NETRI's APP NOTE

HIGH-THROUGHPUT MICROFLUIDIC DEVICE FOR LONG-TERM CULTURE AND FUNCTIONAL ACTIVITY RECORDING OF ORGANOIDS AND EXPLANTS

- Seed of an one organoid/explant (with an initial diameter <500 µm) per chamber with reliable positioning protocol.
- Long-term culture in chip up to a diameter of 4 000 µm.
- Recording of functional activity on the entire surface of the culture chamber.
- Compatibility with High-Throughput Assays – up to 48 organoids/explants multiplexed.

OVERVIEW

Advances in 3D-cell culture and particularly in organoids have shown their potential to recreate physiologically relevant *in vitro* models for studying biological mechanisms and neurological disorders. They offer the possibility to mimic the *in vitro* architecture of the human brain thanks to a cellular diversity and self-organization in three-dimensions (3D). Moreover, neuroscience applications require the possibility to explore the functional activity of the brain with neuronal network recordings.

NETRI has recently developed a technology that allows any 3D-cell culture, whose initial diameter is smaller than 500 µm, to be position in a microfluidic chamber and grown up to 4000 µm under physiological conditions (Figure 1). Such chips can be bonded to a Micro Electrode Array (MEA), which enables the recording of the functional activity of the entire grown 3D-cell culture.

In this application note, we describe a methodology to carefully position a 3D-cell culture (a Dorsal Root Ganglia as a use case) within the microfluidic chamber to perform electrophysiological recording.

RESULTS

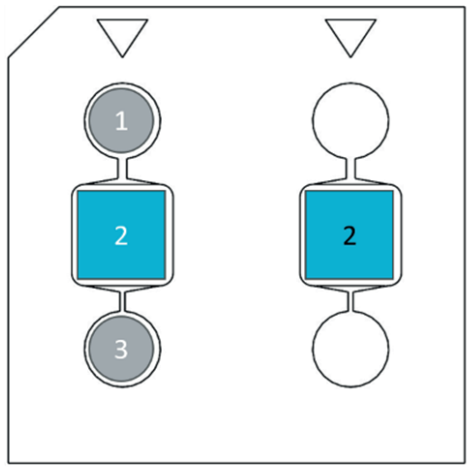
Microfluidic device compatible with 3D-cell culture

Designing a device compatible with seeding and culturing 3D-cell systems

The chip (reference number EX_1_CD_4M2) is composed of two fluidly isolated functional chambers (noted 2 in Figure 1). Each deposition chamber is linked to individual inlet (1 in Figure 1) and outlet (noted 3 in Figure 1) by embedded channels. (cf. Technical Sheet DR_4A_027). The height of the inlet reservoir is 510±10 µm, which allows 3D-cell culture with initial diameter ≤500 µm to be seed (Figure 1B). This dimension is consistent with the size of embryoid bodies.

The width of the deposition chamber is 4,000 µm making it possible for 3D-cell culture to growth up to ≤4,000 µm (Figure 1B). This size is consistent with long-term organoid growth and maturation.

A



B Description	Characteristics (µm)
Inlet (1) and outlet (3) reservoirs	510±10 *H*
Deposition chambre (2)	4 000 x 4 000 x 4 000 ±0.05 *L x W x H*

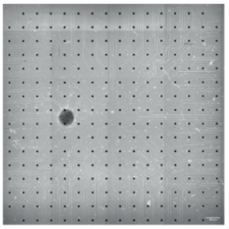
Figure 1. Advantages of NETRI's technology for 3D- cell culture.
(A) Schematic representation of EX_1_CD_4M2 chip.
(B) Characteristics of EX_1_CD_4M2

Using microfluidics to fine position 3D-cell culture

Thanks to the microfluidic control of hydrodynamic flows in the chip, the 3D cell culture can be dragged along with flow streamlines and positioned precisely within the deposition chamber.

To exemplify, we have seeded a rat DRG explant from 18-day old embryos (Sprague Dawley, Janvier Labs) using our Operating Protocol DR_4A_025.01. Conditions were tested while varying seeding media volume (Figure 2C). Final DRG positions were monitored using quantification of x/y coordinates with Fiji software [1] (Figure 2B).

