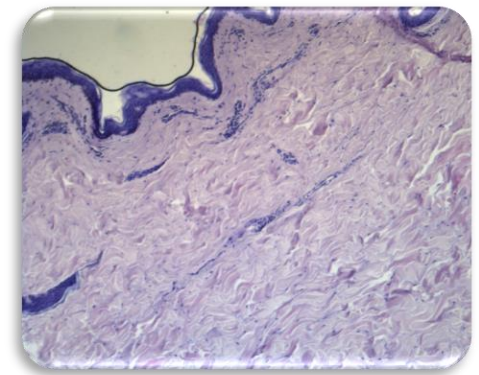
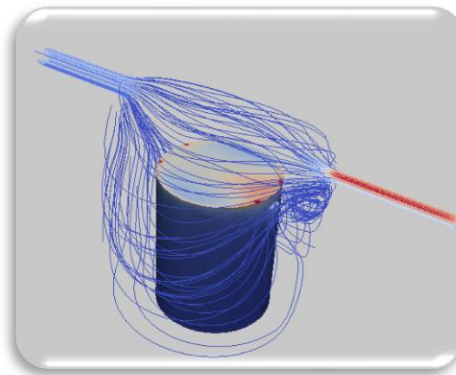




"a new HIGHLY representative in-vitro
model of **Human SKIN**"

...in collaboration
Prof. Calderan, UNIVR



Tommaso Sbrana, PhD
T.Sbrana@IVTech.IT
www.IVtech.IT



Problem

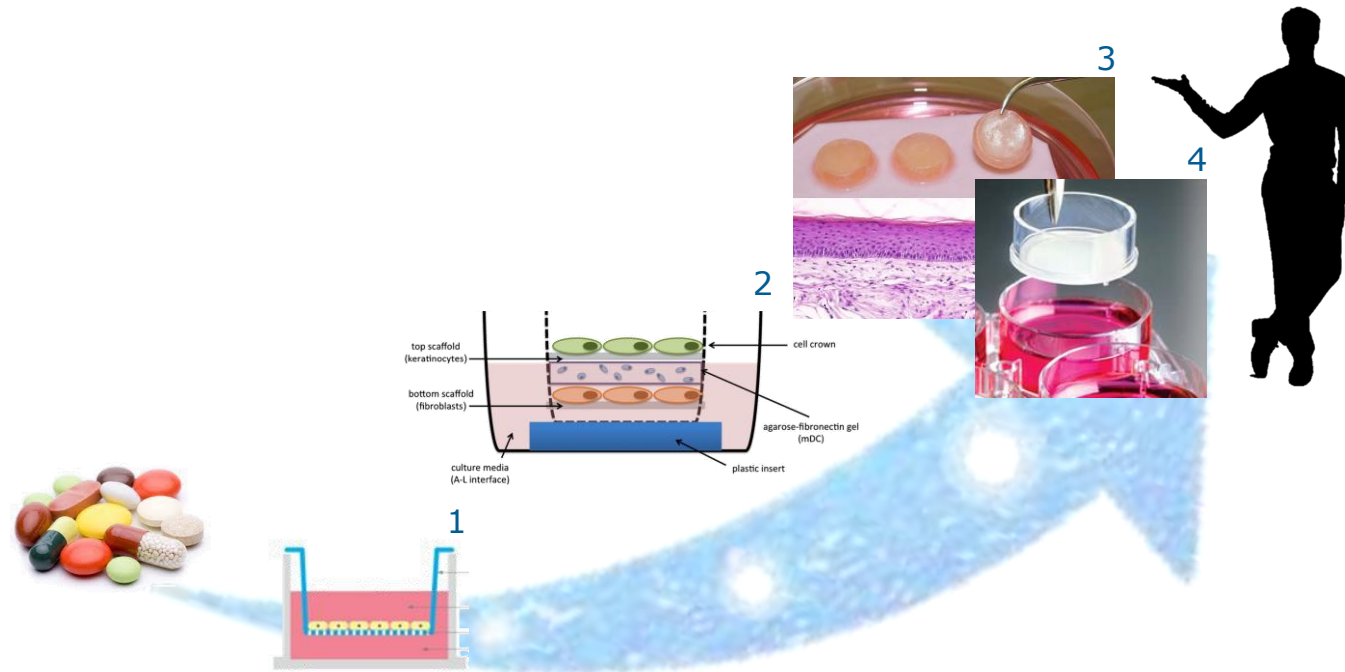
Drug test -> end-user?



We need to evaluate the predictivity of our model
and if this parameter is enough for our goal

Problem

Are we close to the reality?



1. <https://www.corning.com/au/en/products/life-sciences/products/permeable-supports/permeable-support-systems.html>
2. D. chau, *et al.*, The development of a 3D immunocompetent model of human skin, *Biofabrication*, 2013 Sep;5(3):035011. doi: 10.1088/1758-5082/5/3/035011
3. <https://www.phenion.com/products/reconstructed-tissues>
4. <https://www.mattek.com/products/epiderm/>

We need to evaluate the predictivity of our model
and if this parameter is enough for our goal

Incubation under fluid dynamic conditions markedly improves the structural preservation *in vitro* of explanted skeletal muscles

Flavia Carton, Laura Calderan,
Manuela Malatesta

Department of Neurosciences,
Biomedicine and Movement Sciences,
Anatomy and Histology Section,
University of Verona, Italy

Abstract

Explanted organs and tissues represent suitable experimental systems mimicking the functional and structural complexity of the living organism, with positive ethical and economic impact on research activities. However, their preservation in culture is generally limited, thus hindering their application as experimental models for biomedical research. In the present study, we inves-

of new drugs or materials requires experimental models able to mimic efficiently the complex structural and functional features of living organisms or organs. Unfortunately, the currently available *in vitro* systems, from the conventional 2D cell cultures to the 3D co-cultures, can be suitably used only in basic or early-phase applied research because they are not able to reproduce the systemic milieu. On the other hand, the promising technology of microfluidic organs-on-chips, intended to simulate levels of tissue and organ functionality not possible with 2D or 3D culture systems,² is still far from reliably mimicking tissue and organ physiology.

It is therefore necessary to develop alternative experimental models characterized by a functional and structural complexity similar to the living organism. In this view, explanted organs and tissues could represent a suitable and relatively easy solution: surgical and bioptic explants from human or animal subjects could be used for scientific purposes thus drastically reducing tests on laboratory animals. However, the preservation in culture of explanted organs and tissues is generally limited, thus hinder-

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Keywords: Skeletal muscle; organ culture; bioreactor; morphology; ultrastructure.

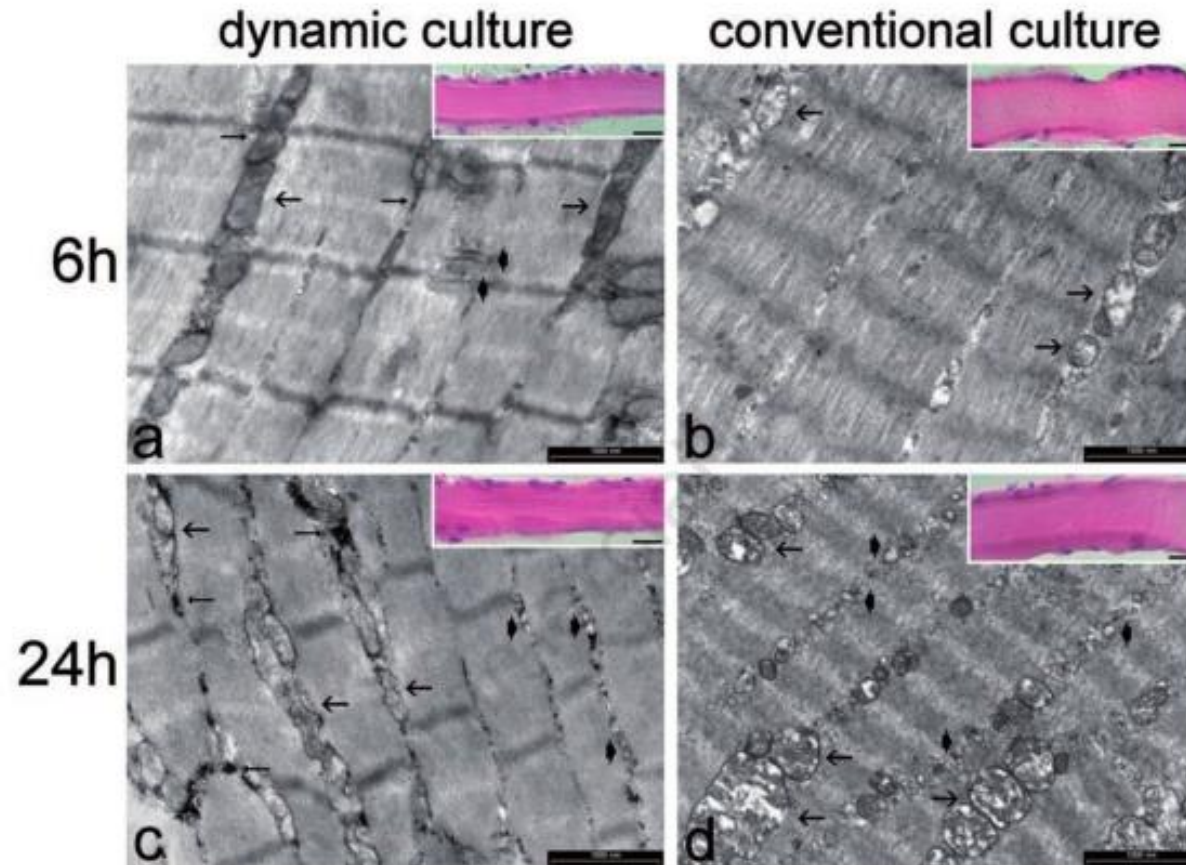
Acknowledgments: This work was supported by I-Care Foundation. F.C. is a PhD student in receipt of a fellowship from the Doctoral Program "Nanoscience and Advanced Technologies" of the University of Verona.

