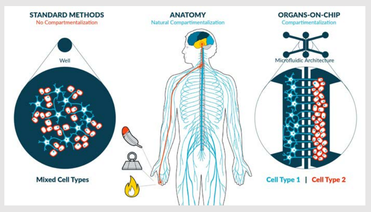
#65 - FUNCTIONAL SKIN-ON-CHIP
A RELEVANT IN-VITRO PLATFORM TO REPLACE ANIMAL MODELS IN DRUG AND COSMETIC DEVELOPMENT

Alexandre Guichard1*, Helene Gautier1, Thomas Bessy1, Louise Dubuisson1, Sebastien Cadau2, Charlene Prier2, Valérie Andre-Frei2, Thibault Honegger1
1 NETRI, Lyon, France, 2 BASF Beauty Care Solutions, Lyon, France, Contact: alexandre.guichard@netri.com

BACKGROUND



Topical drugs and cosmetic developments are limited by in-vitro models, which are not always relevant either because they use rodent cells, banned in the field of cosmetic, or because the co-cultures of skin and neurons do not fully recapitulate the anatomical structure.
To address these limitations, we developed different microfluidics devices using human cells to recapitulate a skin compartment with keratinocytes and nerve endings and a spinal cord compartment with neuronal cell nucleus.
We present here two different chips and their respective applications.

MATERIAL & METHODS

TWO DIFFERENT ARCHITECTURES.

Neuroskin chip *(in collaboration with BASF)*
- Consists of four compartments connected (Fig. A)
- To create a large innervated reconstructed epidermis
- Suitable for topical application of final products, study the mechanism of action and fundamental research

DuaLink MEA chip
- Consists of two compartments connected (Fig. B)
- To create an innervated living epidermis and record electrical activity thanks to MicroElectrode Arrays (MEA)
- Suitable for high throughput screening of dermocosmetic ingredient efficacy or toxicity

A

B

Neuroskin chip
Green: top compartment 8x8x0.6 mm suitable for growing keratinocytes.
Blue: bottom compartment 8x8x0.15 mm suitable for growing endothelial cells or without cells just as a reservoir of medium.
Light green: polycarbonate membrane to separate top and bottom compartments.
Pink: side compartments 26000x1000x200 µm (LxWxH) suitable for growing neurons.
Pink and green compartments are interconnected via 400 microchannels 450x5x3,4 µm (LxWxH) spaced by 20 µm suitable for axons projection.

Dualink MEA chip
Pink and green: side compartments 18800x1000x200 µm (LxWxH) suitable for growing neurons in one compartment and other cell type (e.g. keratinocytes) in the other compartment.
Grey: straight central compartment 6000x200x200 µm (LxWxH) filled with medium only, suitable for increasing the fluidic isolation between side compartments.
The three compartments are interconnected via 200 microchannels 125x6x3,2 µm (LxWxH) spaced by 20 µm suitable for axons projection.
Black lines: MicroElectrode Arrays, in collaboration with Axion BioSystems.

EXPERIMENTAL CONDITIONS.

Cells
- Human iPSC-derived sensory neurons (Axol Bioscience)
- Human primary keratinocytes (PromoCell)

Assessments
- Cellular viability
- Cellular morphology
- Expression of characteristic markers
- Electrophysiological responses (MEA) of human iPS-derived sensory neurones to KCl or temperature stimulations in Dualink MEA chip (Maestro Pro, Axion BioSystems)

RESULTS

NEUROSKIN.
INNERVATED STRATIFIED EPIDERMIS ON CHIP.

Neuronal compartment.
- Long-term viability (28 days)
- Clustering morphology of neurons
- Expression of maturation markers: peripherin, substance P, TrkB, Nav1.8
- Loss of expression of neuronal potency markers: Nestin, Sox2
- At 21 days, neurites have grown up to 33% of the top compartment width

Epidermal compartment.
- High viability (> 80%)
- Up to 5 superposed layers could be observed with a beginning of differentiation observed by filaggrin expression
- Integration of axonal network within keratinocytes
- Sensory neurons are connected with mature keratinocytes

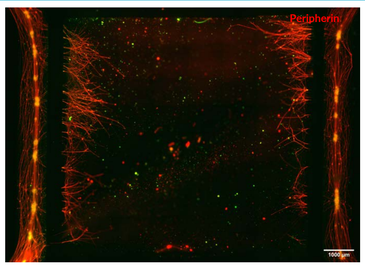


Fig 1. Immunofluorescence image of the top compartment (without keratinocytes) innervated by neurites coming from side compartments (left and right sides of the top compartment) in which human iPSC-derived sensory neurons are organized in clusters.

