NETRI's APP NOTE

INNOVATIVE MICROFLUIDIC DEVICE FOR *IN VITRO* CELL CULTURE: APPLICATION FOR PERIPHERAL PAIN MODELS

- Maintenance and long-term viability of 3D cell culture.
- Projected axons compartmentalization and outgrowth kinetics monitoring using a reproducible semi-quantitative method.
- Dorsal Root Ganglia on a chip as a relevant peripheral pain model.
- Platform compatible with High Throughput Assays and Microelectrode Arrays.

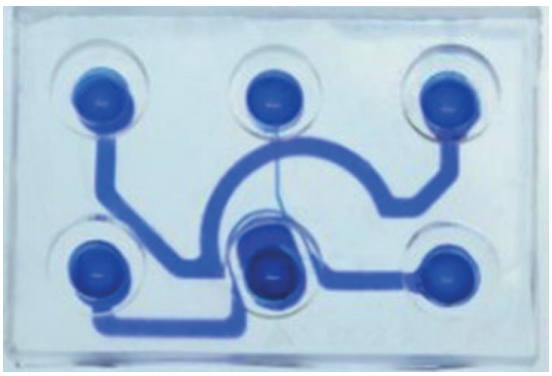
OVERVIEW

2D cell culture is the most spread method to culture mammalian cells. This incapacity to recapitulate 3D environments and cellular connections is however hindering the study of a wide variety of biological or physiological processes. Several methods to overcome this limitation have come under scrutiny in the last decade or so, one of the most promising being microfluidic technology.

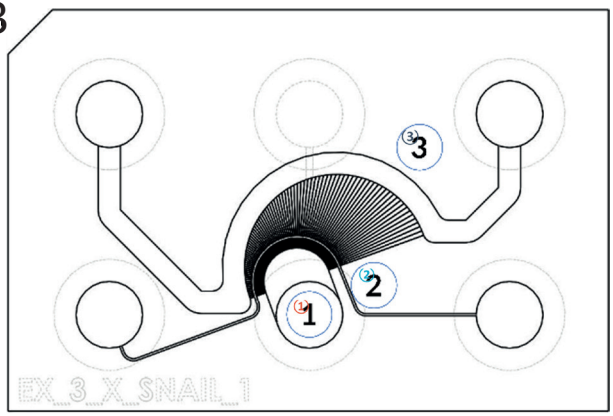
NETRI has recently developed a technology allowing to perform 3D cell culture in a seeding chamber while enabling the investigation of axonal outgrowth kinetics (Figure 1).
As a proof of concept of application of this microfluidic device, we present below a peripheral pain model utilizing such technology. Explant models have already shown their potential to create a relevant *in vitro* model, particularly for neurological aspects as peripheral pain syndromes. They offer the possibility to mimic microenvironments *in vitro* to maintain a complex cellular architecture through time, thus enabling the study of biological physiopathology, such as peripheral neural disorders.

The peripheral pain model presented here relies on the culture of a Dorsal Root Ganglia (DRG) explant in NETRI's recent technology, thus utilizing the advantages of both microfluidic technology and explant models.

A



B



C

Description	Characteristics (µm)
Channels (2) and (3)	100 (2) and 1000 (3)
Microchannels (1) → (2) and (2) → (3)	450 and 200 to 4000
Cell explant chamber (1)	3000 x 3000 x 600 (l x W x H)

Figure 1. Characteristics of NETRI's microfluidic device for 3D cell culture and axonal projection isolation.
(A) Picture of the EX_3_X_SNAIL device.
(B) Schematic representation of theEX_3_X_SNAIL device.
(C) Characteristics of the EX_3_X_SNAIL device.

RESULTS

Snail-shaped device for 3D culture of DRG explant as a peripheral pain model

A device enabling seeding and culturing explants, and axonal projection isolation and growth measurements

In order to develop a peripheral pain on chip model including peripheral level, NETRI has designed a microfluidic device for explant seeding. This snail- shaped microfluidic device is composed of three compartments, namely a seeding chamber for the explant ①, an intermediary channel ② and a distal channel ③ as depicted in Figure 1A. As a proof of concept, rodent primary DRG explants can be seeded in the explant chamber and their axonal projections can travel through microchannels up to the intermediary and distal channels.