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European Journal of Histochemistry 2017; 61:2862
doi:10.4081/ejh.2017.2862

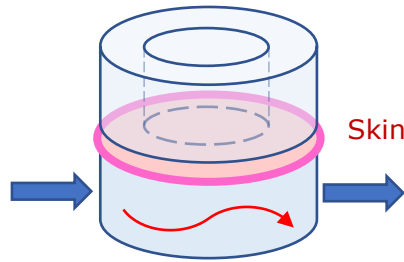
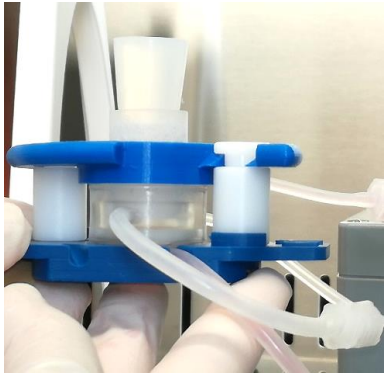
Preliminary results



Carton F. et al., "Incubation under fluid dynamic conditions markedly improves the structural preservation in vitro of explanted skeletal muscles", *European J. Of Histochemistry*, 2017, 61:2862

The main issue with the use of explant as biological material for in-vitro models is related with the loss of phenotype and functionality in a very short time (<24h)

IVTech PLATFORM



- LiveFlow
- LiveBox2 (ALI configuration)
- Fluidic circuit
- Skin explant

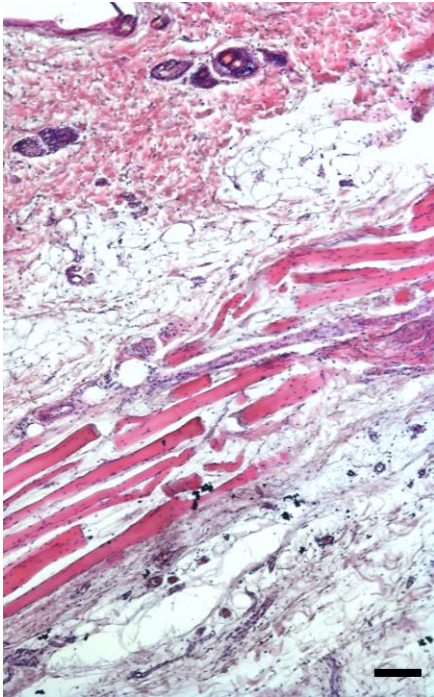


Use of a fluidic platform (IVTech srl) to develop an advanced in-vitro model, allowing for dynamic stimulation and perfusion of tissues during the experiment

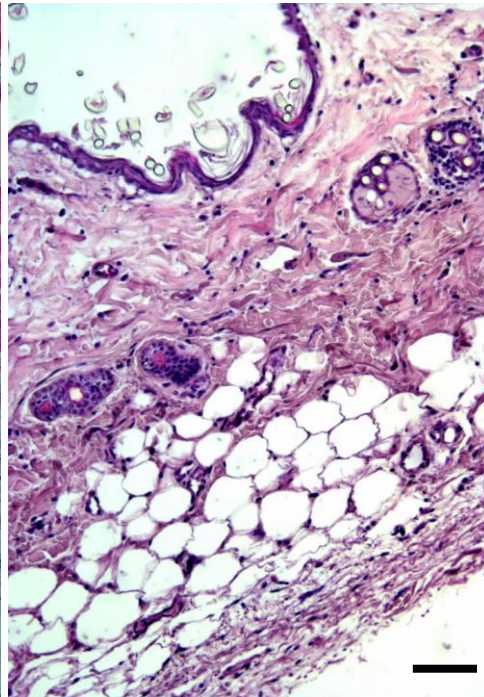
SKIN explant

Rat skin from abdominal region
24 h of incubation in LB2
Hematoxilin-eosin stained section

- Suitable preservation of all the skin layers
- Microstructures (e.g. hair bulb, small vessels, adipocytes, gland) are well preserved



Bar 100 μm



Bar 50 μm

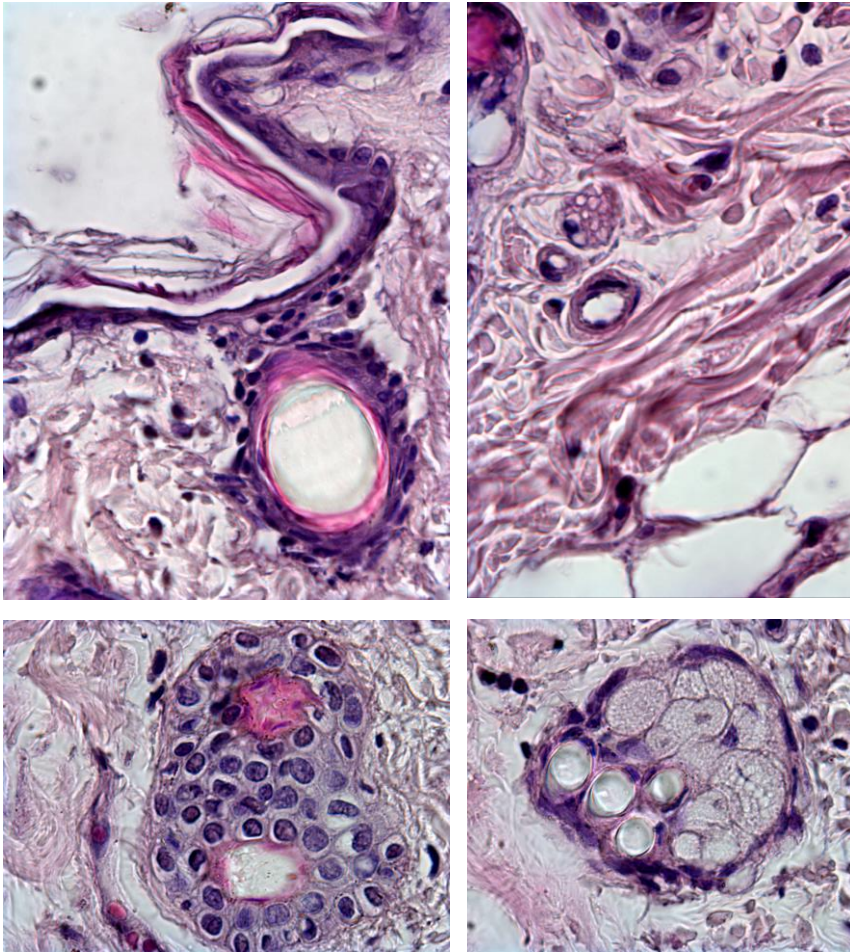
Cappellozza, *et al.*, Integrated Microscopy and Metabolomics to Test an Innovative Fluid Dynamic System for Skin Explants In Vitro, *Microscopy and Microanalysis* (2021), 27, 923–934, doi:10.1017/S1431927621012010

The morphological analysis confirms the well preservation of the biological material if incubated in dynamic conditions (24h, 48h) in a LB2

Skin explant

Rat skin from abdominal region
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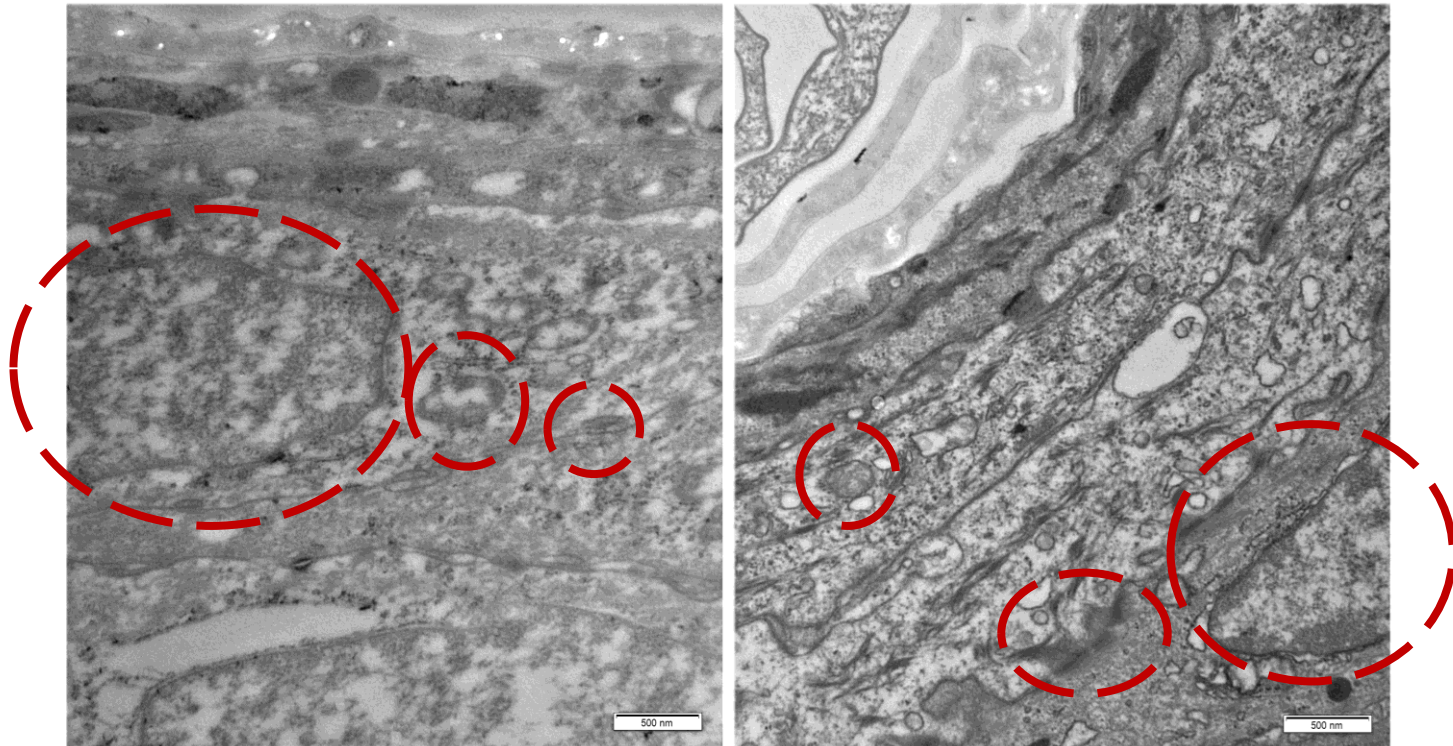


Cappellozza, *et al.*, Integrated Microscopy and Metabolomics to Test an Innovative Fluid Dynamic System for Skin Explants In Vitro, Microscopy and Microanalysis (2021), 27, 923–934, doi:10.1017/S1431927621012010

The morphological analysis confirms the well preservation of the biological material if incubated in dynamic conditions (24h, 48h) in a LB2

TEM ANALYSIS

Skin after 24 hours of incubation in static and in dynamic conditions. Epidermal region.

