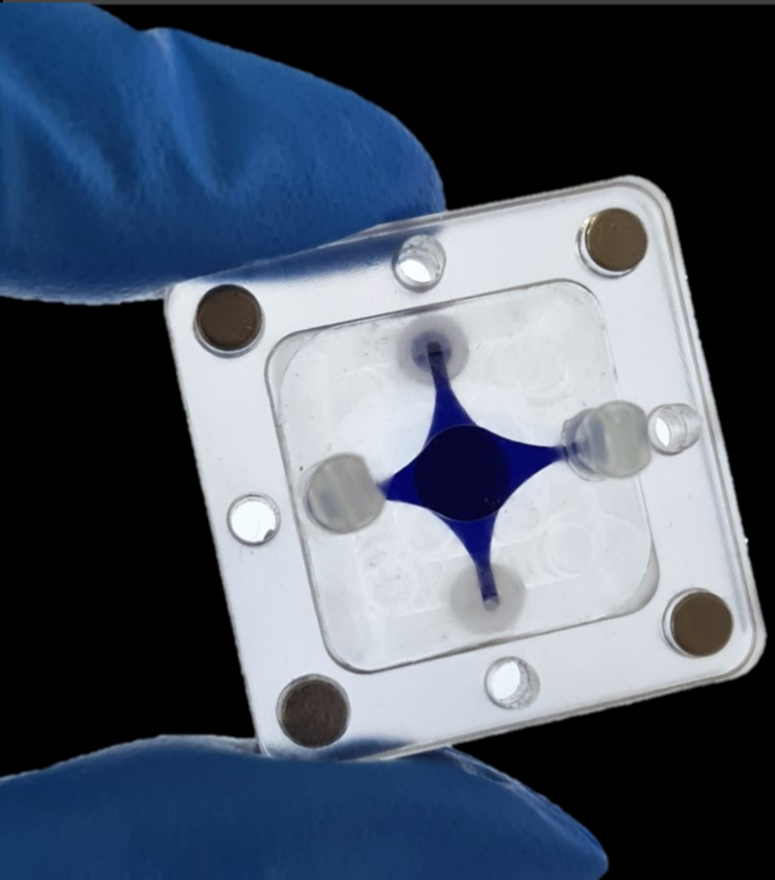
Full-thickness skin-on-a-chip model for *in vitro* drug testing

Patrícia Zoio, PhD Candidate
Abel Oliva, Supervisor



AIM Advanced Integrated Microsystems
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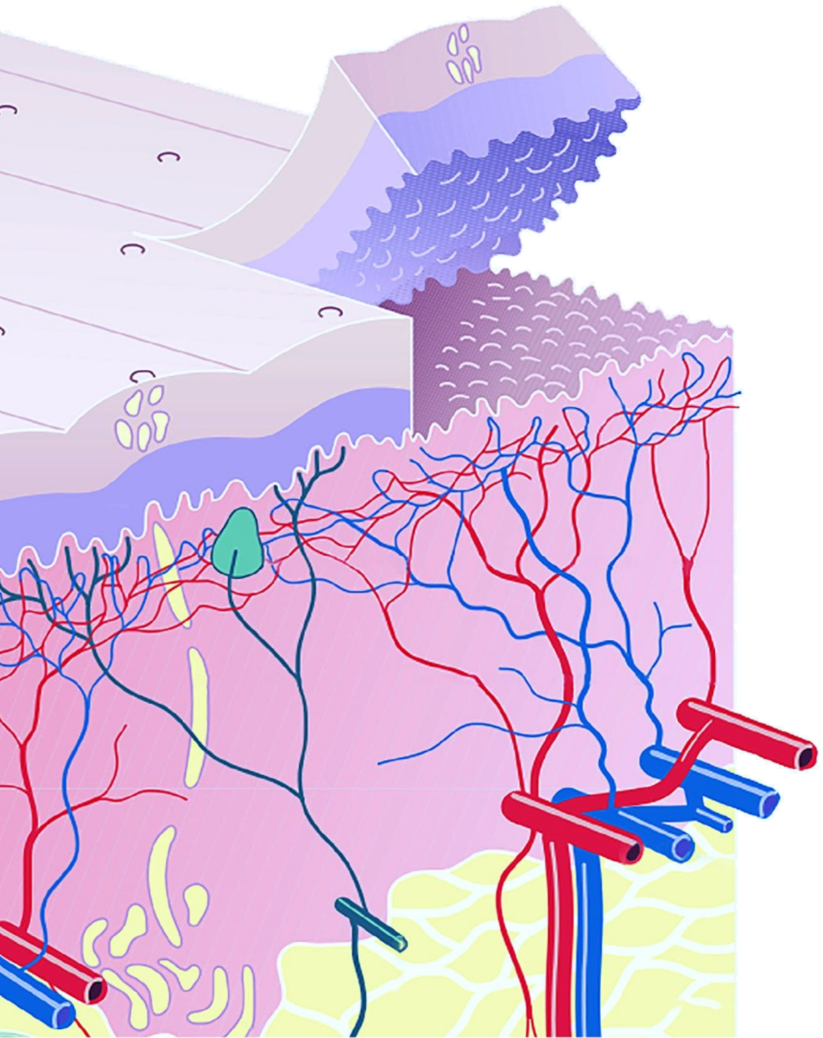
MOTIVATION



Increasing demand for the development of *in vitro* engineered skin models

Ethical regulations

- -3R principles
- -Ban on animal testing (cosmetics)

High prevalence of skin diseases

- -Chronic (e.g., psoriasis, eczema)
- -Malignant (melanoma)

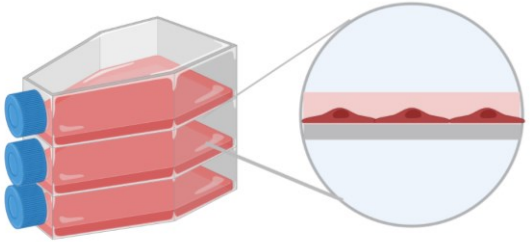
Skin-targeted drug delivery

- -Topical
- -Dermal
- -Transdermal

MOTIVATION

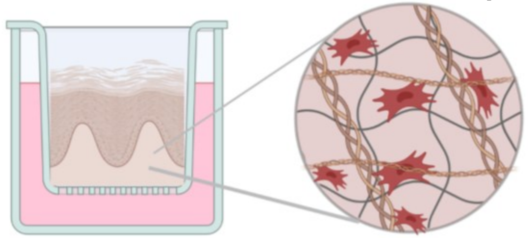
Conventional in vitro skin model

2D cell culture (keratinocytes and/or fibroblast)



- Complexity + reproducibility

Conventional 3D skin models (epidermal or full-thickness)

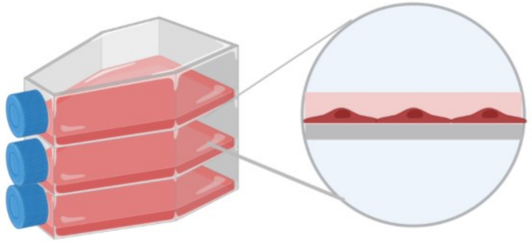


+ Complexity - reproducibility
Weaker barrier than *in vivo* skin
Lack mechanical stability

MOTIVATION

Conventional in vitro skin model

2D cell culture (keratinocytes and/or fibroblast)



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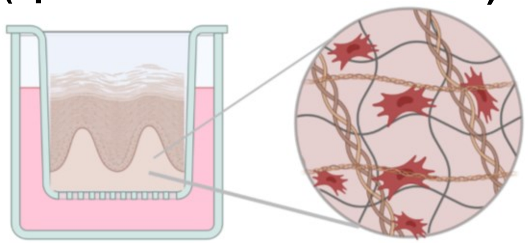


+ Complexity - reproducibility
Weaker barrier than *in vivo* skin
Lack mechanical stability

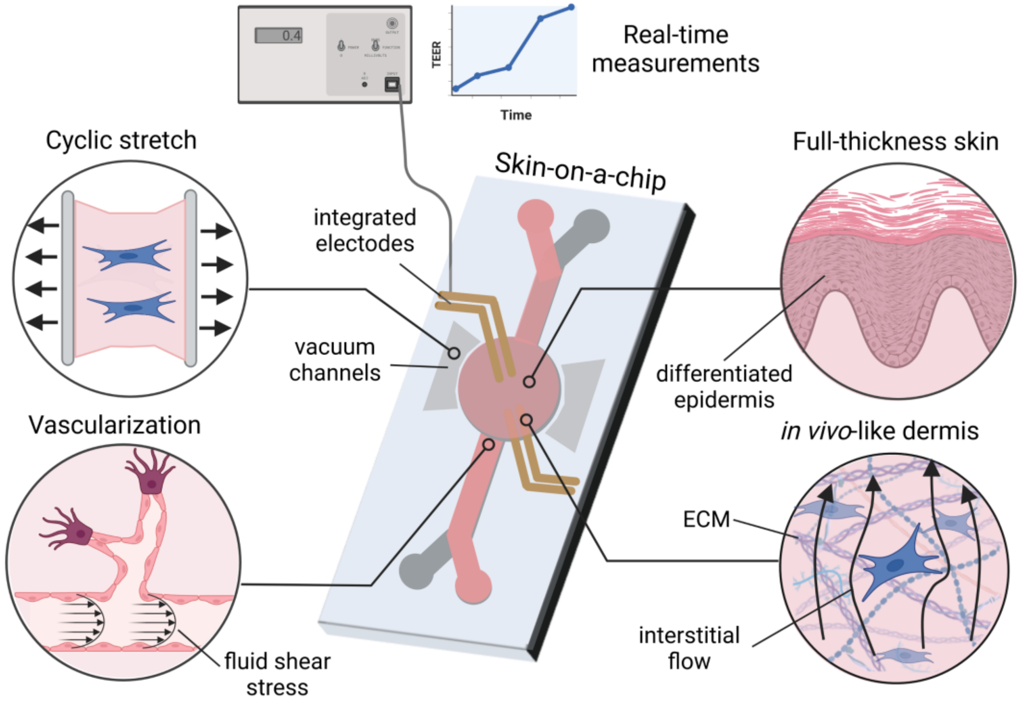
Predictive power of *in vitro* models

→

Physiological relevance

Organ-on-a-chip technology

Combination of cell biology, engineering, biomaterial to simulate the microenvironment of the organ (tissue interfaces and mechanical stimulation)

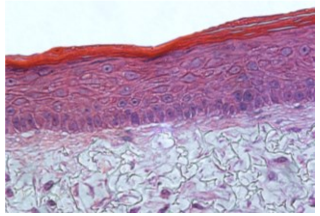


+ Complexity + reproducibility

GOALS

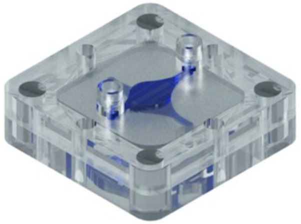
Development of physiologically relevant in vitro full-thickness skin models

Full-thickness skin model under static conditions



- Model with enhanced differentiation and mechanical stability
- Study its applicability for drug testing

