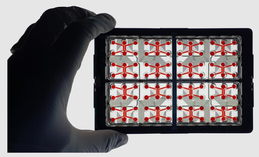
COMBINING SENSORY NEURON ELECTROPHYSIOLOGY AND MICROFLUIDICS TO INVESTIGATE KERATINOCYTE-DERIVED MESSENGERS IN INFLAMMATORY SKIN CONDITIONS

Thomas Bessy[1] & Geoffroy Tousch[2], Camille Baquerre[1], Luiz Motta[2], Céline Blanchard[2] & Alexandre Guichard[1]

[1]NETRI, 76 rue Georges Gouy, 69007 Lyon, France / [2]R&D, SOLABIA Group, Route d'Oulins, 28260 Anet, France

BACKGROUND



The skin is a key sensory organ relying on complex interactions between keratinocytes and sensory neurons especially in the context of skin sensitivity and pruritus. Current *in vitro* models often lack anatomical compartmentalization or fail to provide the neuronal involvement in these mechanistic aspects.
To address these limitations, we developed a fully human in vitro model consisting of a compartmentalized sensory neuron culture integrated into a microelectrode array-based (MEA) microfluidic chip. Within this neurocutaneous communication framework, we investigated the role of keratinocyte-derived secretome under pruritogenic and inflammatory conditions on neuronal activity.
Then, this model was used to evaluate the efficacy of cosmetic compounds.

METHODS

AN INNOVATIVE DEVICE COMBINING PHYSIOLOGICAL CONDITIONS WITH SENSITIVE READOUT

Human iPSC-derived sensory neurons have been cultivated in compartmentalized microfluidic chip (NETRI, DuaLink™ MEA). The design of the chip ensures a fluidic isolation between neurons' soma and neurites endings mimicking actual anatomy.

NETRI chips consist on 3 independant channels interconnected through 200 microchannels (150x6x3.2µm; LxWxH).

NETRI chips integrate microelectrodes array technologies allowing the non-cytotoxic live monitoring of neuronal electrical activity (e.g MFR=Mean Firing Rate=number of spike per time unit).

Immunofluorescence images of iPSC-derived sensory neurons (AXOL Bioscience) cultivated 23 days in Dualink MEA™ chip (NETRI) with a Beta III-tubulin staining. Microelectrodes array (black lines and dots).
The neurons are clustered within the cell body canal (Ch1), and their numerous extensions extend throughout the entire right hand side channel (Ch3).

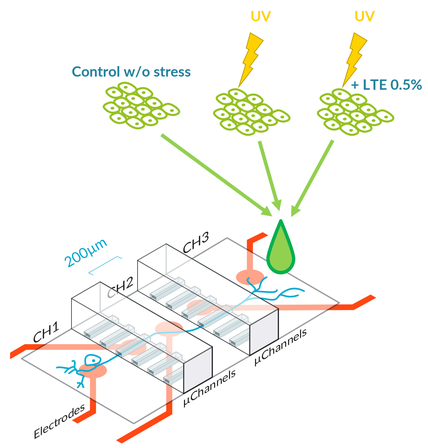
EXPERIMENTAL PLAN

hiPSC derived human sensory neurons (AXOL Bioscience) and adult human primary keratinocytes have been used.
Keratinocytes were stressed by UVB exposure in presence or absence of SOLABIA compounds. SOLABIA compound is an exosome-enriched lemon thyme extract (LTE) obtained through advanced green-biotech phyto-extraction and standardized in rosmarinic acid.

Validation of sensory neuron sensitivity to inflammation. Sensory neurons were exposed to a vehicle or to an inflammatory cytokine cocktail composed of IL1b, IL6 and TNFα at 20ng/mL. MEA activity was recorded before and 24h post treatment.

Stressed keratinocytes conditioned media characterization. ELISA dosage of conditioned media have been performed to assess the release of key inflammatory cytokines.

Sensory neuron response to stressed keratinocytes conditioned media. Neurons' endings have been treated by conditioned media from either non treated keratinocytes or from keratinocytes stressed with UVB in the presence or absence of LTE. MEA recordings have been performed before and at 2h, 24h and 48h post treatment. For each recording, the value of the metric is normalized by its value during the initial recording to account for initial system variability.



