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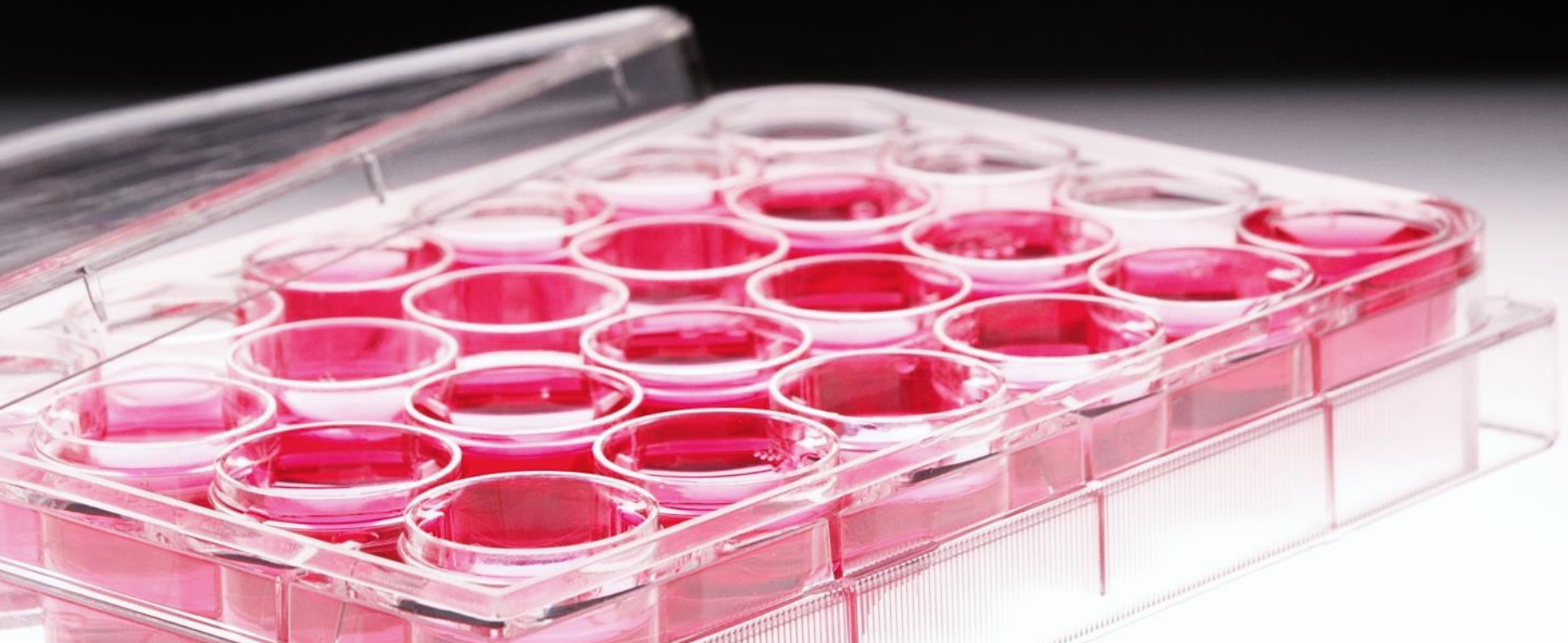
Barrier Function of Mammalian Skin
Gordon Research Conference
August 11 - 16, 2019 • Waterville Valley

Complexification of in vitro 3D skin models, sensory aspects and atopic skin

Dr. Michel SALMON, CEO, Lead Research & Development
StratiCELL, Crealys Science Park, ISNES, Belgium



I. Basic organotypic skin models & applications

II. Increasing complexity – integration of other cell types for particular skin conditions and diseases (non exhaustive state-of-the-art review)

III. Inflammatory skin conditions through compromised in vitro models

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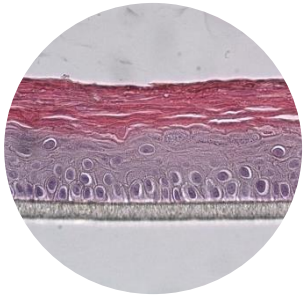
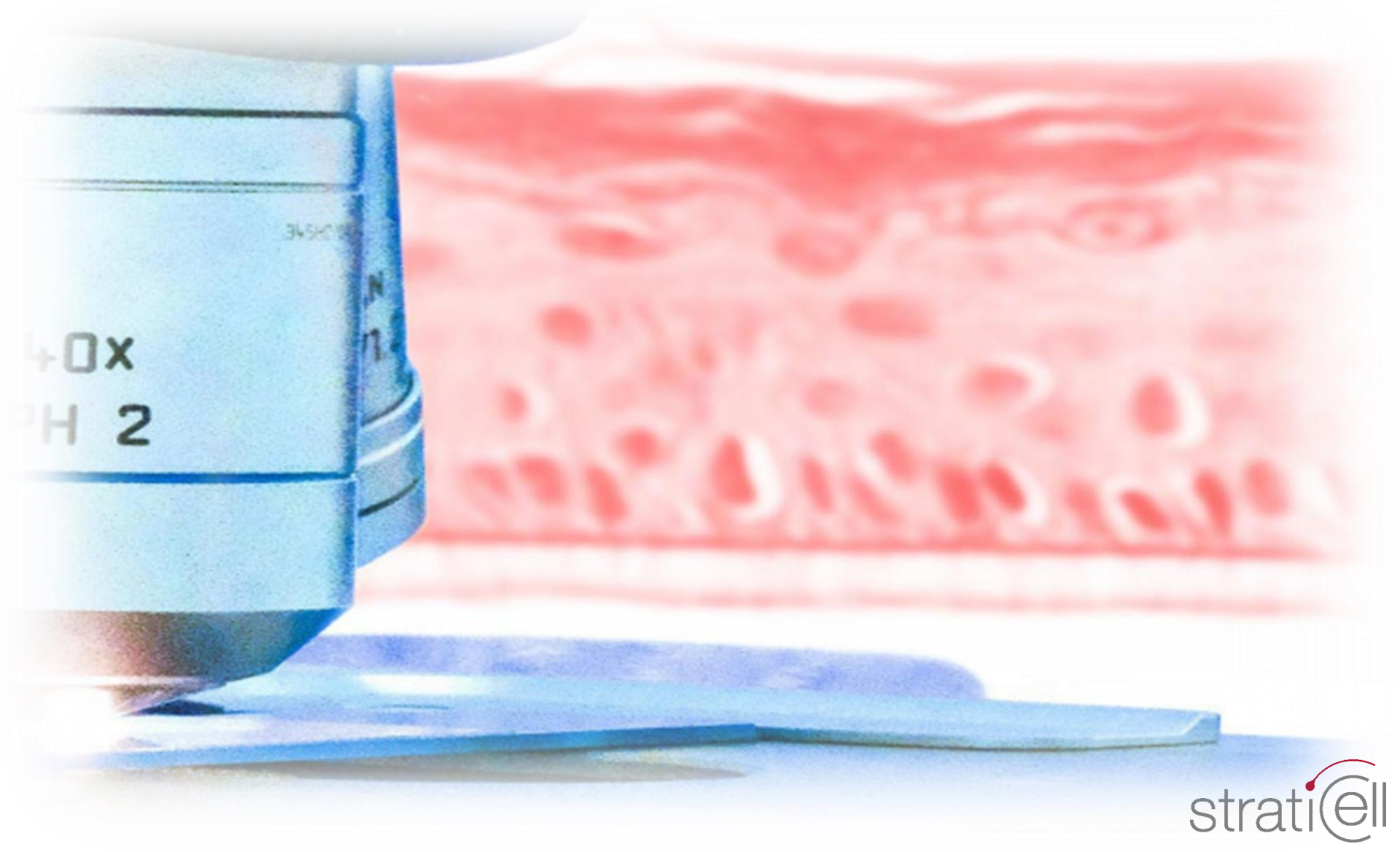
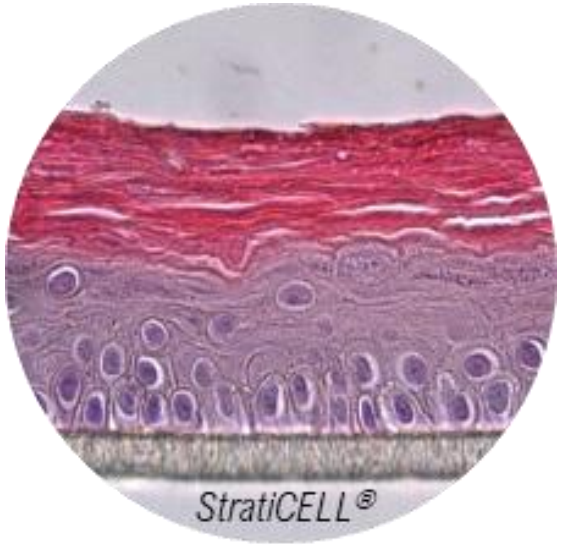


