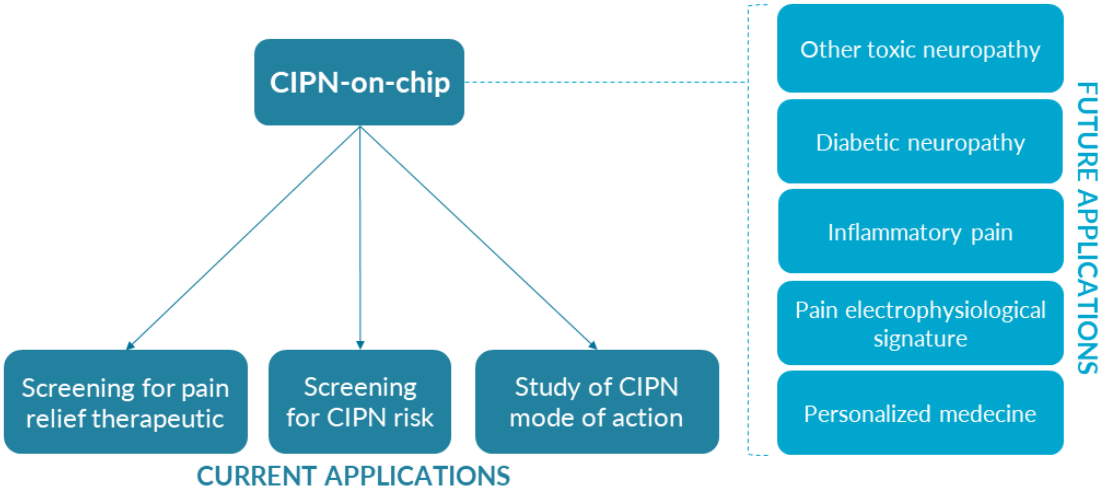
Here we show that compartmentalized microfluidics can be used to recreate two modalities of chemotherapy-induced neuropathic pain. The humanized *in vitro* CIPN models presented here hold potential in a wide variety of applications.

First, they can be used to screen potential drugs or therapeutic interventions for their effectiveness in preventing or treating CIPN. Researchers could test various compounds to identify those that have the least neurotoxic effects on peripheral nerves while still effectively targeting cancer cells. Moreover, understanding the cellular and molecular mechanisms underlying CIPN is crucial for developing targeted therapies. Our CIPN model can help researchers dissect these mechanisms by allowing them to manipulate and observe nerve cells and their interactions with chemotherapeutic agents in a controlled environment, while choosing the site of application of their drugs (axons vs soma) and investigating the intricate cellular and molecular interactions that lead to CIPN. These models can also be used to study potential neuroprotective agents that can shield nerve cells from the damaging effects of chemotherapy. This could lead to the discovery of compounds that mitigate or prevent CIPN symptoms.

It is also essential to keep in mind that animal models are commonly used to study CIPN, but they have limitations in replicating human responses accurately. Our models presented here can help reduce the need for animal testing in drug development, by providing a more relevant alternative to animal studies, thereby contributing to the reduction of ethical concerns and the time and resources required for animal experimentation.

Ultimately, these models also could be created by using patient-derived cells, allowing for personalized medicine approaches. By using cells from individual patients, researchers could assess how specific patients might react to certain chemotherapy drugs and help clinicians to tailor treatment plans accordingly.

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.



RESOURCES

Available upon request

- Operating Protocol Neurofluidics™ – DR_3B_010.07
- Operating Protocol Axol Sensory Neurons - DR_3B_057
- Operating Protocol - Immuno Staining DR_3B_059

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Based on 10 years of scientific research, NETRI has developed a unique know-how in designing organs/organoids-on-Chip by integrating disruptive building blocks into the same microfluidic devices, while maintaining industrial production standards compatible with pharma industry equipments & requirements.

Thanks to our patented technologies, we are capable of manufacturing prototypes and validating their biological function using primary animal or human induced pluripotent stem cells differentiated in our chip. Our unique infrastructure allows us also to scale up chip production for mass production.

Need more information about NETRI's products and services or how Organs-on-Chip revolutionize pre-clinical trials and human *in-vitro* models? Contact us!

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