Utilizing microchannels of varying length between the intermediary and the distal chambers, the snail- shaped device enables the quantification of axonal projections and their maximal length. Moreover, this device, as all NETRI technology, is compatible with standard high throughput screening, thus can be used with every cell culture automate, and with microelectrode arrays (MEA) to enable recordings of electrophysiological activity (c.f. Application Note Organoids - DR_4A_041.00).

The snail-shaped device enables long term culture of DRG *in vitro*

Using the unique design of the seeding chamber, we were able to seed and position a rat DRG explant from a 18-day old embryo (Sprague Dawley, Janvier Labs in the device) using our Operating Protocol Neurofluidics™ – NB1CD4M2 and the methodology described in our Application Note Organoids - DR_4A_041.00. (Figure 2A-C).

DRG and axonal projections were cultured up to 22 days in-vitro (DIV22). NETRI's home made media was changed every two days. Axonal projections grew rapidly in the microchannels, and reached the intermediary channel by DIV8 (Figure 2B). By DIV20, many projections had reached the distal channel (Figure 3).

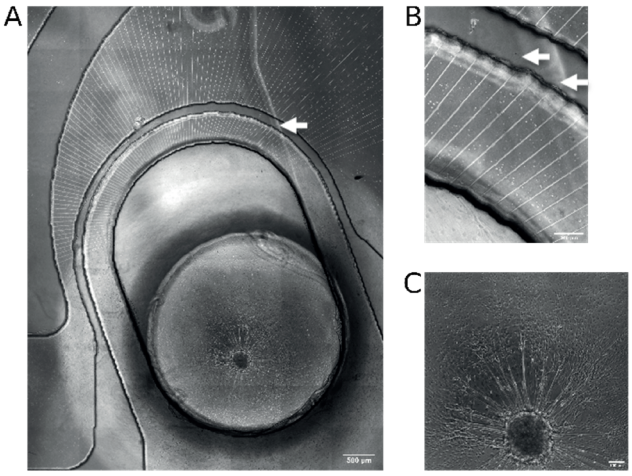


Figure 2. Morphology of rat DRG explant and their axonal projections in the EX_3_SNAIL at DIV8.
(A) Bright-field picture of DRG seeding in EX_3_X_SNAIL device.
(B) Bright-field picture of axonal projections in the intermediary channel (White arrows).
(C) Bright- field picture of axonal projection in the seeding chamber.

DRG staining in the snail-shaped microfluidic device

All standards staining can be performed on 3D cell culture in the presented microfluidic device with classical epifluorescence microscopy acquisition. To exemplify this, we performed both immunostainings and live staining on the DRG explants in culture.

DRG identification in snail-shaped device as a model for peripheral pain

Common immunostainings of the DRG can be performed in the microfluidic device, as shown in Figure 3 (DIV ranging from 20 to 22). We first fully characterized the DRG explants in culture by marking them with a specific neuronal marker (Peripherin (PRPH), in red), a specific marker for peripheral neurons (β-III-tubulin (bIII), in yellow) and a vesicular glutamate transporter (vGlut1, in red).

As the application presented here is a peripheral pain model, we also performed immunostainings targeting the transmission mechanisms of nociceptive signals. We thus marked for a neuropeptide delivered by the DRGs to transmit the nociceptive signal (Calcitonin gene-related peptide (CGRP), in red), a sodium ion channel, (NaV1.8, in green), and a pain receptor present on the surface of axons (Transient receptor potential vanilloid type 1 (TRPV1), in green) as shown in Figure 3.

Finally, we also marked myelin components of the DRG explants using Myelin-associated glycoprotein (MAG, in red) and SRY-Box Transcription Factor 10 (Sox10, in red), which is also a glial cells marker (Figure 3).

The details of the immunostaining process can be found in our Operating_Protocol - Immuno Staining DR_3B_059.