Full-thickness skin-on-a-chip

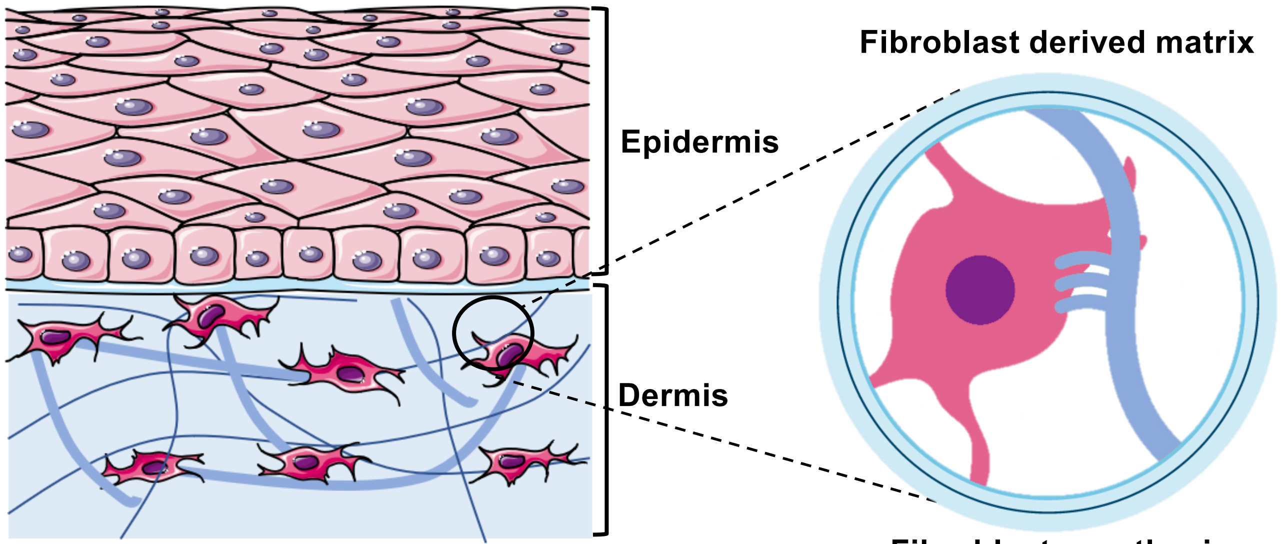


- Design and fabrication of OoC for dynamic perfusion
- Sensor integration for real-time evaluation of the skin barrier

SKIN MODEL

Porous polystyrene scaffold

90% porosity; 42 μm void size



Dermal fibroblasts

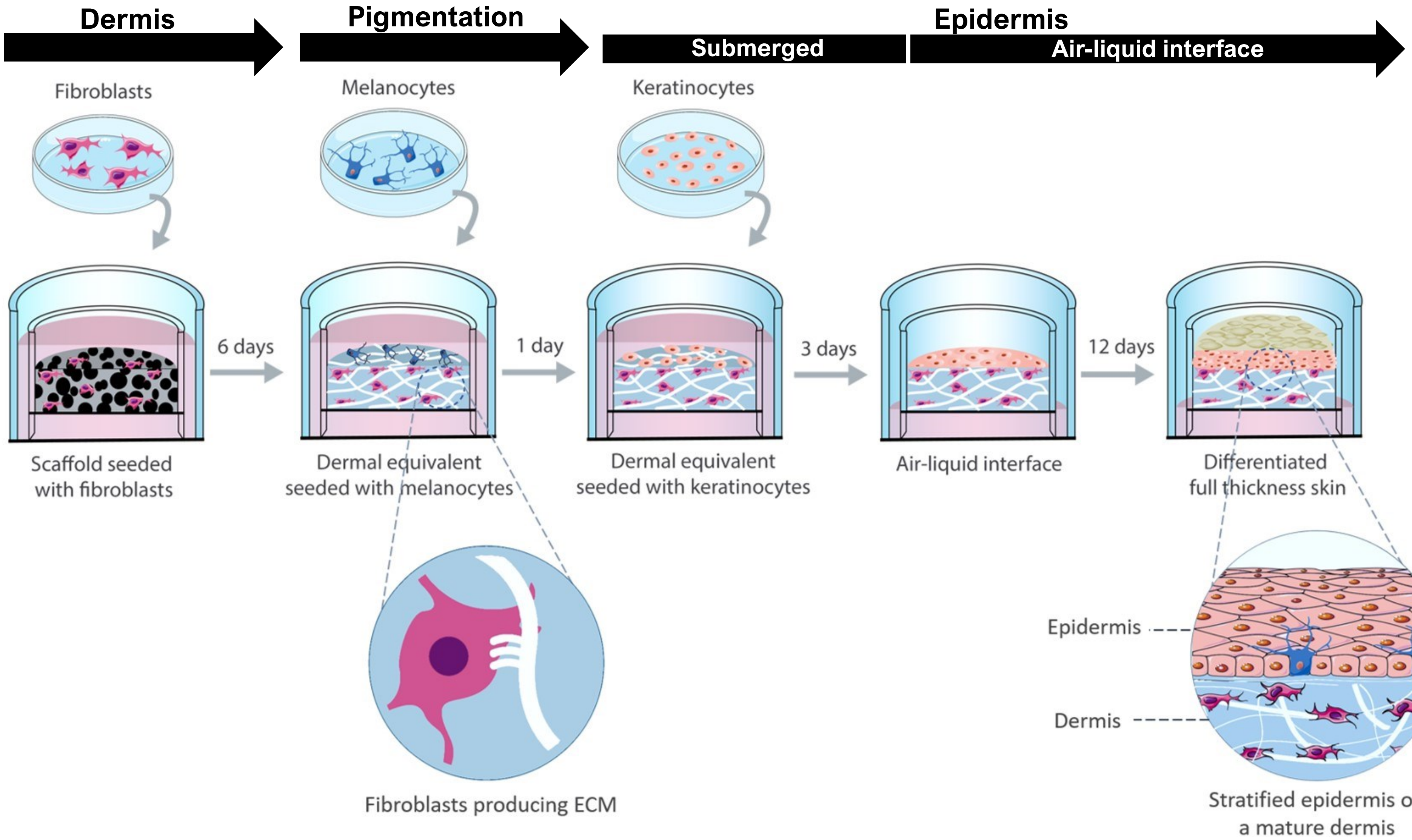
Extracellular matrix (ECM)

Fibroblasts synthesize human ECM

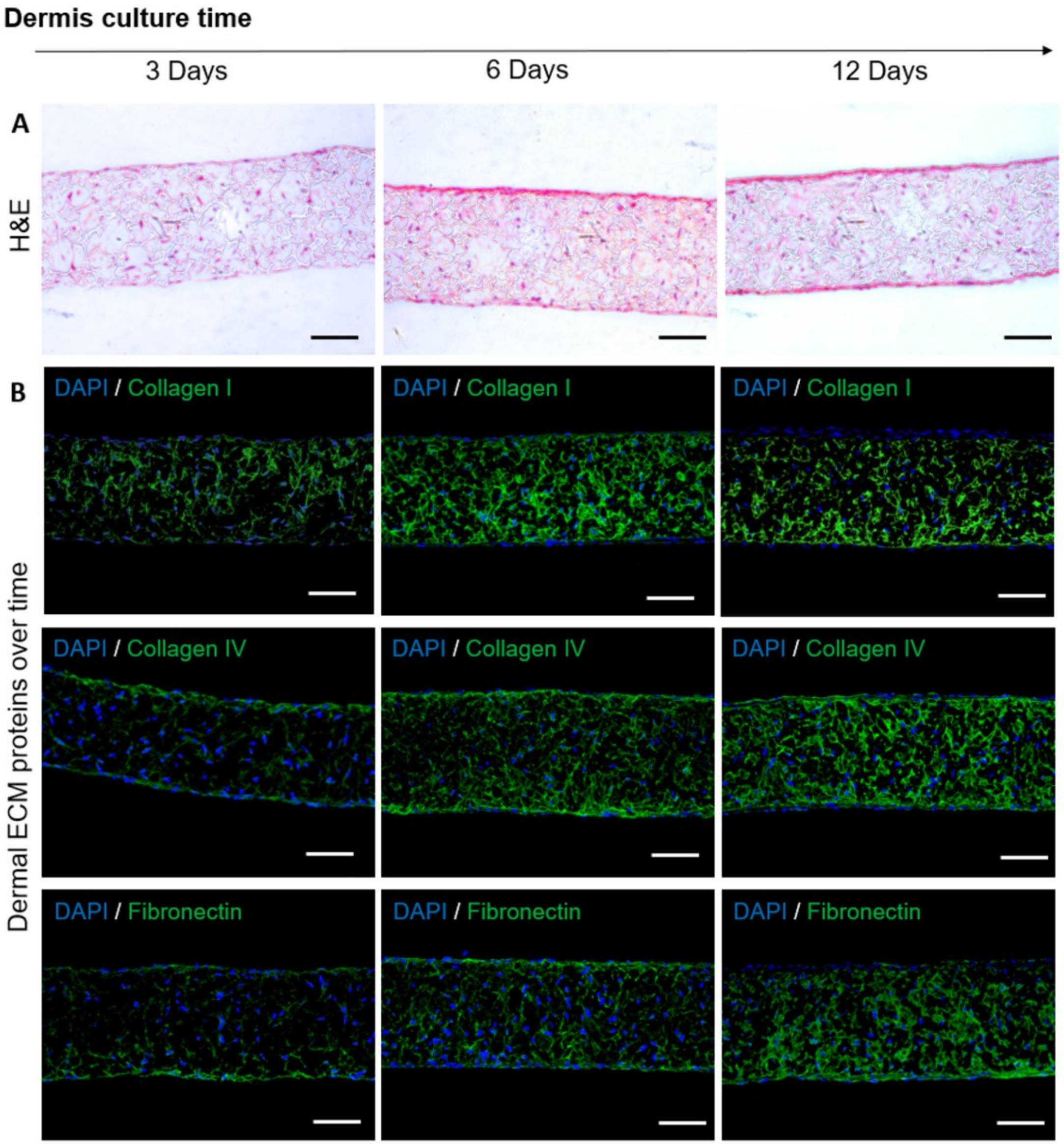
X Animal-derived collagen

SKIN MODEL

Protocol developed to generate a full-thickness skin model based on a fibroblast-derived matrix:



SKIN MODEL

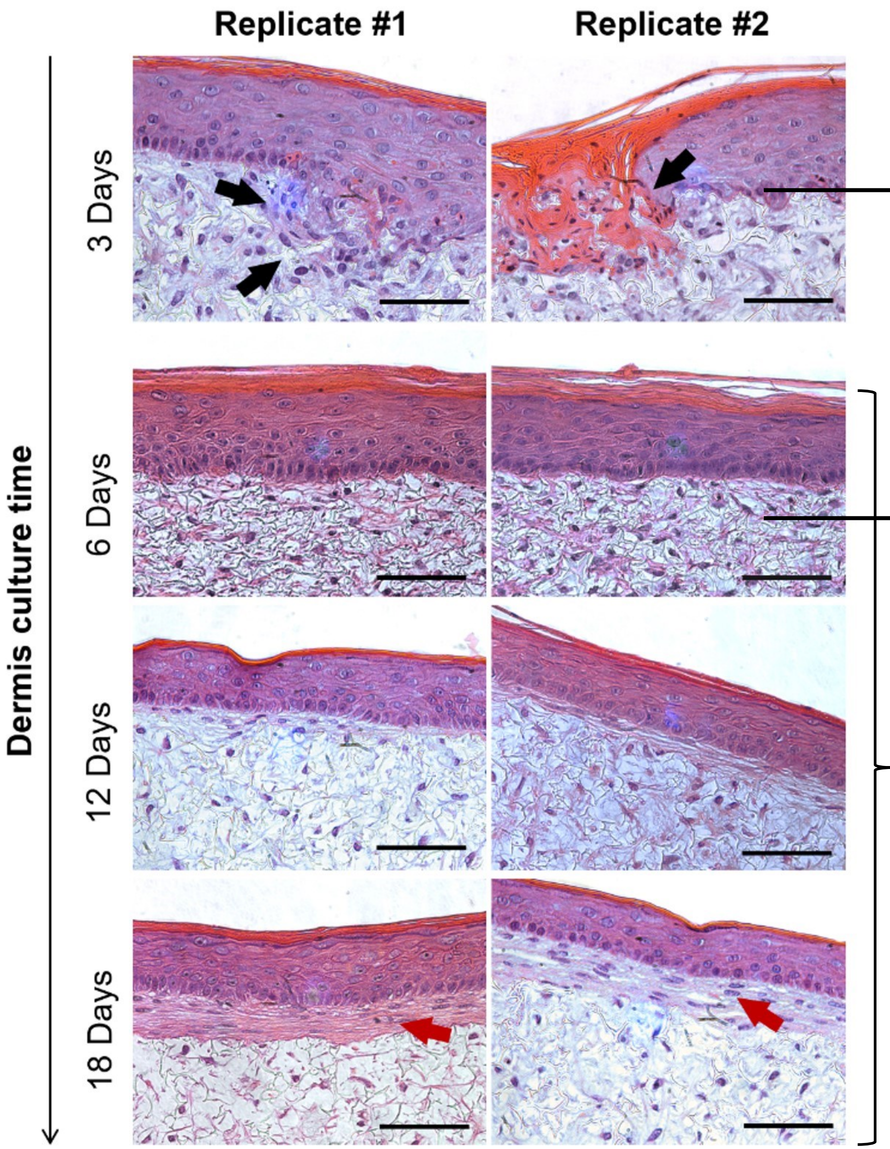


Optimization of culture time:
-Mature dermal equivalent
-Epidermis support
-Keratinocyte infiltration minimization