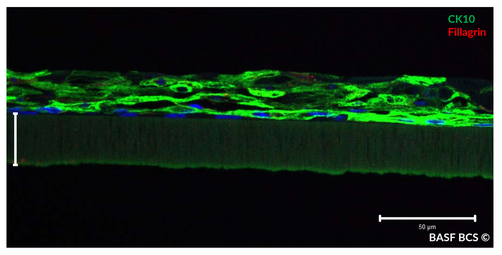


Fig 2. Cross section of top compartment in which keratinocytes were grown on polycarbonate membrane (white line).
Air-liquid interface was created to stratify the epidermis (Fillagrin)

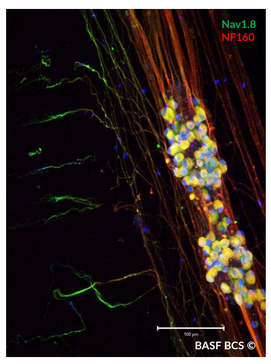
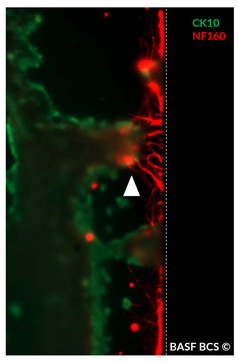


Fig 3. Human iPSC-derived sensory neurons differentiated and organized in cluster into a side compartment. Neurites (green) are migrating into the microchannels to innervate the top compartment (not visible).

A



B

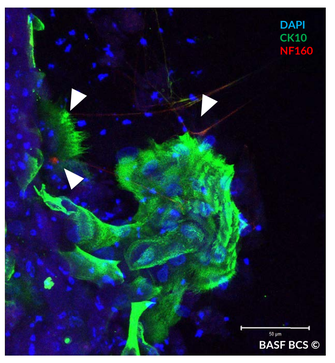


Fig 4. Contact between keratinocytes and neurons.
A. Keratinocytes (green) fully colonized the top compartment (left side of the dotted line).
Neurites (red) coming from side compartment (right side of the dotted line) through microchannels (not visible) innervate the top compartment and connect to keratinocytes (white arrowheads).
B. Neurites (red) projecting into the top compartment and connecting with keratinocytes (green, white arrowheads)

DUALINK MEA.
INNERVATED LIVING EPIDERMIS ON CHIP FOR ELECTROPHYSIOLOGICAL RECORDING.

Neuronal compartment.
- Long-term viability (28 days)
- Clustering morphology of neurons
- Expression of maturation markers: peripherin, substance P, TrkB, βIII-Tubulin, TRPV1, Nav1.7 (Fig. 1)
- Loss of expression of neuronal potency markers: Nestin, Sox2
- Electrophysiological response to KCl or temperature stimulations (Fig. 2)

Epidermal compartment.
- High viability (> 80%)
- Confluent monolayer of keratinocytes
- Expression of specific markers: K14, K10, TRPV1, CGRP (Fig. 2)

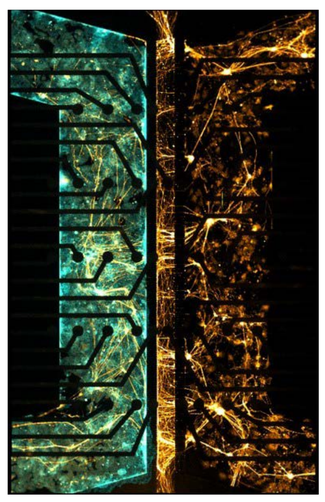


Fig 1. Coculture of human primary keratinocytes (left, green=Phalloidin) with human iPSC-derived sensory neurons (right, yellow=Peripherin) at day 23 on Dualink MEA chip (electrodes = black lines)