- 2D cell culture are simple to use and low cost, but are not always translatable to physiological *in vivo* systems
- They do not allow the application of liposoluble compounds
- Lack environmental factors associated with a 3D *in vivo* environment, *i.e.*
    - Mechanical forces
    - Spacial orientation
    - Signaling gradients
    - Physiological oxygen
    - Cell-to-cell and cell-to-matrix interfaces
- Need for more physiological and complex 3D skin models to better predict clinical outcomes in drug development, dermo-cosmetics and toxicology profile assessment

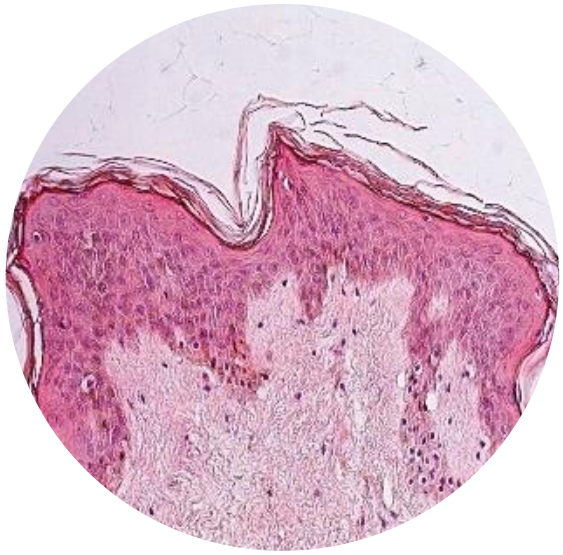
I. Basic organotypic skin models & applications





- Culture of human keratinocytes on a **porous polycarbonate membrane** at the **air-liquid interface** for 11-17 days, high calcium, VitC
- Commercially available
- Sourcing : **NHEK**s, **iPSC-derived** keratinocytes, **h-TERT/p16$^{-/-}$**, **patient-derived** keratinocytes, **canine/murine** keratinocytes, **genetically-modified** keratinocytes, etc.
- Although protocols are fully available, **standardization remain an issue** (sourcing, media, etc.)

- Differ by **the composition of the dermal matrix** (collagen, fibrin, chitosan/collagen/HA, hydrogels, etc.)
- Allow **paracrine communication** between the dermal and epidermal compartments
- Commercially available
- Time-consuming and quite expensive to produce



- Closely **representative of skin physiology**
- Commercially available or supplied by hospitals (might be an issue)
- Explants are in **survival conditions**, readily in inflammatory state
- Tissues were treated with antiseptics before surgery
- **Variability** from one individual to another, and from different body sites

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3D skin models – applications in testing and research

- **Proof of efficacy, mechanistic studies, claim identification for dermo-cosmetic products**
- **Biomedical research in skin physiology**
- **Preclinical drug development, metabolism and drug delivery studies**
- **Cytotoxicity studies**
- **Regulatory toxicology**
  - ***Skin corrosion OECD431***
  - ***Skin irritation OECD439***
  - ***Dermal absorption OECD 428***
  - ***Genotoxicity (micronucleus test)***
  - ***Skin sensitization (SENS-IS)***
  - ***Phototoxicity***

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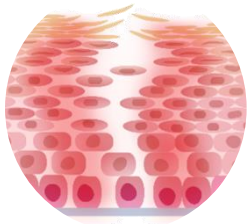
Challenging skin models with environmental or endogenous stressors

Solar radiations (UV, IRA, blue light)

- *Tissue morphology*
- *P-p53, caspase activation*
- *DNA damage (CPDs, 8-oxodG)*
- *Inflammatory response*
- *Lipid and protein oxidation*
- *ECM degradation*

Pollutants (individual pollutants, urban dust, cigarette smoke, VOCs, etc.)

- *ROS production*
- *Inflammation*
- *Barrier defects*
- *Matrix modeling*
- *AhR activation*



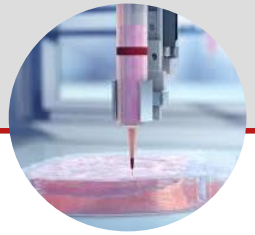
Pro-inflammatory stimulation (PMA, LPS, cytokines, bacteria, etc.)

- *Cytokine & PG release*
- *Signal transduction*
- *NFκB activation*
- *Inflammatory skin diseases*

Barrier disruptors (organic solvants, detergents)

- *TEER, TEWL, biotin & LY labeling*
- *CE components*
- *FLG processing*
- *Cell-cell junctions*

and many others, to everyone's imagination…



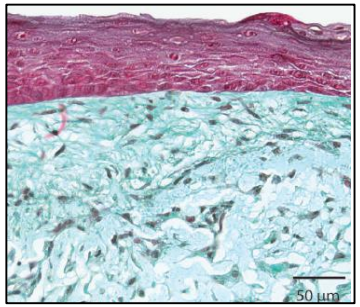
Bioimpression of 3D skin models

ADVANCED SCIENCE NEWS
www.advancedsciencenews.com

ADVANCED HEALTHCARE MATERIALS
www.advhealthmat.de

Human Skin 3D Bioprinting Using Scaffold-Free Approach

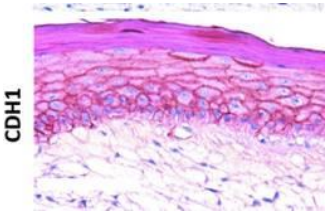
*Léa J. Pourchet, Amélie Thepot, Marion Albouy, Edwin J. Courtial, Aurélie Boher, Loïc J. Blum, and Christophe A. Marquette**



* 3D-Fab/LabSkin, Lyon, France

Fully Three-Dimensional Bioprinted Skin Equivalent Constructs with Validated Morphology and Barrier Function

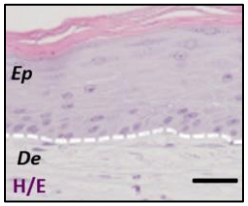
Kristy Derr, BS,[1] Jinyun Zou, MS,[2] Keren Luo, BS,[2] Min Jae Song, PhD,[3] G. Sitta Sittampalam, PhD,[1] Chao Zhou, PhD,[2,4] Sam Michael, BS,[1] Marc Ferrer, PhD,[1] and Paige Derr, PhD[1]



*NIH, MD

3D bioprinting of functional human skin: production and *in vivo* analysis

Nieves Cubo[1,5], Marta Garcia[1,2,3,5], Juan F del Cañizo[4], Diego Velasco[1,3] and Jose L Jorcano[1,2]



* U. Madrid, Spain

Direct 3D bioprinted full-thickness skin constructs recapitulate regulatory signaling pathways and physiology of human skin

Prasad Admane[a], Abhishak C. Gupta[a], Prashanth Jois[b], Subhadeep Roy[a], Chittur Chandrasekharan Lakshmanan[b], Gurpreet Kalsi[b], Balaji Bandyopadhyay[b,**], Sourabh Ghosh[a,*]

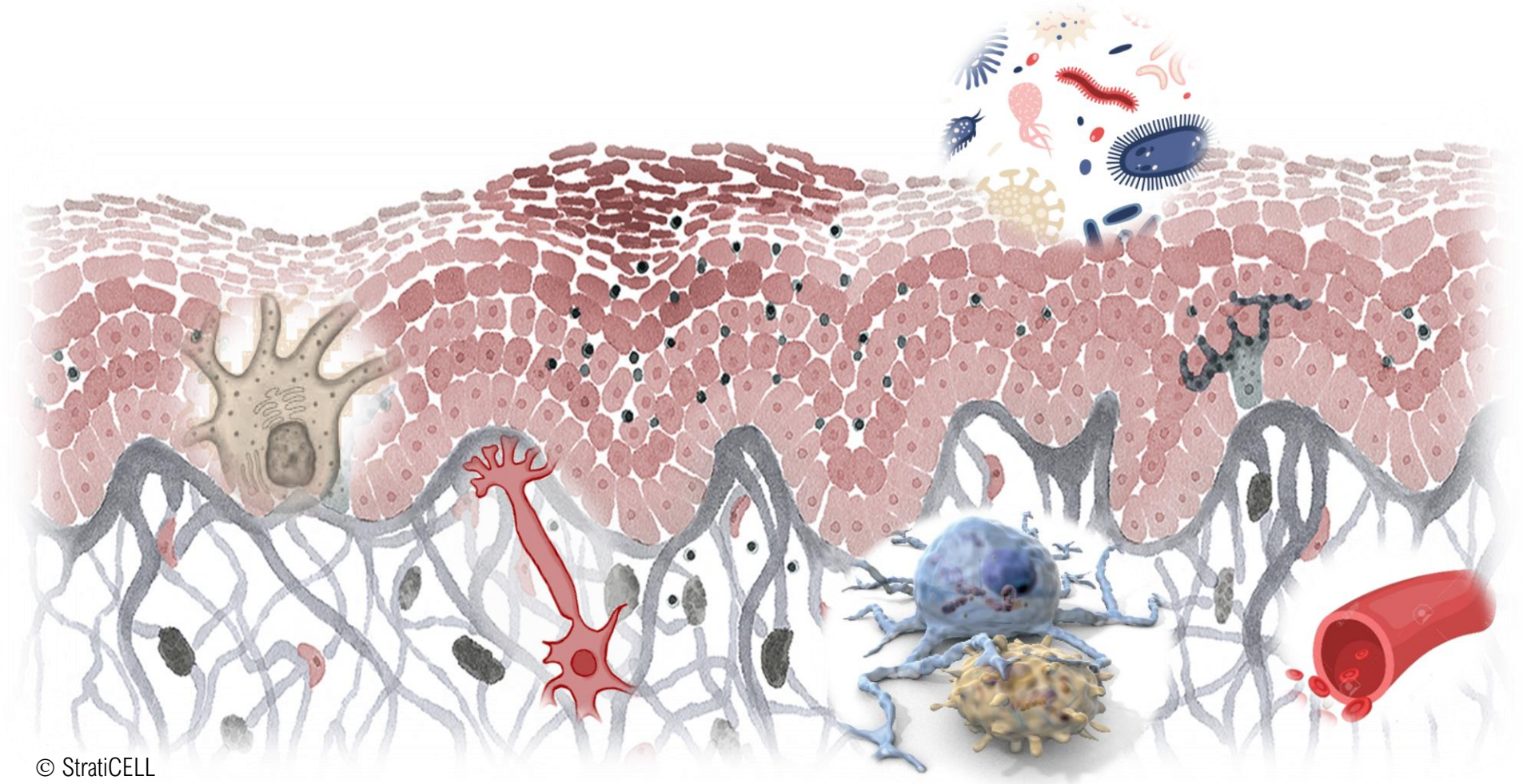
* Indian Institute of Technology, Delhi, India