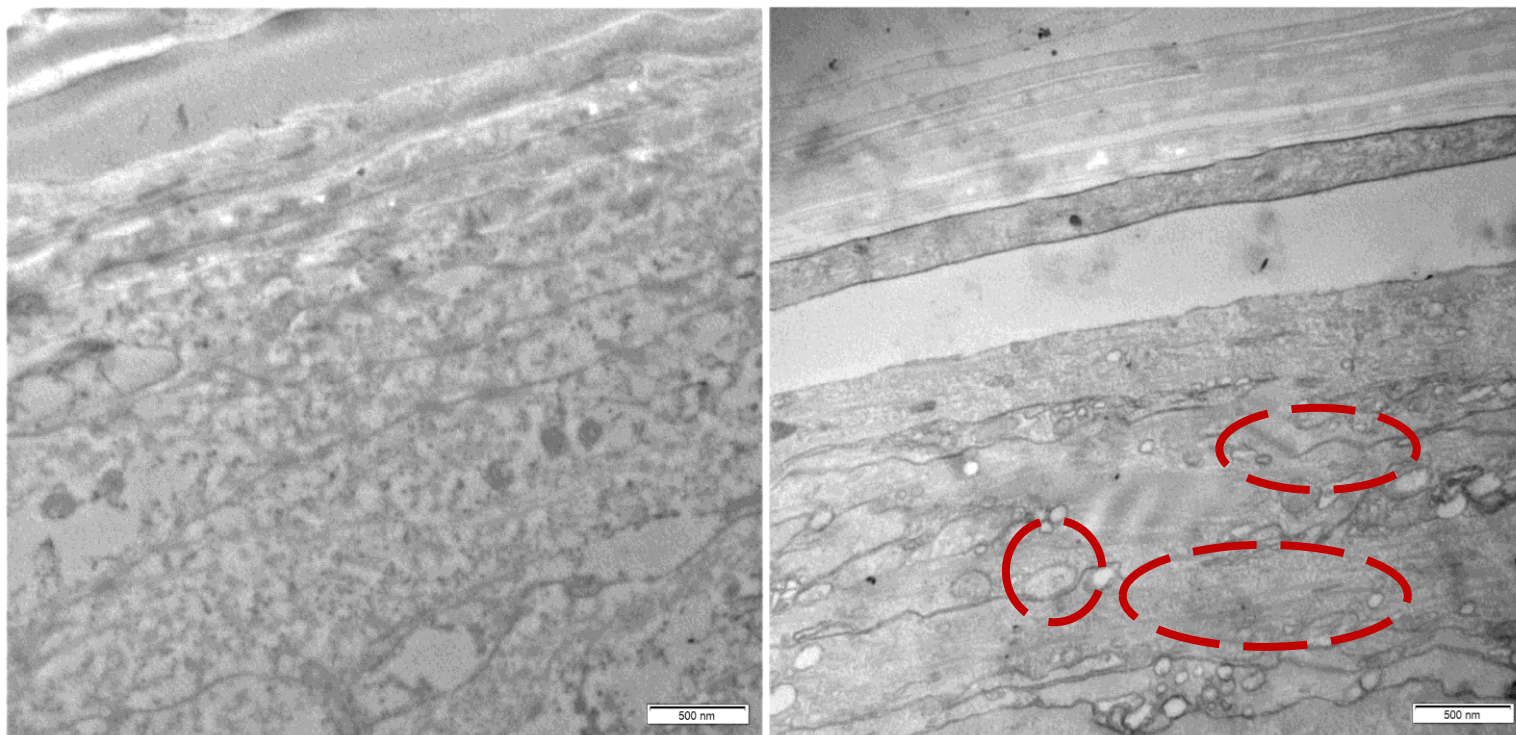


Cappellozza, *et al.*, Integrated Microscopy and Metabolomics to Test an Innovative Fluid Dynamic System for Skin Explants In Vitro, *Microscopy and Microanalysis* (2021), 27, 923–934, doi:10.1017/S1431927621012010

The ultrastructural analysis is important to evaluate the functionality of the tissue.
Differences at 24h between static and dynamic conditions.

TEM ANALYSIS

Skin after 48 hours of incubation in static (A) and in dynamic (B) conditions.
Bar 500 nm

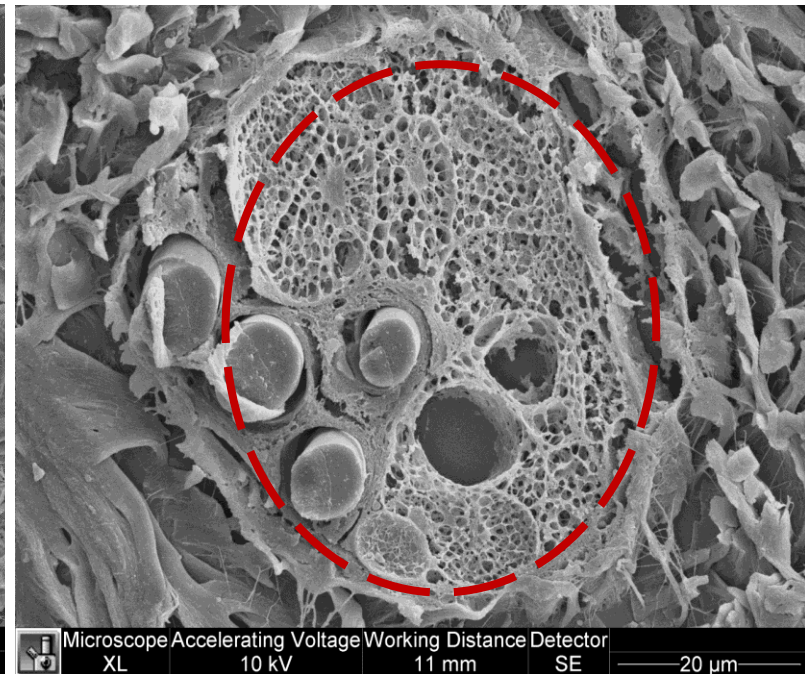
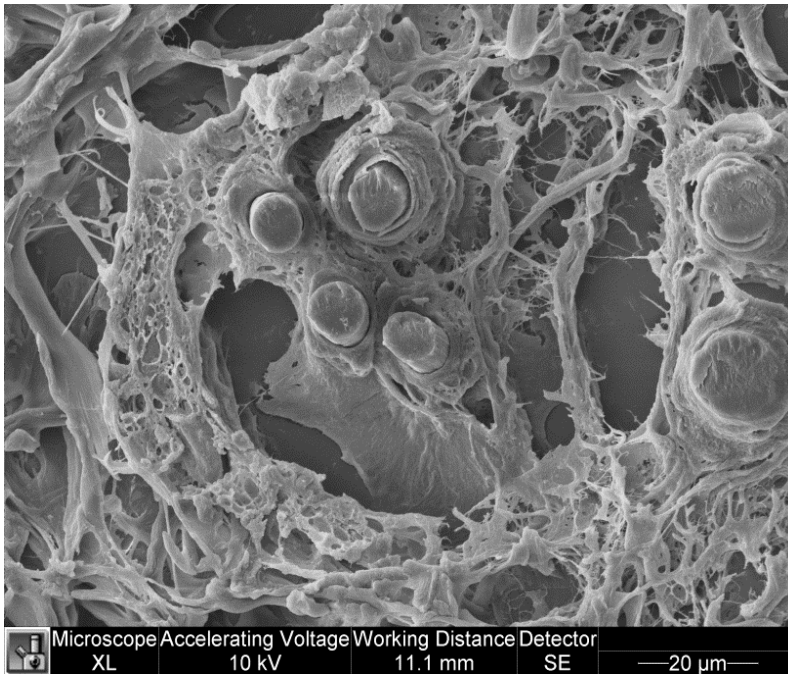


Cappellozza, *et al.*, Integrated Microscopy and Metabolomics to Test an Innovative Fluid Dynamic System for Skin Explants In Vitro, *Microscopy and Microanalysis* (2021), 27, 923–934, doi:10.1017/S1431927621012010

At 48h the microstructure in static condition is degenerated. Advanced necrotic phenotype. In dynamic condition, we still recognize the main characteristics.

