ENGINEERING OF HUMAN 3D VASCULARIZED TISSUES INCLUDING DISEASE MODELS

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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
Our innovation chain – from basics to industrial applications

**Fundamental research**
- Universität Stuttgart
- 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
The Translational Center Würzburg
A joint research center by Fraunhofer and the University Hospital Würzburg

Translational Center Würzburg

Applied research

- Biomaterials Department
- Testsystem Department
- Bioreactor Department

Clinical application

- Implant Department
- Theranostics Department
# In-vitro-Testsystems

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<th>Advanced tissue models</th>
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Generating complex tissues

Biological vascularized scaffold (BioVaSc®)

Porcine jejunum

Porcine cells

Collagen scaffold

Vascular structures

Acellularization

Vital cells                   Necrotic cells

BioVaSc® seeded with endothelial cells

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
Unique technology: BioVaSc® – Platform for vascularized tissue models

- PanVaSc
- BoneVaSc
- KidVaSc
- LiVaSc
- MenVaSc
- OncoVaSc
- TraVaSc
- GutVaSc
- SkinVaSc
- AdiVaSc

*EP 2 029 186
*EP 2 089 509
*EP 2 430 147
*EP 2 429 707
*WO2010130303
Research & development activities at Fraunhofer

HUMAN AIRWAY MUCOSA MODEL
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
In vitro- in vivo correlation

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

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*

Sample processing for transmission electron microscopy

Steinke M et al., An engineered 3D human airway mucosa model based on an SIS scaffold. Biomaterials 35:7355-7362
Ultrastructural analysis after infection with *B. pertussis*
Research & development activities at Fraunhofer

LUNG TUMOR MODEL
Establishment of decellularized matrix

Native

Decellularized

Fecher et al., under review
Recellularization of lung scaffold

Tumor cells

Fecher et al., under review
Tumor cells on the lung scaffold

A549

H441

HCC827

Fecher et al., under review
3D lung matrix induces a more *in vivo*–like phenotype

**A549**

**HCC827**

**Ki67**

**Fecher et al., under review**
3D lung matrix induces a more *in vivo* – like phenotype

A549

HCC827

Fecher et al., under review
Effect of dynamic culture on tumor tissue formation

Tumor cells
Research & development activities at Fraunhofer

INTESTINAL MODEL
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% CO₂, 21 days
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
Results – immunohistological characterisation

- Ki67
- Mucin2
- ZO1
- Chromogranin A
- Lysozyme
- Villin
Results – electron microscopy
Research & development activities at Fraunhofer

SKIN MODEL
Three dimensional skin models

Epidermal model

Full thickness skin model

Vascularized skin model

- Lack of key cellular components
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
Full thickness skin model
Non-contracting collagen hydrogel

*In vivo skin*  
*In vitro skin*
Full thickness skin model
Wound model

- **Features:**
  - Reproducibility (shape and depth)
  - Sterility

- **Applications**
  - Efficacy testing for wound healing

**Comparison with other methods**

**Wounded skin equivalent**
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
Helminth infection studies

- Strongyloides ratti

- Schistosoma mansoni

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
Infection Studies – Trypanosoma
Collaboration Prof. Engstler  University of Würzburg

*T. brucei* life cycle

Tsetse fly infecting a full thickness skin equivalent

*T. brucei* larvae

*T. brucei* larvae after infection
Vascularized skin model

1. Foreskin biopsy
2. Dynamic culture in a bioreactor system at the air-liquid interface for 14 days
Vascularized skin model

▲: Vessels
ED: Epidermis
D: Dermis
GL: Vessel lumen
hEK: Human epidermal keratinocytes
hDF: Human dermal fibroblasts
hDMEC: Human microvascular endothelial cells
Assessment of mild irritative effects via impedance spectroscopy

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

12.5 Hz

Assessment of mild irritative effects via impedance spectroscopy

MTT

Viability [%]

42 h

PBS SDS 2-propanol

Impedance spectroscopy

Ohmic resistance [Ω]

PBS SDS 2-propanol

Capacitance [µF]

PBS SDS 2-propanol

N=3

Epidermal model automated production

Cell Extraction

Cell Expansion

Tissue Culture
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!