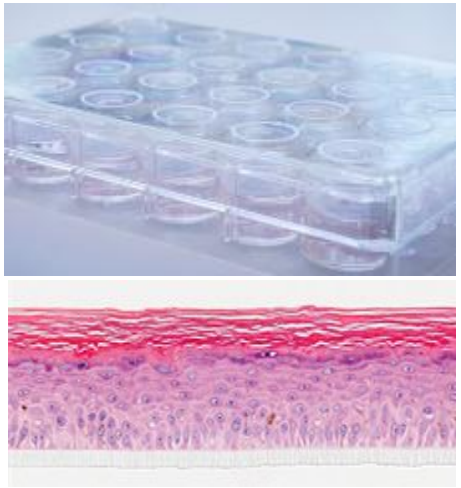
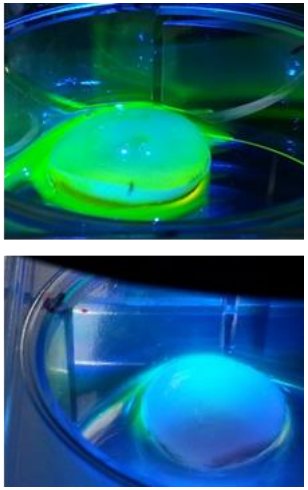
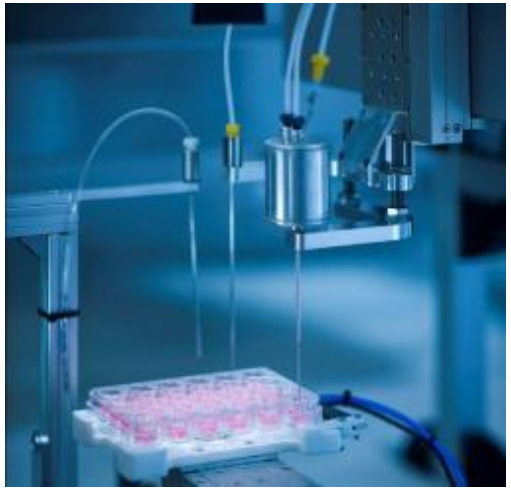
ENGINEERING OF HUMAN 3D VASCULARIZED TISSUES INCLUDING DISEASE MODELS

Angela Rossi, Florian Groeber, Maria Steinke, Marco Metzger, Heike Walles







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Fraunhofer IGB

The Fraunhofer-Gesellschaft

Locations in Germany

- 67 institutes and research units
- more than 23,000 staff
- Annual research budget of
  2 billion euro

Translational Center Würzburg

Fraunhofer IGB

Our innovation chain – from basics to industrial applications

Fundamental research

Universität Stuttgart
Institut für Grenzflächenverfahrenstechnik und Plasmatechnologie

EBERHARD KARLS
UNIVERSITÄT TÜBINGEN

Julius-Maximilians-
UNIVERSITÄT WÜRZBURG

Technische Universität München

Applied research

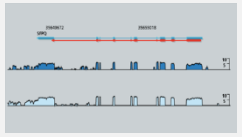
1 
Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB

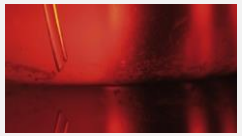
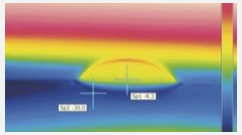
2 
Fraunhofer-Zentrum für Chemisch-Biotechnologische Prozesse **CBP**

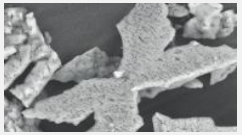
3 
Bio, Electro and Chemocatalysis BioCat, Straubing branch

4 
Translational center "Regenerative therapies for Oncology and Musculoskeletal Diseases **TLC**

Industrial applications


BioEconomy Cluster




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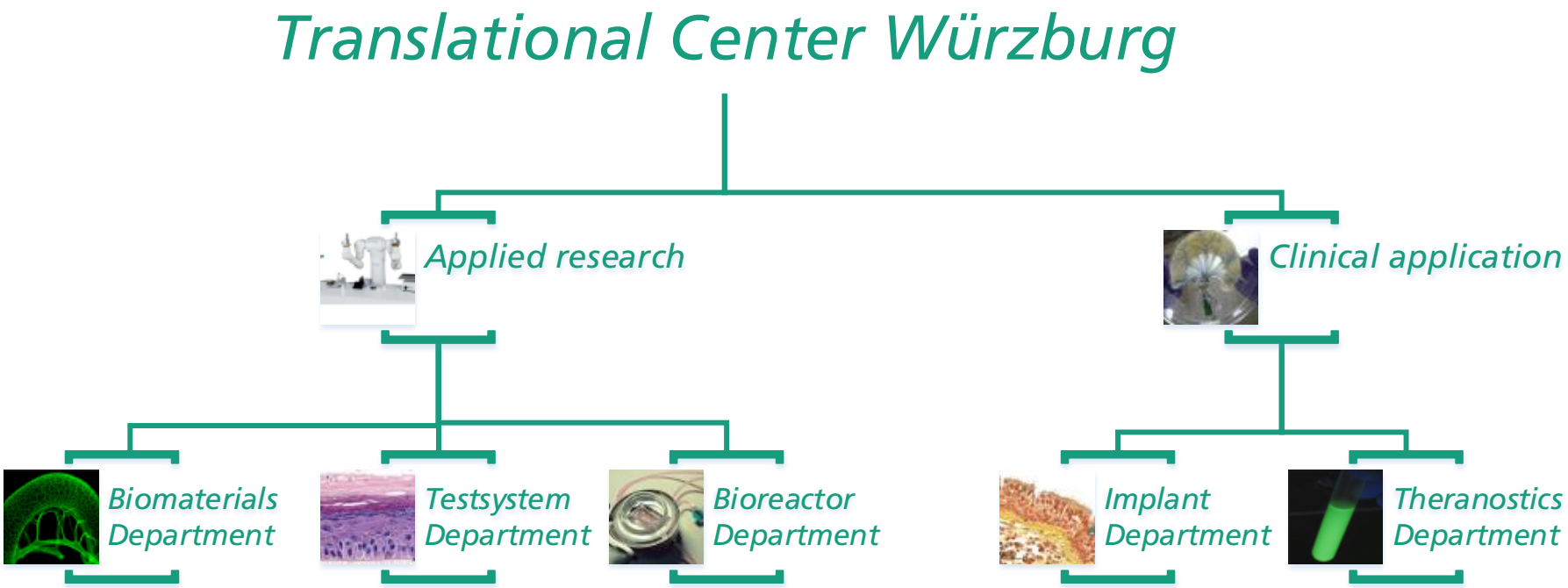
The Translational Center Würzburg

A joint research center by Fraunhofer and the University Hospital Würzburg



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In-vitro-Testsystems

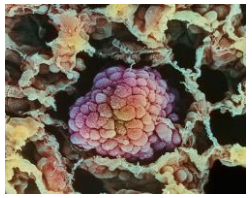
BioVaSc®

Human airway mucosa model

Lung tumor model

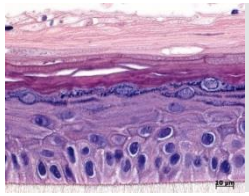
Intestinal model

Skin model



Tests Procedures

Technologies



Advanced tissue models

Oral mucosa, Blood-Brain-barrier (BBB), Bone, Cardiac tissue

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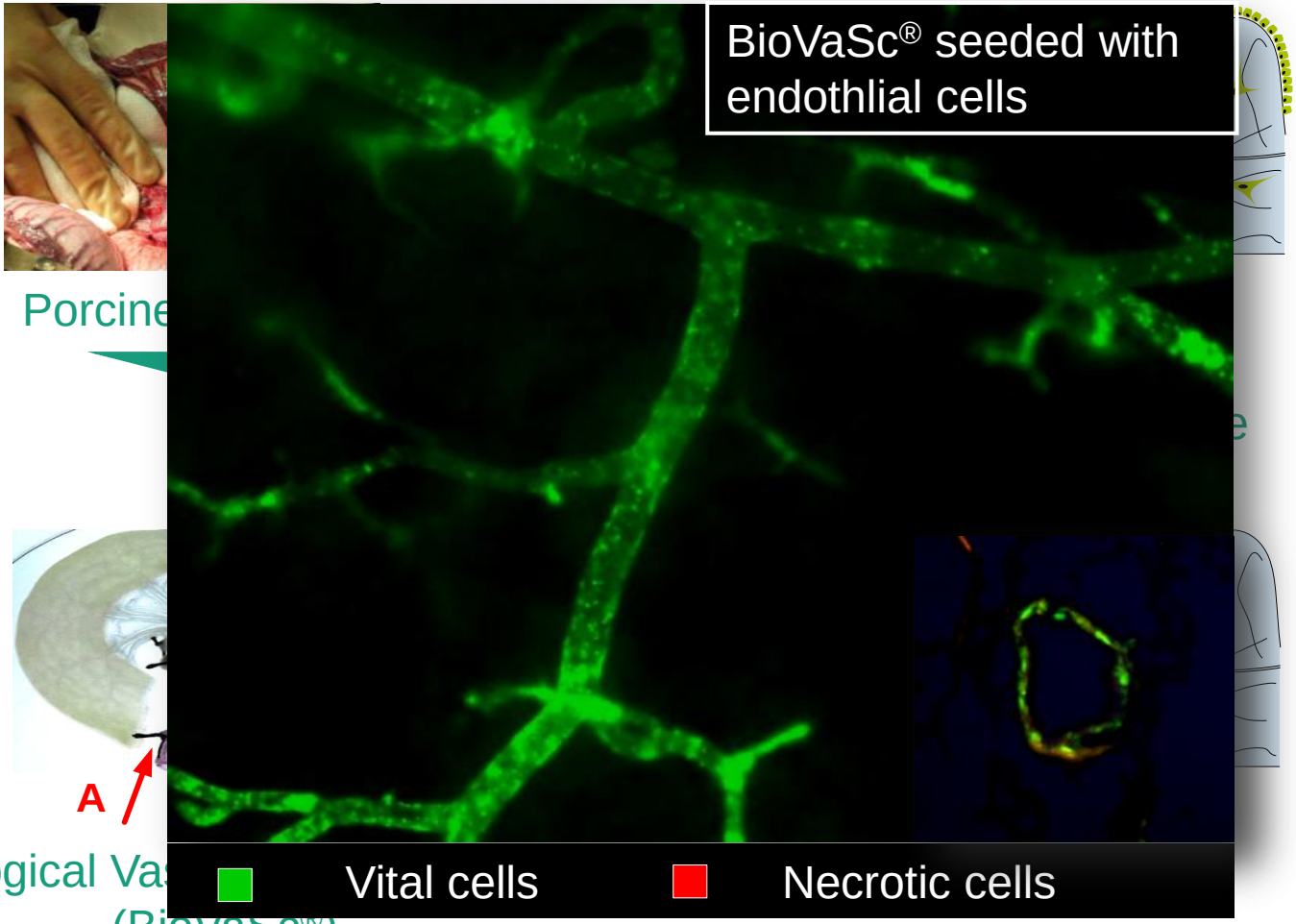
Generating complex tissues
Biological vascularized scaffold (BioVaSc®)



Mertsching H, Schanz J, Steger V, Schandar M, Schenk M, Hansmann J, Dally I, Friedel G, Walles T. Generation and transplantation of an autologous vascularized bioartificial human tissue. Transplantation. 2009 Jul 27;88(2):203-10.

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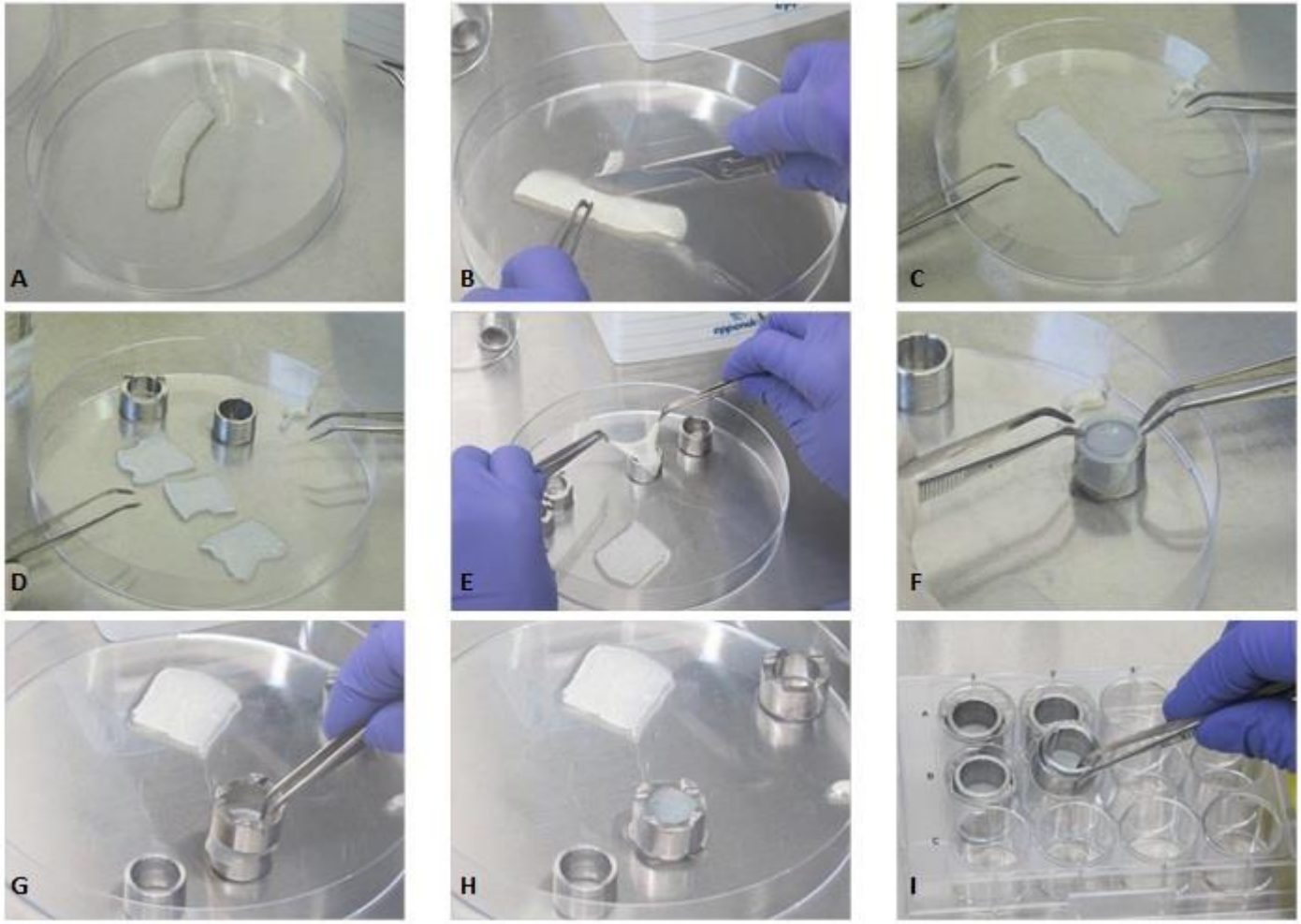
Static culture conditions



Stratmann AT et al., Establishment of a human 3D lung cancer model based on a biological tissue matrix combined with a Boolean in silico model. Molecular Oncology, 2014 Mar;8(2):351-65