In Situ Bioprinting of Autologous Skin Cells Accelerates Wound Healing of Extensive Excisional Full-Thickness Wounds

Mohammed Albanna[1], Kyle W. Binder[1], Sean V. Murphy[1,5], Jaehyun Kim[1], Shadi A. Qasem[2], Weixin Zhao[1,3], Josh Tan[1], Idris B. El-Amin[1], Dennis D. Dice[1], Julie Marco[1], Jason Green[1], Tao Xu[1], Aleksander Skardal[1,5], James H. Holmes[4], John D. Jackson[1], Anthony Atala[1] & James J. Yoo[1]

* Wake Forest Inst., NC

II. Increasing complexity and relevance – integration of other cell types for particular skin conditions and diseases



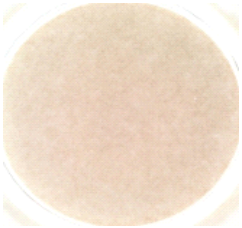
Integration of human melanocytes in 3D reconstituted epidermis (RHE)

Dermoscopy (C-Cube)

Fontana Masson staining

RHE-MEL (human primary Mc)

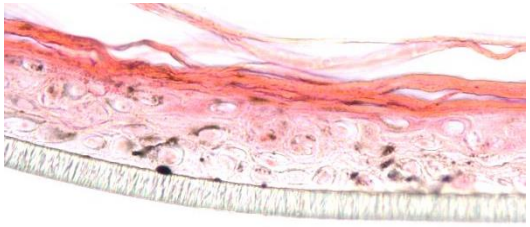
No melanocytes



NHEMs (III-IV)

NHEMs (V-VI)

No challenge



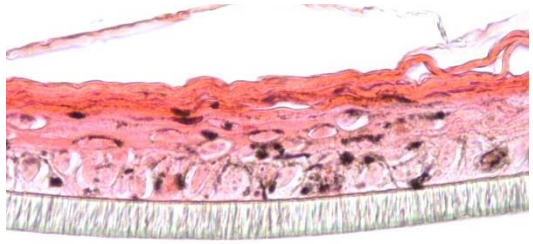
RHE-MEL iPSC (iPSC-derived Mc)

iPSC-derived Mc, Caucasian (III-IV)

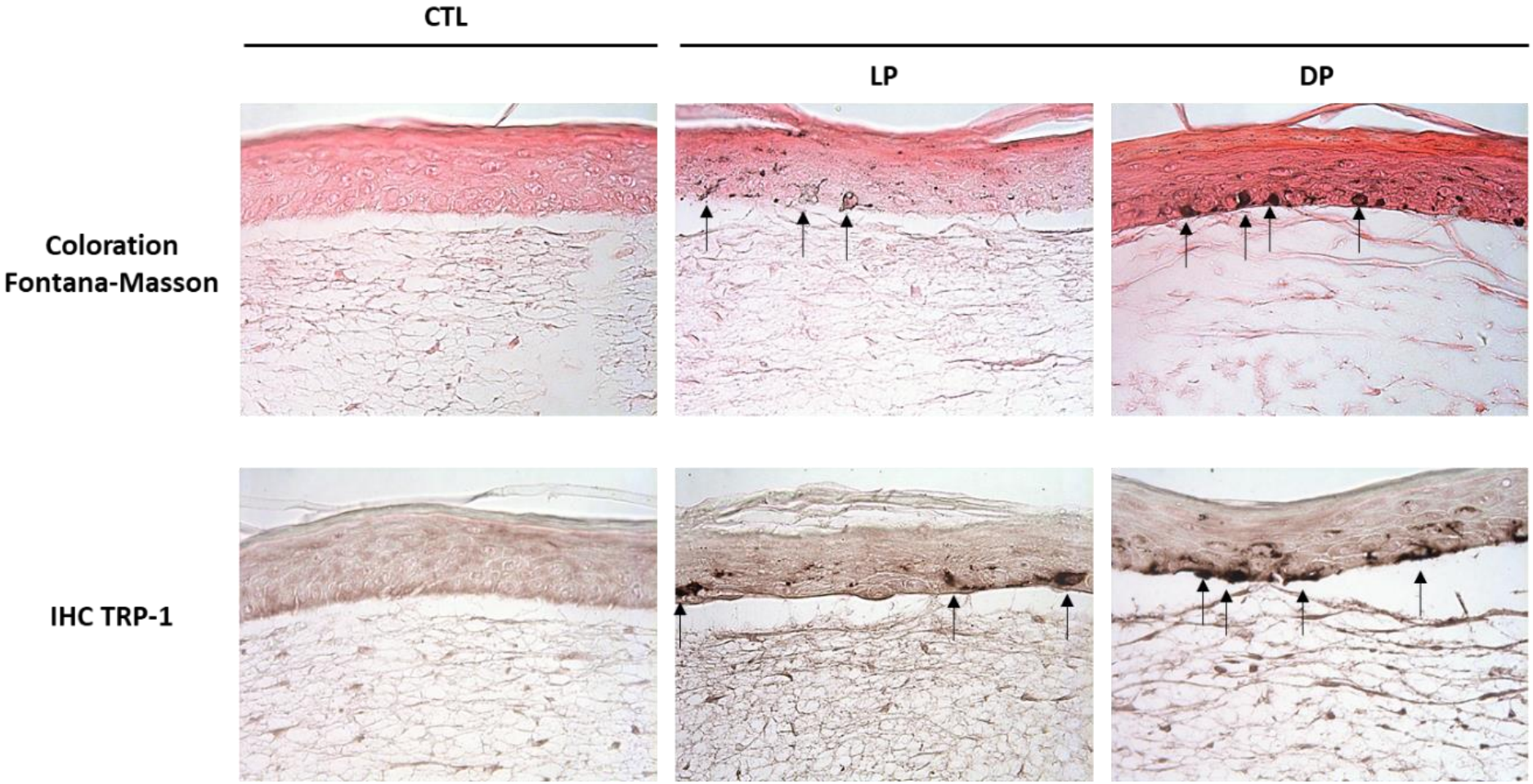
iPSC-derived Mc, Asian (IV-V)

iPSC-derived Mc, oculocutaneous albinism type-1 (OCA1)

Challenge : UVA 1J/cm² + UVB 50mJ/cm² in presence of α-MSH (1µM)



Integration of human melanocytes in human skin equivalents (HSE)



HSE (StratiCELL, BE) – human lighly-pigmented (LP) or darkly-pigmented (DP) melanocytes seeded with NHEKs on a fibrin-based dermal matrix

Pigmentation disorders – RHE model for *solar lentigines*

(StratiCELL, based on Chen et al, 2010)

Dermoscopy (C-Cube)