Increased expression of colagens and fibronectin with culture time

SKIN MODEL

Dermal maturation affects epidermal development



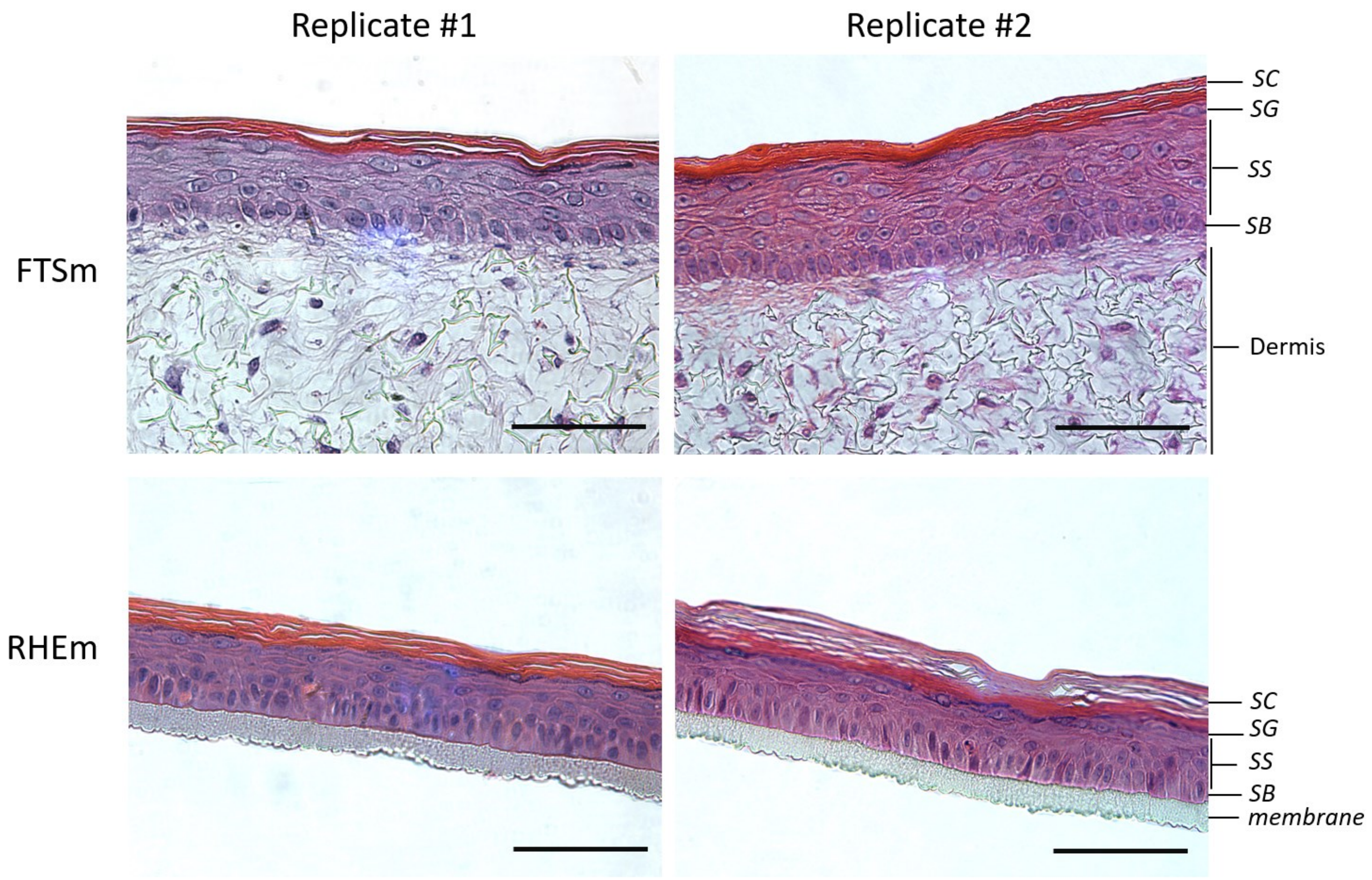
HEKns seeded on top of dermal equivalents cultured for different times

Keratinocyte infiltration; Epidermal disorganization

Epidermis support; No ECM accumulation

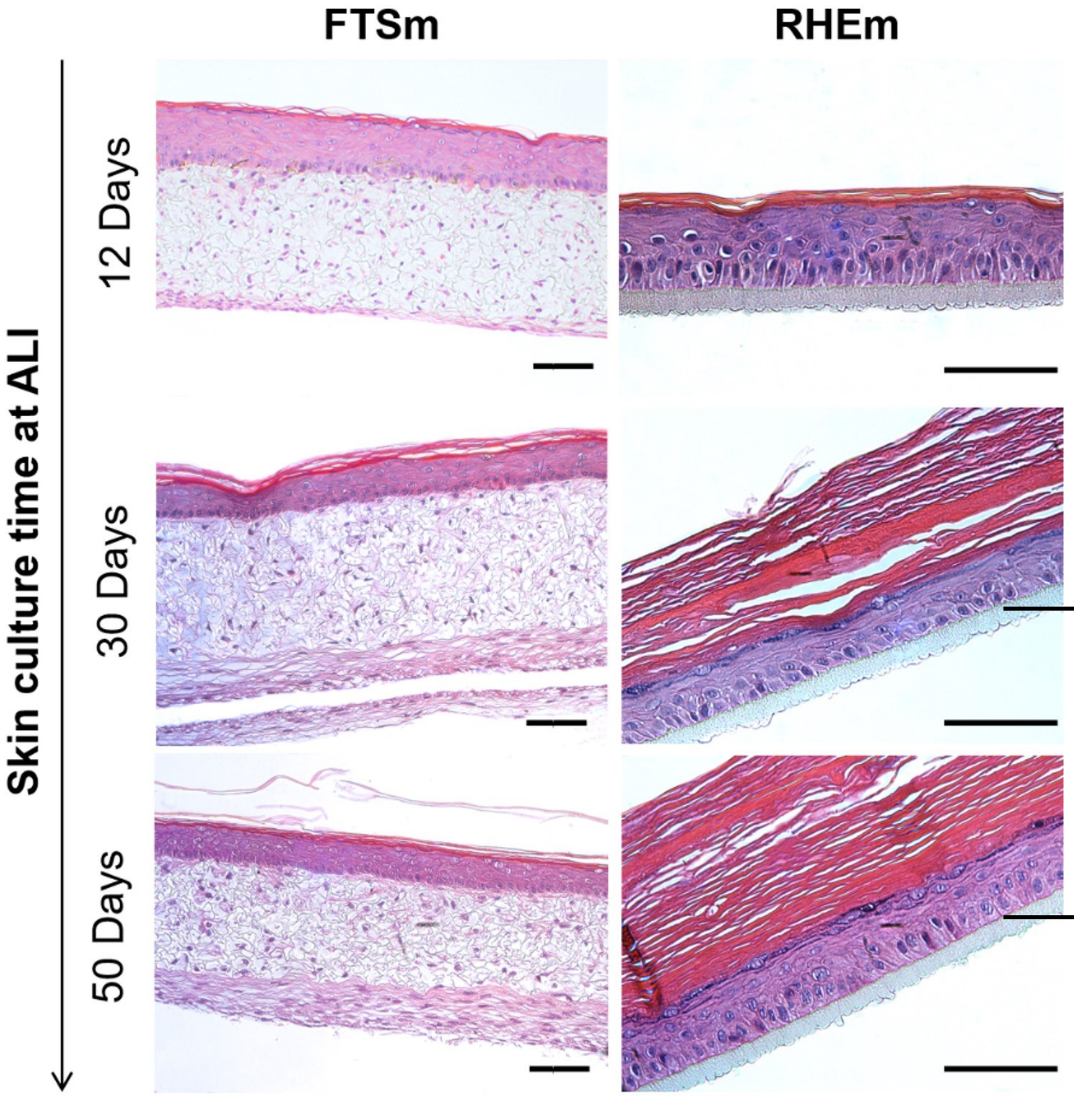
No signs of keratinocyte infiltration; Differentiated and organized epidermis

SKIN MODEL



Stratified squamous epithelium structured in basal, spinous, granular and corneal layers

SKIN MODEL



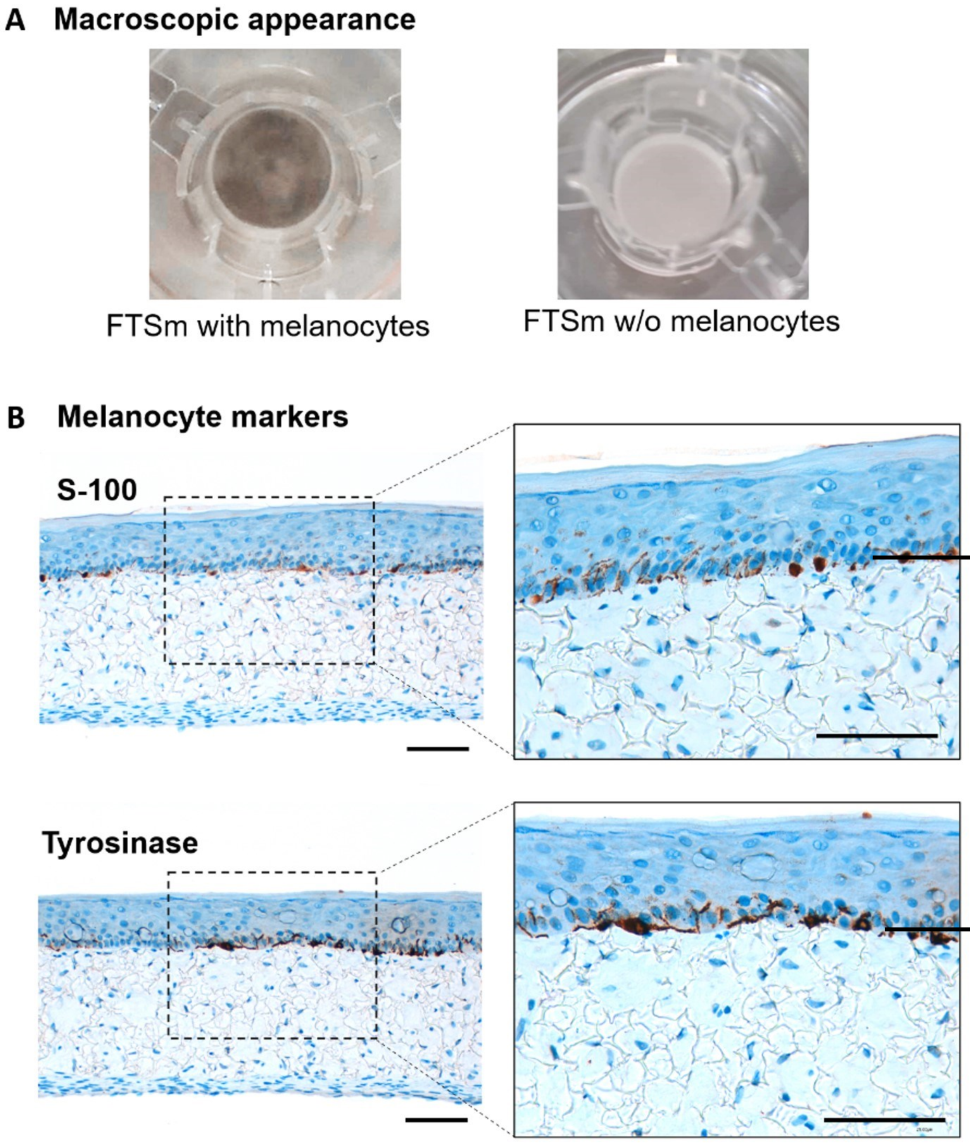
Models were kept at ALI for up to 50 days and its structure monitored over time