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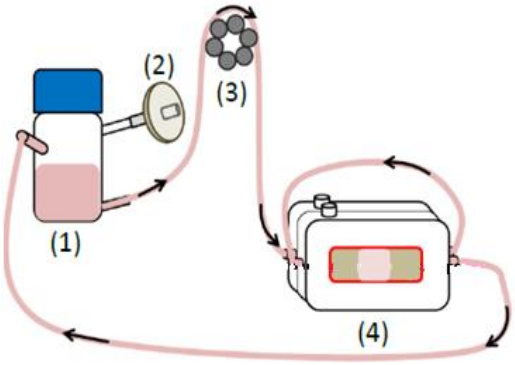
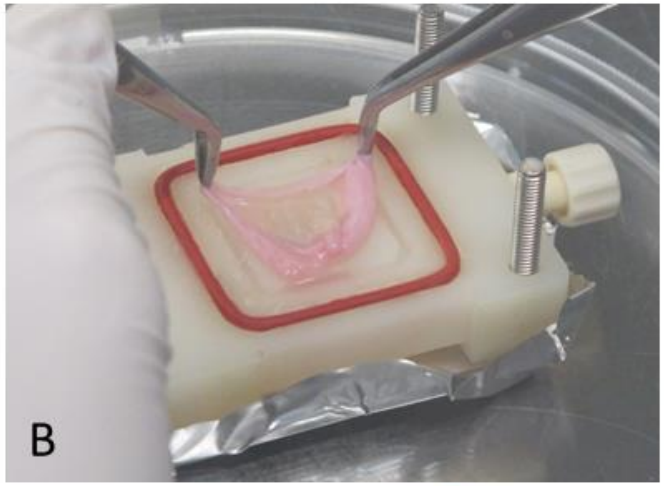
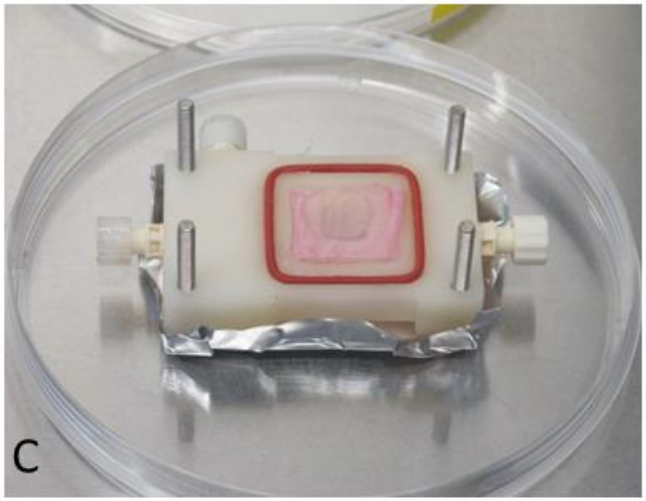
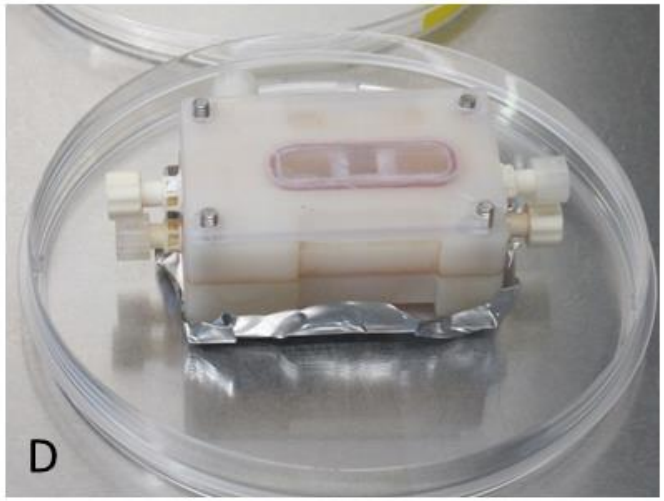
Dynamic culture conditions









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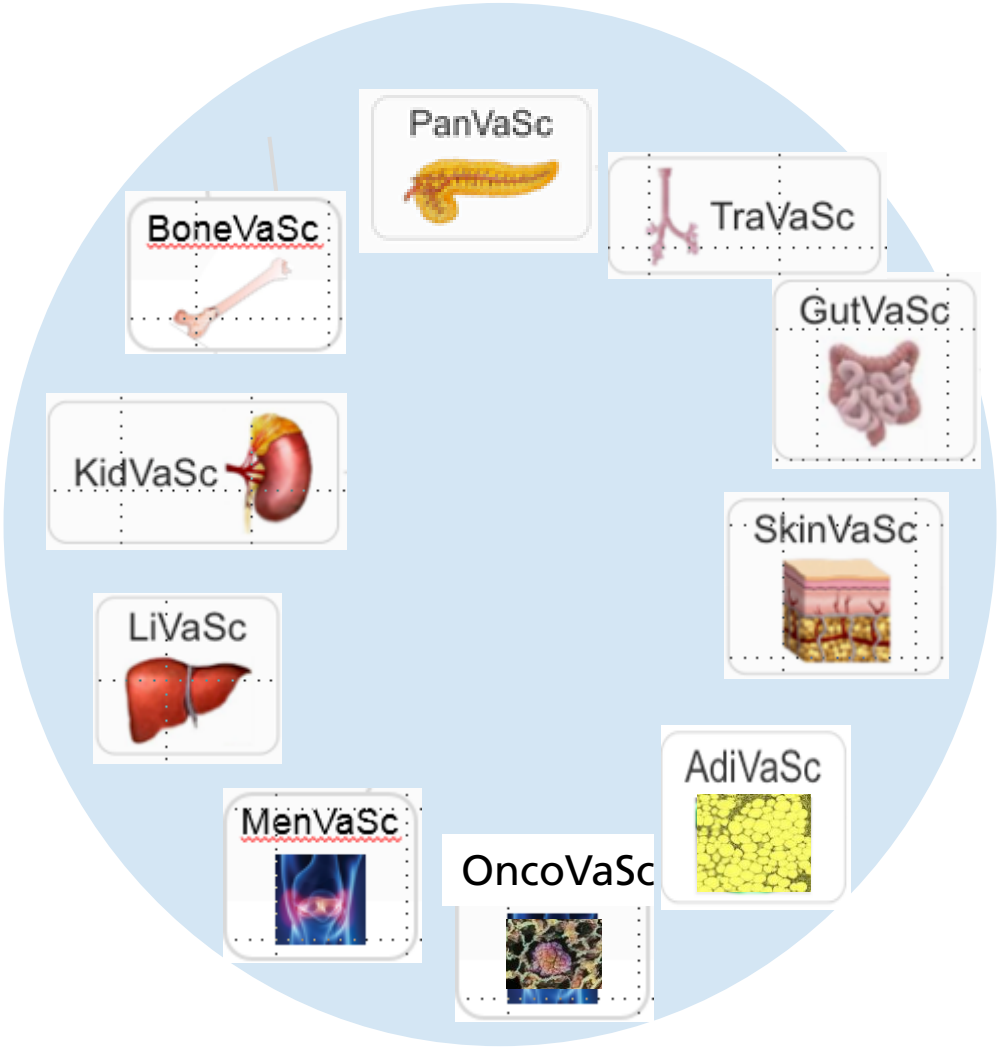
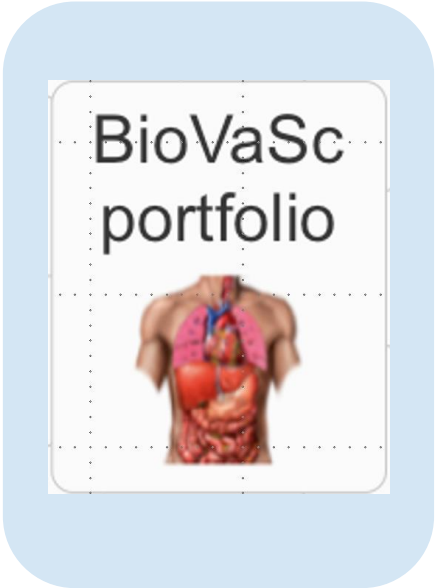
Unique technology: BioVaSc® – Platform for vascularized tissue models





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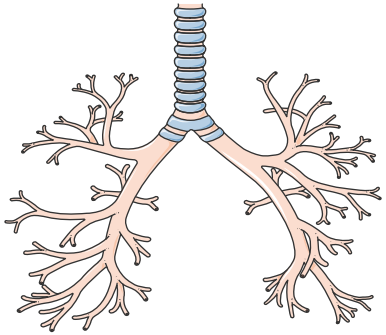

Research & development activities at Fraunhofer

HUMAN AIRWAY MUCOSA MODEL

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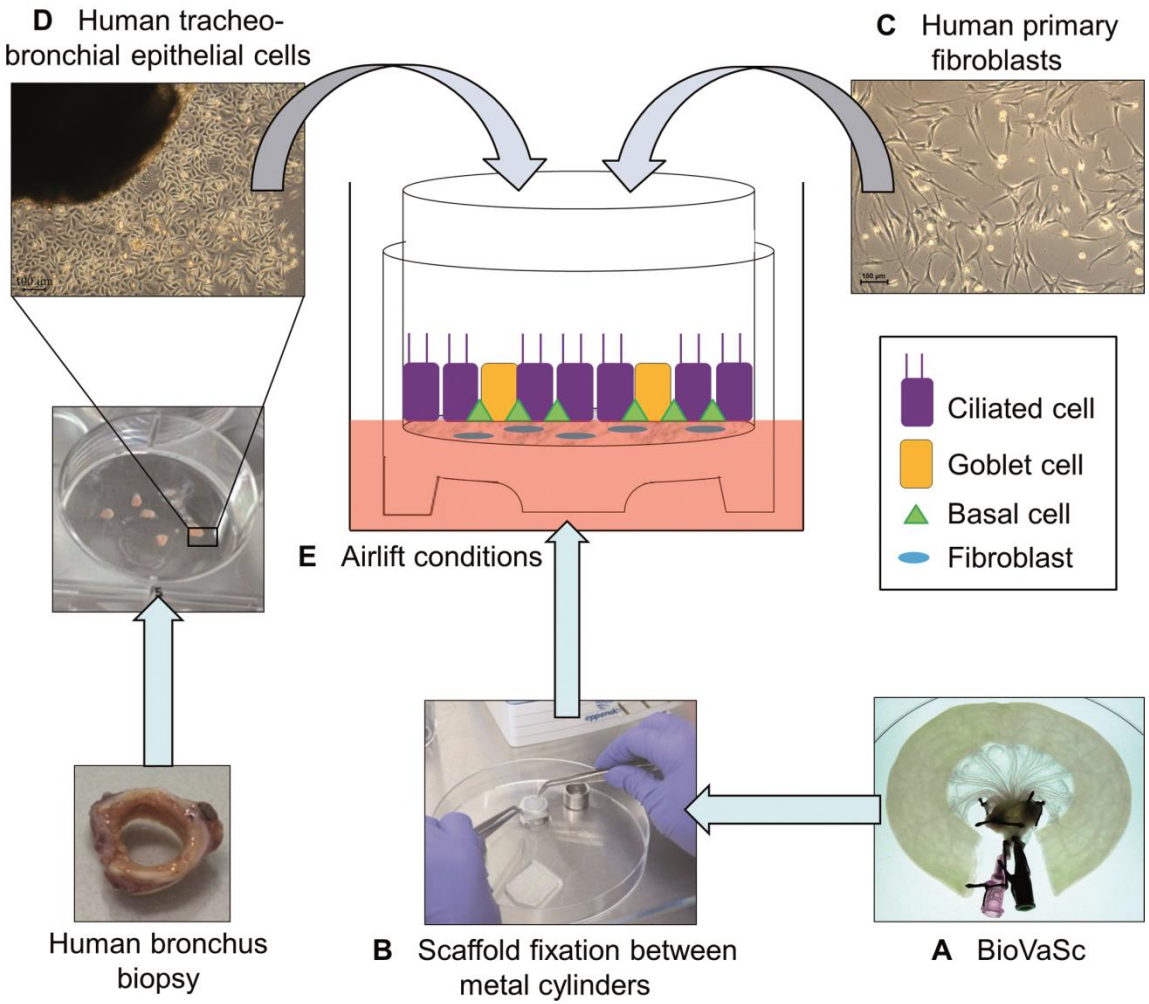
Generation of a 3D tissue model of the human airway mucosa



Steinke M et al., An engineered 3D human airway mucosa model based on an SIS scaffold. Biomaterials 35:7355-7362

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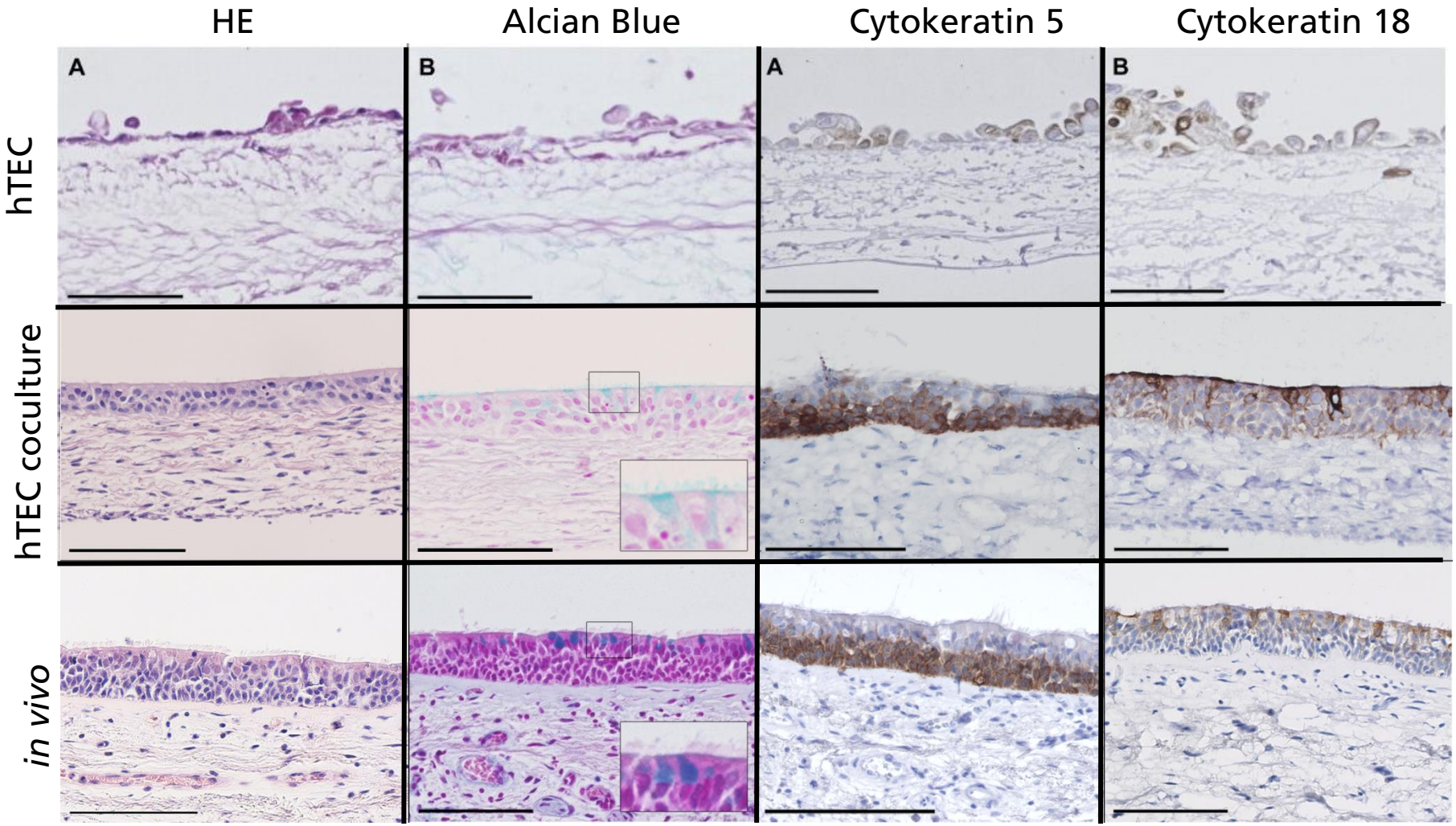
In vitro- in vivo correlation