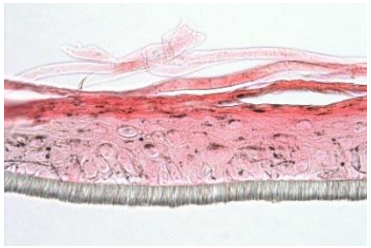
RHE-MEL

RHE-SL (fibroblast-derived melanogenic factors

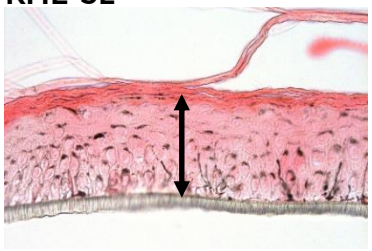
RHE-SL + kojic acid

Fontana Masson & melanin deposition

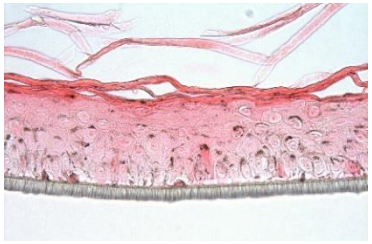
RHE-MEL

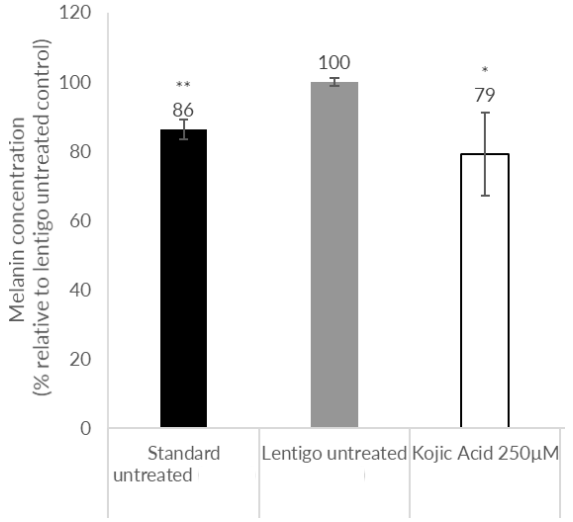


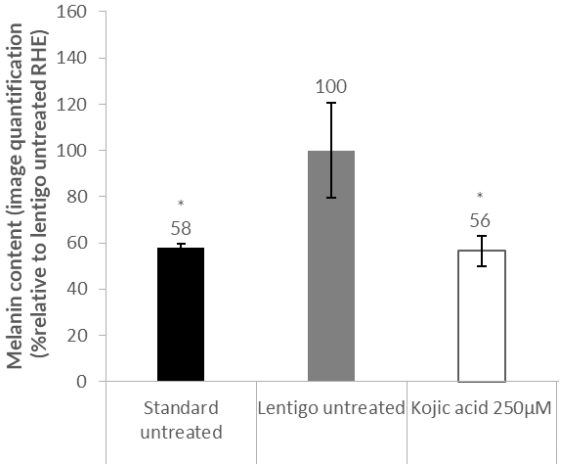
RHE-SL



RHE-SL + KA 250 µM







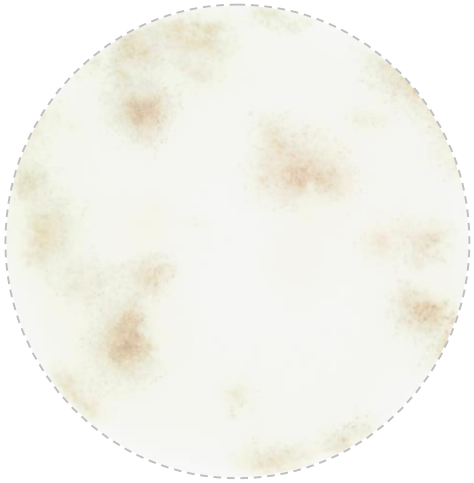
RHE model for *solar lentigines* with individual spots

(StratiCELL, based on Chen et al, 2010)

© StratiCELL

RHE-MEL

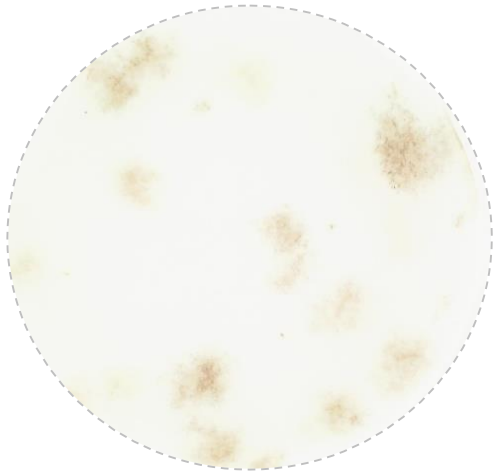
Standard RHE with melanocytes (J17)



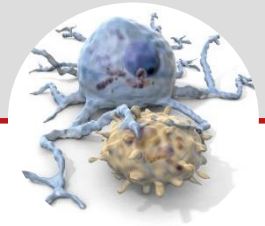
+Kojic Acid 250µM

RHE- SL spots

Extended (J17) culture with a cocktail of fibroblast-derived melanogenic factors



+Kojic Acid 250µM

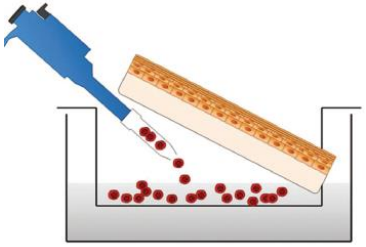


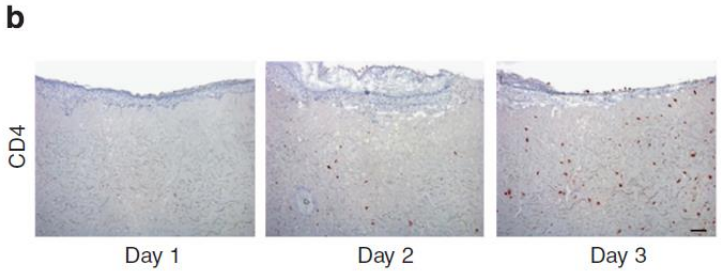
Immunocompetent skin models

ORIGINAL ARTICLE

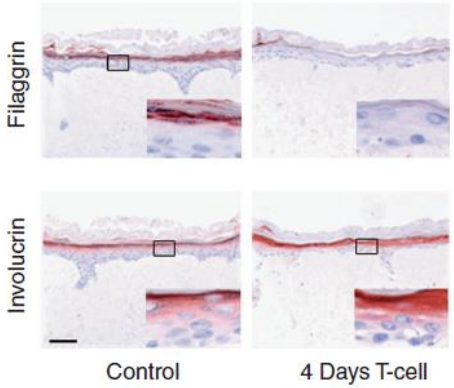
Crosstalk between Keratinocytes and T Cells in a 3D Microenvironment: A Model to Study Inflammatory Skin Diseases

Ellen H. van den Bogaard[1,3], Geuranne S. Tjabringa[1,3], Irma Joosten[2], Mieke Vonk-Bergers[1], Esther van Rijssen[2], Henk J. Tijssen[2], Mirthe Erkens[1], Joost Schalkwijk[1,4] and Hans J.P.M. Koenen[2,4]





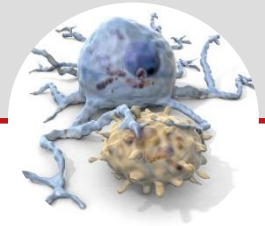
« Anti-CD3/CD28 activated CD4+ T cells are placed between the transwell membrane and the HSE. They migrate to the dermis after 2 days »



« Activated CD4+ T cells induces a psoriasiform phenotype »

« Th-1 or Th-17 polarized CD4+ T cells induces a psoriasis-like inflammatory phenotype and disturbed epidermal differenciation »

« The model responds to ATRA and CsA with a down-regulation of DEFB4, PI3, LC3A and S100A8 »



Immunocompetent skin models

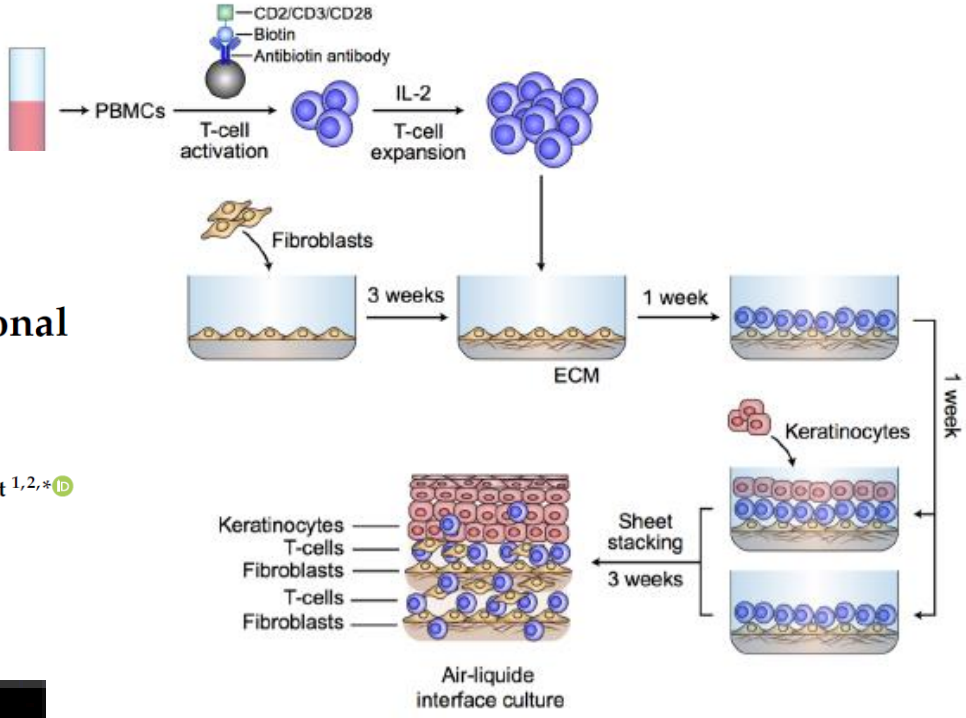
International Journal of *Molecular Sciences*