Sem analysis

Skin after 24 hours of incubation under dynamic inside the bioreactor (A) and static (B) conditions (SEM XL30 scanning electron microscope)



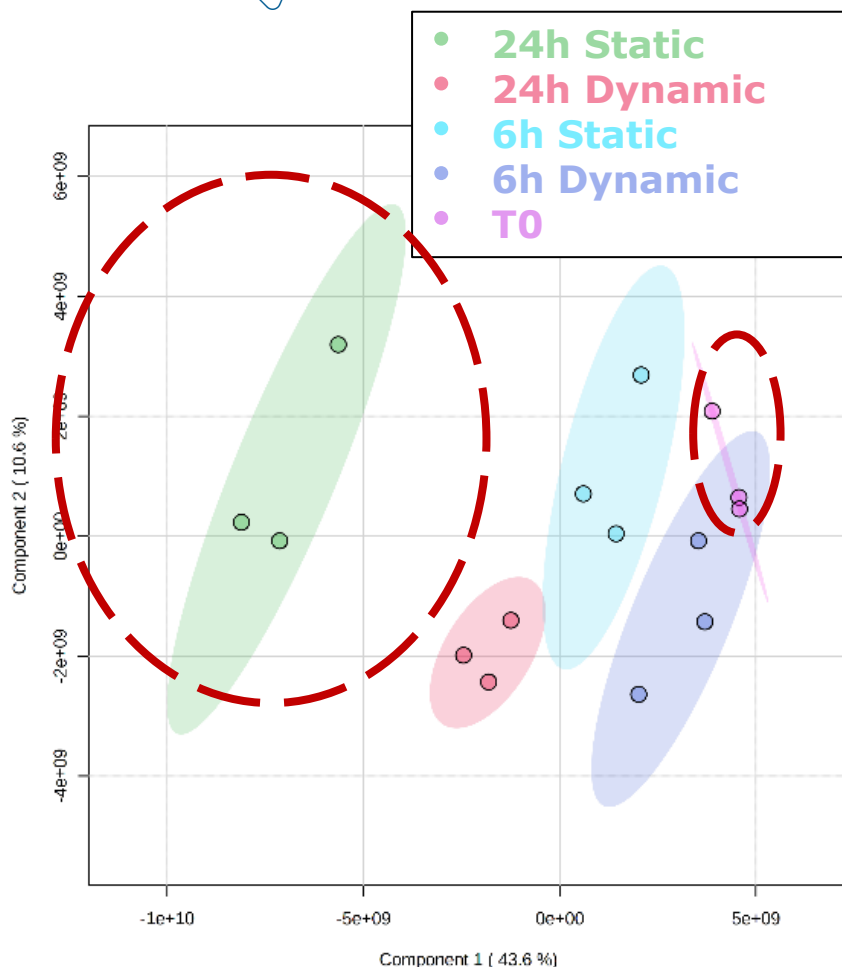
Cappellozza, *et al.*, Integrated Microscopy and Metabolomics to Test an Innovative Fluid Dynamic System for Skin Explants In Vitro, *Microscopy and Microanalysis* (2021), 27, 923–934, doi:10.1017/S1431927621012010

3D structure of hair bulb and sebaceous gland. The differences in 24h are evident if static is compared to the dynamic conditions sample

MeTAbOLOmICS ST.

Cell culture medium was sampled at 6h and 24h and analyzed by NMR
Multivariate statistical analysis was performed using MetaboAnalyst 4.0

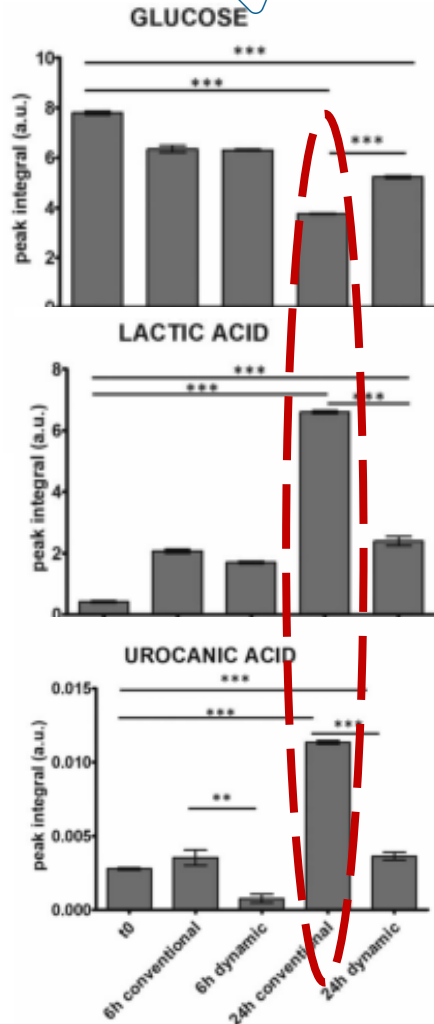
The Principal Component Analysis (**PCA**) was performed to generate an overview of trends, grouping, and identify significant loadings in the NMR data set.



Cappellozza, *et al.*, Integrated Microscopy and Metabolomics to Test an Innovative Fluid Dynamic System for Skin Explants In Vitro, Microscopy and Microanalysis (2021), 27, 923–934, doi:10.1017/S1431927621012010

Analysing the NMR spectrum -> differences between cell culture medium composition of static condition vs the t0 or the dynamic condition sample

MeTAbOLOmICS ST.



In static condition, the glucose is consumed, and Lactic acid is produced. This is a stressed condition.

UA is expressed in the apical side of skin. It has been found in medium -> skin barrier functionality lost

The static conditions, at 24hm show metabolomics pathway compatible with a stressed condition for the skin explant



Eco. evaluation

In vivo

Animals: purchase 30,00 €/animal
maintenance 100,00 €/animal

Reagents and disposable: 100,00 €

Total: 3000-4000,00 €

Advanced In-vitro model

Amortization of the instrument,
reagents and disposable ...

Total: 100,00 €

Ideal experiment: 1 drug to be tested on rodent skin

- test at least 2 dosages
- kinetics: 3 time points
- subject variability (n=5).

Total number of rodents = 30.

More characterizations published on:

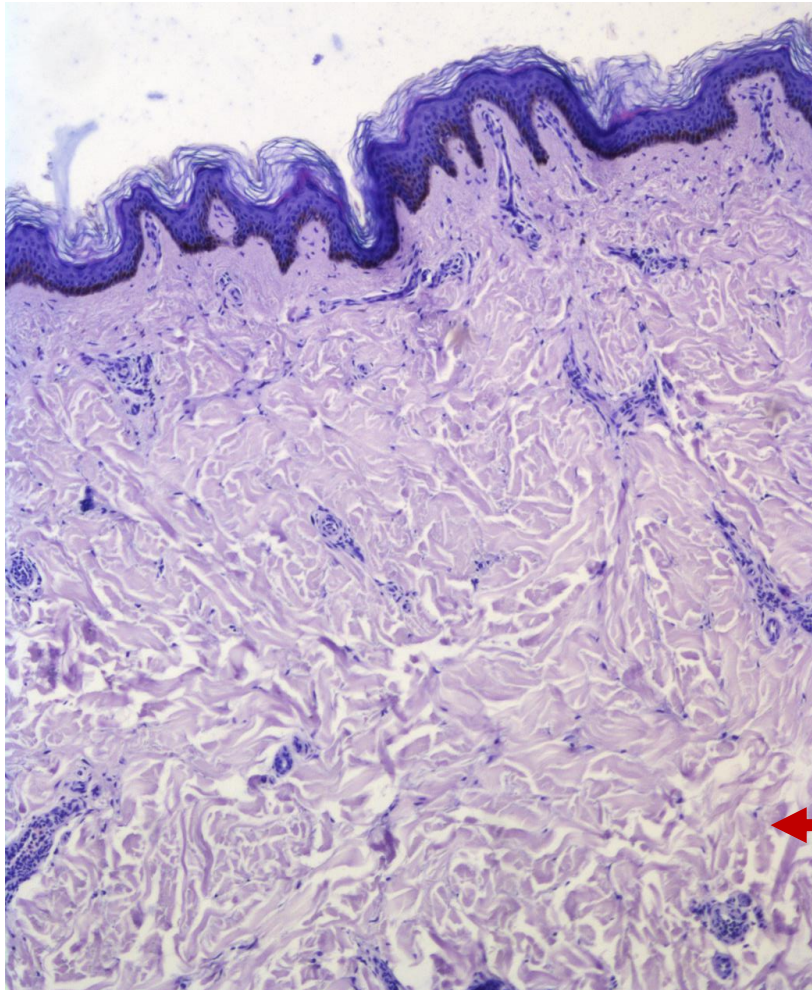
Cappellozza, *et al.*, A spectrofluorometric analysis to evaluate transcutaneous biodistribution of fluorescent nanoparticulate gel formulations, European Journal of Histochemistry 2022; 66:3321, doi:10.4081/ejh.2022.3321

The comparison between in-vivo and in-vitro can be based also on economical aspect.
It is confirmed that an advanced in-vitro model is cheaper than in-vivo tests

Human SKIN BIOPSY

Human skin is incubated in dynamic conditions in the bioreactor (t exp = 0-72h)

- Whole thickness skin. Breast surgery. Woman 20-40 y. No pathological conditions
- Abdominal surgery. Man-woman. No pathological conditions