Growth kinetics measures of projected axons

As depicted in Figure 3, by DIV20-22, a large amount of growth is observed, with projections reaching the distal channel. It is also possible to monitor and study axonal projections growth kinetics in the snail-shaped device, as demonstrated using live staining.

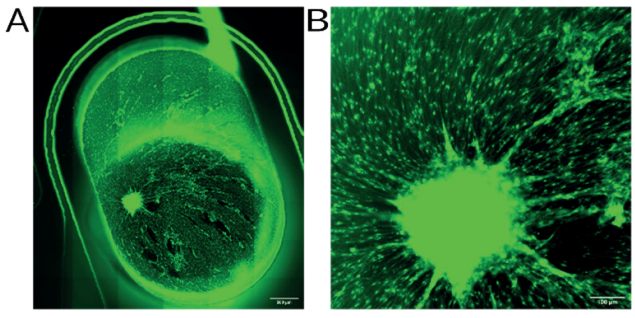


Figure 3. Live Staining of a DRG explant at DIV13 in EX_3_X_SNAIL.
(A) Fluorescent picture of a DRG explant seeded in an EX_3_X_SNAIL device.
(B) Fluorescent picture with x5 zoom of DRG in the explant chamber.

To do so, we used a lipophilic membrane Live Staining assay to stain axonal projections of the DRG explants. This staining method is not toxic, which enables to study axonal growth at various time points using the same device. Figure 4 shows an example of such staining procedure at DIV13.

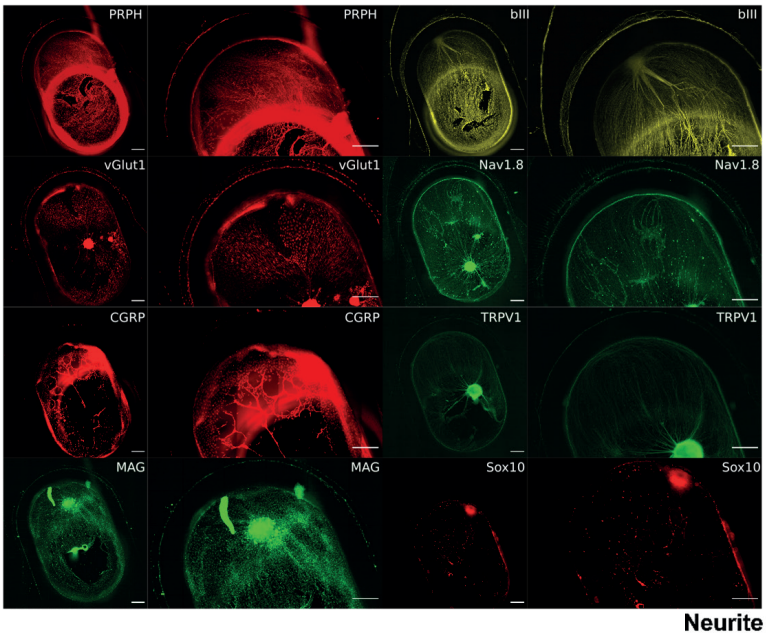


Figure 4. Immunofluorescence characterization of DRG explant in a snail-shaped chip (EX_3_X_SNAIL). DRG explant seeded and cultured up to DIV20-DIV22 and marked with PRPH (in red) β-III-tubulin (bIII, in yellow), vGlut1 (in red), Nav1.8 (in green), CGRP (in red), TRPV1 (in green), MAG (in green) and Sox10 (in red).

Live staining of DRG explant's axonal projections coupled with the specific snail shaped architecture of this device enabled us to quantify the growth of the projections from the DRG. Using the Operating Protocol DR_3B_009.03, the maximum length of axonal projections from the core of the DRG explant is measured over time. A steady growth of DRG explant's axonal projections through time was observed, reach up to 4525 µm at DIV20 (Figure 5).