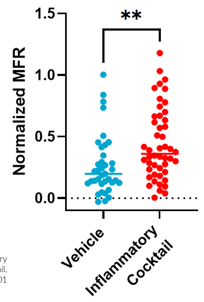
Schematic view of Dualink MEA® chip and experimental plan

RESULTS

hiPSC SENSORY NEURONS IN MICROFLUIDIC DEVICES ARE SENSITIVE TO INFLAMMATORY CYTOKINES

MFR has been recorded before and 24h after the addition of either a vehicle or an inflammatory cocktail

The inflammatory cocktail induced an increase of the neuronal activity in 24h.



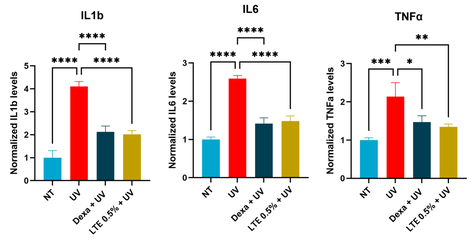
Electrical activity (MFR=mean firing rate) of hiPSC-derived sensory neurons with or without inflammatory cytokines cocktail.
Statistical test : Mann Whitney test ** = p<0.01

LTE PREVENTS INFLAMMATORY STATE INDUCED BY UVB IN KERATINOCYTES

UV exposure increased significantly the release of pro-inflammatory cytokines by keratinocytes +310%, +159%, +114%, respectively for IL-1b, IL-6 and TNFα.

Keratinocyte treatment with a potent anti-inflammatory reference compound (Dexamethasone) showed a significant reduction of pro-inflammatory cytokines release (-48%, -45%, -45%).

Keratinocyte treatment with LTE showed also a significant reduction pro-inflammatory cytokines release comparable to Dexamethasone (-51%, -43%, -43%)

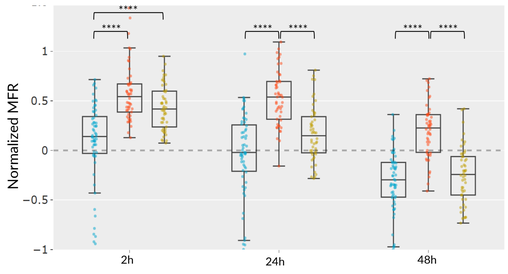


Cytokines released in keratinocytes' conditioned media after treatments (ELISA dosage).
NT= Non treated keratinocytes, Dexa = Dexamethasone.
Statistical test : ANOVA-Dunnett * = p<0,05, ** = p<0,01, *** = p<0,001 **** = p<0,0001

hiPSC SENSORY NEURONS IN MICROFLUIDIC DEVICES IS A RELIABLE METHOD TO MEASURE THE ANTI-INFLAMMATORY EFFECT OF LTE

The conditioned media of keratinocytes exposed to UV induced an increase of neuronal activity (MFR) (red) compared to neurons exposed to untreated keratinocytes medium (blue).

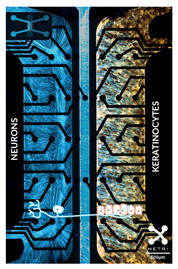
0,5% LTE (yellow) reduced progressively this hyper activity until reaching a total and significant recovery at 24h.



Electrical activity (MFR=mean firing rate) normalized to baseline of sensory neurons with or without treatments after 2h, 24h and 48h.
Blue=non treated keratinocytes, orange=UV, yellow=LTE.
Statistical test : mixed-effects statistical model with False Discovery Rate (FDR) correction * = p<0.05, **** = p<0.0001

CONCLUSION & PERSPECTIVES

1. This first-in-class study demonstrated the feasibility and relevance of using:
   - NETRI microfluidic devices to mimick actual anatomy
   - Neurons as biosensors
   - Electrophysiology (MEA) to measure neuronal responses to stimuli
   - Conditioned media to investigate indirect keratinocyte-derived messengers in inflammatory skin conditions
2. Conditioned media of keratinocyte stressed by UV induced an increase of neuronal excitability.
3. LTE showed a protective effect against UV stress by reducing neuronal excitability.
4. MEA data were correlated with mediators release dosage (ELISA).
5. This study showed similar effect and protection on LPS and another SOLABIA compound (data not shown, patent pending).



Furthermore, NETRI is developing machine learning algorithm to analyze other MEA metrics (which are not limited to only MFR) in order to better discriminate compounds' effects and establish the unique digital signature of compounds i.e the NaaS concept (Neuron-as-a-Sensor) (*Bessy et al. bioRxiv 2025.09.25.678500).*

Finally, NETRI also developed a compartmentalized 2D coculture of innervated-keratinocytes monitored by MEA on which direct effect of compounds can be evaluated *(Bessy et al. Lab Chip, 2026, 26, 248.)*

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Chicago, US

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