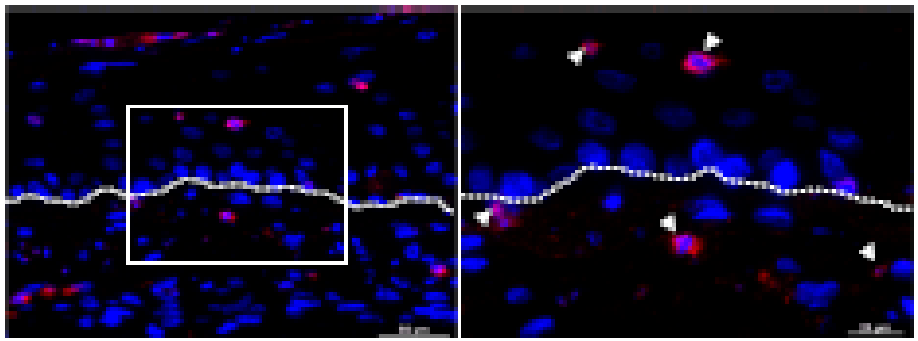
Article

Infiltration of T Cells into a Three-Dimensional Psoriatic Skin Model Mimics Pathological Key Features

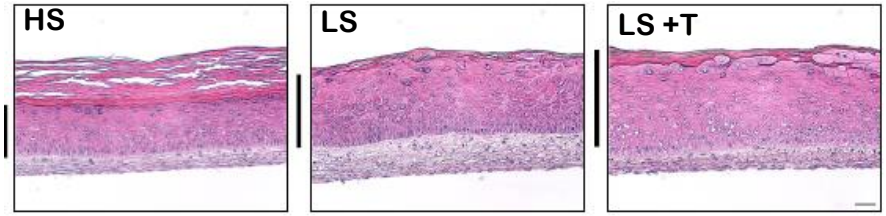
Isabelle Lorthois [1,2], Mélissa Simard [1,2], Sophie Morin [1,2] and Roxane Pouliot [1,2,*]

- **Comparison between HS, LS, LS+T**

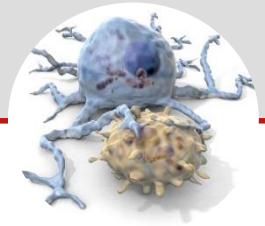




- T cells migrate within the dermis and towards the epidermis of lesional skin (CD3 labeling)



- T cell infiltration in lesional HSE increases tissue thickness, hyperproliferation (PCNA) and release of pro-inflammatoty factors
- The model responds to MTX with an improved morphology and reduced T cell inflammation



Immunocompetent skin models

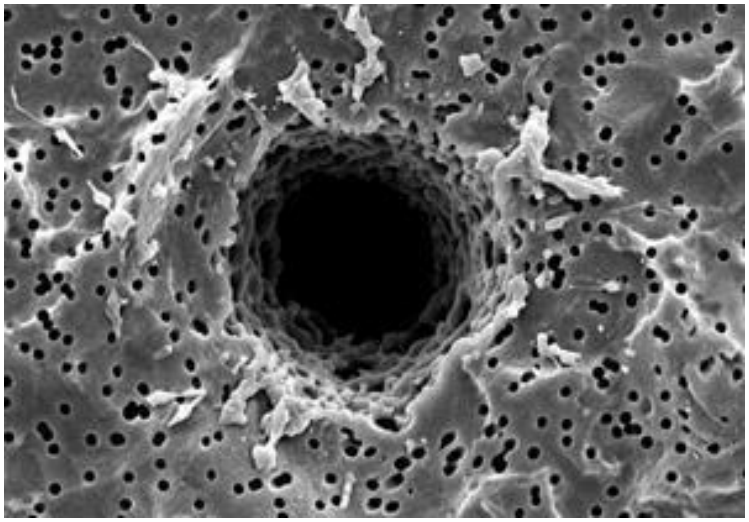
The 8th International Congress of ASIATOX

Adding the immune component in reconstructed human skin and eye epithelia models

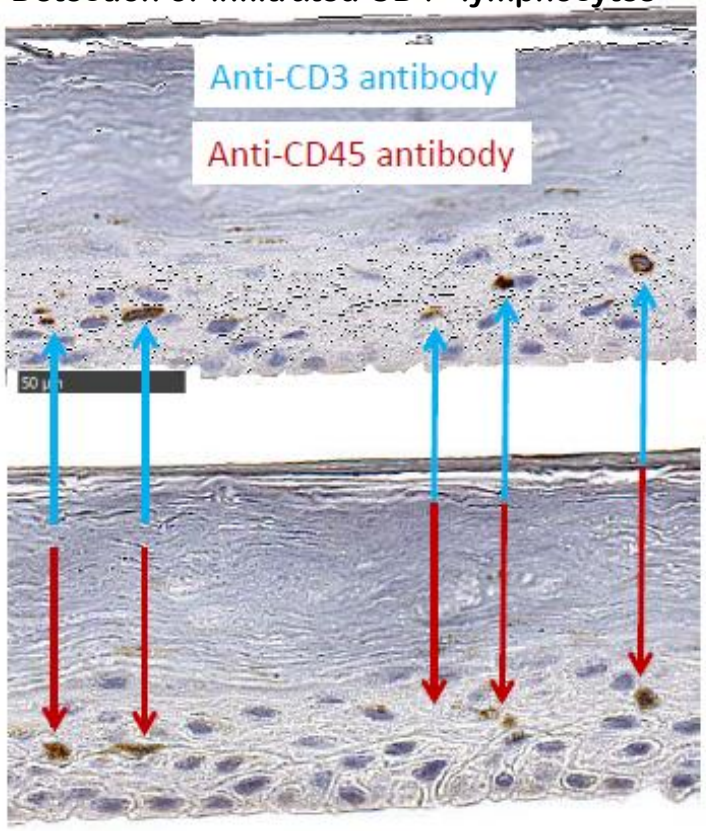
Florent Sahuc[1], Damien Benas[1], Mélanie Ligouis[2], Lucie Mondoulet[2], Marisa Meloni[3], Gaëlle Douillard[4], Anne Sophie Rigaudeau[1], Valéry Segaud[1], Jean Marc Ovigne[1], Christian Pellevoisin[1]

**EpiSkin, Lyon*

CMM technology : cell migration model (EpiSkin)



Detection of infiltrated CD4+ lymphocytes



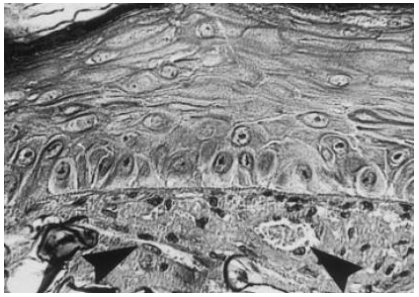
3D skin models with blood & lymphatic capillary-like network

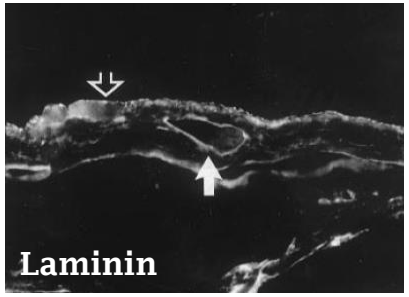
In vitro reconstruction of a human capillary-like network in a tissue-engineered skin equivalent

ANNIE F. BLACK,[1] FRANÇOIS BERTHOD, NICOLAS L'HEUREUX,[2] LUCIE GERMAIN, AND FRANÇOIS A. AUGER[3]

*LOEX, Québec

- Chitosan-linked collagen-GAG matrix
- Seeded with HUVECs (31d)
- BM deposition around capillary-like structures



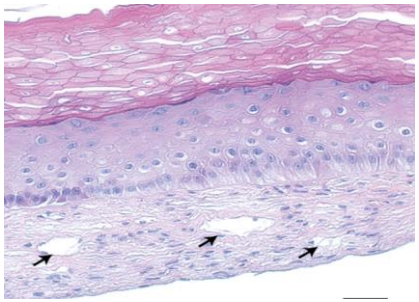


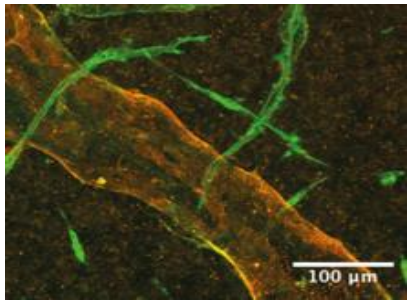
Tissue-engineered 3D melanoma model with blood and lymphatic capillaries for drug development

Jennifer Bourland[1,2,3], Julie Fradette[1,2,3] & François A. Auger[1,2,3]

