3D nerve-on-a-chip model effectively screens neurotoxic compounds

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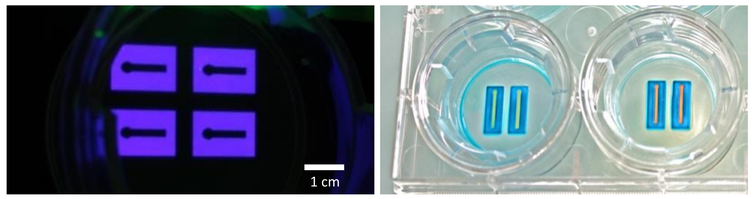
Overview

Organ-on-a-chip devices that mimic in vivo physiology have potential to identify chemical toxicity for early preclinical development, relying less heavily on animal models. We have developed a nerve-on-a-chip construct to culture animal and human neural tissue in a dual hydrogel system that promotes axon growth analogous to mature nerve anatomy.

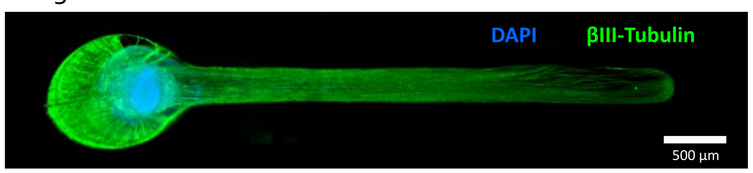
Here we culture rat sensory dorsal root ganglia (DRG) in the construct to demonstrate preclinical screening of nerve dysfunction by measuring electrical signals and imaging structural changes in tissue exposed to 4 chemotherapy drugs known to cause peripheral neuropathy.

Methods

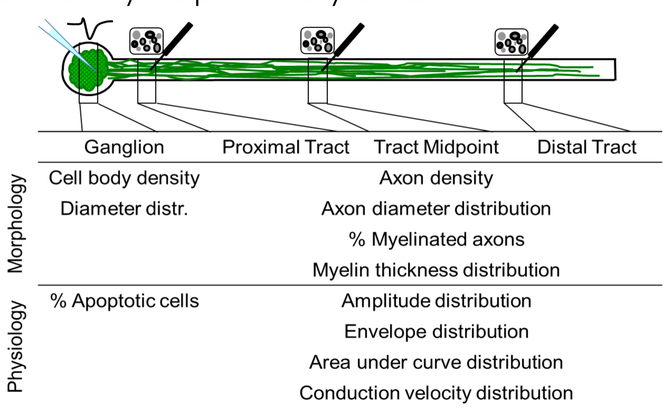
Fabrication: PEG hydrogel scaffolds are printed onto semi-permeable membranes using photolithography.



Tissue culture: Embryonic rat DRG explants are inserted into the inner Matrigel area and mature for 4 weeks, where confined 3D axon growth mimics nerve fiber tract.



Test Metrics: Robust nerve growth facilitates morphological and physiological outputs that can be measured as a screening assay for neurotoxicity and pharmacodynamics.



Results

Toxicity screening: DRG sensory nerves embedded in the hydrogel system are dosed for 7d with chemotherapeutics known to cause neuropathy. Cell viability is measured with CCK-8 assay (*# of live cells measured by metabolic output*) and LDH assay (*# of dead cells measured by release of LDH in media*). Electrophysiology tests measure nerve conduction velocity (NCV) and signal amplitude (Amp).

- *Bortezomib: Proteasome inhibitor*
- *Oxaliplatin: Creates platinum-DNA adducts, inhibits replication*
- *Paclitaxel: Microtubule stabilizer, prevents mitosis*
- *Vincristine: Vinca alkaloid inhibits microtubule formation*