Extended lifespan compared to conventional models

Reduction of epidermal thickness; Preserved architecture;

Models remain stable, maintaining the same thickness and epidermal layers;

SKIN MODEL



Development of a pigmented version of the FTSm with active melanocytes at the dermo-epidermal junction

S100 marker, highly sensitive for melanocytes, expressed at the basal layer

Tyrosinase, a key enzyme for controlling the production of melanin, expressed in the basal layer

SKIN MODEL

TISSUE ENGINEERING: Part C
Volume 27, Number 7, 2021
Mary Ann Liebert, Inc.
DOI: 10.1089/ten.tec.2021.0069

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Tissue Engineering
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International Society

METHODS ARTICLE

Pigmented Full-Thickness Human Skin Model Based on a Fibroblast-Derived Matrix for Long-Term Studies

Patrícia Zoio, MSc,[1] Sara Ventura, MSc,[1] Mafalda Leite, BSc,[1] and Abel Oliva, PhD[1,2]

FTSm with increased mechanical stability, excluding the use of animal-derived hydrogels;

Dermis formation in one week whereas other fibroblast-derived matrix approaches need 4 weeks;

FTSm with extended lifespan compared to conventional models;

Pigmented version of the FTSm with active melanocytes at the dermo-epidermal junction

<u>accepted</u>

ALTEX
ALTERNATIVES TO ANIMAL EXPERIMENTATION

Open-source human skin model with an *in vivo*-like barrier for drug testing

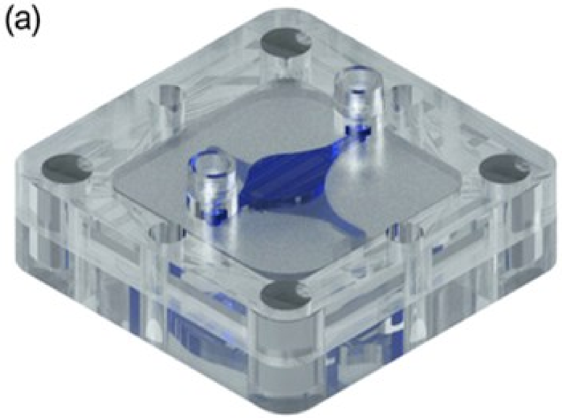
Patrícia Zoio[1], Sara Lopes-Ventura[1], Joana Marto[2], Abel Oliva[1,3]

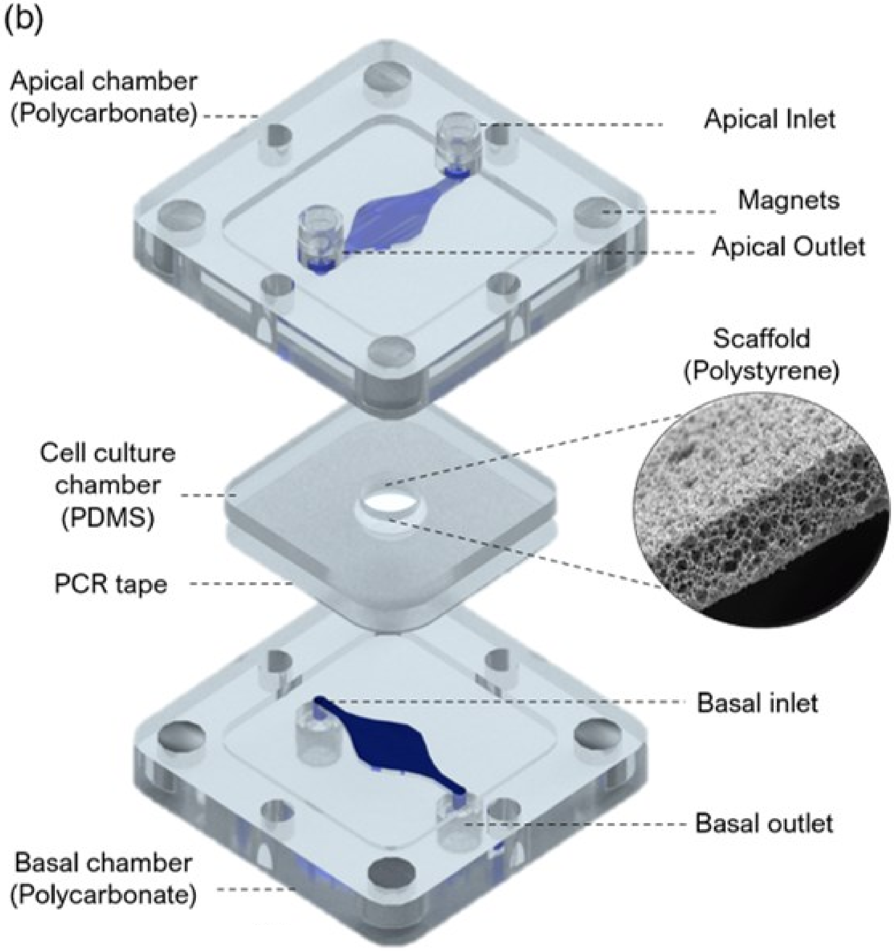
In-depth analysis of the skin barrier function;

Drug testing performed according OECD TG;

SKIN-ON-A-CHIP

Combining the FTSm based on a fibroblast-derived matrix with OoC technology





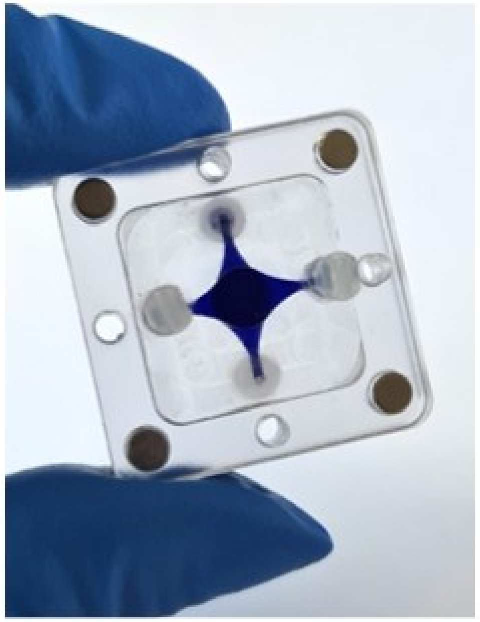
SoC designed to ensure **stability** during long-term culture while providing a **modular geometry**

Double perfusion system: Continuous supply of nutrients and removal of metabolic waste products