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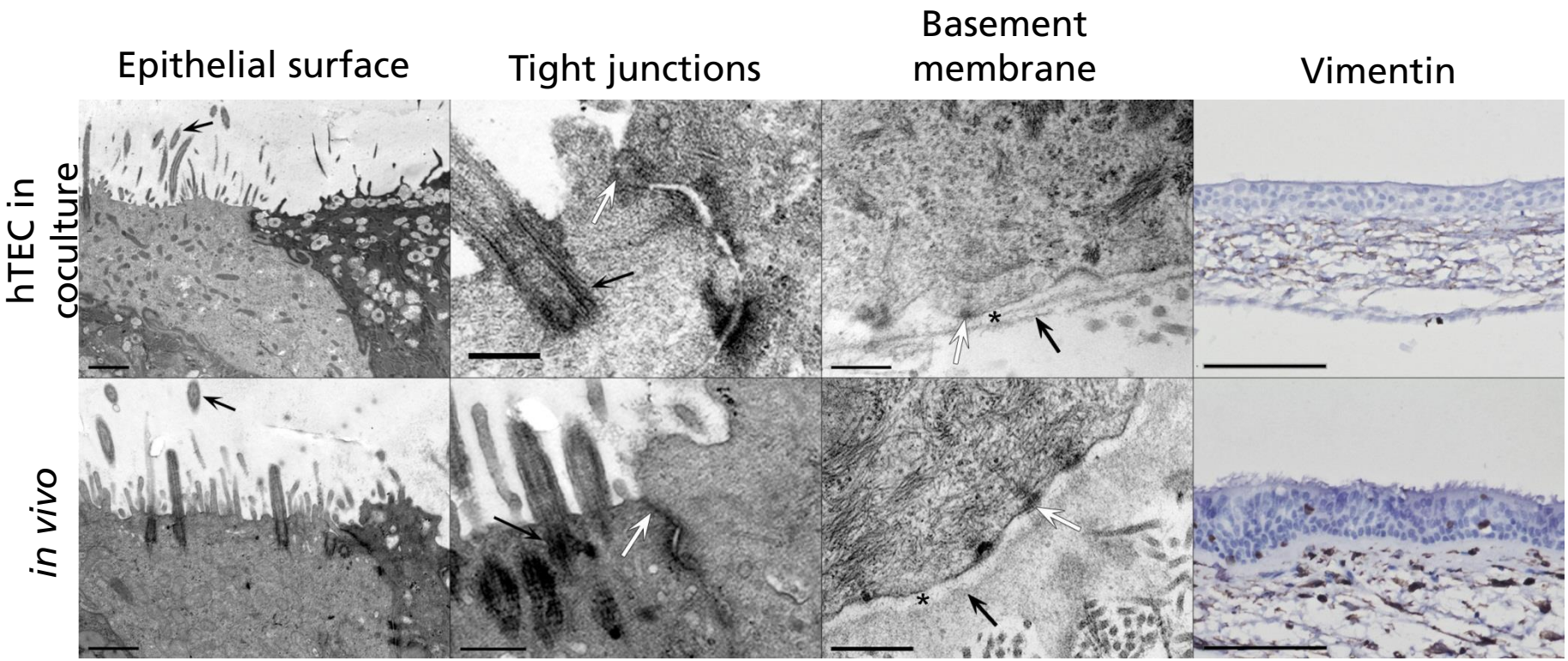
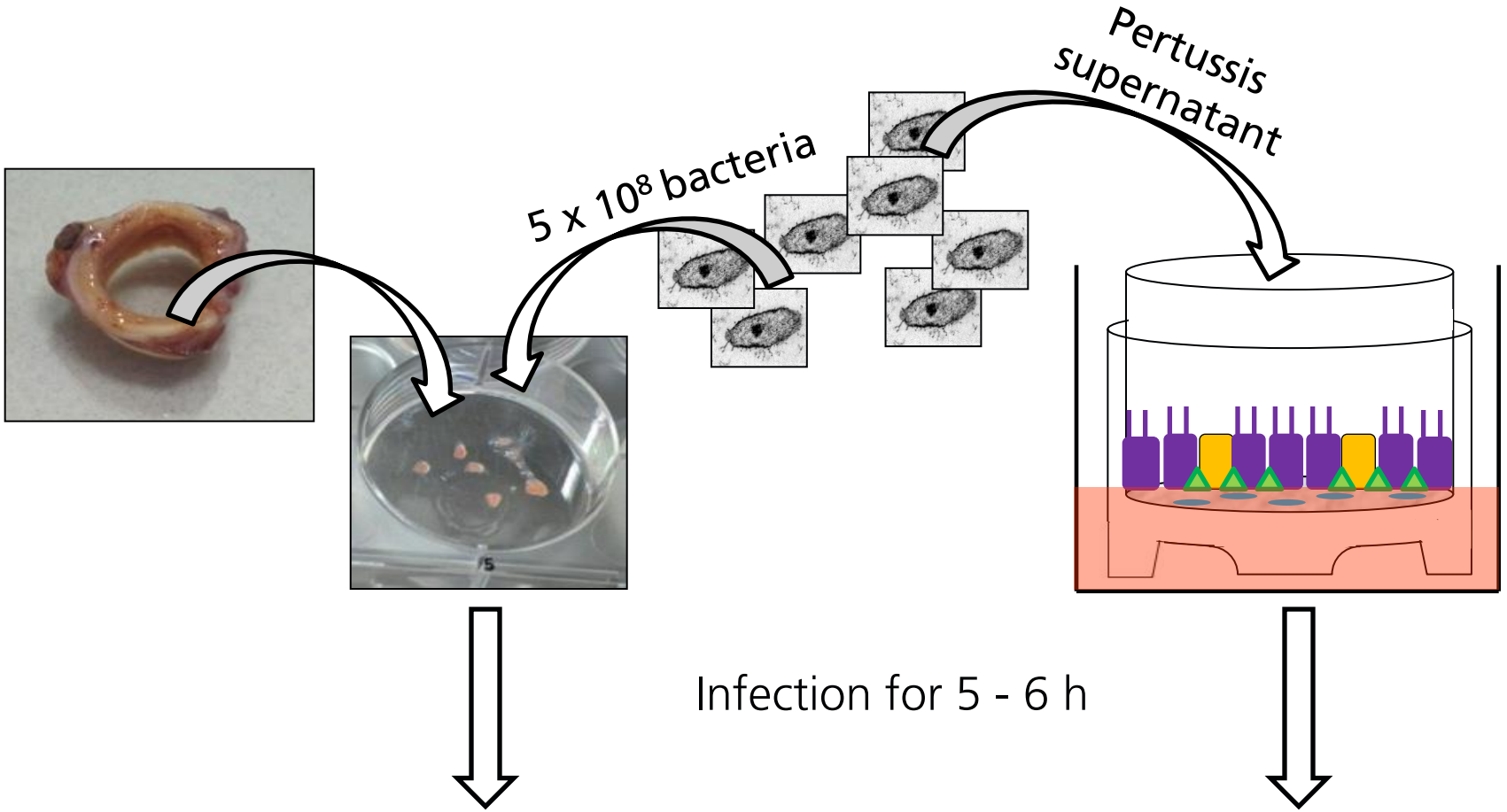


Steinke M et al., An engineered 3D human airway mucosa model based on an SIS scaffold. Biomaterials 35:7355-7362

Infection studies with *B. pertussis*



Steinke M et al., An engineered 3D human airway mucosa model based on an SIS scaffold. Biomaterials 35:7355-7362

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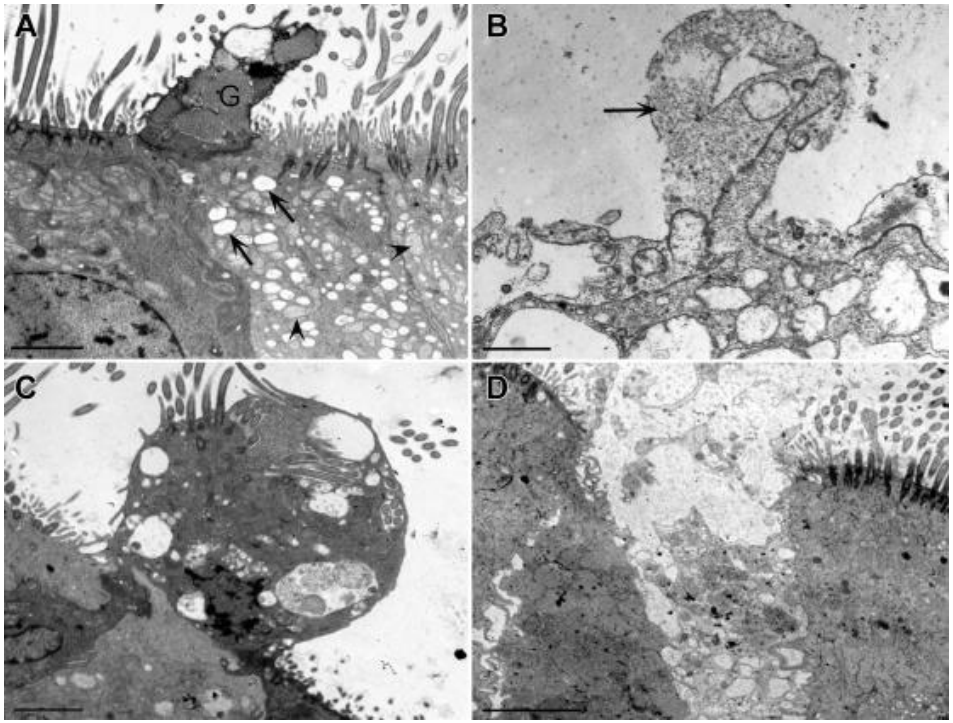
Ultrastructural analysis after infection with *B. pertussis*



Research & development activities at Fraunhofer

LUNG TUMOR MODEL

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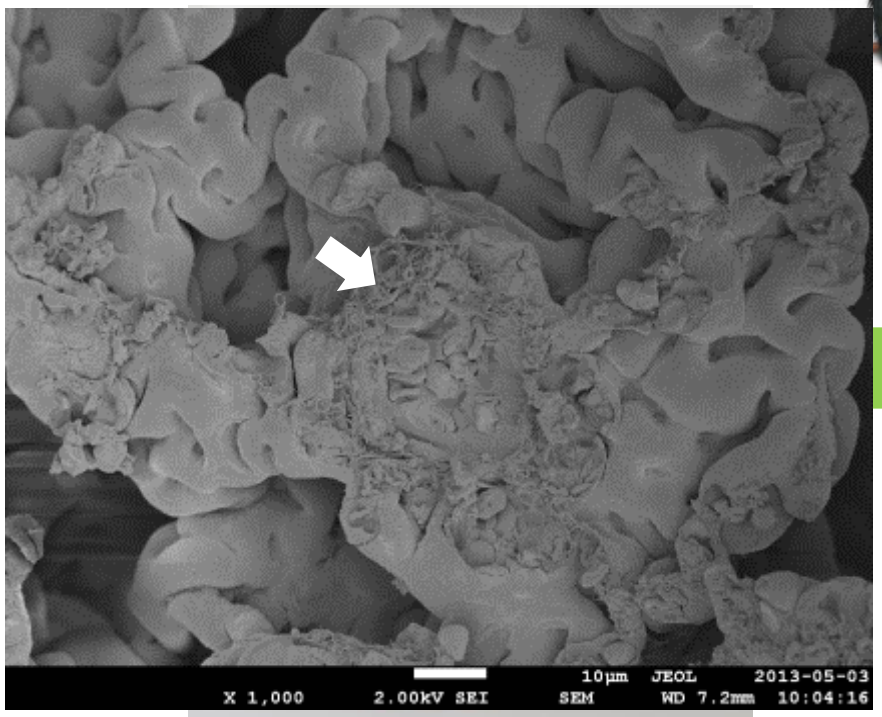
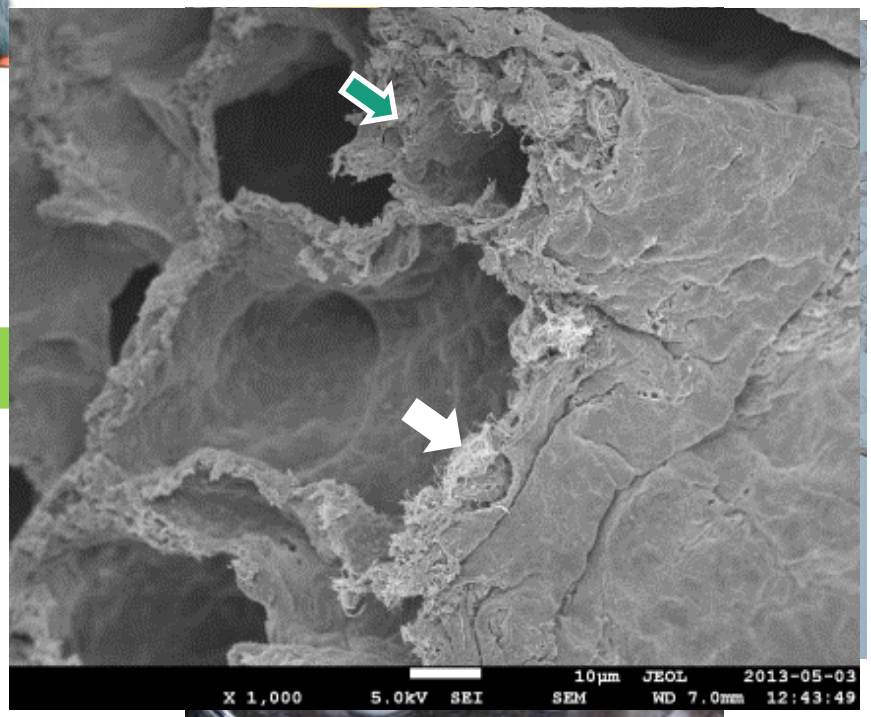
Establishment of decelluarized matrix