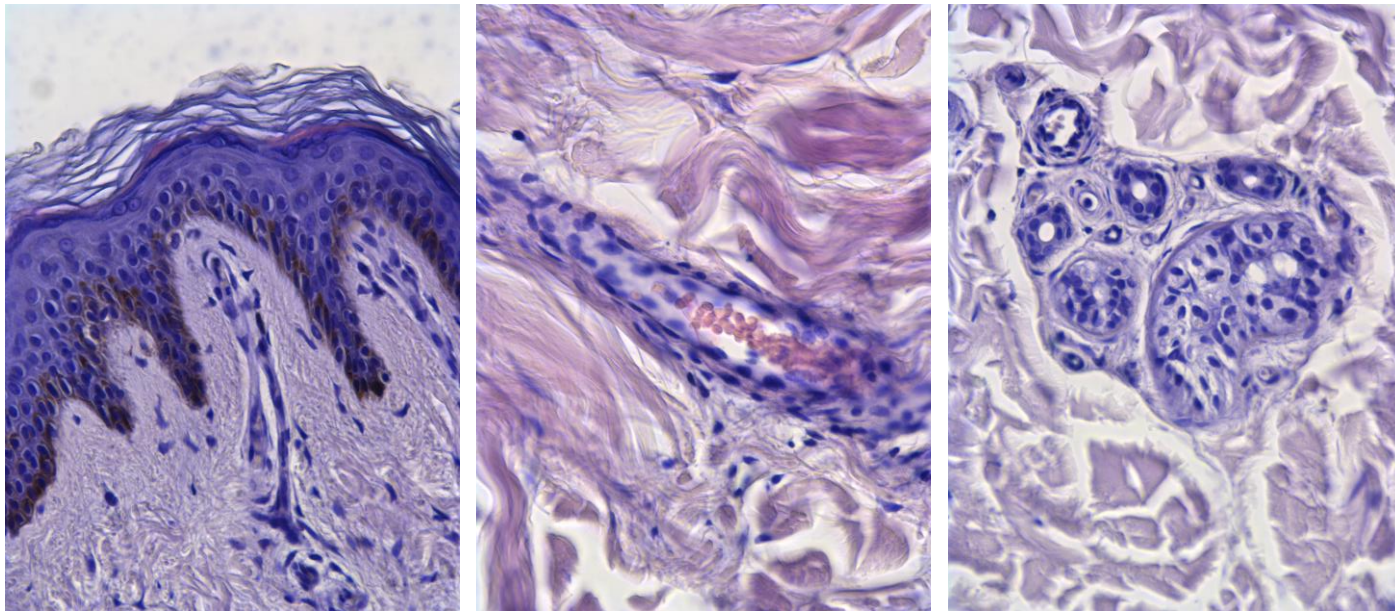
← Capillaries endothelium

← Convoluted fibers in derma

The morphological analysis confirms the well preservation of the biological material if incubated in dynamic conditions (24h, 48h, 72h) in a LB2

Human SKIN BIOPSY

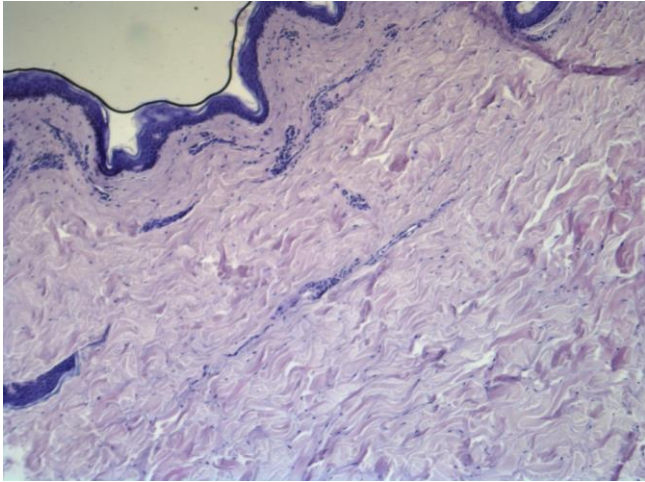
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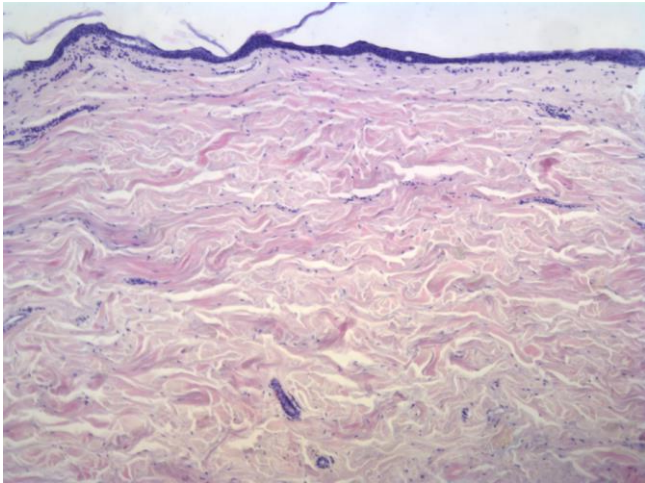
The morphological analysis confirms the well preservation of the biological material if incubated in dynamic conditions (24h, 48h, 72h) in a LB2

Human SKIN BIOPSY

Human skin (24h) treated with
Hyaluronic Acid (t=0)



a



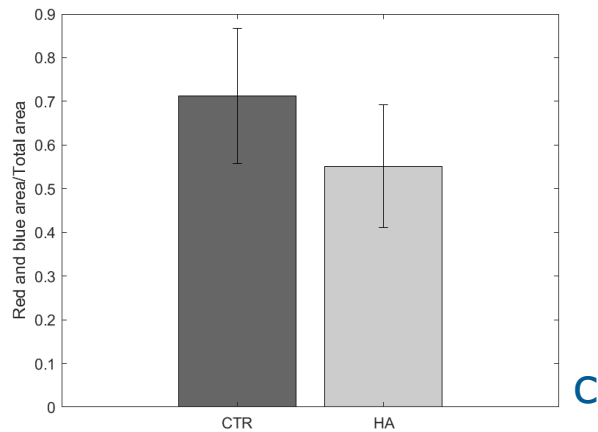
b

- Hyaluronic Acid (HA) -> more space between fibers of derma (fig. b) -> swollen fibers organization than the control (fig. a)
- Fibers seem to be more aligned if compared with the control (fig. a)

This platform allows to test the effect of a drug (e.g. hyaluronic acid), basing on human skin expant

Human SKIN BIOPSY

Human skin (24h) treated with
Hyaluronic Acid (t=0)

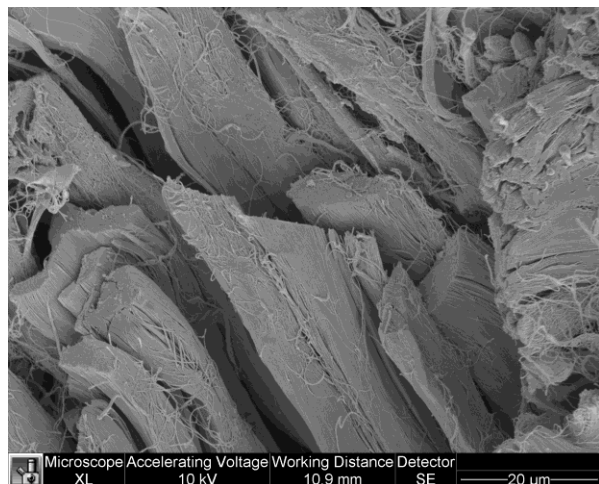


- Hyaluronic Acid (HA) -> more space between fibers of derma (fig. b) -> swollen fibers organization than the control (fig. a)
- Fibers seem to be more aligned if compared with the control (fig. a)
- The % space occupied by fibers is reduced in the HA treated sample (fig. c)

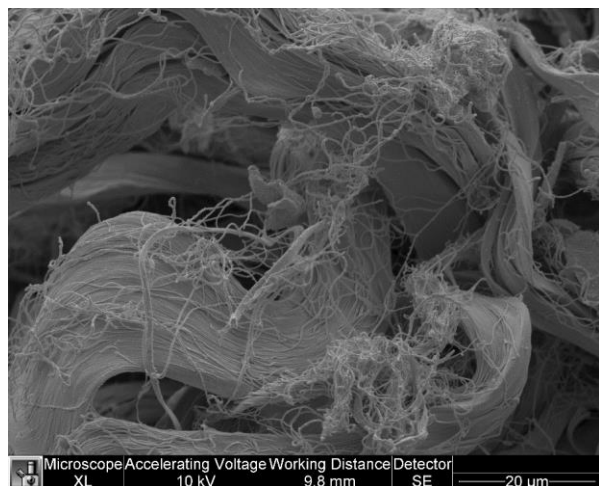
This platform allows to test the effect of a drug (e.g. hyaluronic acid), basing on human skin expant

Human SKIN BIOPSY

Human skin (24h) treated with
Hyaluronic Acid (t=0)



d



e

- Hyaluronic Acid (HA) -> more space between fibers of derma (fig. b) -> swollen fibers organization than the control (fig. a)
 - Fibers seem to be more aligned if compared with the control (fig. a)
 - The % space occupied by fibers is reduced in the HA treated sample (fig. c)
 - SEM analysis has confirmed a change in the organization of the fibers (fig. d vs e)
- ↓
- Turgid behaviour of the tissue (?)