Fabricated using CNC micromachining and laser cutting

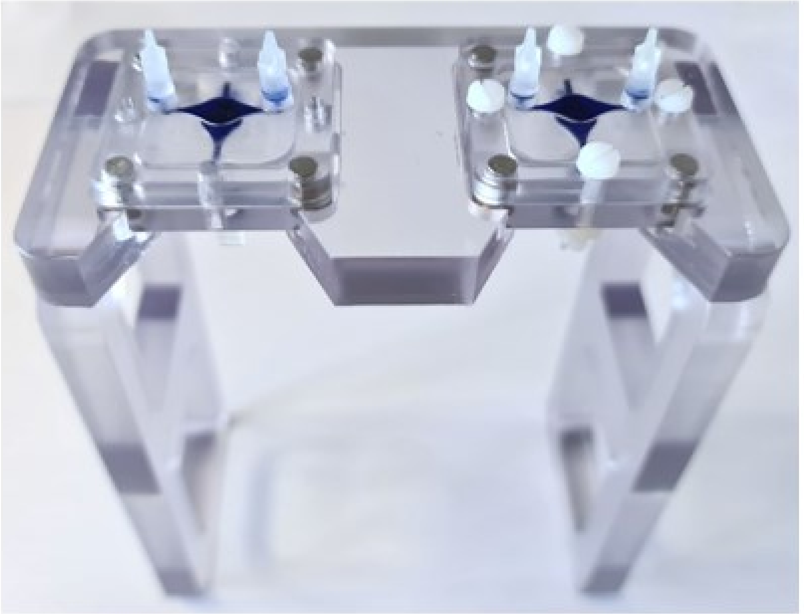
SKIN-ON-A-CHIP

Assembled SoC device

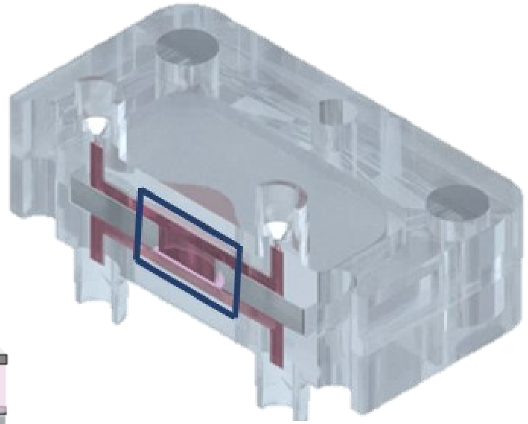


PDMS cell culture layer

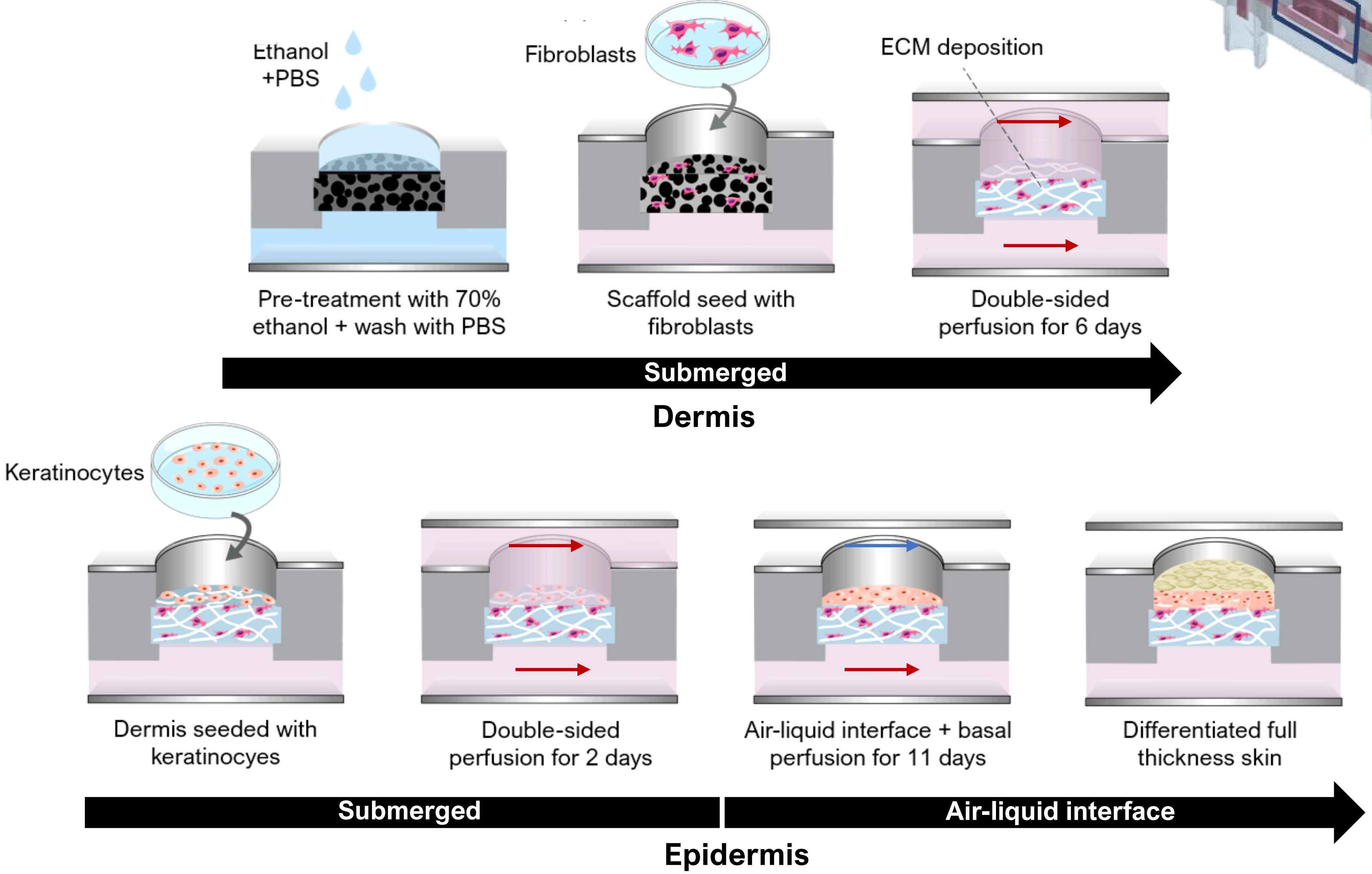
SoC devices placed in dedicated support



SKIN-ON-A-CHIP



Protocol developed to generate a skin-on-a-chip model:



SKIN-ON-A-CHIP

Reservoir with cell medium

Peristaltic pump

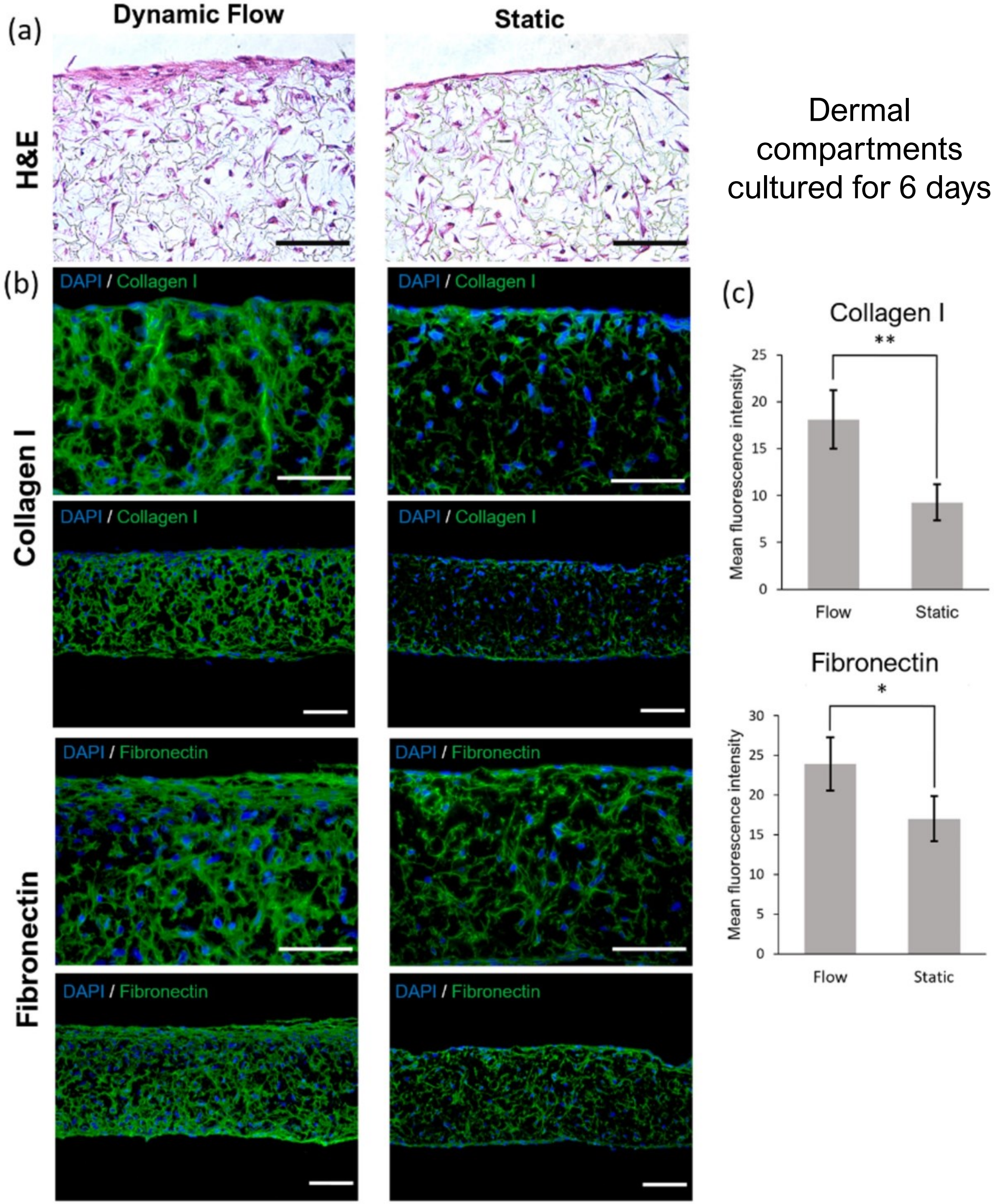
Reservoir with perfusate

SoC devices

Dynamic perfusion was achieved by connecting the inlet ports to a multichannel peristaltic pump. Flow rate 1.5 ul/min – 2 ul/min

SKIN-ON-A-CHIP

Dynamic flow stimulates the production of endogenous FDM. Compared to the static culture, the dermis produced using dynamic flow resulted in increased deposition of ECM



Dermal compartments cultured for 6 days

SKIN-ON-A-CHIP

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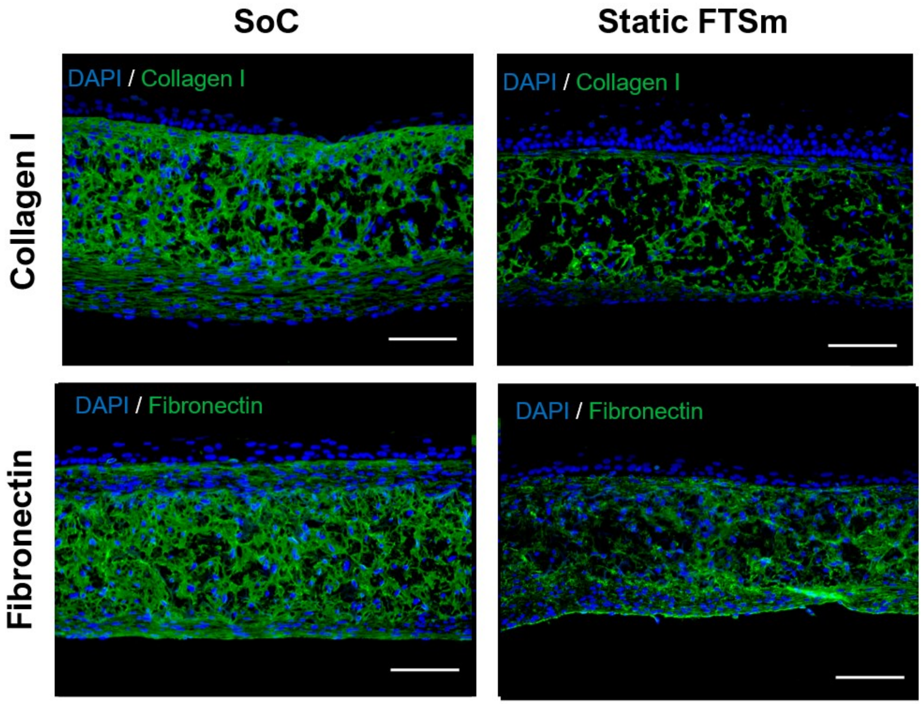
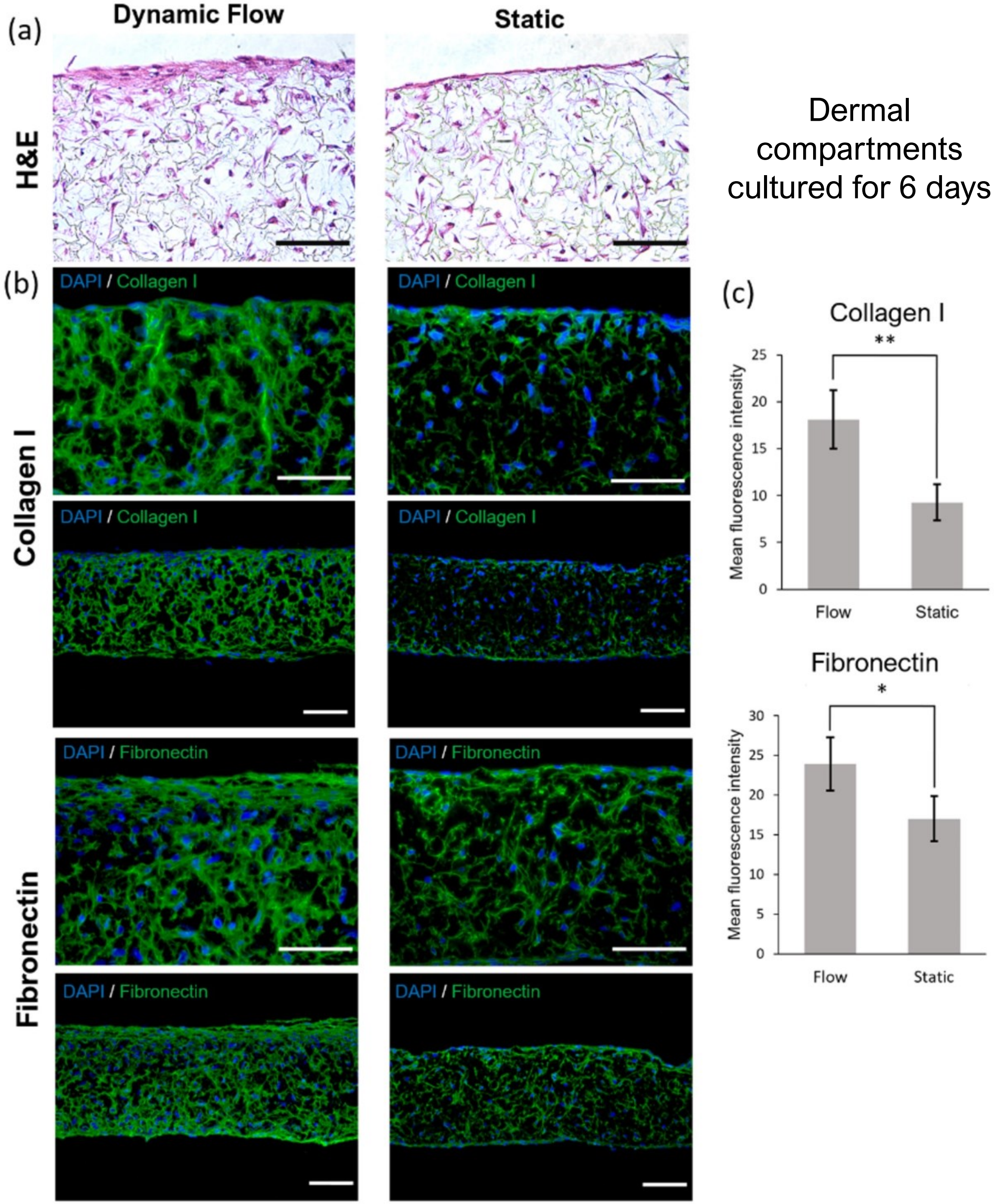
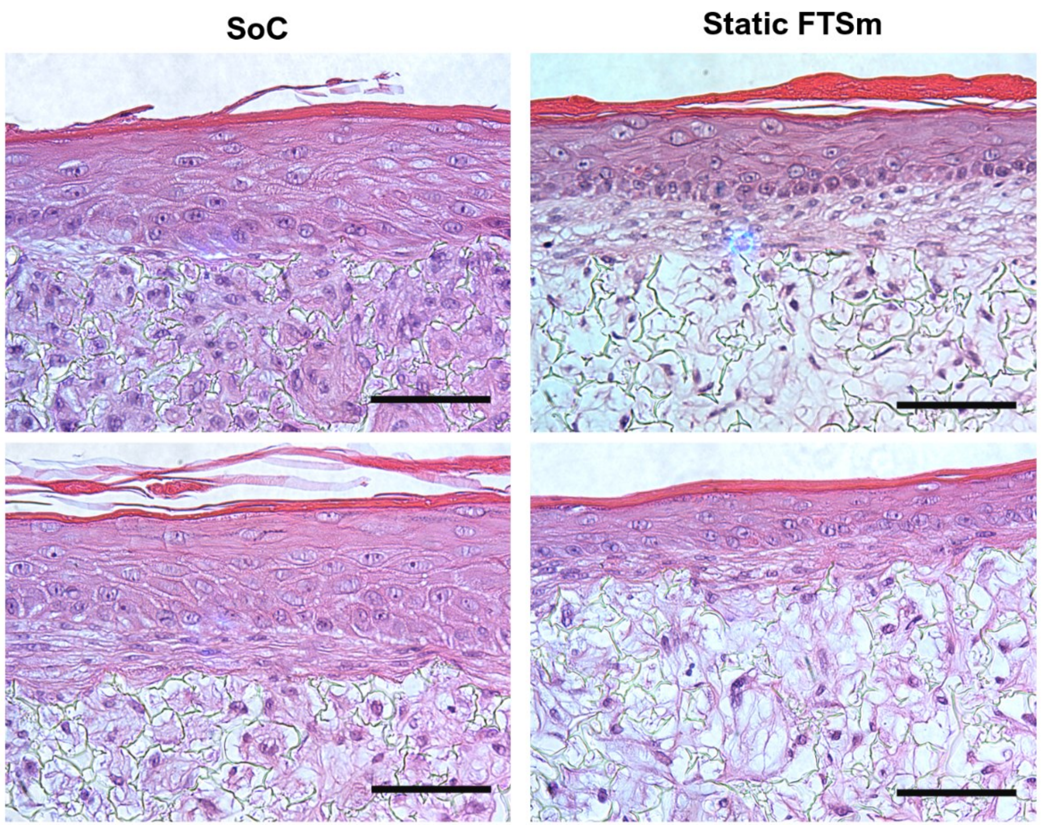