A



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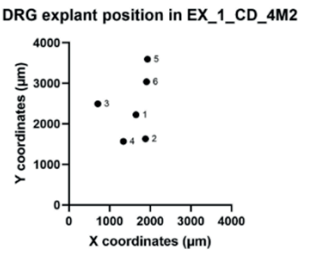

C

Conditions	% of positionning DRG in the microfluidic chamber (total assays)
1	25 (8)
2	38 (8)
3	13 (15)
4	88 (8)
5	21 (14)
6	25 (12)
7-12	0

Figure 2. Optimized positioning protocol for 3D-cell culture in NETRI's technology.
(A) Picture of DRG seeding in EX_1_CD_4M2 device coupled with MEA electrodes.
(B) Graph DRG coordinates according to the tested conditions.
(C) Table of condition tested with successful DRG positioning in deposition chamber. Dorsal Root Ganglia: DRG.

We target the most reproducible position at the center of the microfluidic device and on MEA electrodes (Figure 2A). Condition 04 corresponds to (i) fill the outlet with 100 µl of media and add 50 µL media containing DRG in the inlet reservoir and (ii) aspirate 50 µL from the outlet reservoir. This procedure was implemented into the Operating Protocol DR_4A_025.01.

In this study case, the chip was previously coated with Poly-D-Lysin (PDL, 0.01 mg/mL, Sigma, P6407) / Laminin (0.1 µg/mL, Corning, 354232 coating). Our devices are compatible with scaffold coating (i.e. low density Matrigel), low-attachment coating and/ or the absence of coating.

Using microfluidics to fine tune media replacement

Certain types of 3D cell culture require specific media perfusion over space (spatial distribution of the media perfusion) and time (duration of media renewal within the chip). Media renewal in the chips is ensured by aspirating or filling inlet and outlet reservoirs, without the need of external pumping systems. We have used a fluorescein solution as a media renewal tracker within the chamber while varying inlet and outlet filling/aspirating.

After one washing step, 60% of culture media is renewed and 90% if the washing step is repeated four times as shown on Figure 3.

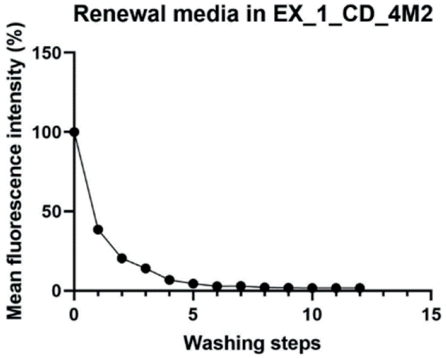

Figure 3. Variations of mean fluorescence intensity according to number of washing steps (n=2).

Spontaneous activity recording of 3D-cell culture using MEA

The fine positioning of the 3D-cell cultures in our chips allows a reliable recording of spontaneous activity, thus reducing the probable failure using in- wells classical approaches.
We report here the spontaneous extra-cellular activity of 7 DRG explants (Figure 4); both on the core explant and its subsequent projections prolongation over time (Figure 5).

All data were recorded with a MEA2100-256- Systems commercially available from MultiChannel Systems (Reutlingen, Germany) composed of a 256-channels amplifier head-stage. Recording was performed using the commercially available software (Multi Channel Experimenter, MultiChannel Systems). All of the experiments were carried out with 256MEA100/30iR-ITO-w/o (MCS) that consist of 30-µm-diameter electrodes spaced by 100 µm. Raw data were off-line filtered by a Bandpass Butterworth filter 2nd order (with cut-off frequencies of 100 Hz and 2500 Hz) and plotted with a homemade program based on Matlab (The Mathworks co.).