Native

Decellularized





Fecher et al., under review

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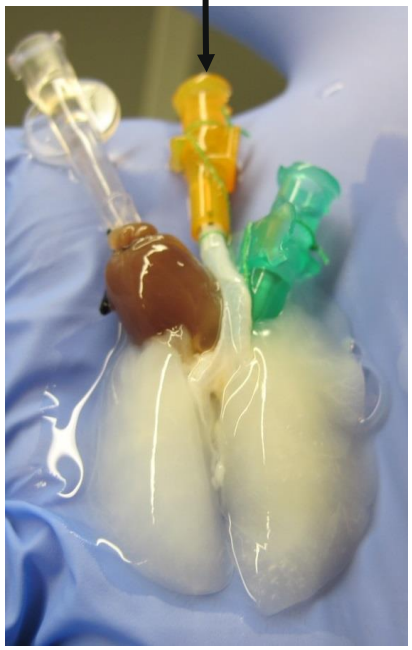
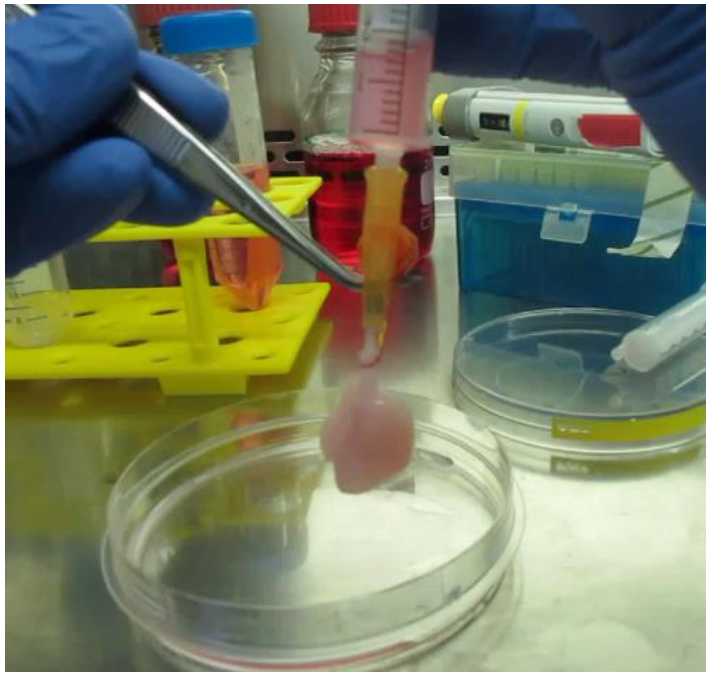
Recellularization of lung scaffold

Tumor cells





Fecher et al., under review

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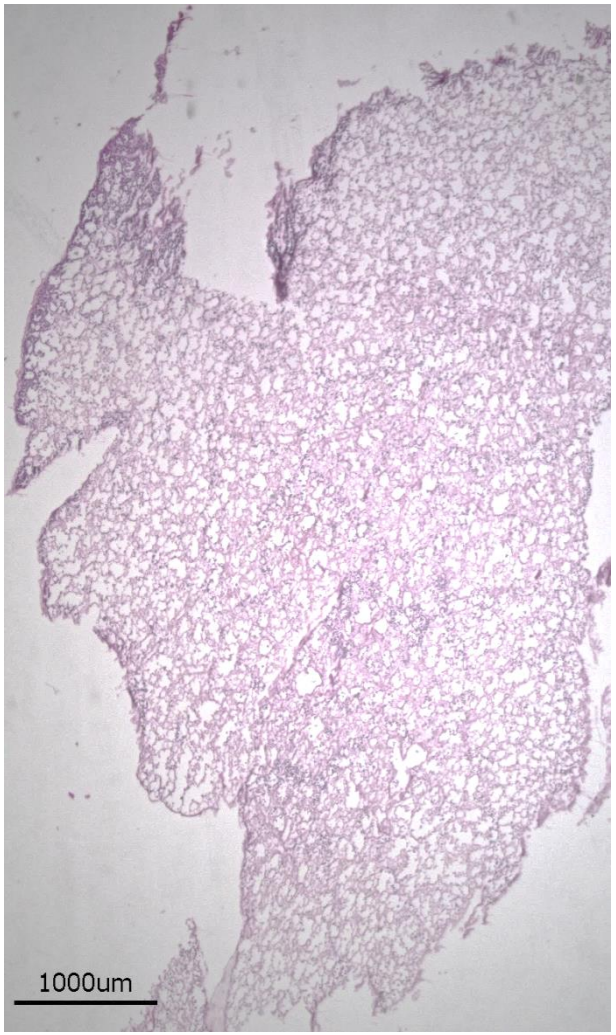
Tumor cells on the lung scaffold

A549



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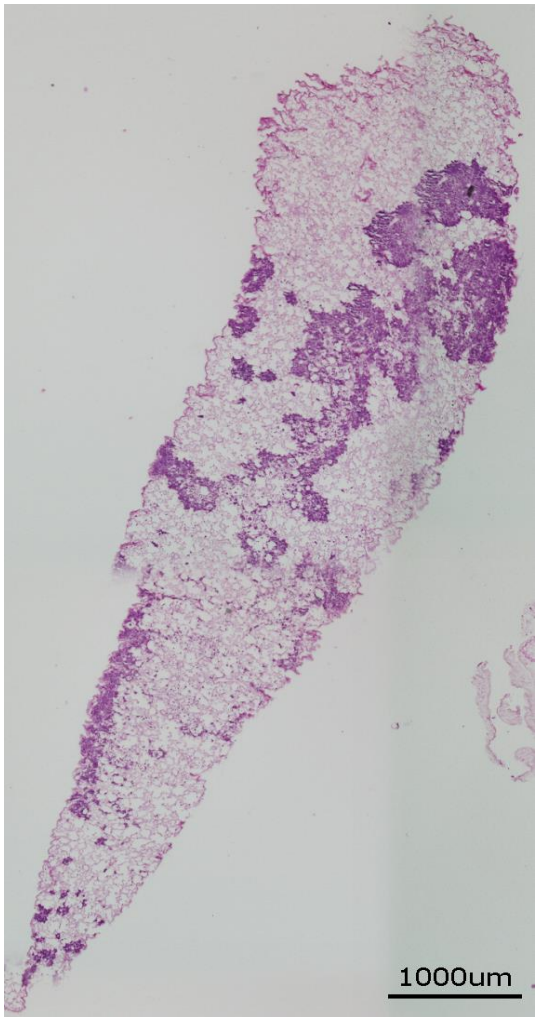

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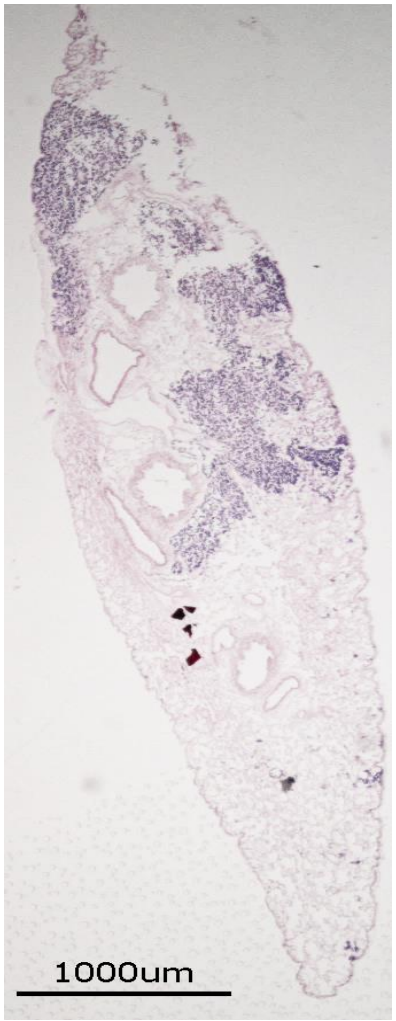

Fecher et al., under review

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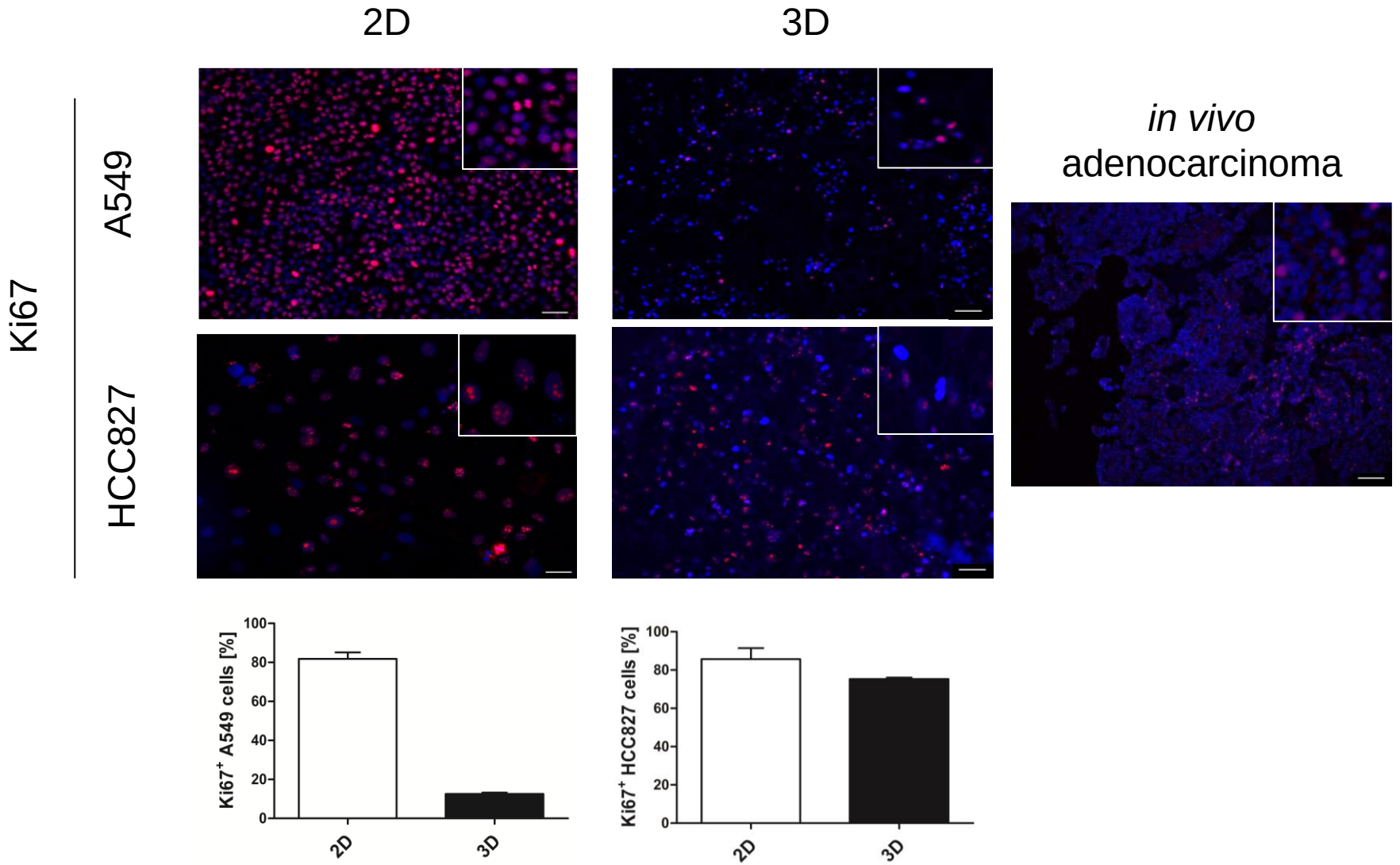
3D lung matrix induces a more *in vivo* – like phenotype



Fecher et al., under review

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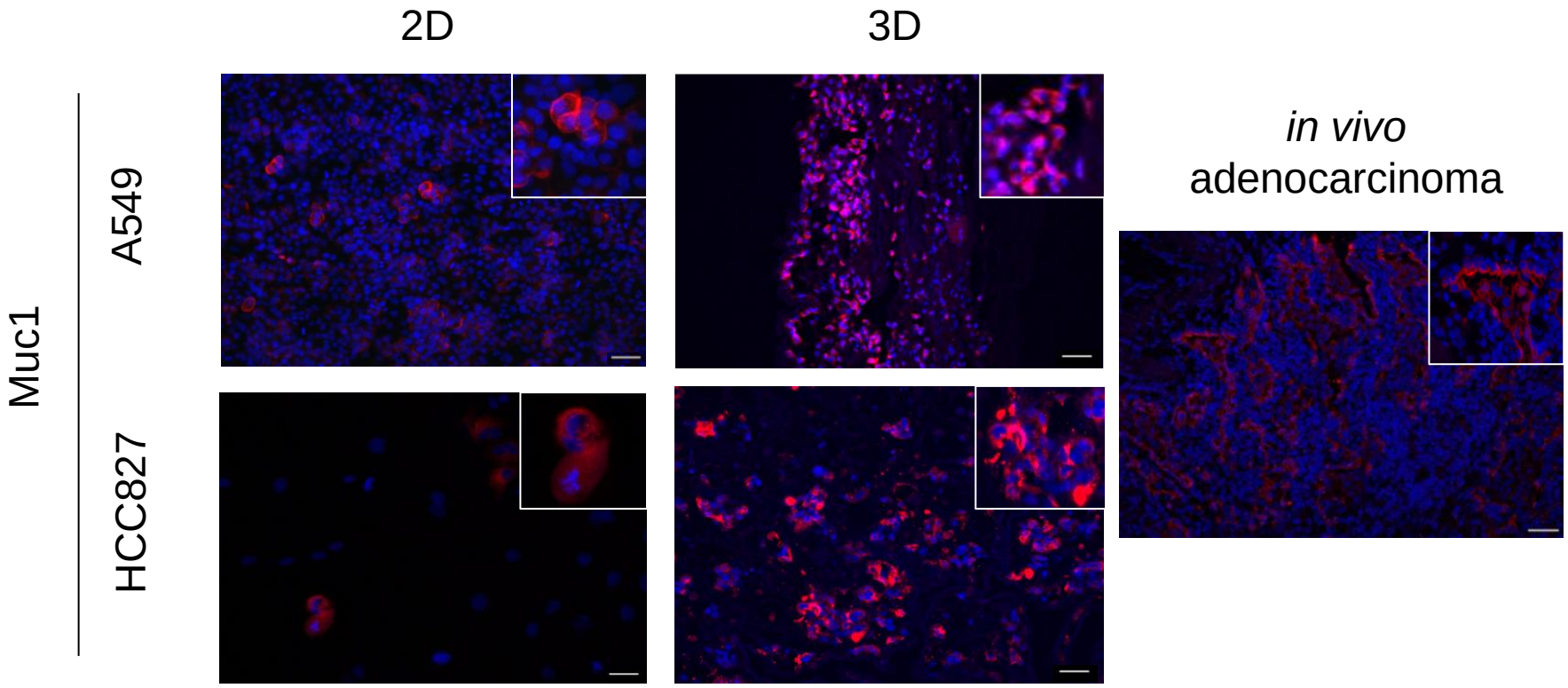