*LOEX, Québec

- HMVEC (BEC + LEC) seeded on a dermal sheet (self-assembly)
- Inclusion of melanoma spheroids
- Cells assemble into blood and lymphatic capillaries
- BRAF+ melanoma respond to vemurafenib (↘ Ki67, ↗ apoptosis)





- HE staining of HMVEC-enriched skin sections
- Merge of **CD31** (green signal) and **LYVE-1** (red signal) immunostaining. LYVE-1 stains the lymphatic structures, while CD31 identifies both blood and lymphatic structures

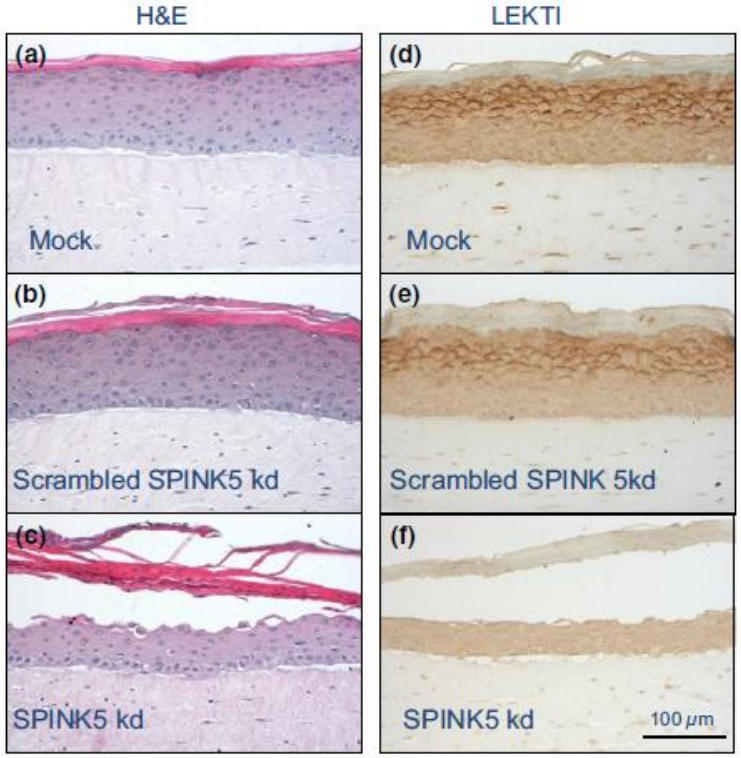
Genodermatoses, one example based on Netherthon syndrome

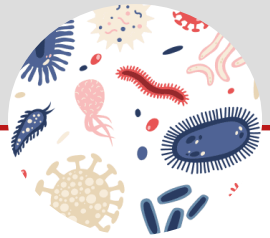
SPINK5 knockdown in organotypic human skin culture as a model system for Netherton syndrome: effect of genetic inhibition of serine proteases kallikrein 5 and kallikrein 7

Shirley Wang, Sabine Olt, Nicole Schoefmann, Anton Stuetz, Anthony Winiski and Barbara Wolff-Winiski

**Novartis, Vienna*

- **NS : mutations in SPINK5 with loss of the serine-protease inhibitor LEKTI**
- **Deregulation of KLK5 and KLK7 that degrade corneodesmosomes in the SC**
- **Tissue reconstruction from SPINK5 siRNA transfected keratinocytes**
- **Loss of the granular layer**
- **Thicker and parakeratotic SC, detached from the epidermis**
- **Decrease of DSC1, DSG1 and FLG**
- **KLK5 and KLK7 KD restore DSC1, DSG1, and FLG**





Integration of microbiota components in 3D skin models – the rational in skin care

- **Demonstrate the harmlessness of skin care products to the microbiota**
  "*Preserve microbial biodiversity*"
  "*Do not disturb the balance between strains*"

- **Beneficial or protective effect of an exogenous strain (probiotics)**

- **Beneficial effect of a product on the balance and diversity of the microflora (prebiotics and postbiotics)**

- **Beneficial action against a pathology or a condition related to an imbalance of the bacterial ecosystem or dysbiosis ?**

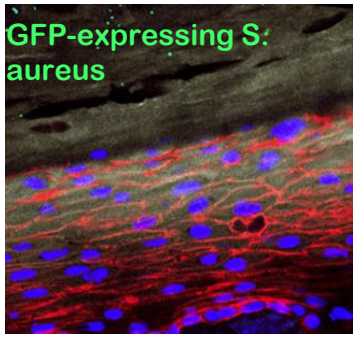
Skin disorders linked to dysbiosis or opportunistic infections

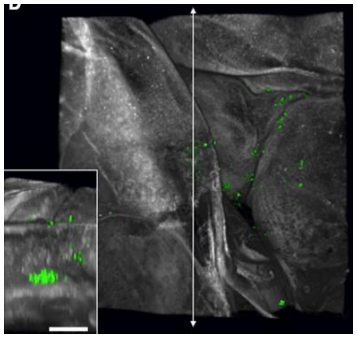
Integration of microbiota components into HSE & RHE

Three-dimensional human skin models to understand *Staphylococcus aureus* skin colonization and infection

Lauren Popov[1], Joanna Kovalski[2], Guido Grandi[3], Fabio Bagnoli[3] and Manuel R. Amieva[1,4]*

[1] Microbiology and Immunology, Stanford University School of Medicine, Stanford, CA, USA
[2] Program in Epithelial Biology, Stanford University School of Medicine, Stanford, CA, USA
[3] Novartis Vaccines, Siena, Italy
[4] Pediatrics, Stanford University School of Medicine, Stanford, CA, USA





Staphylococcus epidermidis Activates Aryl Hydrocarbon Receptor Signaling in Human Keratinocytes: Implications for Cutaneous Defense

Franziska Rademacher Maren Simanski Bettina Hesse Gregor Dombrowsky
Nikolas Vent Regine Gläser Jürgen Harder

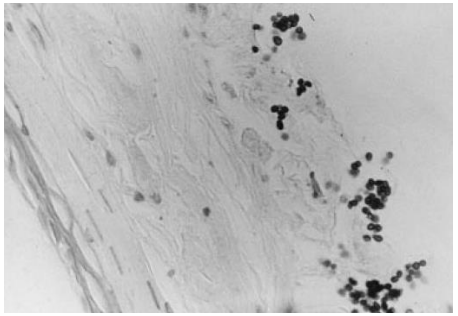
SCIENTIFIC REPORTS

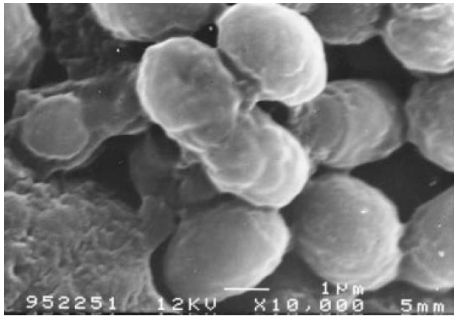
RNase 7 participates in cutaneous innate control of Corynebacterium amycolatum

Stephanie Walter, Franziska Rademacher, Nicole Kobinger, Maren Simanski, Regine Gläser & Jürgen Harder

Colonization of living skin equivalents by *Malassezia furfur*

T. BHATTACHARYYA,* M. EDWARD,* C. CORDERY† & M. D. RICHARDSON*
*Regional Mycology Reference Laboratory, Department of Dermatology, University of Glasgow, UK; and †Unilever Research, Port Sunlight Laboratories, Wirral, UK





Experimental Dermatology ADF

ORIGINAL ARTICLE

Lactobacillus reuteri DSM 17938—A comparative study on the effect of probiotics and lysates on human skin

Ia Khmaladze, Éile Butler, Susanne Fabre, Johanna M. Gillbro

Development of a 3D Skin Model Colonized with an Uncultured Skin Microbiota

Valérie Cenizo, Leslie Landemaine, Géraldine Lemaire, Tarik Badre, Bénédicte Le Bris and Pascal Portes
L'OCCITANE Group, Laboratoires M&L, Z.I Saint-Maurice, 04100 Manosque, France

Dermatophyte infection model based on RHE

Mycosis triggered by filamentous keratinophilic fungi, i.e. dermatophytes
20-25 % in global population, up to 70 % in regular sport and swimming
50-90 % in humans due to *Trichophyton rubrum* species

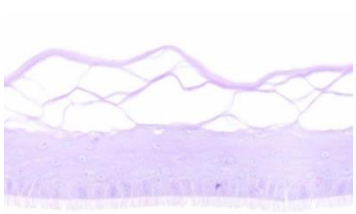
Modeling dermatophytosis in reconstructed human epidermis: A new tool to study infection mechanisms and to test antifungal agents

Émilie Faway[1], Ludivine Cambier[2], Bernard Mignon[2], Yves Poumay[1,*] and Catherine Lambert de Rouvroit[1]

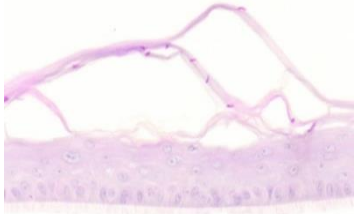
[1]URPHYM-NARILIS, University of Namur, Namur, Belgium and [2]FARAH, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium