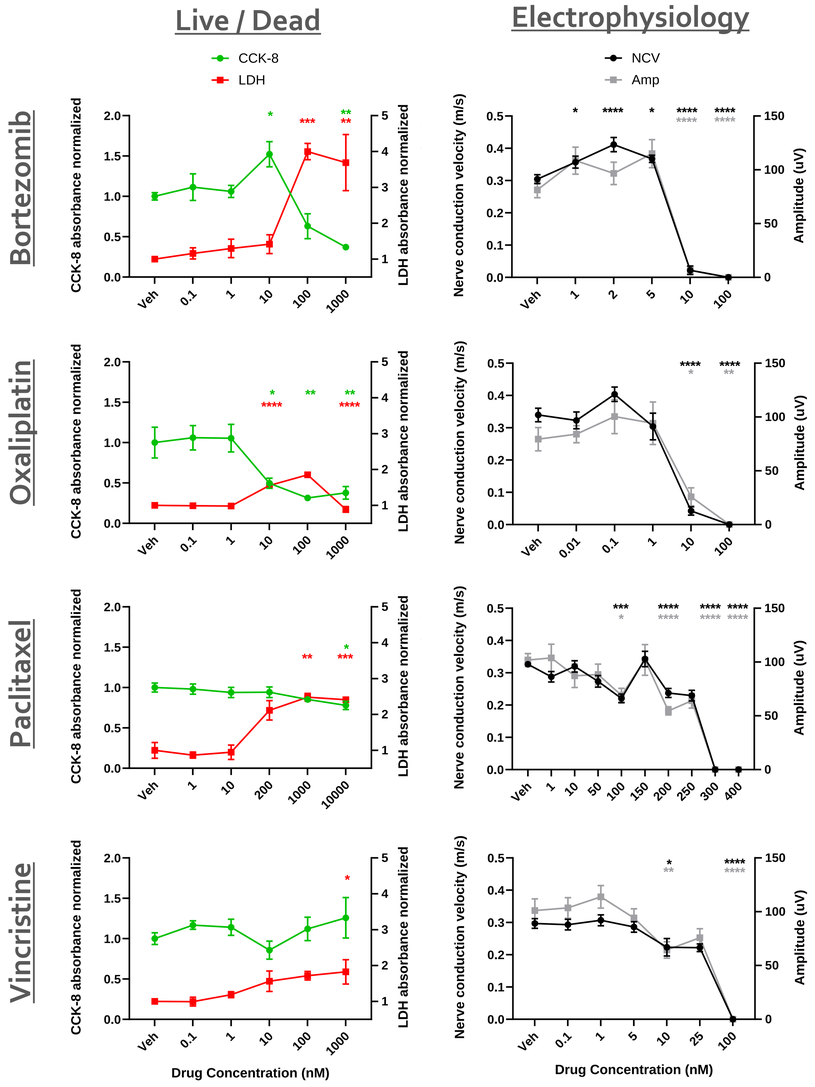
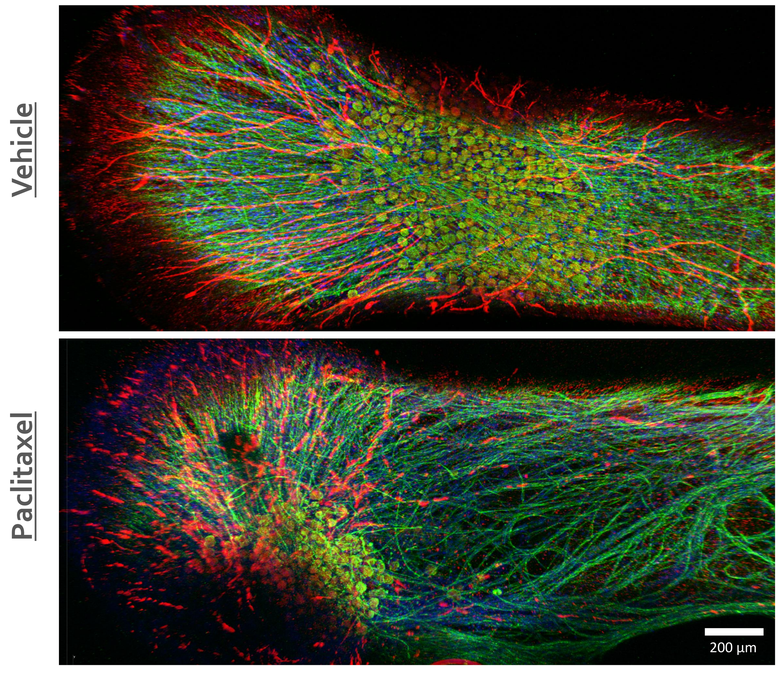


Table 1. Cell viability and electrophysiological measurements were collected and are each normalized to the vehicle.

Results



MBP – myelin, **DAPI** – nucleus, **βIII-tubulin** – neural marker

Figure 1. Confocal images of the DRG in hydrogel constructs after 1-week exposure to neurotoxic compounds demonstrate a significant reduction in myelination while cell and axon count remain intact.

Summary

Dose response curves of electrically stimulated neurons show significant decreases in NCV and amplitude occur before significant decreases in cell viability. This demonstrates that functional measurements represent a more clinically-relevant, sensitive metric. Furthermore, immunostaining reveals that some of these drugs have deleterious effects on myelination.

Future work will include (1) expanding on sensitivity testing of the assay and (2) improving electrode design to record cell activity. AxoSim's Nerve-on-a-Chip technology mimics in vivo animal models of chemotherapy-induced peripheral neuropathy, both functionally and structurally, providing an innovative platform for neurotoxicity screening.

Acknowledgements

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