This platform allows to test the effect of a drug (e.g. hyaluronic acid), basing on human skin explant



EXPERTS' FEEDBACK



Visionaries Speak
virtual roundtable series

Tuesday, January 26

7 AM PST | 10 AM EST | 4 PM CET

Animal-free Drug Safety Testing: Challenges and Opportunities



Prof. Thomas Hartung

Center for Alternatives
to Animal Testing



Dr. Stefan Platz

Senior Vice President Clinical
Pharmacology & Safety
Sciences, BioPharmaceuticals
R&D, AstraZeneca



Prof. Armin Wolf

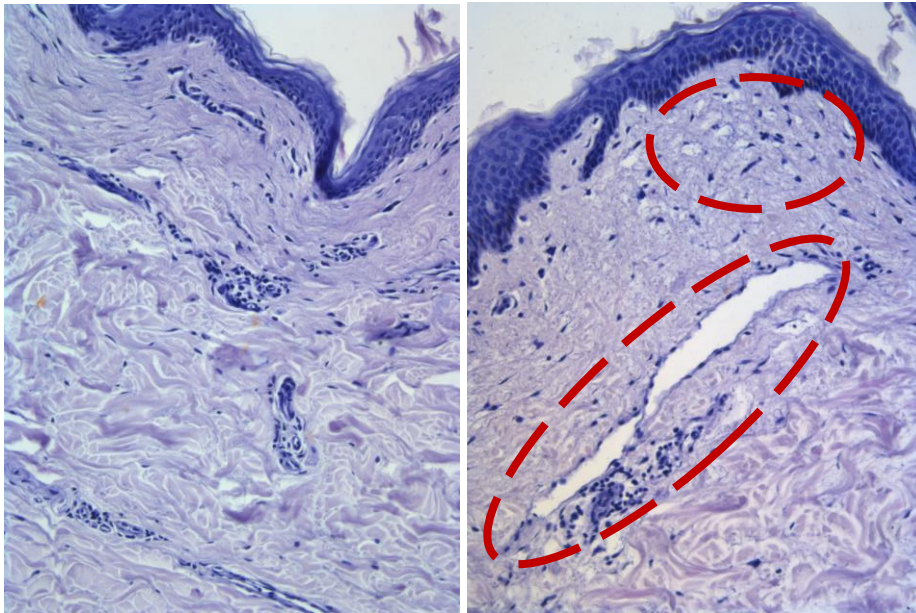
InSphero AG

Experts' vision (2021):
we need standardized in-vitro models of pathological human reality

InFLamed SKIN

The use of Substance P is accepted to induce inflammation¹.
This inflammation is induced mixing Substance P in the medium

- Vasodilatation highlighted in inflamed skin (fig. b), if compared with control (fig. a)
- Preliminary Immunohistochemical data seem to suggest a macrophages activation in treated samples



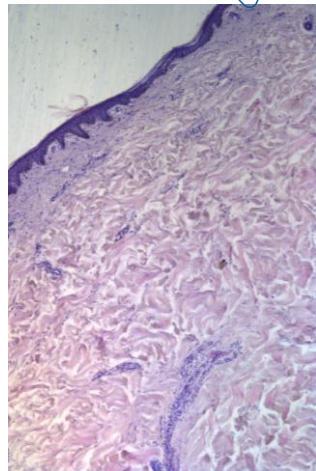
a

b

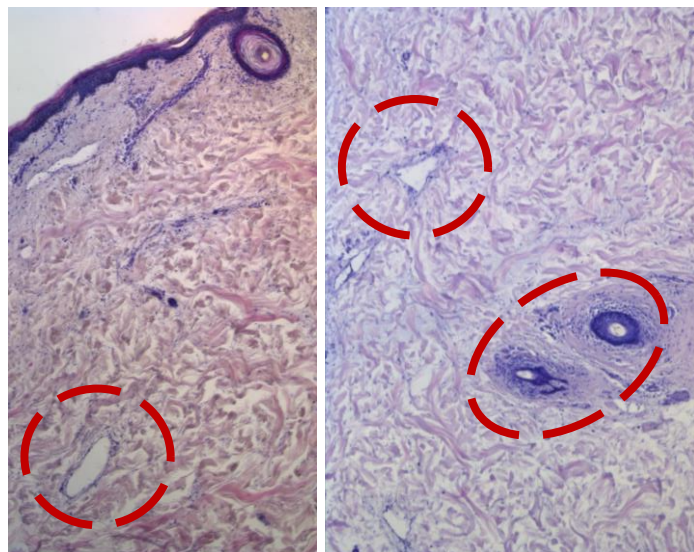
1. Goodarzi, *et al.*, The potential of probiotics for treating acne vulgaris: A review of literature on acne and microbiota, *Dermatologic Therapy*. 2020;33:e13279, <https://doi.org/10.1111/dth.13279>

The test of drugs can be performed in a pathological scenario.
It is possible to simulate inflammation disease

Dermatitis Disease



a

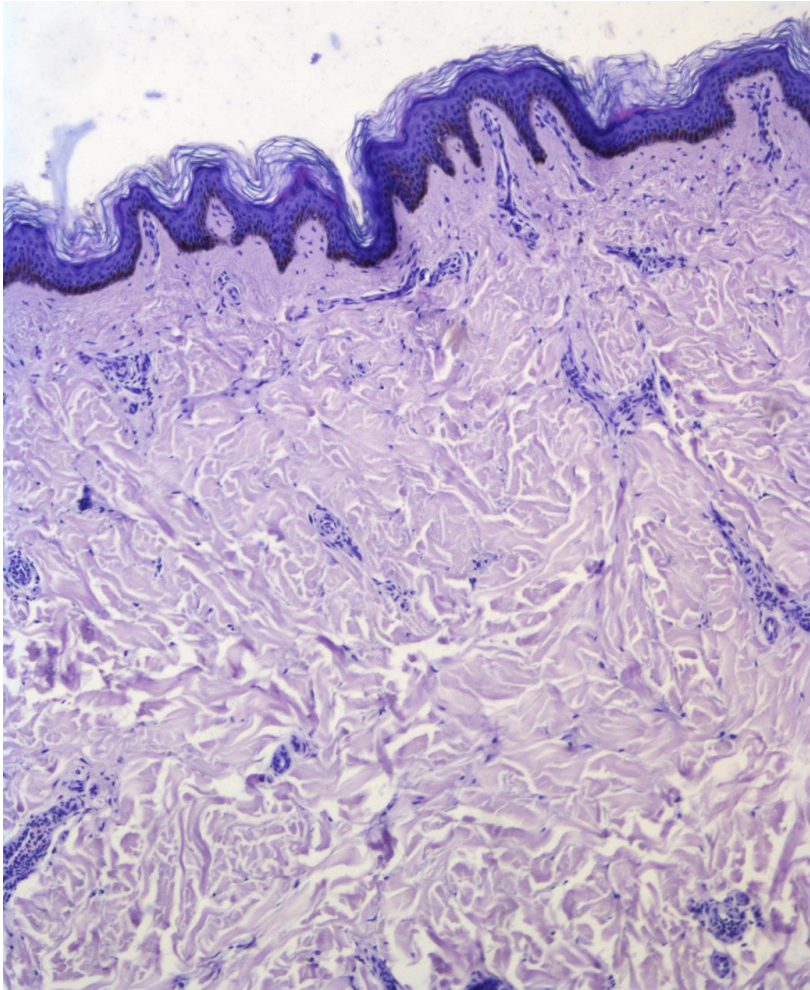


b

The contact dermatitis induced can be simulated using of Dithranol. This inflammation is induced by a topic administration of irritant molecule (t exp = 0-24h)

- Vasodilatation highlighted in inflamed skin (fig. b), if compared with control (fig. a)
- Preliminary Immunohistochemical data seem to suggest a macrophages activation in treated samples

The test of drugs can be performed in a pathological scenario.
It is possible to simulate contact dermatitis disease



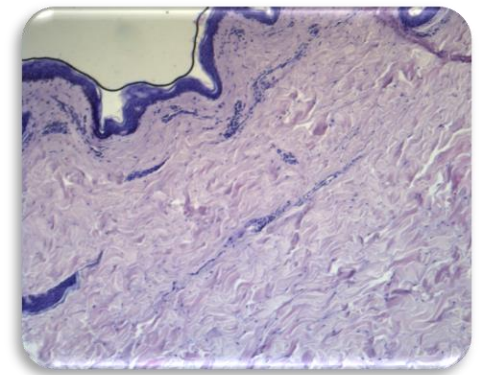
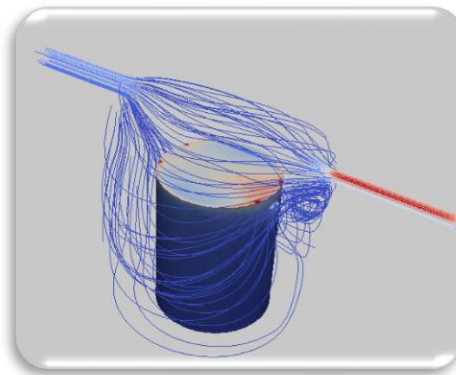
Conclusion

- The use of human skin explant allows to refine the in-vitro model
- The use of IVTech products allows to perform test at 24, 48 and 72 h
- The inflammation can be induced using substance P
- Contact dermatitis disease can be induced using dithranol
- The use of a pathological in-vitro model of skin allows to test the effect of a drug in a highly representative model

The platform is ready to be used in the test of new drug. We offers this kind of service in collaboration with our partner at the University of Verona



“IVTech: advanced TECHNOLOGY FOR
representative IN-VITRO MODELS OF
Human reality”



TOMMASO SBRANA, PHD
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WWW.IVTECH.IT





IVTech way

Goal:

We want to become your reference partner to develop advanced in-vitro models

2014:

- UNIPi spin-off
- Ita market

2016:

- First StarterKit
- Ita distributor

2018:

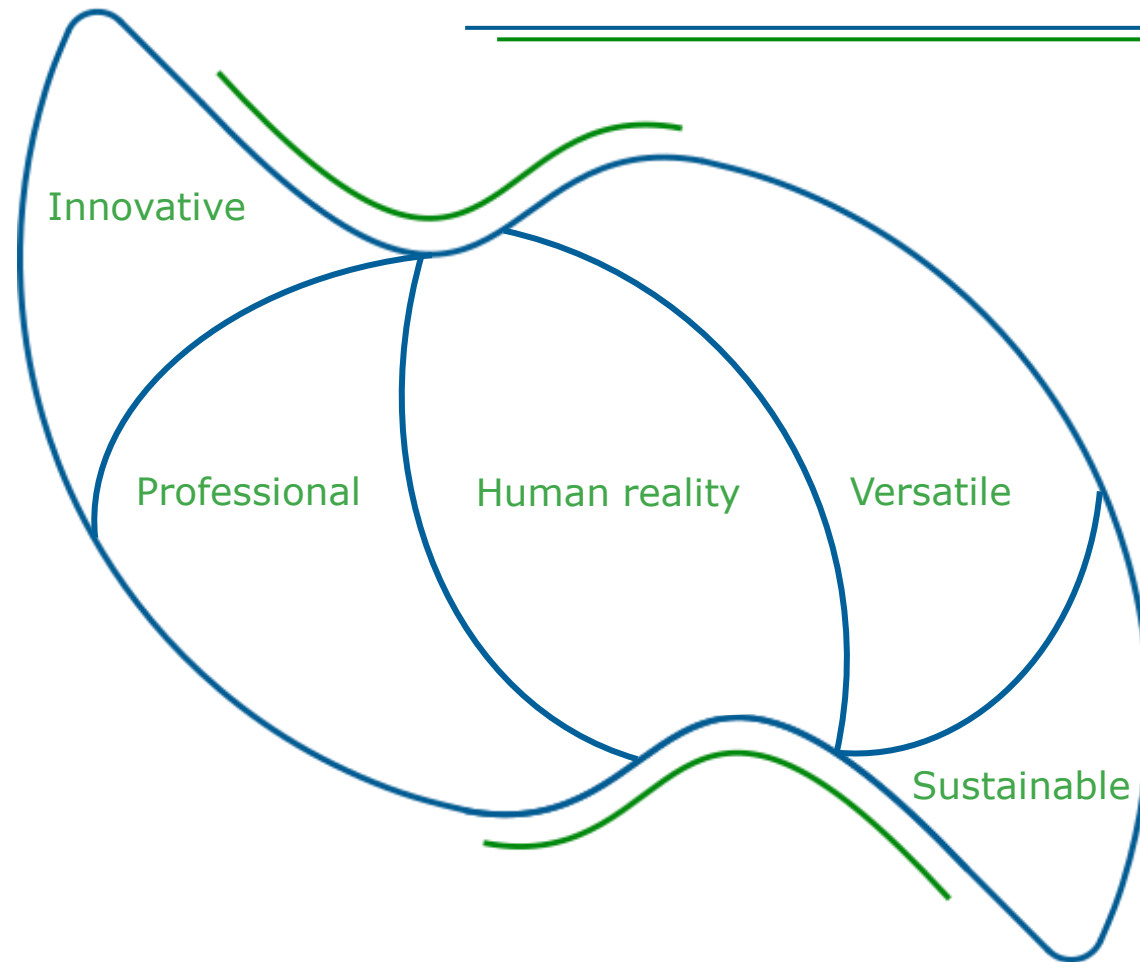
- LiveFlow
- Ger distributor

2021:

- 7 chambers
- 3 pumps
- International Distributors

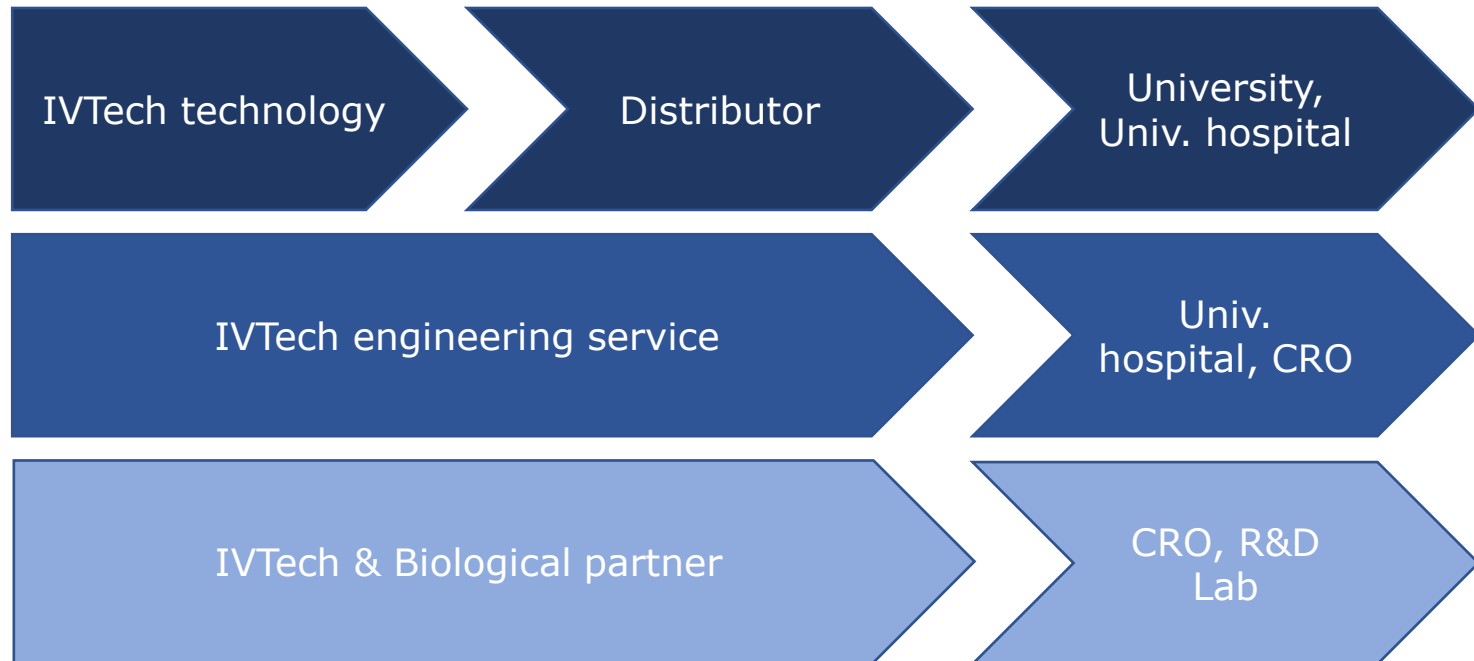
We offer technology, service and collaboration as a crucial step to increase the correlation of adv. in-vitro models with the human reality

Core values



Aim -> to increase the predictivity of human reality,
maintaining a versatile and sustainable approach

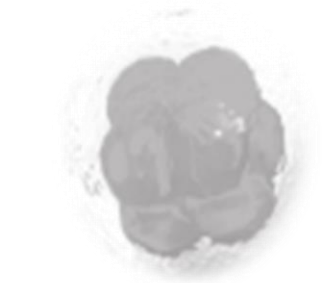
Business model



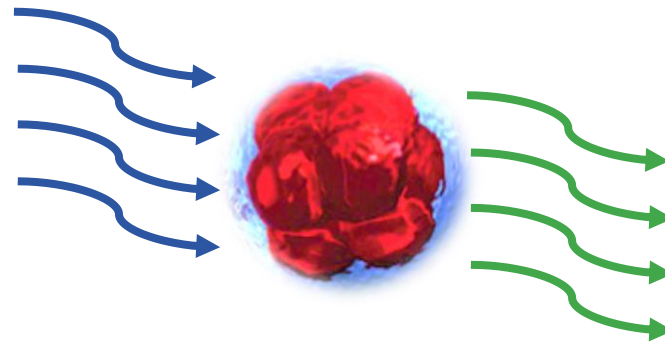
Different value propositions -> different leads



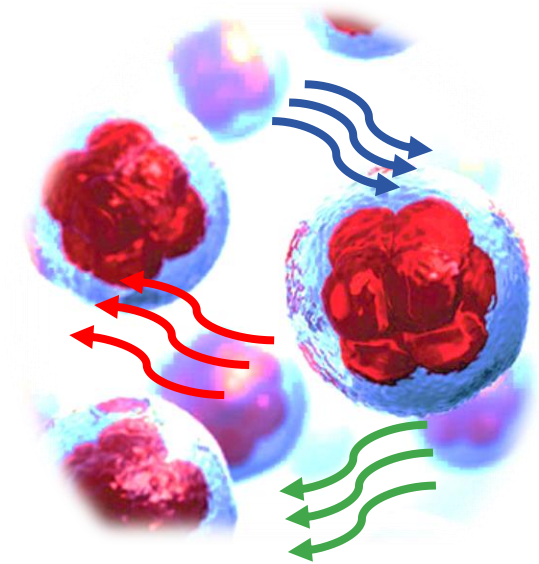
IVTech mission



3D static model



3D Dynamic model



3D Dynamic Pathway

IVTech -> technology and service
for a more representative in-vitro model (5Ds) of human reality

Tech chain



Cell culture
chambers



Tools for
dynamicity



Environmental
control tools

IVTech offers a compact fluidic platform,
compatible with the incubator environment

Overview: THE app.