Fecher et al., under review

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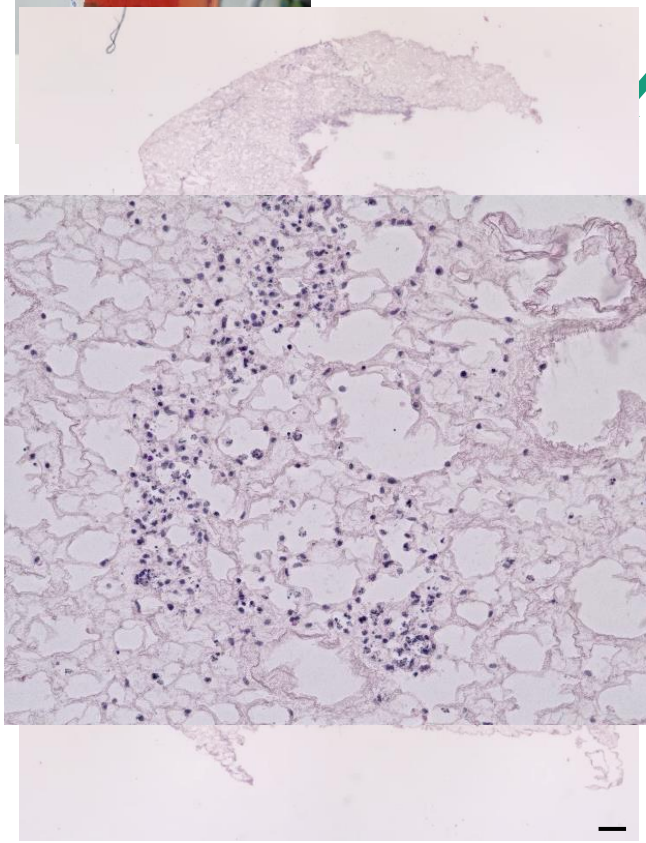
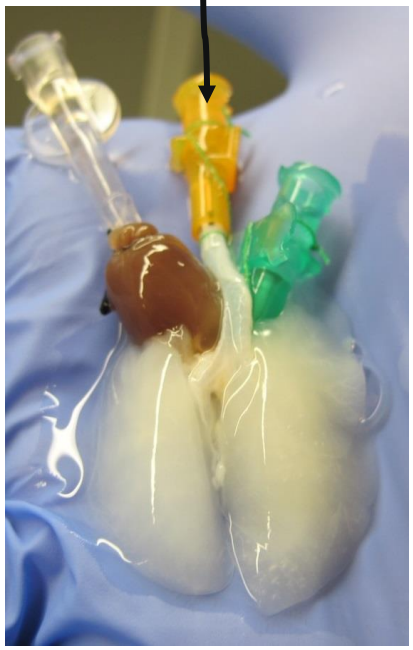
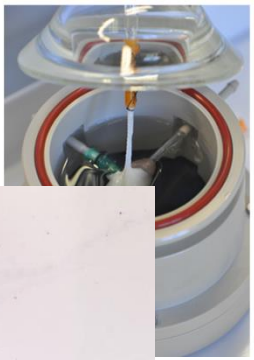
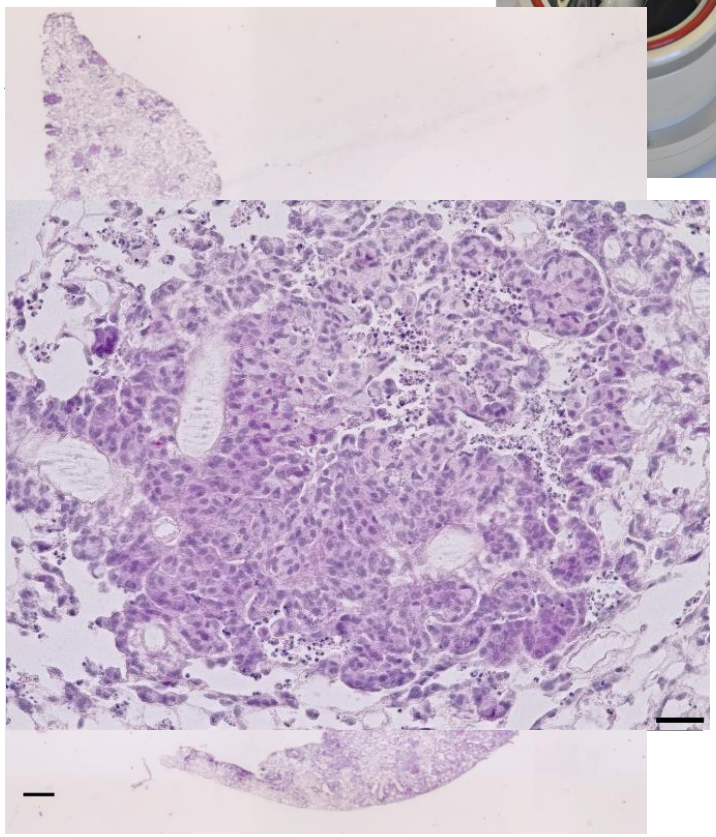
Effect of dynamic culture on tumor tissue formation



Tumor cells







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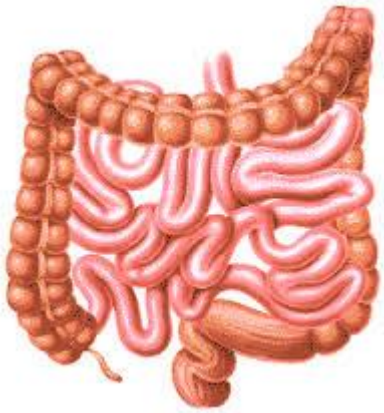

Research & development activities at Fraunhofer

INTESTINAL MODEL

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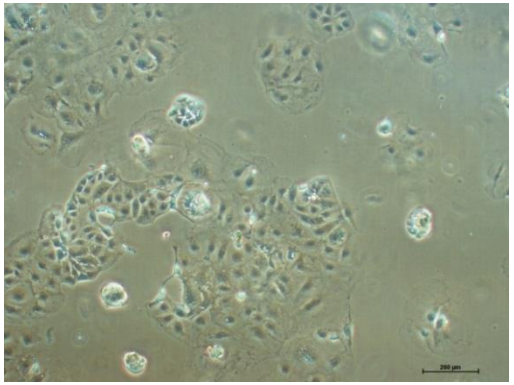
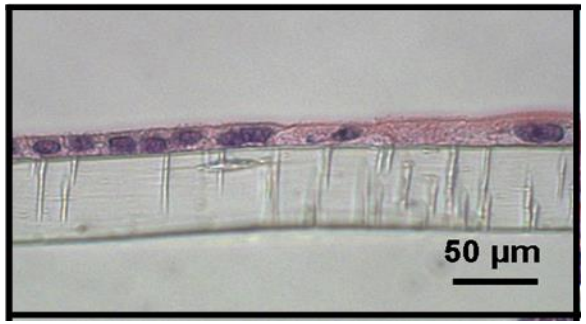
Intestinal Barrier - State of the art - Caco-2 Test



CaCo-2

PET-Insert with defined pore size (1,0 µm)

Culture conditions static bei 37° C, 5% CO_2, 21 days



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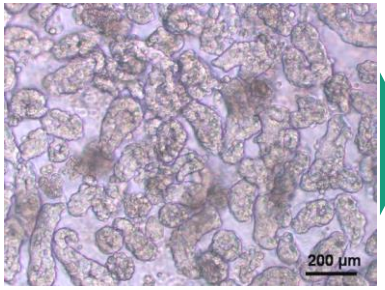
Development of primary intestinal model

Cell isolation from small intestinal tissue (jejunum)

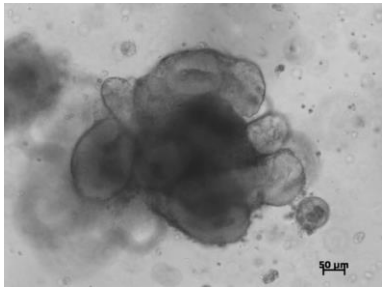
Cell expansion in matrigel (2-4 weeks)

3D-culture for 7 or 14 days in monoculture and co-culture with intestinal fibroblasts

Analysis:
(i) histology
(ii) qPCR
(iii) functional assays



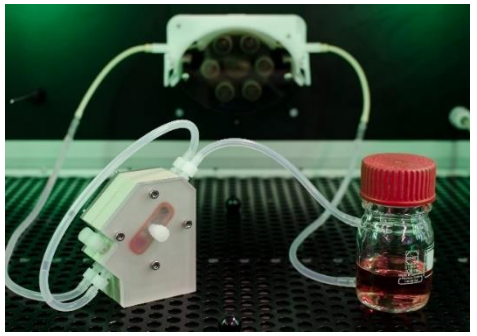
Crypts



Organoid culture

BioVaSc

Static culture



Dynamic culture

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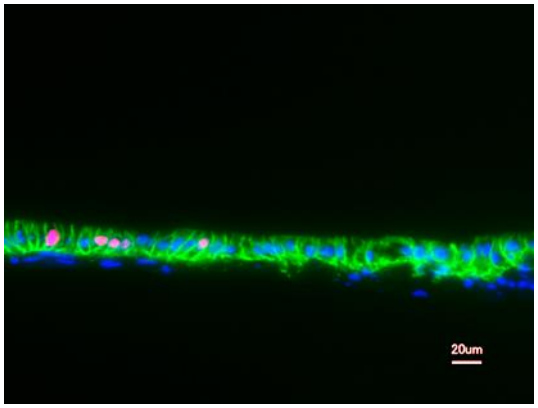
Results – immunohistological characterisation



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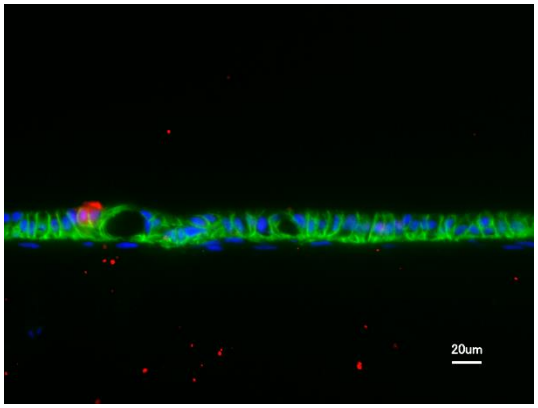
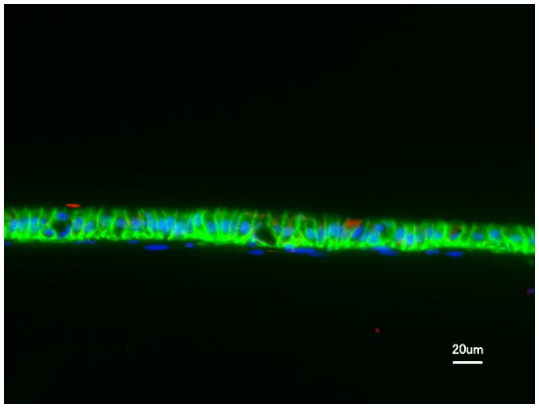
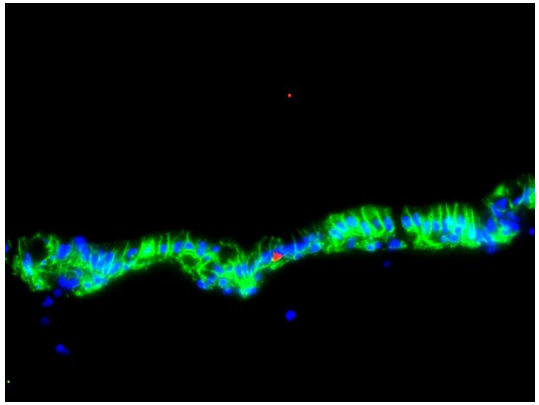


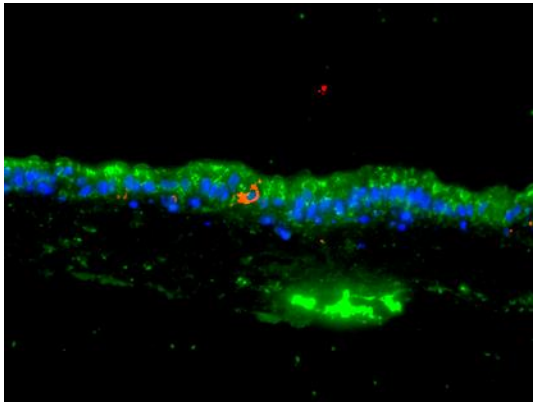
Mucin2



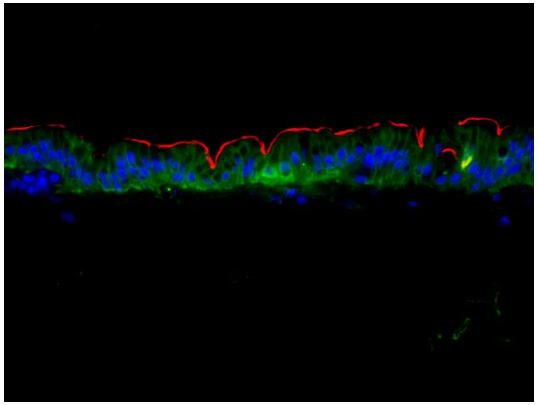
ZO1



Chromogranin A



Lysozyme



Villin

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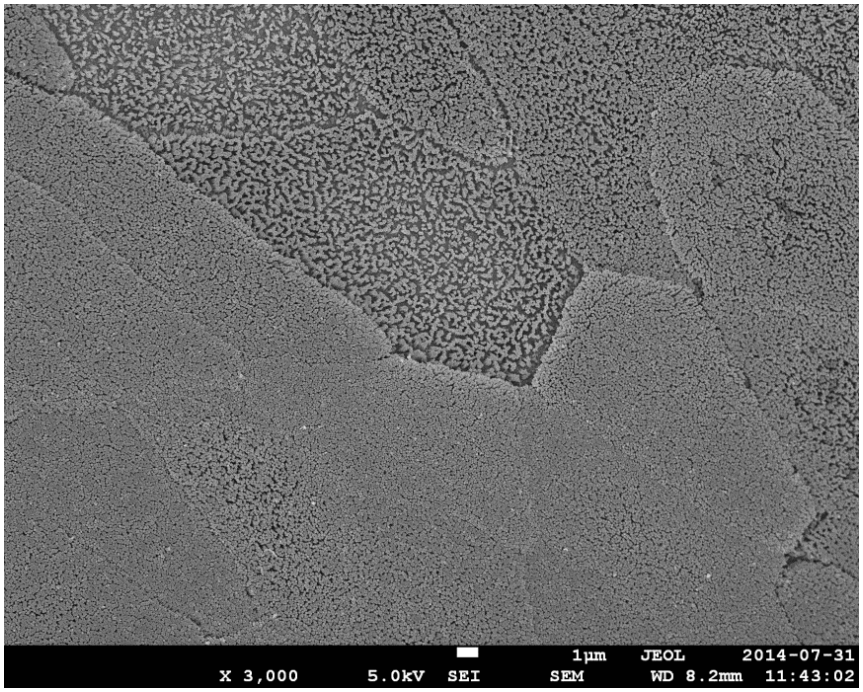
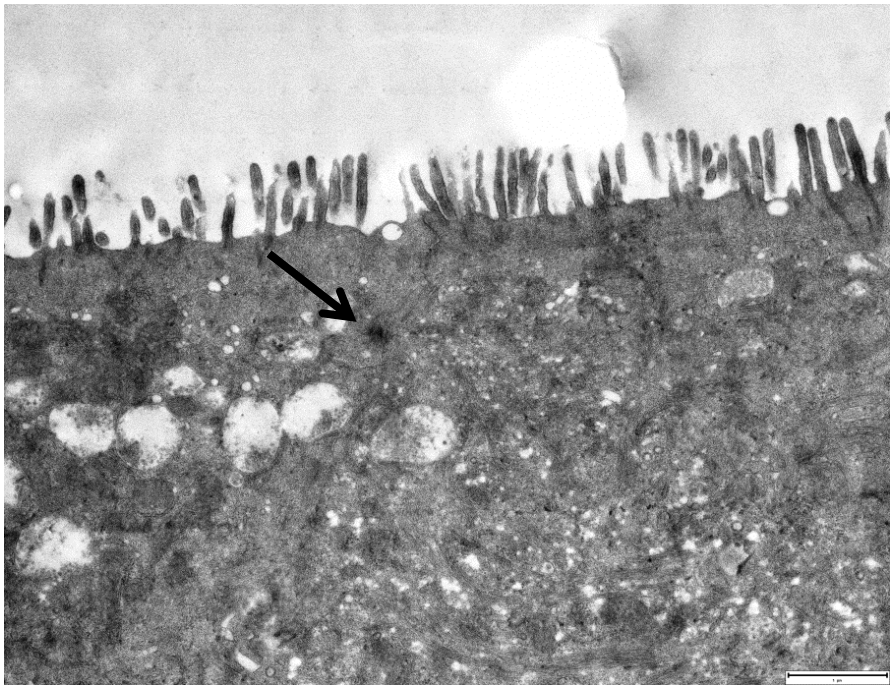
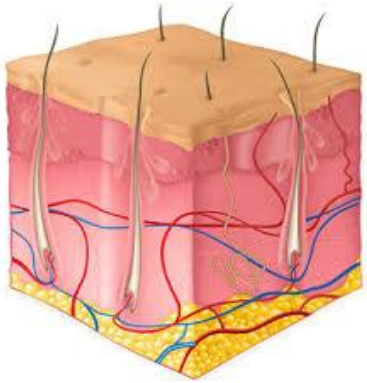
Results – electrone microscopy