Full-thickness models cultured for 20 days



Dermal compartments cultured for 6 days

SKIN-ON-A-CHIP



Epidermal thickness 70±30 μm

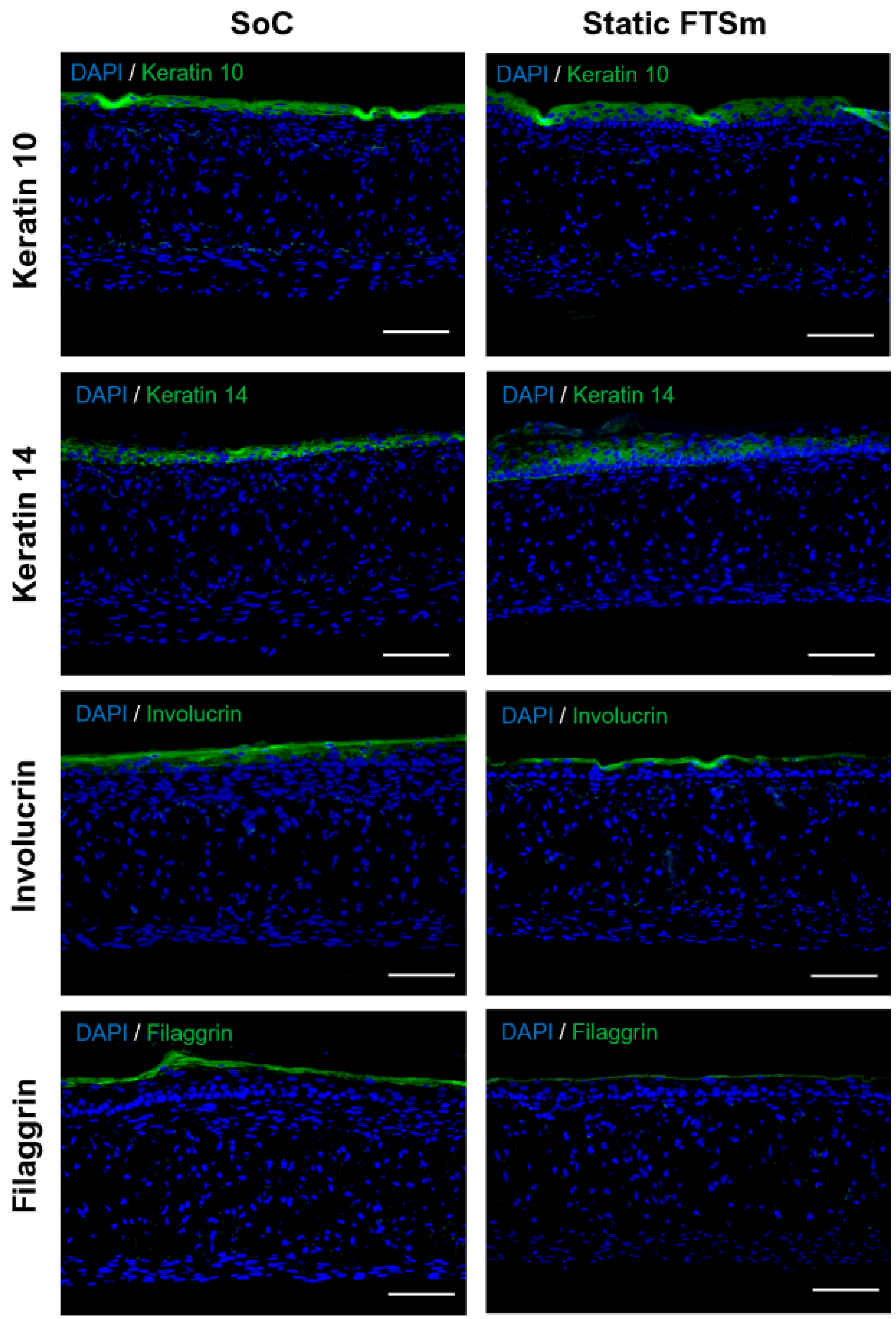
Epidermal thickness 41±15 μm

Both models show a stratified and differentiated epidermis after 11 days at ALI. No significant differences were detected concerning the thickness of the corneal layers

From the histological analysis, the major difference was the increased thickness of the epidermal compartment

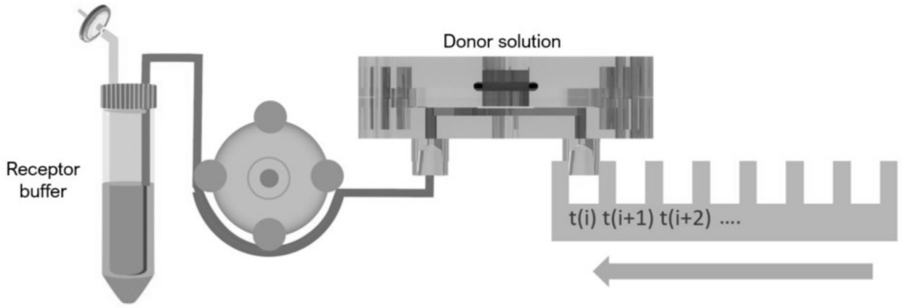
SKIN-ON-A-CHIP

SoC shows increased filaggrin expression, a skin barrier protein and differentiation marker, and increased involucrin expression. This points to an enhanced barrier function

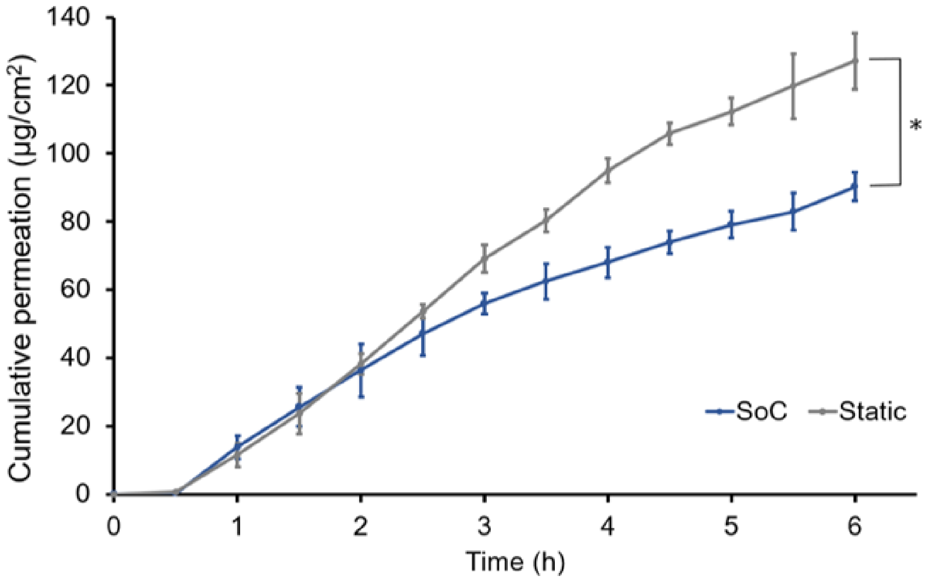


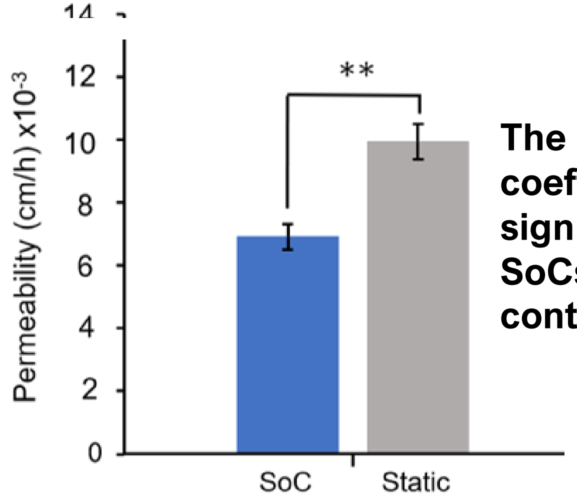
SKIN-ON-A-CHIP

Permeation studies were performed directly on-chip



The SoC models presented significantly lower mean FITC-dextran cumulative amounts