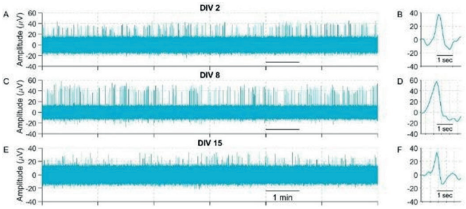

Figure 4. NETRI's technology is compatible with electrophysiological analysis of 3D-cell culture.
Recordings with a Multi Electrode Array (MEA) system at (A) 2, (C) 8, (E) and 15 Days In Vitro (DIV) of spontaneous activity. As example, a single peak signal – that reflects the induction of an action potential – is presented on the right panel for same experiments (respectively in B, D and F).

DRG explants show spontaneous activity in vitro (Figure 4). A drop of electrical activity was observed until 15 DIV on the DRG itself (Figure 4) while monitoring increasing spontaneous activity on prolongations (Figure 5). These results are consistent with previous reported work [2,3,4,5,6]. We observed a strong activity from the DRGs neurites at DIV 15 suggesting long-term DRG explant viability in NETRI's technology.

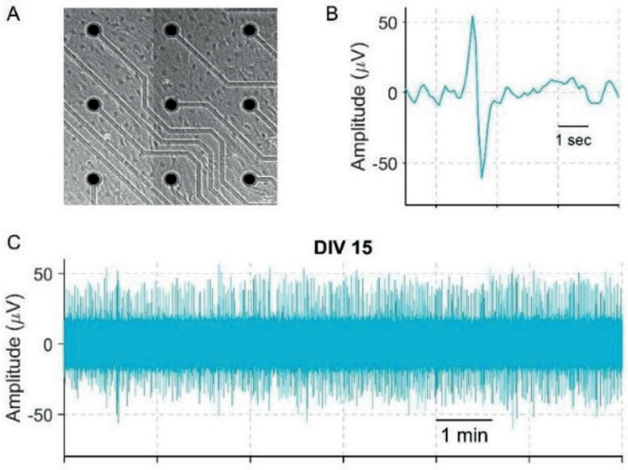

Figure 5. MEA electrodes cover all the NETRI's device.
(A) *Picture of neurites at 15 DIV localized on electrodes into microfluidic chamber. Scale bare represent 30 µm.*
(B) *A single peak signal and plot.*
(C) *Recorded using MEA system and taken from a 10 minutes recording by an electrode located far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

CONCLUSION

This Application Note reports a use case of the chip referenced EX_1_CD_4M2 in which:

- 3D-cell cultures can be positioned and maintained under physiological conditions. The dimensions of the chip can let them sprout or grow until 4,000 µm.
- Electrophysiological recordings can be performed over time with a high probability successful recording rate. Coupled to MEA, the chip offers the opportunity to characterize 3D-complex structures (such as human cerebral organoids).
- In mid-perspectives, limited neuronal maturation caused by insufficient oxygen/nutrient and lack of vasculature in cerebral organoids could be assess by microfluidic systems with adaptation of air-liquid interface culture.
- Application of this method will provide new insights into brain understanding and generalize the regulatory acceptance of Human cerebral Organoid-on-Chips in drug testing.

RESOURCES

Available upon request

- Technical Sheet Neurofluidics™ – NB8L1CD4M2 DR_4A_027
- Operating Protocol Neurofluidics™ – NB8L1CD4M2 DR_4A_025.01

1. Schindelin, J. et al. Fiji: An open-source platform for biological-image analysis. Nature Methods vol. 9 676–682 (2012).
2. Ma, C. & LaMotte, R. H. Multiple sites for generation of ectopic spontaneous activity in neurons of the chronically compressed dorsal root ganglion. J. Neurosci. 27, 14059–14068 (2007).
3. Xie, W., Strong, J. A. & Zhang, J. M. Increased excitability and spontaneous activity of rat sensory neurons following in vitro stimulation of sympathetic fiber sprouts in the isolated dorsal root ganglion. Pain 151, 447–459 (2010)
4. McConnell, E. R., McClain, M. A., Ross, J., LeFew, W. R. & Shafer, T. J. Evaluation of multi- well microelectrode arrays for neurotoxicity screening using a chemical training set. Neurotoxicology 33, 1048–1057 (2012).
5. Newberry, K. et al. First published April 6. J Neurophysiol 115, 3217–3228 (2016).
6. 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!

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