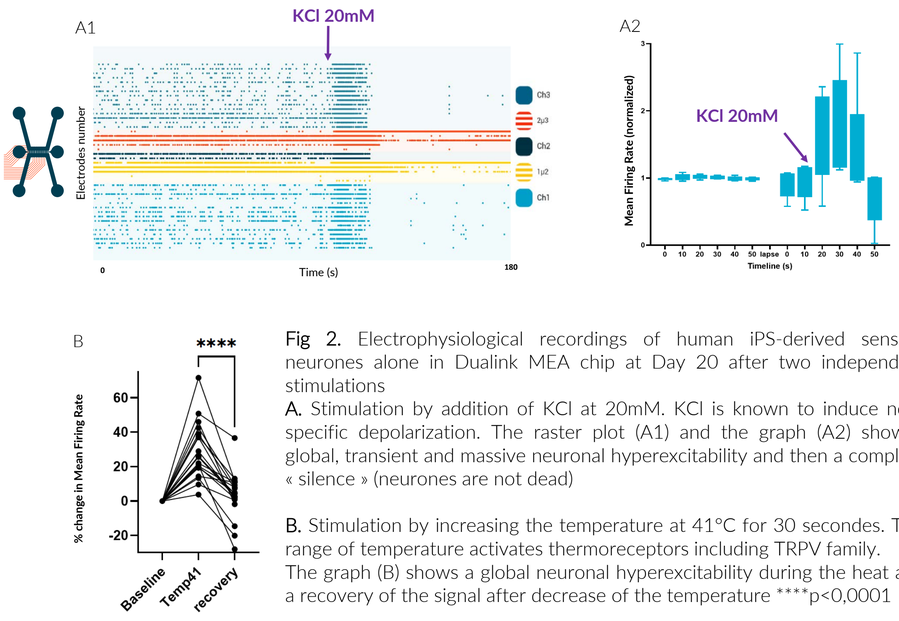


Fig 2. Electrophysiological recordings of human iPS-derived sensory neurones alone in Dualink MEA chip at Day 20 after two independant stimulations
A. Stimulation by addition of KCl at 20mM. KCl is known to induce non-specific depolarization. The raster plot (A1) and the graph (A2) show a global, transient and massive neuronal hyperexcitability and then a complete « silence » (neurones are not dead)

B. Stimulation by increasing the temperature at 41°C for 30 secondes. This range of temperature activates thermoreceptors including TRPV family.
The graph (B) shows a global neuronal hyperexcitability during the heat and a recovery of the signal after decrease of the temperature ****p<0,0001

CONCLUSION & PERSPECTIVES

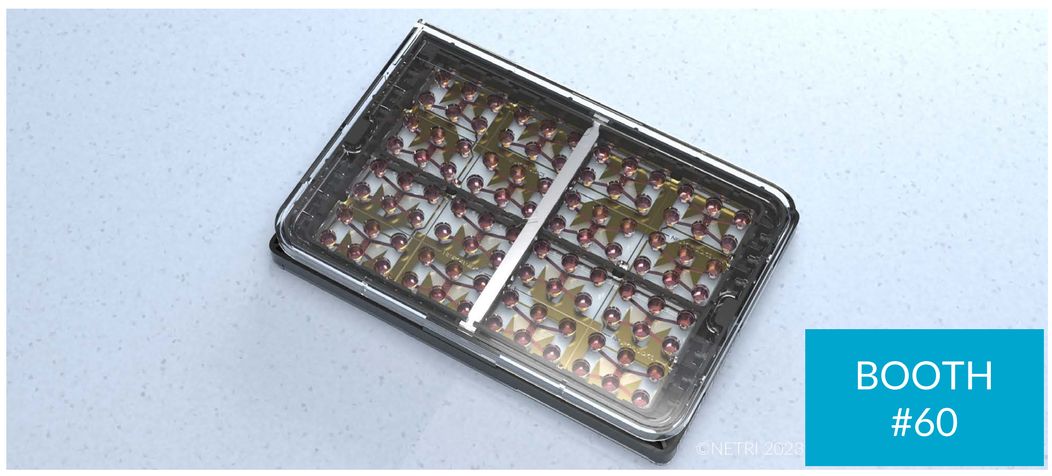
We **succeed to develop and grow an innervated epidermis** close to the actual physiology with **viability, maturation, and functionality** successfully proved. **Neuroskin and DuaLink MEA chips** have both advantages and are complementary.

Coupling NETRI's technology with **MEA technology** allows **electrical signals recording** of soma after axonal stimulation, leading to **innovative readout** for the dermo-cosmetic research. The **applications are broad**: sensitive skin, itching, skin ageing, wound healing, inflammatory dermatosis (psoriasis, eczema, rosacea…).

Our **compartmentalized and fully human organs-on-chip devices** are used to **accelerate preclinical phases**, **decrease the rate of clinical failure** and **minimize animal testing**.

Compartmentalization and fluidic isolation have several advantages:
- Cells are cultivated with their respective culture medium allowing optimal growth
- Application of a stimulus or an ingredient specifically on one cell type
- Supernatant can be sampled in a specific compartment

Thanks to the **industrial** production and a **strict quality control**, NETRI provides repeatable and reproducible devices in a high-throughput format, **NeoBento™**, and are therefore compatible with:
- High-throughput MicroElectrode Array systems
- High Content Screening imaging systems & microscopy
- Liquid handling robots



MPS WORLD SUMMIT
CONNECT, EXCHANGE, EDUCATE

CONTACT
www.netri.com
Phone: +33 4 78 23 08 66
Email: contact@netri.com