U Namur/U Liège, Belgium

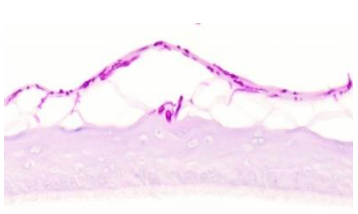
***T. rubrum* arthroconidia infection (**PAS staining)



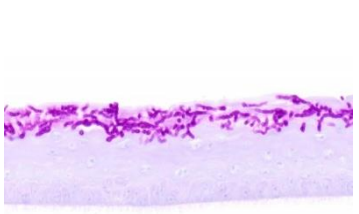
Day 1 post-infection



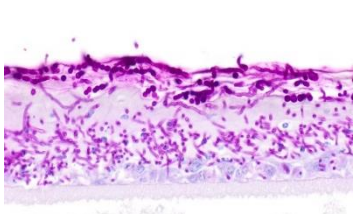
Day 2 post-infection



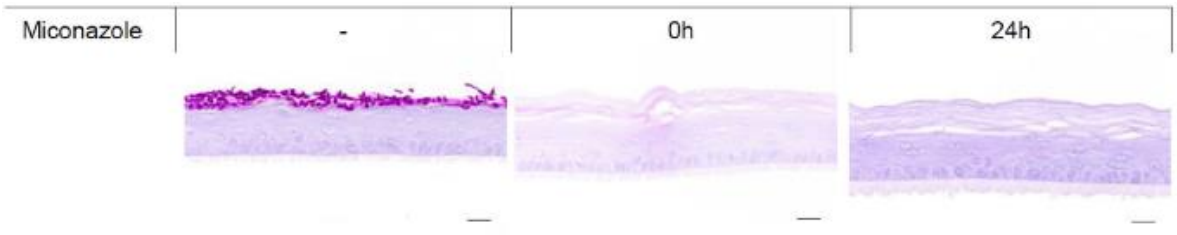
Day 3 post-infection

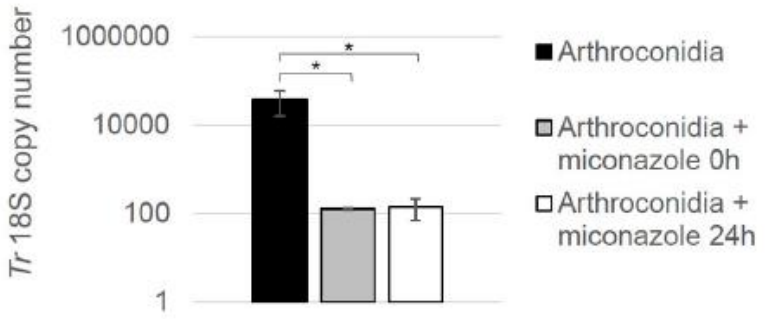


Day 4 post-infection



Day 5 post-infection





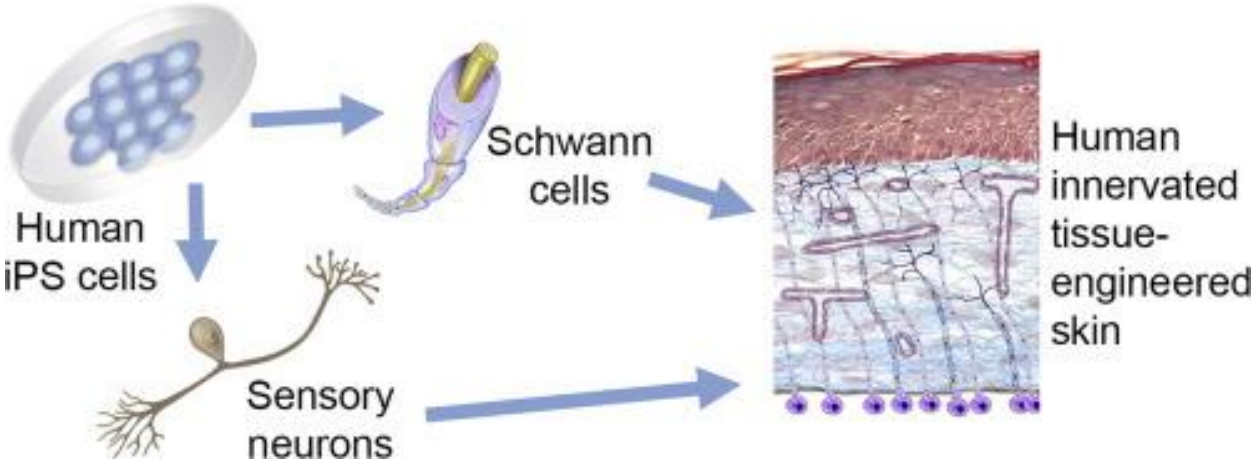
Inhibitory activity of miconazole on *T. rubrum* arthroconidia seeded on RHE. RHE were infected by arthroconidia of T. rubrum only, or in presence of miconazole applied at the same time or applied 24h after infection. Four days after inoculation, RHE were stained by PAS. Measurement of T. rubrum 18S rDNA gene copy number was then performed by qPCR.

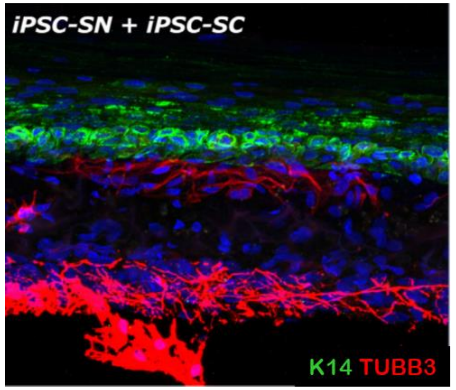
Combining 3D skin models with sensory neurons

Development of an innervated tissue-engineered skin with human sensory neurons and Schwann cells differentiated from iPS cells

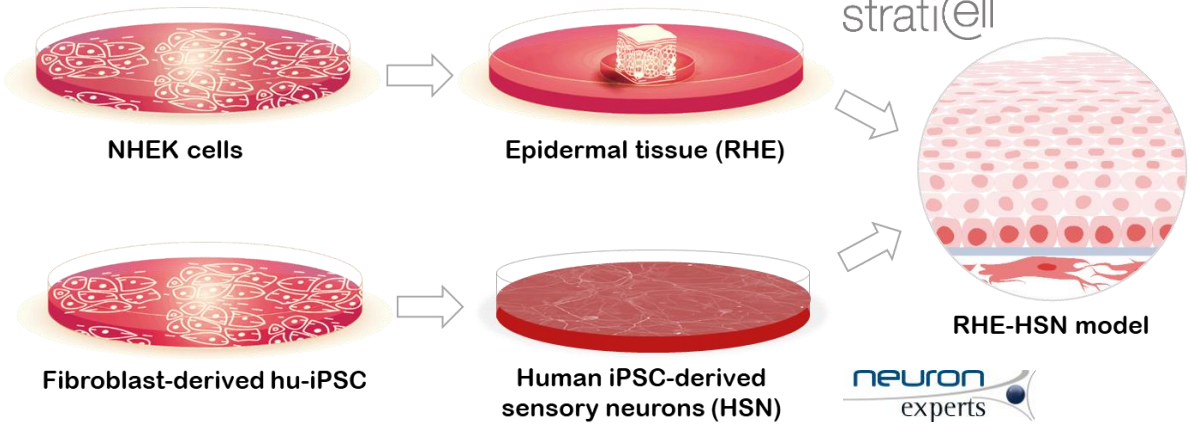
Quentin Muller [a,b,1], Marie-Josée Beaudet [a,1], Thiéry De Serres-Bérard [a], Sabrina Bellenfant [a], Vincent Flacher [b], François Berthod [a,*]