SEM



TEM



Research & development activities at Fraunhofer

SKIN MODEL

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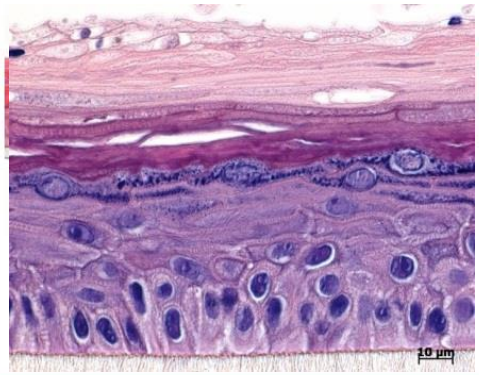
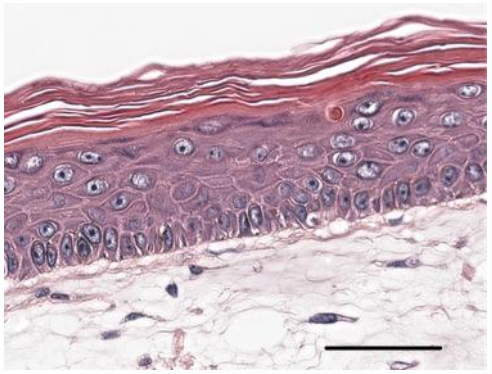
Three dimensional skin models

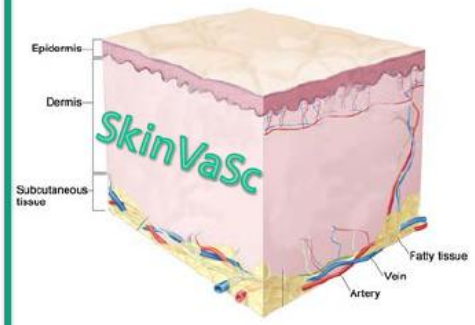
Epidermal model

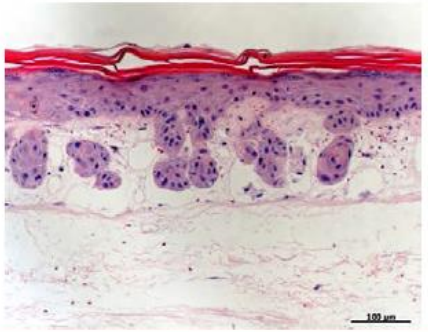


Full thickness skin model



Vascularized skin model





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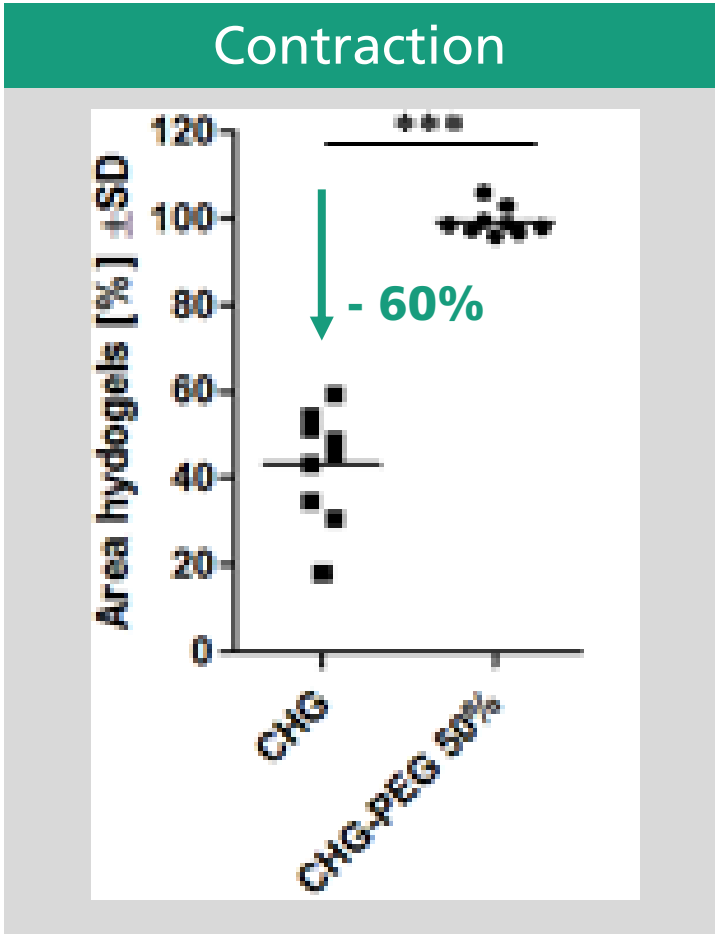
Full thickness skin model



Full thickness skin models

- Fibroblast mediated contraction of full-thickness skin models up to 60%
- Limitation to industrial applicability and life span
- Chemical crosslinking to reduce contraction with PEG
- Long term culture
- Repeated application of test substances

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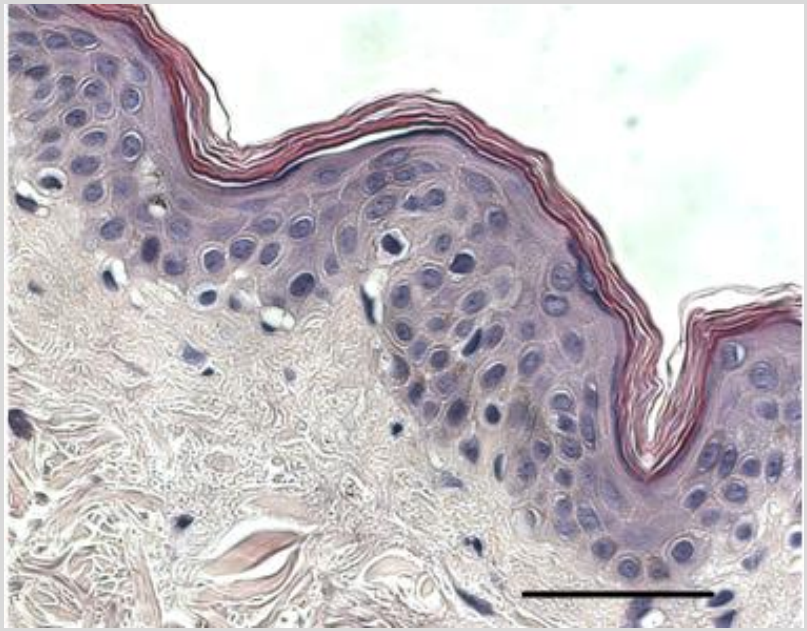
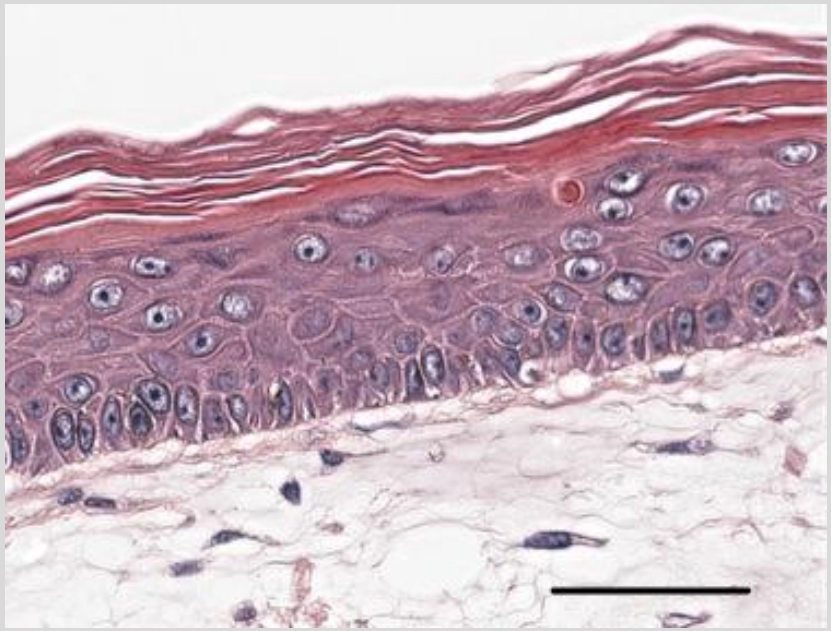
Full thickness skin model

Non-contracting collagen hydrogel

In vivo skin	*In vitro* skin
	

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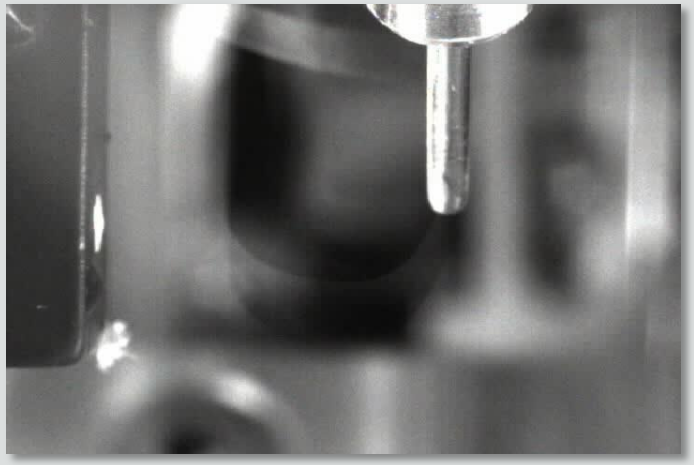
Full thickness skin model

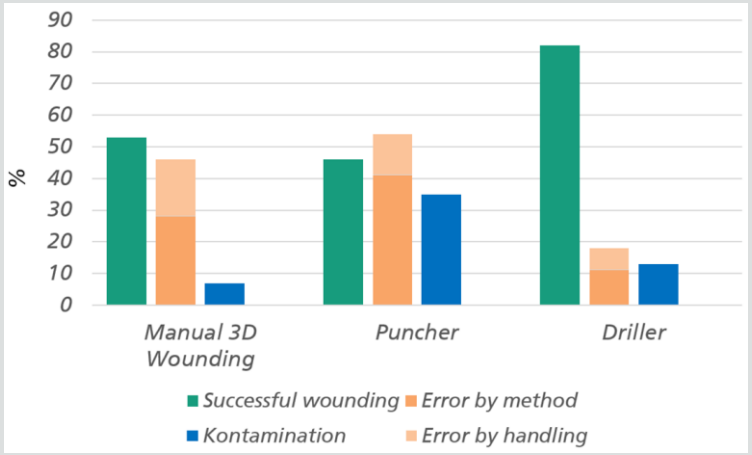
Wound model

- **Features:**
  - Reproducibility (shape and depth)
  - Sterility
- **Applications**
  - Efficacy testing for wound healing

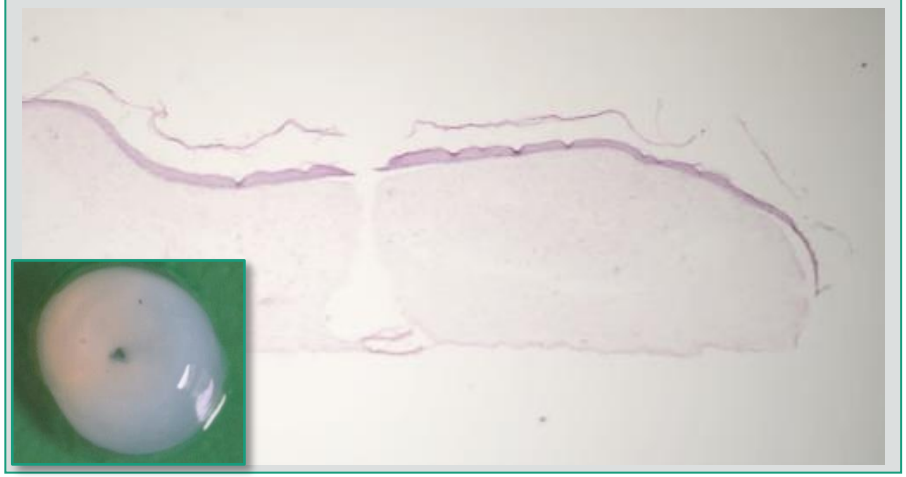
Wounding (video)



Comparison with other methods



Wounded skin equivalent



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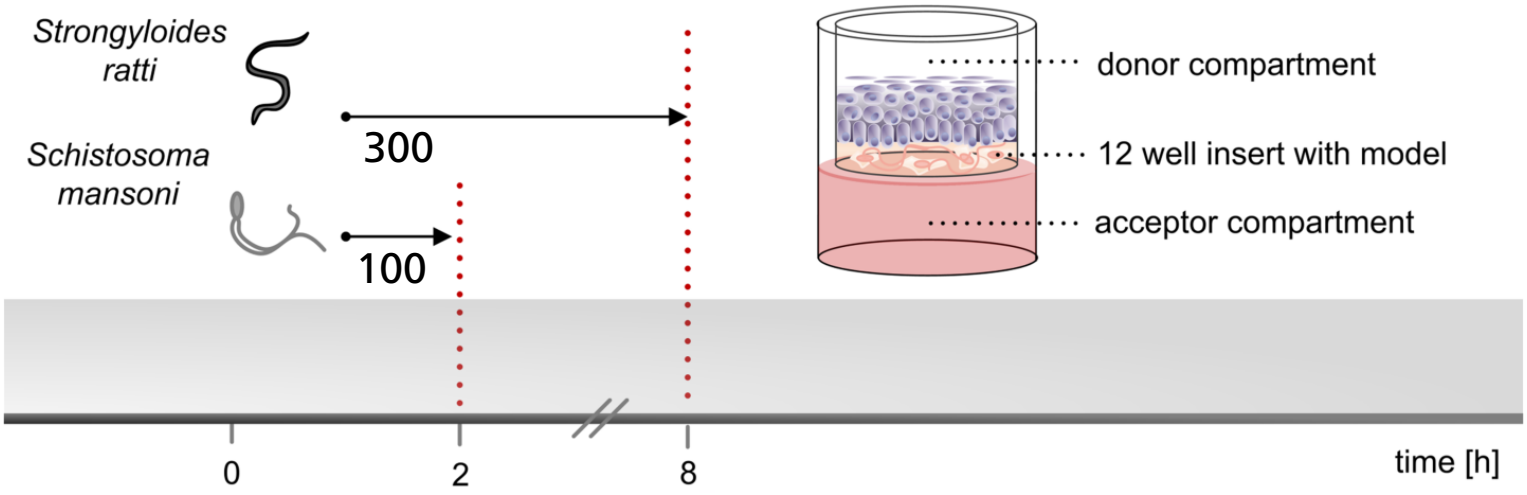
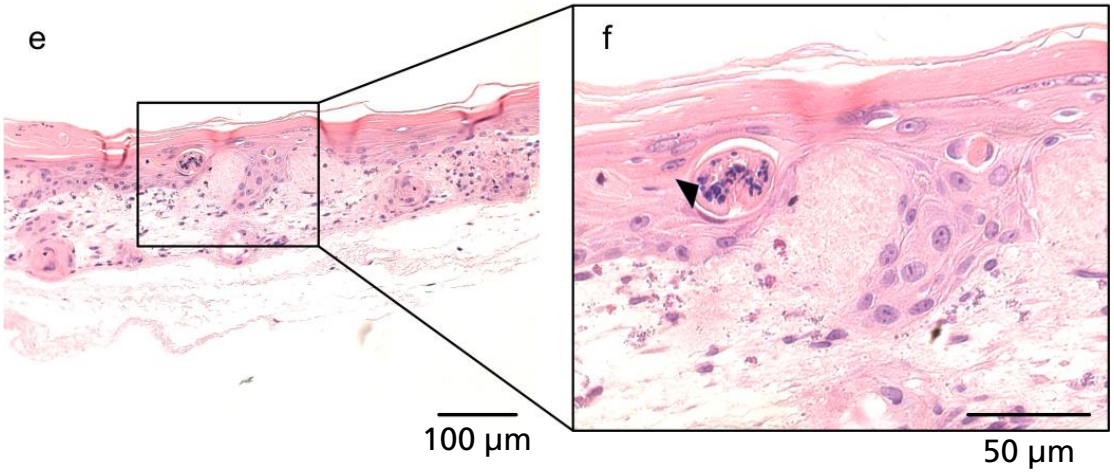
Helminth infection studies





larvae of *S. mansoni* (arrow) found in the reconstructed human skin (RhS)