Metabolism:

- ✓ Stomach
- ✓ Liver
- ✓ Fat tissue

Permeability studies:

- ❖ Lung
- ✓ Intestine
- ✓ Skin

Test of new materials:

- ✓ Drug release
- ✓ Mech. Prop. of scaffolds
- ✓ Surface treatments

3D - stem cells:

- ✓ Bone

3D - tissue explant:

- ✓ Nail
- ✓ Muscle
- ✓ Skin

Disease models:

- ❖ Leukaemia
- ✓ Glaucoma
- ✓ Obesity
- ✓ Breast cancer
- ❖ Bone cancer

Different fields of application -> different tissues
(✓ -> available publications; ❖ -> on-going projects)



IVTech PRODUCTS



LiveBox1 Suite

- single flow bioreactors (1 inlet – 1 outlet)
- attached cell culture models



LiveBox2 Suite

- double flow bioreactors (2 inlets – 2 outlets)
- equipped with a porous membrane
- physiological barriers simulation



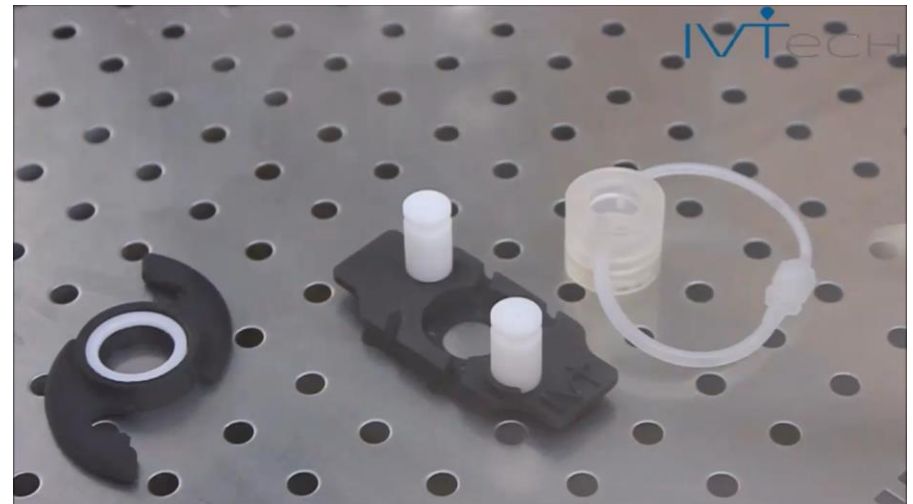
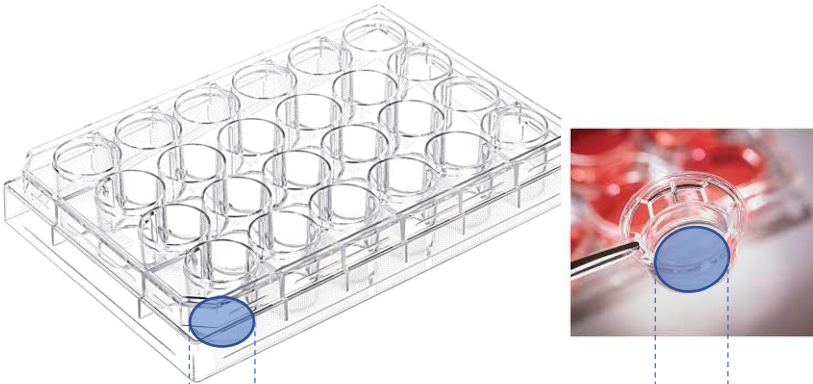
LiveFlow Suite

- compatible with the incubator environment
- 4 independent fluidic circuits
- 2 versions: standard and programmable

2 different chamber types and a peristaltic pump suite
to design your pathway in a Lego approach

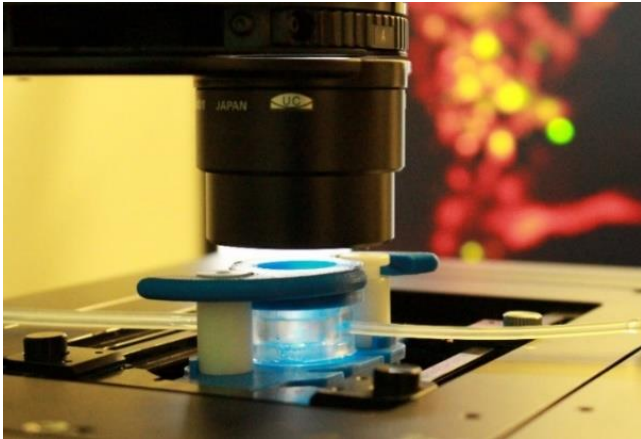
STD. PROTOCOLS

- Cell growth area compatible with plate slots or transwell
- Seeding protocol -> similar to the std.

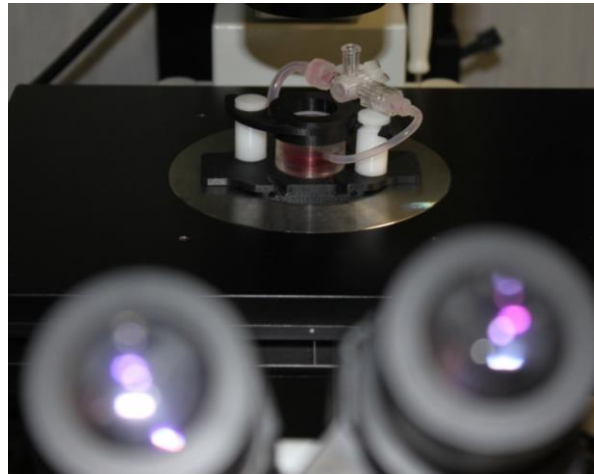
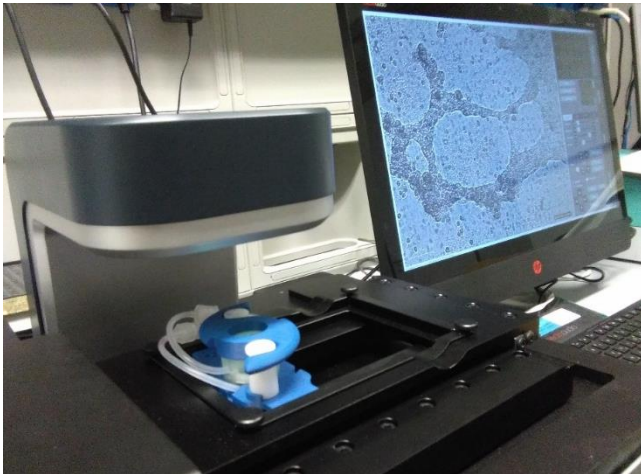


The use of standard cell culture protocols is assured by the compatibility between our chambers and the standard devices

STD. PROTOCOLS

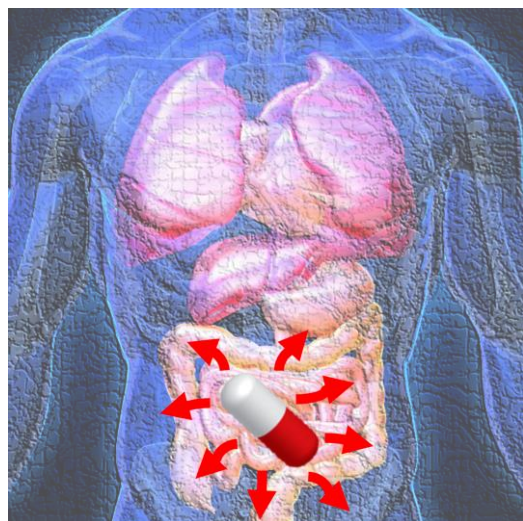


- Removable glass coverslip
- Clamp design -> slide or plate reader
- Compatibility with adv. opt. platform
- Standard monitoring protocols

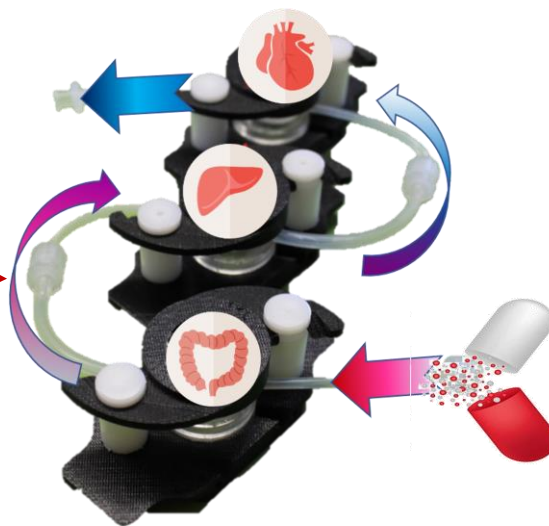


The use of standard monitoring protocols is assured by the transparency of our chambers

MULTI-organ appr.



- Simulation of dynamic pathway
- Signalling monitoring
- Evaluation of principle and side effects of a drug



The multi-organ approach is crucial
if a disease model has to be evaluated

Disease models

- Simulation of a disease scenario (i.e. hypertension, glaucoma)
- Evolution of the environmental conditions (i.e. food ingestion)



Dimensions [cm]	8.5×2.8×1.9
Compatible with LF drawer	✓
# P incremental steps	10
Battery	9V

The disease conditions related with an increase of pressure can be simulated using our LivePa

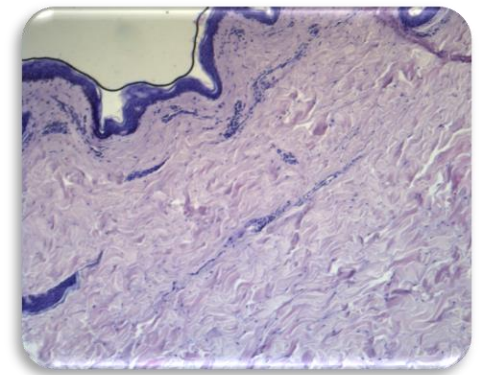
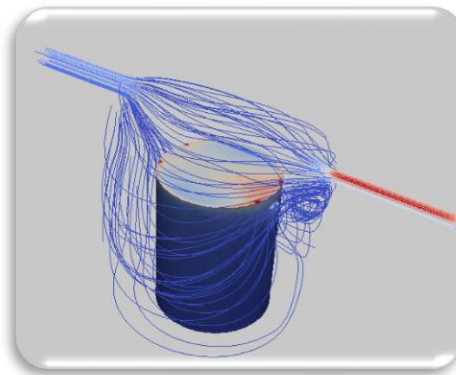


Conclusion

- IVTech products allow to use std. cell culture and monitoring protocols
- Possibility to re-collect the biological material at the end of an experiment
- Compatibility with the incubator environment
- Possibility to develop a multi-organ approach (MoA)
- Possibility to recreate the environmental stimulation of a disease (e.g. hypertension, glaucoma)



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