Spontaneous activity recording of 3D-cell culture using MEA

The intermediary channel of the device can also be used to perform an axotomy on 3D cell culture projections, and investigate axonal projections regeneration. To do so, 0.5% of Triton can be added on the axonal projections in the intermediary channel during 30 seconds. Thanks to this reproducible axotomy protocol in microfluidic devices (data available on the ISSCR 2021 poster), axonal projections regrowth for ten days after injury was monitored.

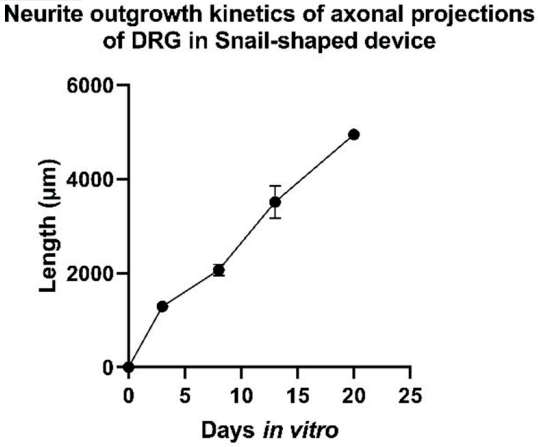


Figure 5. MEA electrodes cover all the NETRI's device.
(A) Picture of neurites at 15 DIV localized on electrodes into microfluidic chamber. Scale bare represent 30 µm.
(B) A single peak signal and plot.
(C) Recorded using MEA system and taken from a 10 minutes recording by an electrode located far from the DRG explant particularly on the neurites of an DRG.

CONCLUSION

This Application Note presents a snail-shaped microfluidic device referenced EX_3_X_SNAIL in which:

- 3D cell culture can be seeded and maintained for extended periods of time.
- Immunostaining and live staining can be performed on 3D cell culture.
- Axotomy protocols can be performed, and axonal projection growth (and/or re-growth) can be monitored.
- A peripheral pain model has been developed, using a DRG explant.
- Functional activity with Microelectrode Array (MEA) can be carried out (c.f. Application Note Oragnoids – DR_4A_041.00)
- Drug application/screening can be performed as High Throughput Assays, such as:
  - BDNF, a growth factor for study the influence on growth kinetic.
  - Capsaicin, Lidocaine: for modelling "Peripheral Pain on a chip".

RESOURCES

Available upon request

- Operating Protocol Neurofluidics™ – NB2XTR DR_3B_009.03
- Operating Protocol Neurofluidics™ – NB1CD4M2 DR_3B_025.02
- Operating_Protocol - Immuno Staining DR_3B_059
- Application Note Organoids – DR_4A_041.00
- Poster: Microfluidic high-thoughput screening platform to screen pre-clinical stage compound effects on neurite outgrowth of human Motor Neurons post-injury – ISSCR 2021

1. Schindelin, J. et al. Fiji: An open-source platform for biological-image analysis. Nature Methods vol. 9 676–682 (2012).
2. Tsantoulas, C. et al. Probing functional properties of nociceptive axons using a microfluidic culture system, PLoS ONE, 8(11), pp. 1–17 (2013).
3. Vysokov, N., Mcmahon, S. B. and Raouf, R. The role of NaV channels in synaptic transmission after axotomy in a microfluidic culture platform, Scientific Reports. Springer US, 9(1), pp. 1–13 (2019).
4. Viana, F. Nociceptors: thermal allodynia and thermal pain, in Handbook of Clinical Neurology. Elsevier B.V., pp. 103–119 (2018).
5. Johnstone, A. F. M. et al. Acute in vitro effects on embryonic rat dorsal root ganglion (DRG) cultures by in silico predicted neurotoxic chemicals: Evaluations on cytotoxicity, neurite length, and neurophysiology. Toxicol. Vitr. 69, 104989 (2020).

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!

sales@netri.com **+33 4 87 75 63**

www.netri.com
NETRI SAS - 321 Avenue Jean Jaures 69007 Lyon - FRANCE
Bat.A - 213 Rue de Gerland 69007 Lyon - FRANCE
Phone: +33 4 87 65 75 63 - Email: contact@netri.com