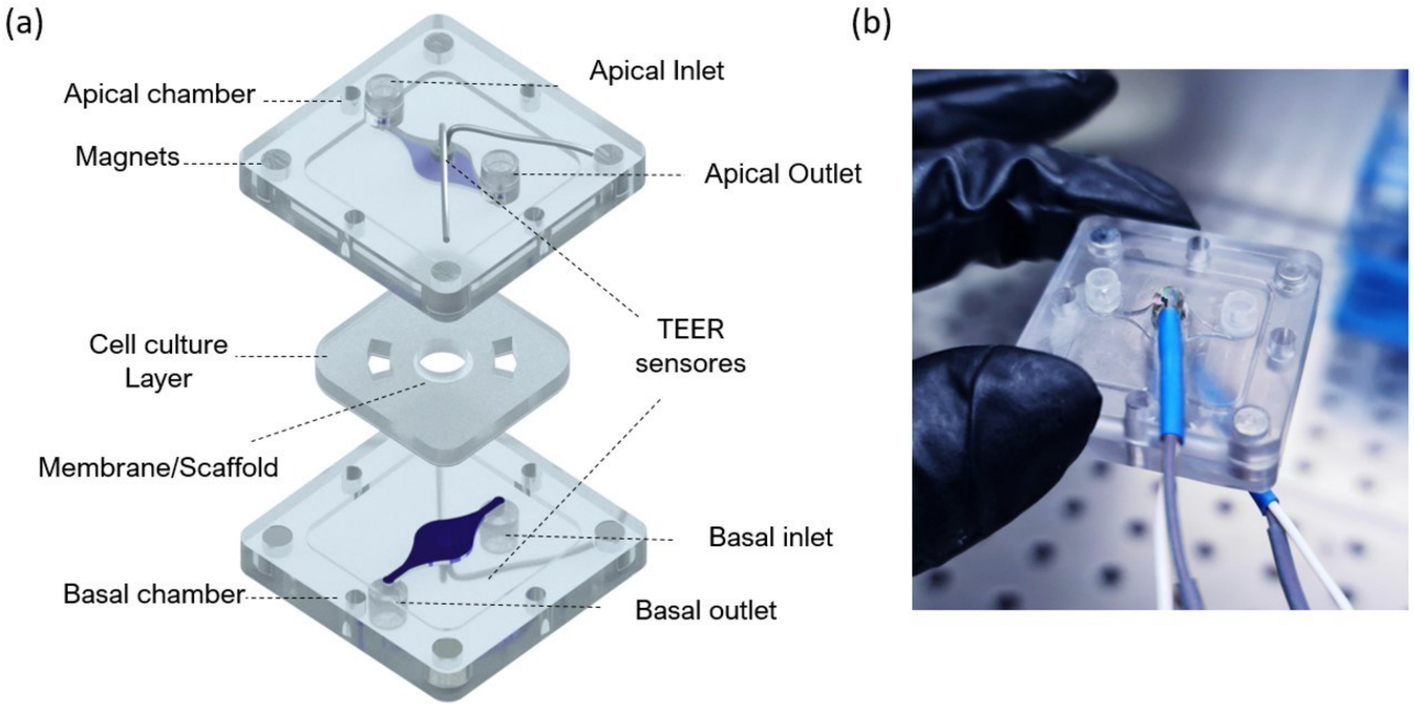


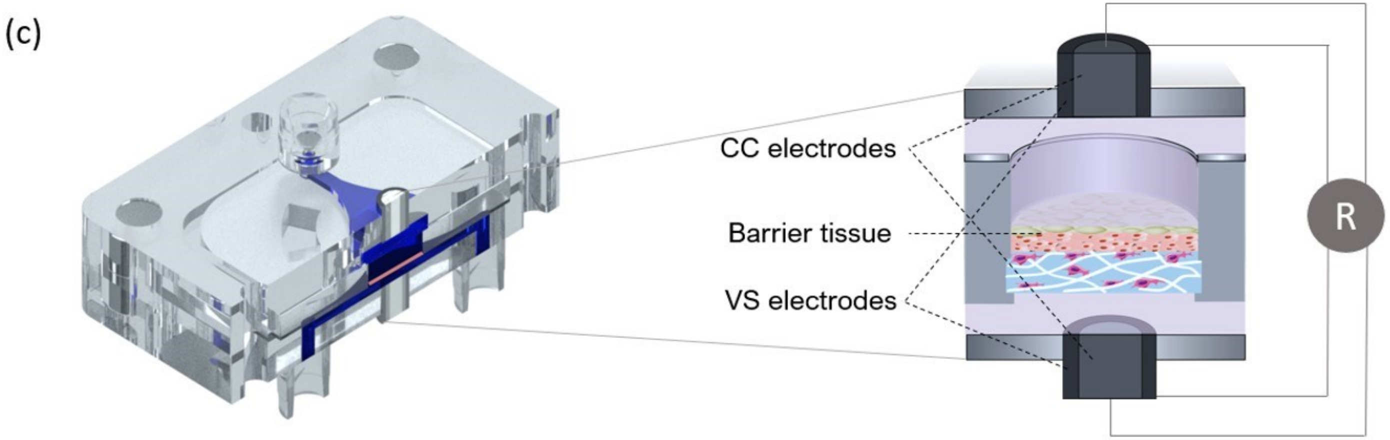


The permeability coefficient was significantly lower for SoCs compared to controls

OoC produces skin with increased barrier properties

SKIN-ON-A-CHIP



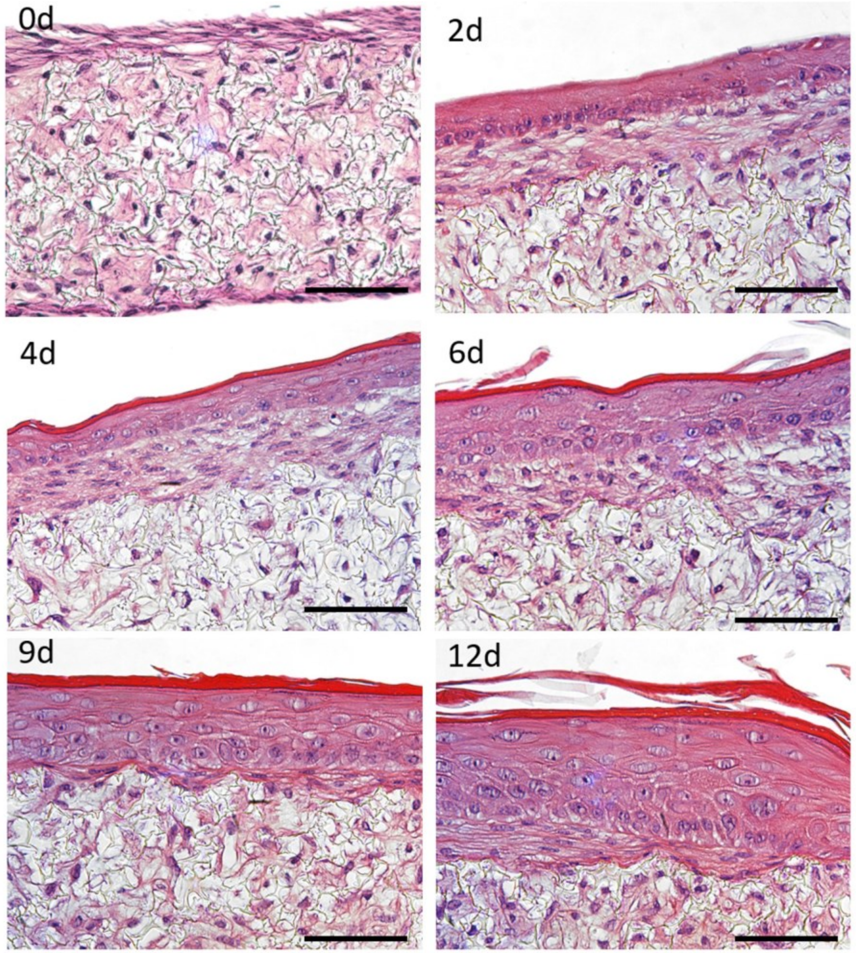


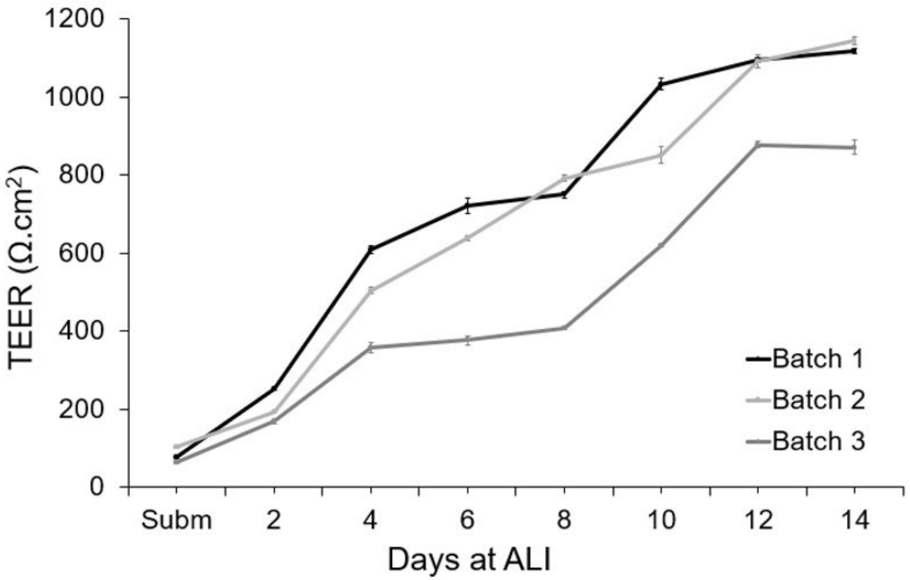
Sensors were integrated into the OoC device for real-time TEER measurement during tissue formation

TEER measurements as a non-destructive technique to quantify the skin barrier. TEER reflects skin barrier functionality by measuring the overall barrier to ions.