Jannasch M., Groeber F., Brattig N., Hoffmann W. , Walles H., Hansmann J.; Three dimensional skin equivalents as an in vitro test system for percutaneous worm infection; Experimental Parasitology, 2015

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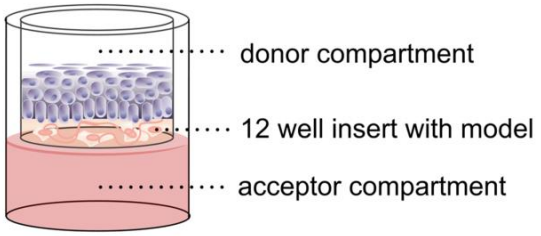
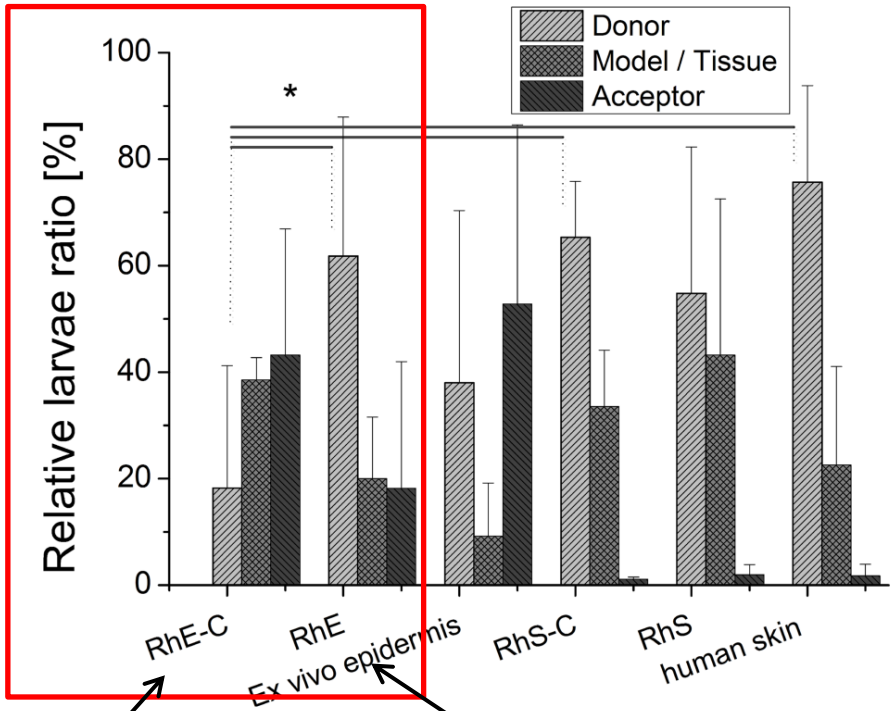
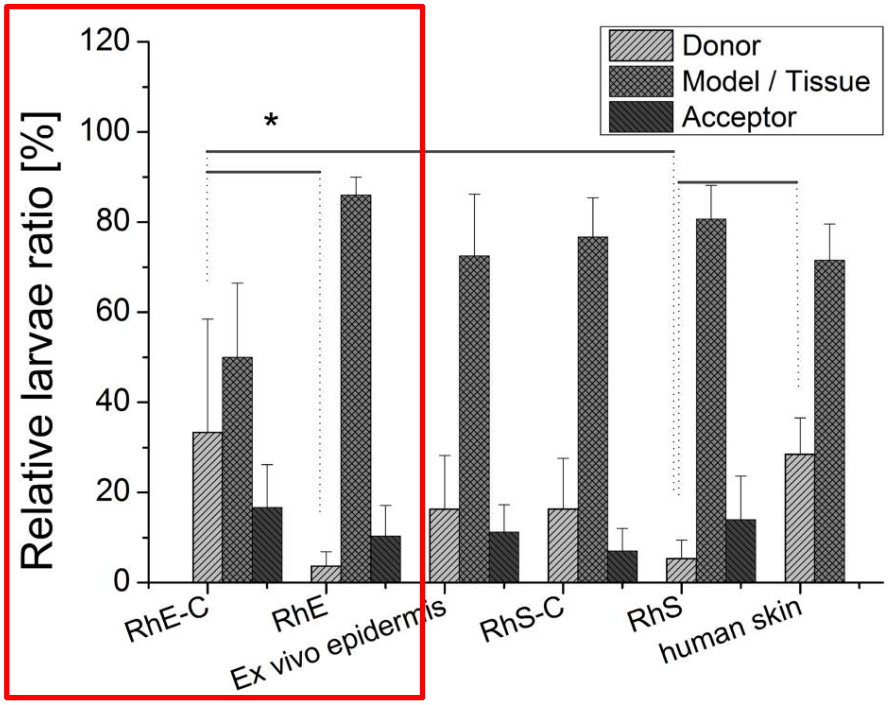
Helminth infection studies



- Strongyloides ratti



- Schistosoma mansoni



Cell-free collagen carrier

Reconstructed epidermis

Jannasch M., Groeber F., Brattig N., Hoffmann W. , Walles H., Hansmann J.; Three dimensional skin equivalents as an in vitro test system for percutaneous worm infection; Experimental Parasitology, 2015

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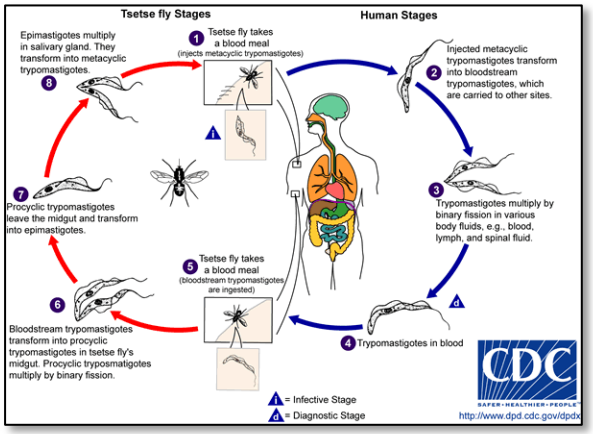
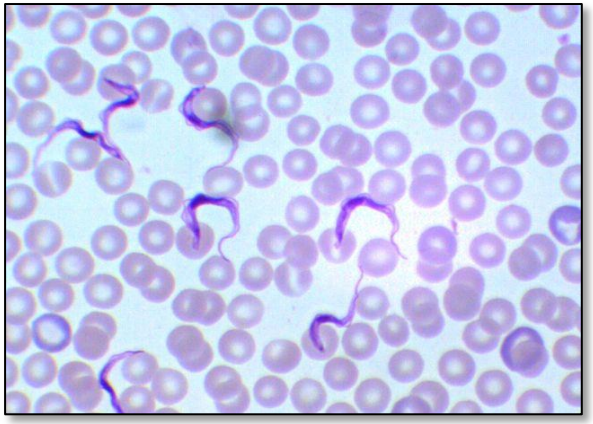
Infection Studies – Trypanosoma

Collaboration Prof. Engstler University of Würzburg

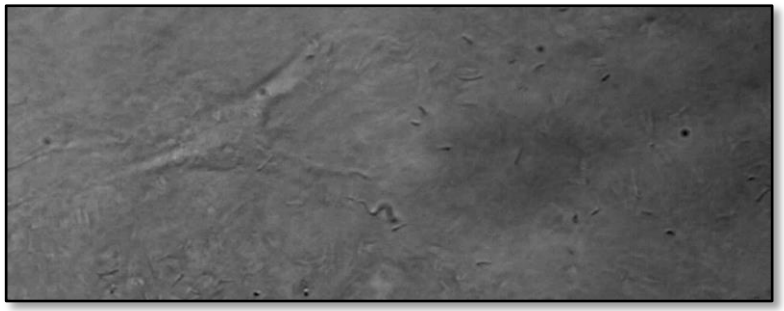


T. brucei life cycle



T. brucei larvae

Tsetse fly infecting a full thickness skin equivalent



T. brucei larvae after infection

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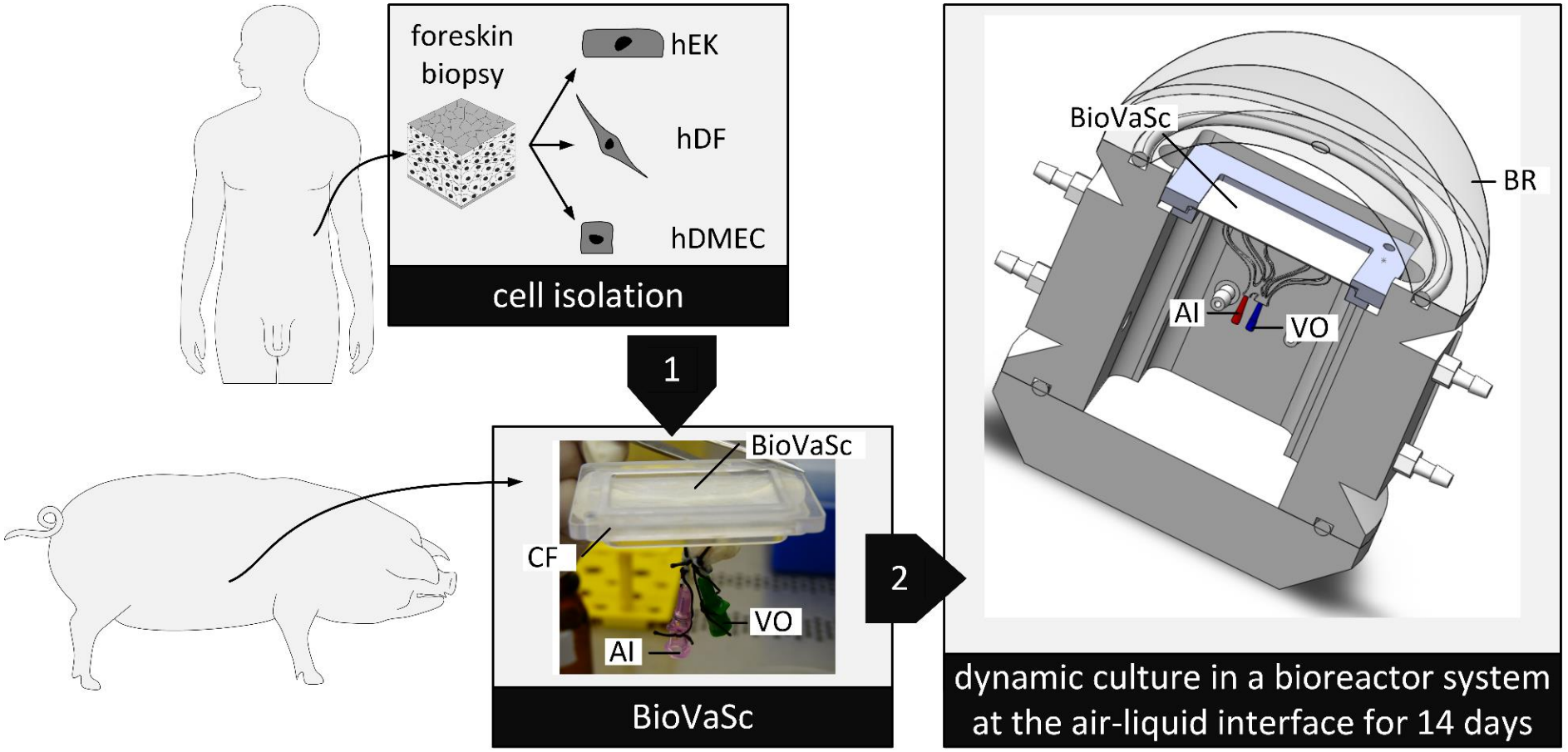
Vascularized skin model



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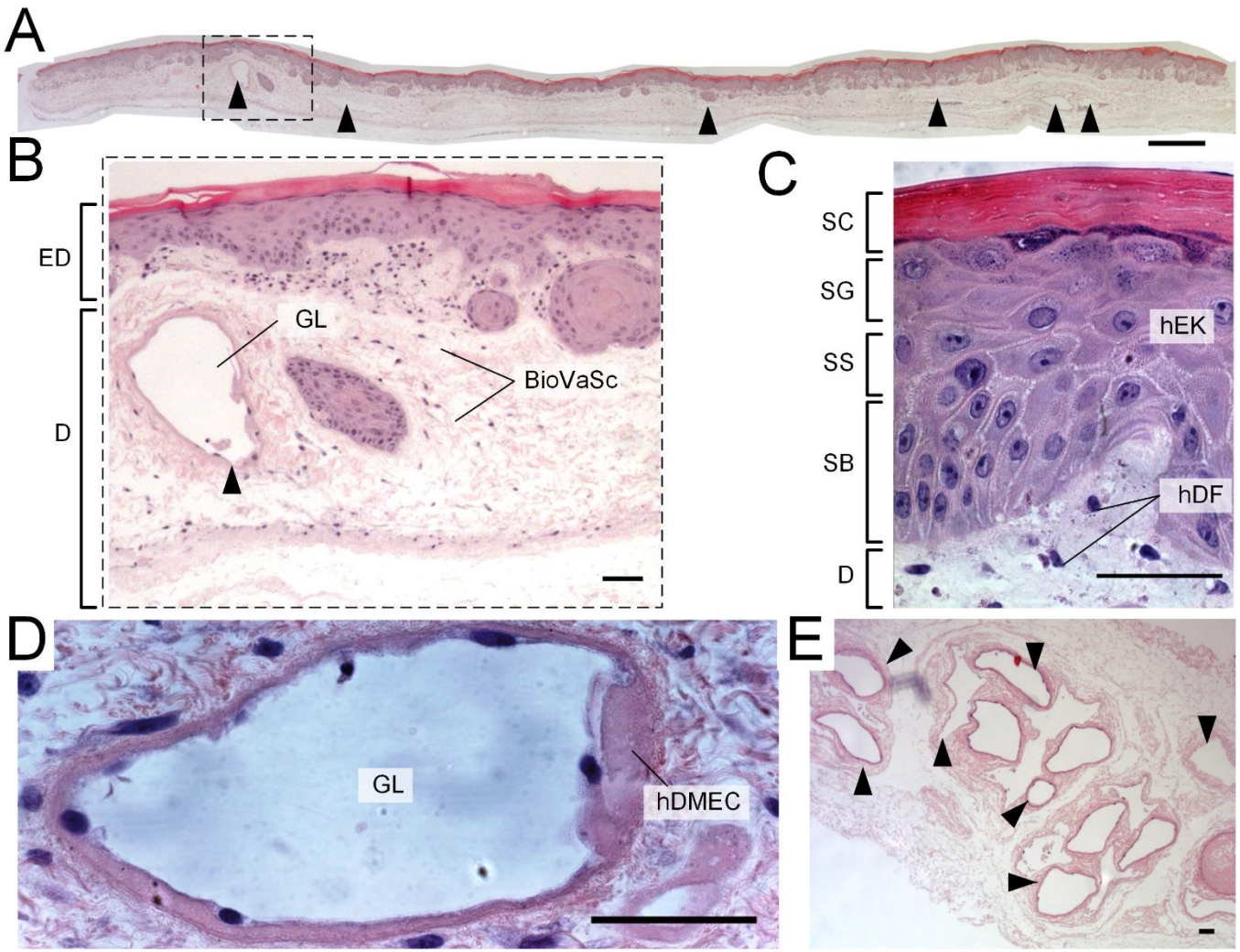
Vascularized skin model