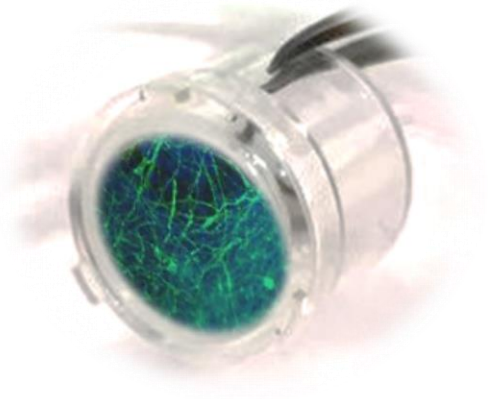
*U Stratsbourg, LOEX, Québec



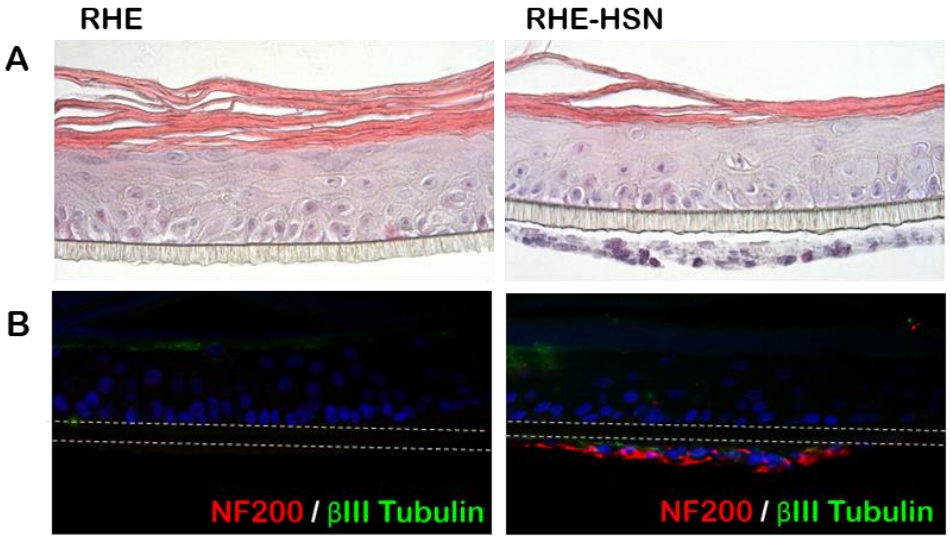


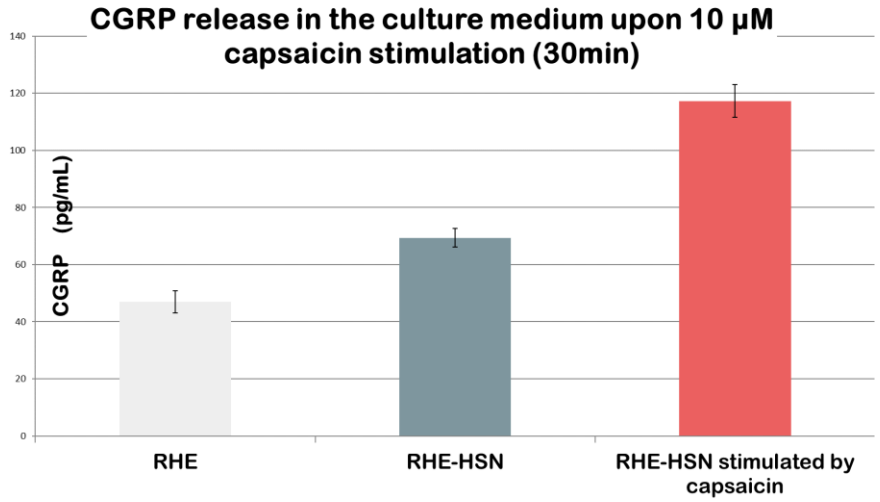
Development of a compartmentalized 3D model combining RHE and human iPSC-derived sensory neurons (*StratiCELL and Neuron Experts*)





Upper view of the iPSC-derived neuron network on the PC filter





Proteomic characterisation of the RHE-HSN model

(nano LC-MS/MS)

The presence of neurons in the RHE-HSN tissues induces significant differences in the relative abundance of 84 proteins associated with significantly enriched terms linked to the nervous system. 75 are at least 2 fold more abundant and 9 are at least 2 fold less abundant

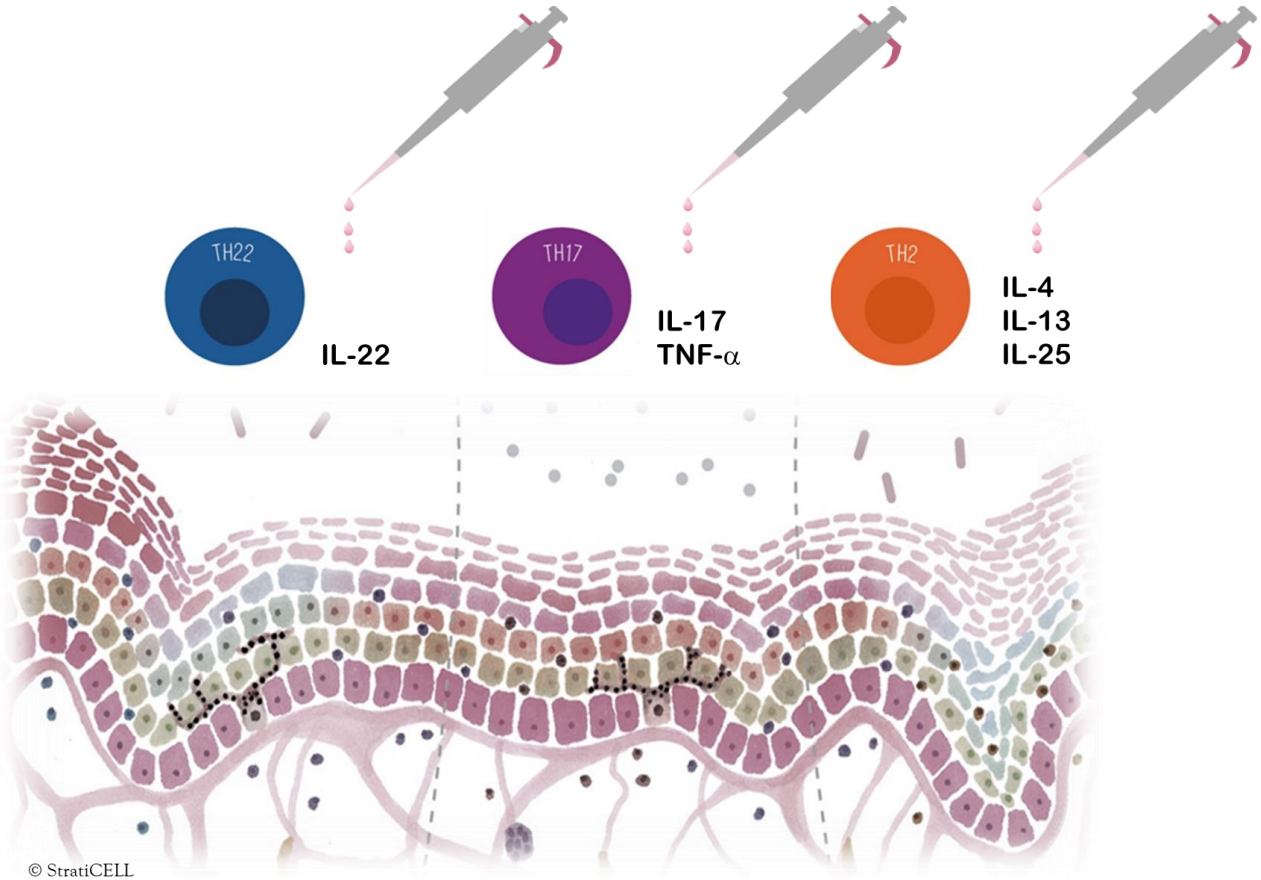
STRUCTURE/CYTOSKELETON		
TBB3	neuron-specific beta-tubulin isotype III	Axon guidance and maintenance (Tischfield *et al.*, Cell 2010)
TBA1A	Tubulin alpha- 1A chain	Differentiated neurologic cells
NFM	Neurofilament medium polypeptide	Maintenance of neuronal caliber
MTAP2	Microtubule-associated protein 2	Microtubules stabilization
STMN1	Stathmin	Microtubules depolymerization
MAP1B	Microtubule-associated protein 1A	Neurite extension
H7BY57	Neurofascin	Synapse formation
J3KN01	Afadin	GO term "cerebral cortex development"
CADH2	Cadherin-2	
NFL	Neurofilament light polypeptide	
TRANSPORT		
SNP25	Synaptosomal- associated protein 25	Neurotransmitters release and transport
SYUA	Alpha-synuclein	
KIF5C	Kinesin heavy chain isoform 5C	Dendritic trafficking

Proteome signature show that neurons are still active

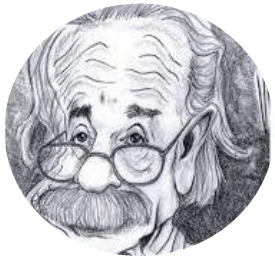
30TH IFSCC CONGRESS
18 – 21 SEPTEMBER 2018

EFFECTIVE *IN VITRO* 3D SKIN MODELS AND GENE EXPRESSION ASSAYS TO STUDY SKIN INFLAMMATORY DISORDERS AND BARRIER RESILIENCE

C. Lancelot*, R. Hubaux, A. Chrétien, C. Bastin, M.Salmon | *corresponding author: clancelot@straticell.com



Conclusion



Progresses in stem cell research, knock-down technologies and tissue engineering allow to integrate other cell types (*i.e.* melanocytes, immune, sensory and endothelial cells, etc.) as well as microbiota components

Accordingly, 3D models have become essential tools for **dermo-cosmetic research** and **pre-clinical *in vitro* studies for** skin pathologies, *i.e.* inflammatory skin diseases, pigmentation disorders, itch, genodermatoses, dysbiosis, etc.

A major concern remains from the industrial stakeholders : **what is the best balance between biological relevance, scalability, standardization, manufacturing issues…and price !**

neuron experts

temisis therapeutics

eurostars™

Avec le soutien de la DGO6
Département du développement Technologique

Wallonie

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Service public de Wallonie

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Thank you for your attention !