SKIN-ON-A-CHIP

TEER analysis used to evaluate the stage of skin differentiation

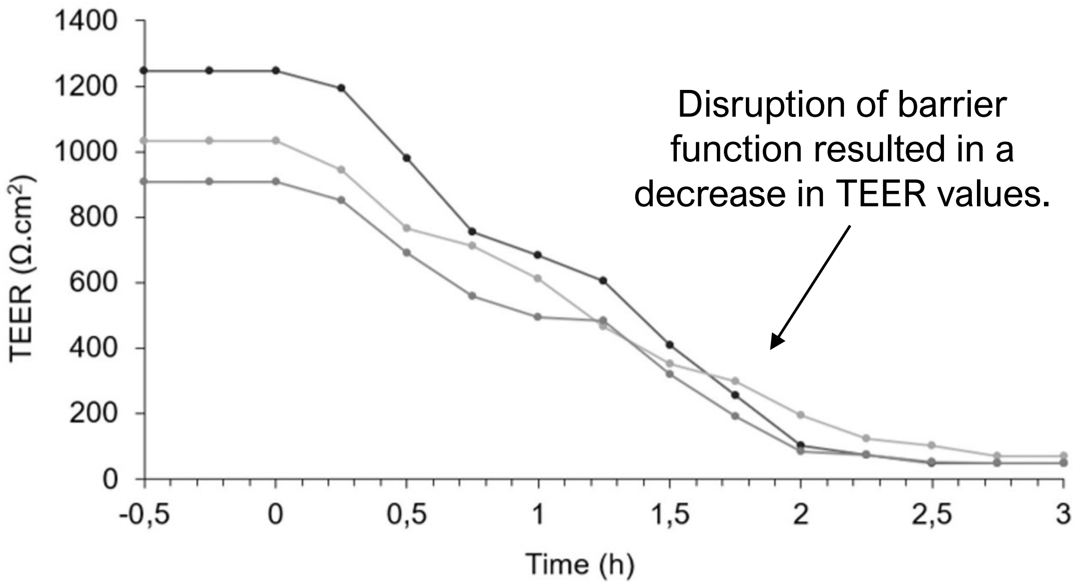


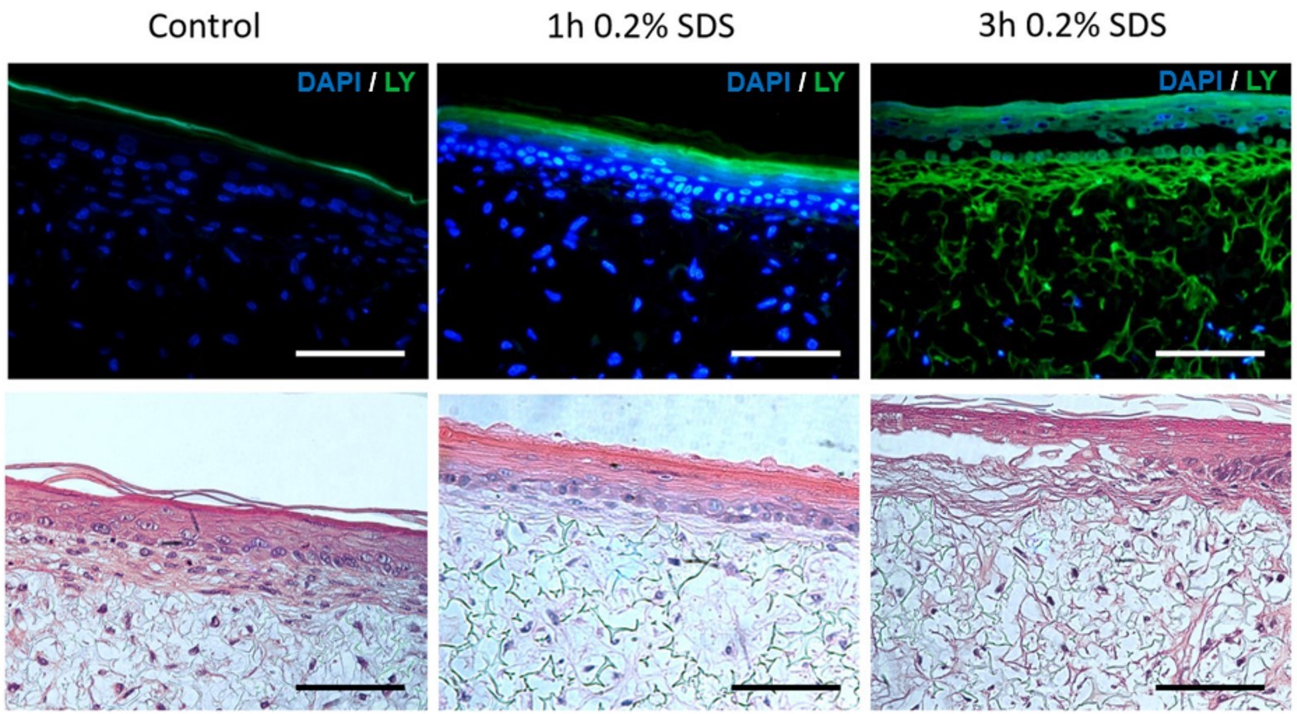


During culture for 2 weeks at ALI, there is an increase in TEER values, reflecting progressive SC accumulation and TJ formation; Values consistent with barrier function integrity

SKIN-ON-A-CHIP

Skin toxicity testing: TEER measurements were performed while exposing the skin to a benchmark irritant for 3 h.





The impact of the irritant was also shown by the penetration of the LY

Drug testing can directly be performed on the platform, with key diagnostic parameters being monitored in real-time.

SKIN-ON-A-CHIP

micro MDPI

Article

Biomimetic Full-Thickness Skin-on-a-Chip Based on a Fibroblast-Derived Matrix

Patrícia Zoio [1], Sara Lopes-Ventura [1] and Abel Oliva [1,2,*]

Platform with a modular architecture that allows the leakage-free integration of a porous scaffold;

Dynamic flow applied during dermis generation increased ECM deposition;

Well-differentiated and organized epidermis with increased thickness and enhanced barrier function;

Permeation assays performed in situ;

micromachines MDPI

Article

Barrier-on-a-Chip with a Modular Architecture and Integrated Sensors for Real-Time Measurement of Biological Barrier Function

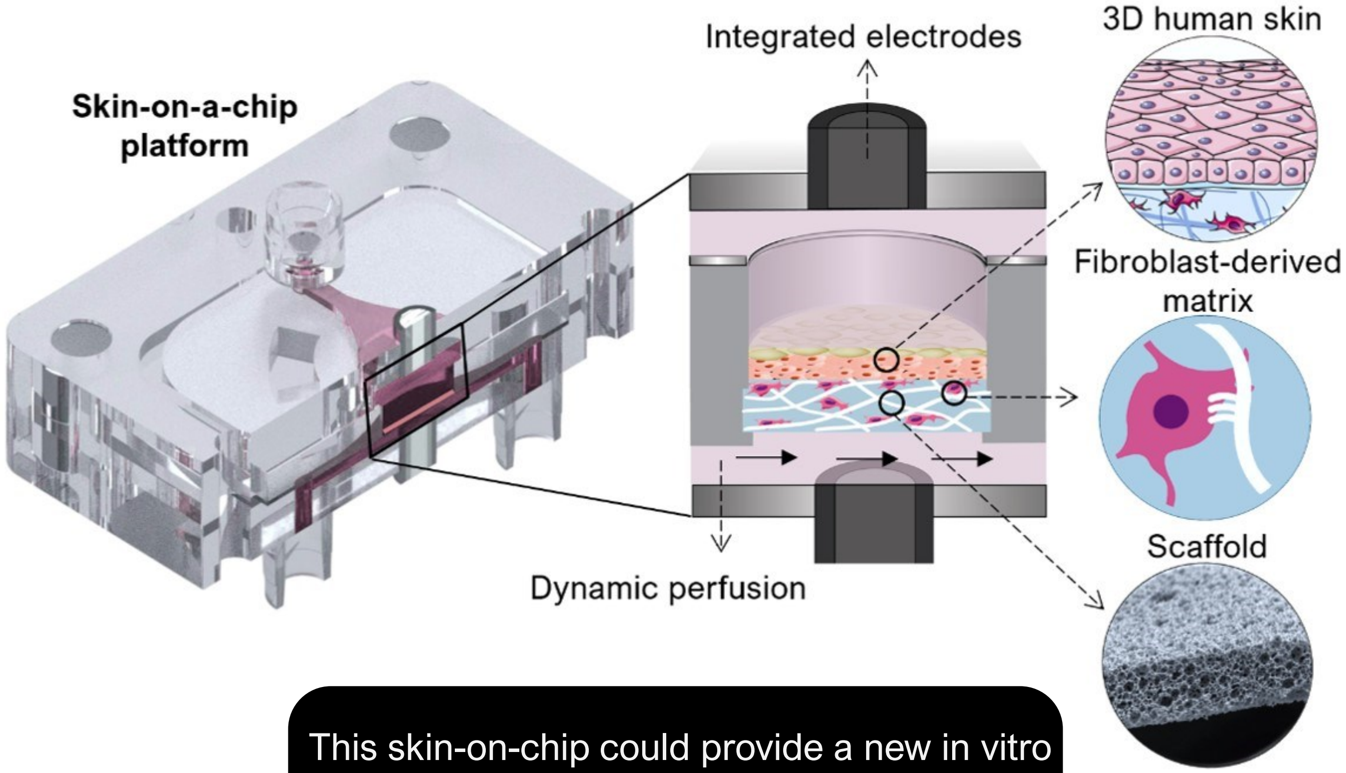
Patrícia Zoio [1], Sara Lopes-Ventura [1] and Abel Oliva [1,2,*]

The integrated tetrapolar electrode configuration resulted in a more homogenous sensitivity distribution along the culture area

Integrated electrodes for real-time, non-destructive TEER measurements to monitor the tissue development;

Skin toxicity assays performed in situ;

CONCLUSIONS



This skin-on-chip could provide a new in vitro tissue system compatible with long-term studies to study skin diseases and evaluate the safety and efficacy of novel drugs.

ACKNOWLEDGMENTS

THANK YOU

ITQB
INSTITUTO DE TECNOLOGIA QUÍMICA E BIOLÓGICA ANTÓNIO XAVIER /UNL
Knowledge Creation

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AIM
Advanced Integrated Microsystems

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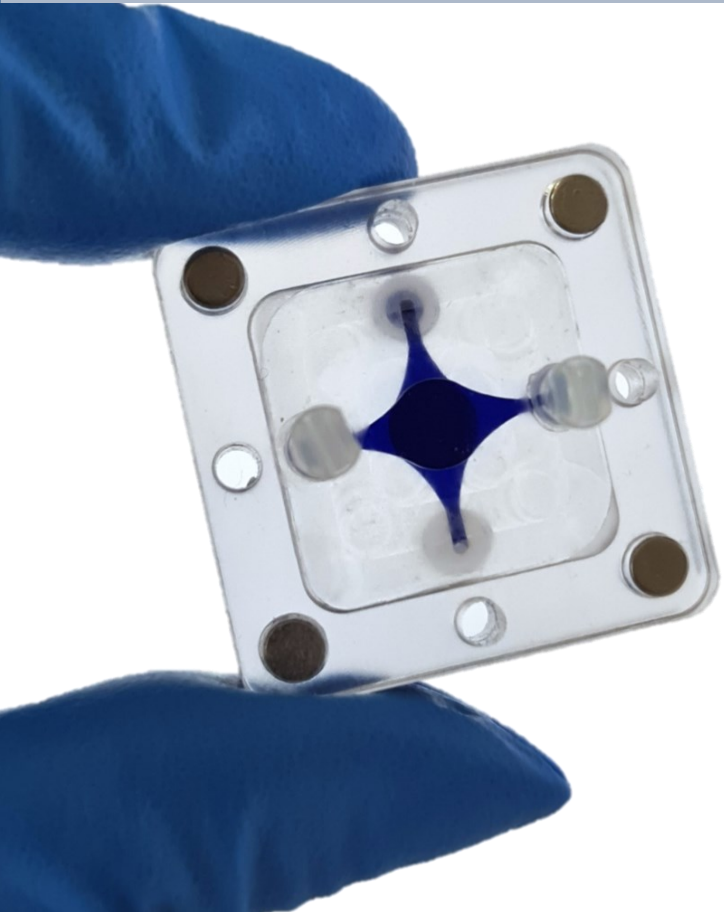
MINISTÉRIO DA EDUCAÇÃO E CIÊNCIA

iMed.ULisboa
Instituto de Investigação do Medicamento

CONTACTS

THANK YOU



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