▲: Vessels

ED: Epidermis

D: Dermis

GL: Vessel lumen

hEK: Human epidermal keratinocytes

hDF: Human dermal fibroblasts

hDMEC: Human microvascular endothelial cells

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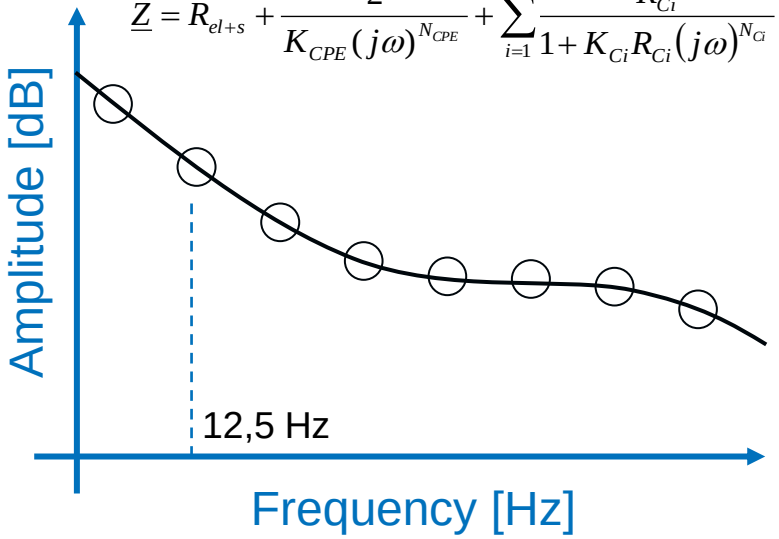
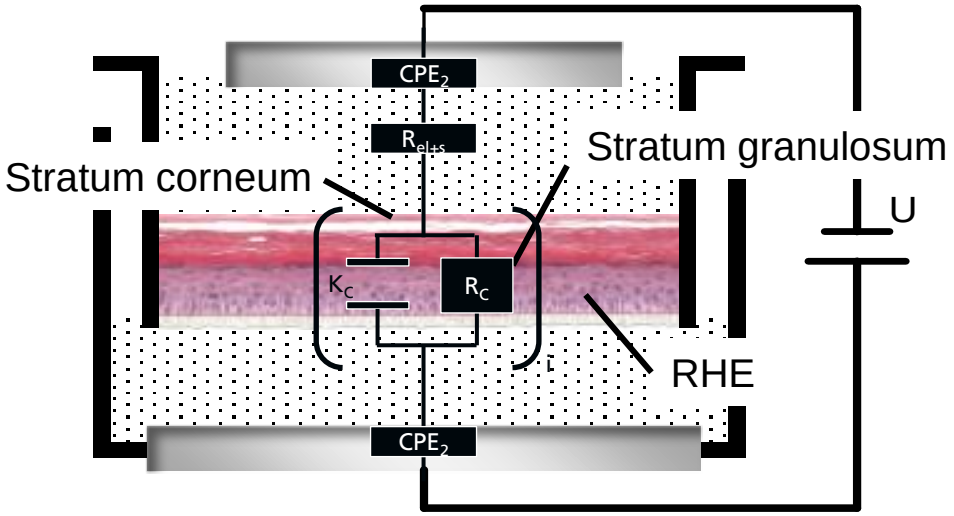
Assessment of mild irritative effects via impedance spectroscopy

$$\underline{Z} = R_{el+s} + \frac{2}{K_{CPE}(j\omega)^{N_{CPE}}} + \sum_{i=1}^{n} \frac{R_{Ci}}{1 + K_{Ci} R_{Ci} (j\omega)^{N_{Ci}}}$$





F. Groeber, L. Engelhaldt, S. Egger, H. Werthmann, M. Monaghan, H. Walles, J. Hansmann. Impedance spectroscopy for the non-invasive characterization of in vitro epidermal models.

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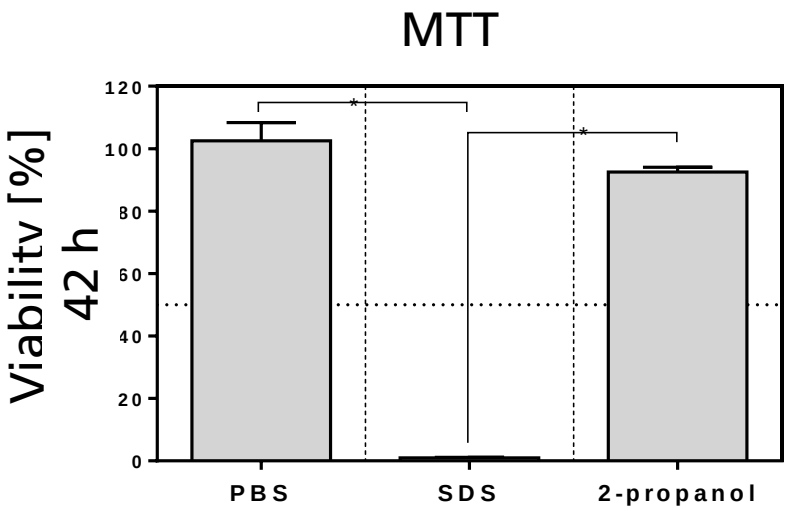
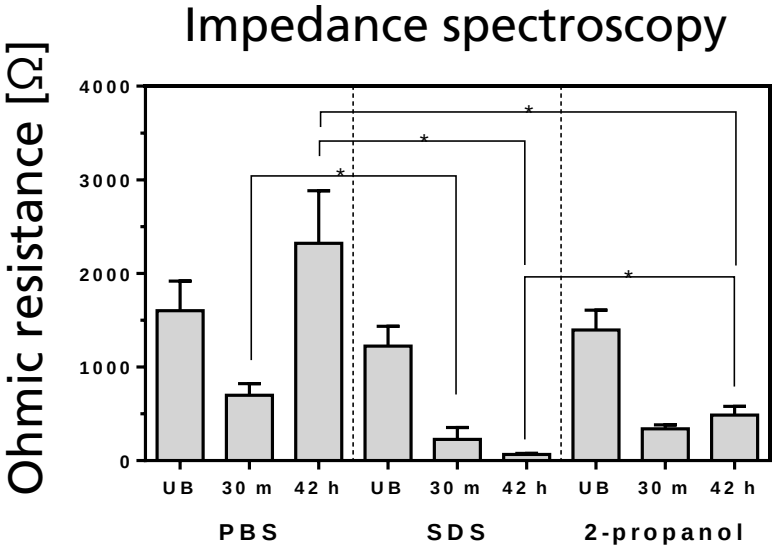
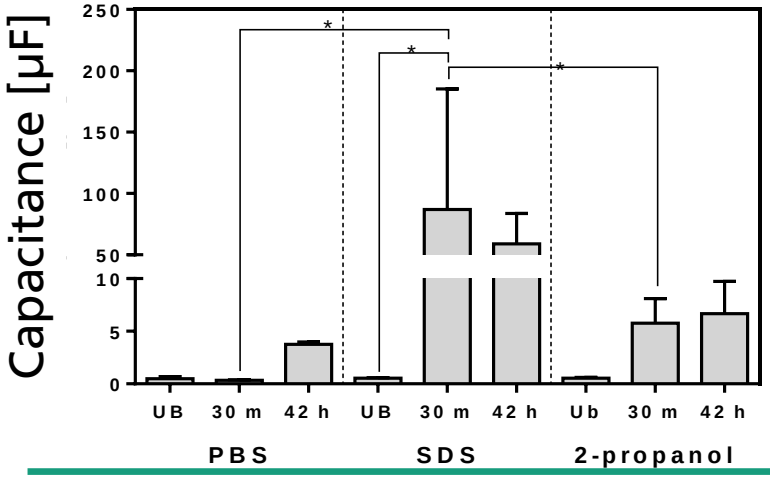
Assessment of mild irritative effects via impedance spectroscopy







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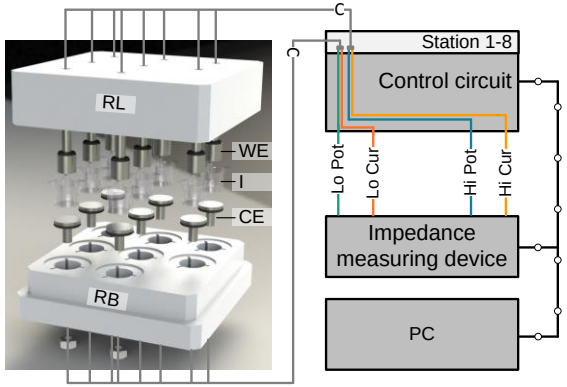

F. Groeber, L. Engelhaldt, S. Egger, H. Werthmann, M. Monaghan, H. Walles, J. Hansmann. Impedance spectroscopy for the non-invasive characterization of in vitro epidermal models.

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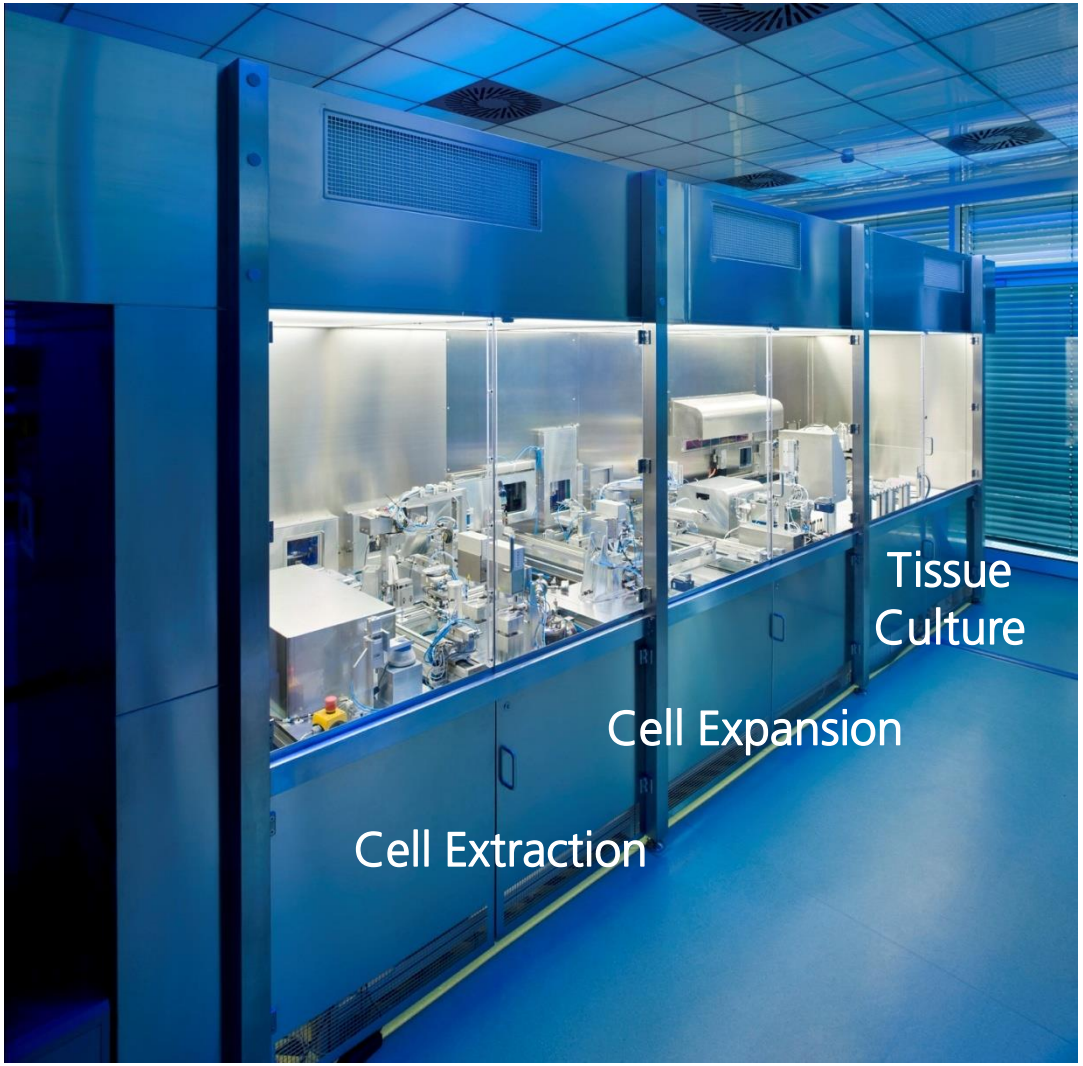
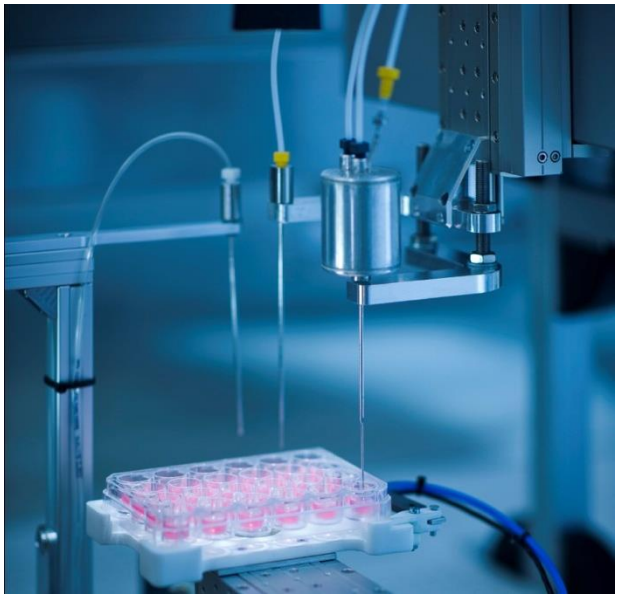
Epidermal model automated production





AUTOMATED TISSUE ENGINEERING ON DEMAND

Fraunhofer

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Process automation of down stream analysis

Schmid F., Schwarz T., Schuberthan W., Klos M., Walles H., Hansmann J., Groeber F.; Automated assessment of the barrier function of in vitro epidermal models using a dual-arm robotic system; Biotechnology Journal; submitted

Research & development activities at Fraunhofer

THANK YOU FOR YOUR ATTENTION!

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