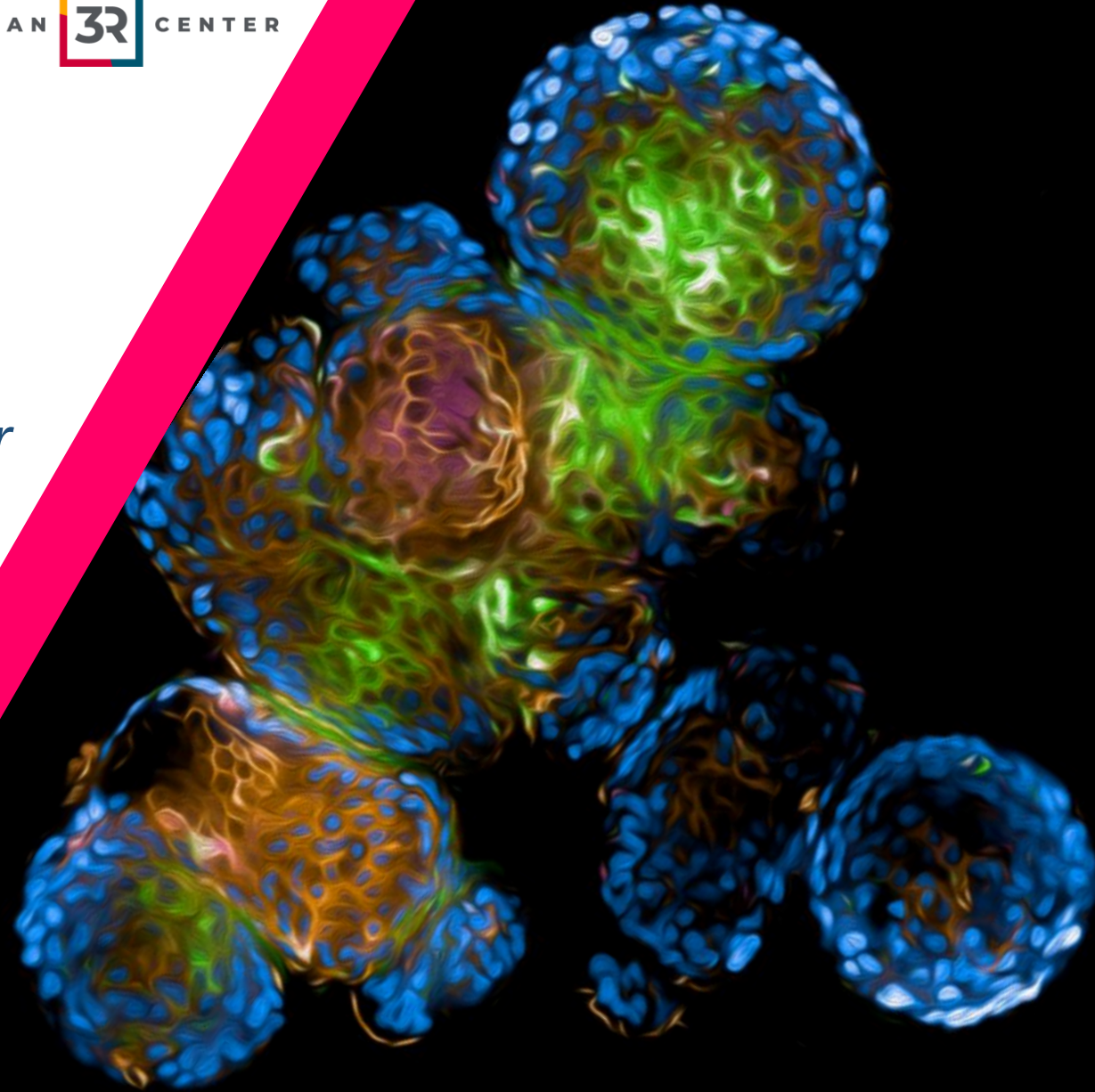
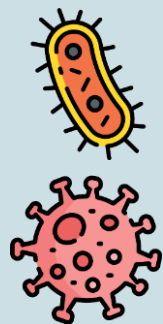


The breathtaking world of animal-free immune-competent respiratory barrier models

Univ.-Prof. Doris Wilflingseder
Stefanie Dichtl, PhD
Viktoria Zaderer, PhD

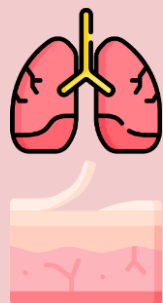


Research Interests



Pathogens

HIV-1 / HIV-2
SARS-CoV-2 / Influenza / RSV
Other Respiratory Challenges
Aspergillus spp.
Co-Infection Models



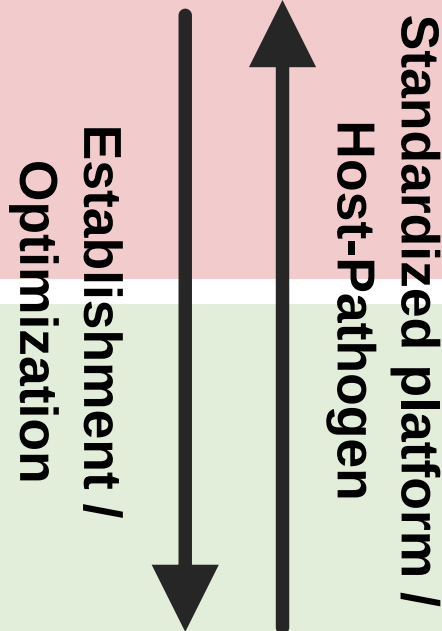
Barrier models

Mucosa
Lung
[Skin (*ex vivo*)]



Immune system

Dendritic cells (DCs)
Macrophages
Complement system
T-cells



WGs Wilflingseder and Posch:

Elliott Lafon (PhD student), Marta Bermejo-Jambrina (Post Doc), Viktoria Zaderer (Post Doc), Viktoria Kozubowski (Technician), Sophie Erckert (PhD Student), Paul Schweighofer (MSc Student), Doris Wilflingseder, Andrea Windisch (Technician), Wilfried Posch (Assoc.-Prof.), Hussam Abd-El-Halim (PhD Student), Michael Jäger (PhD Student), Gabriel Diem (PhD Student)

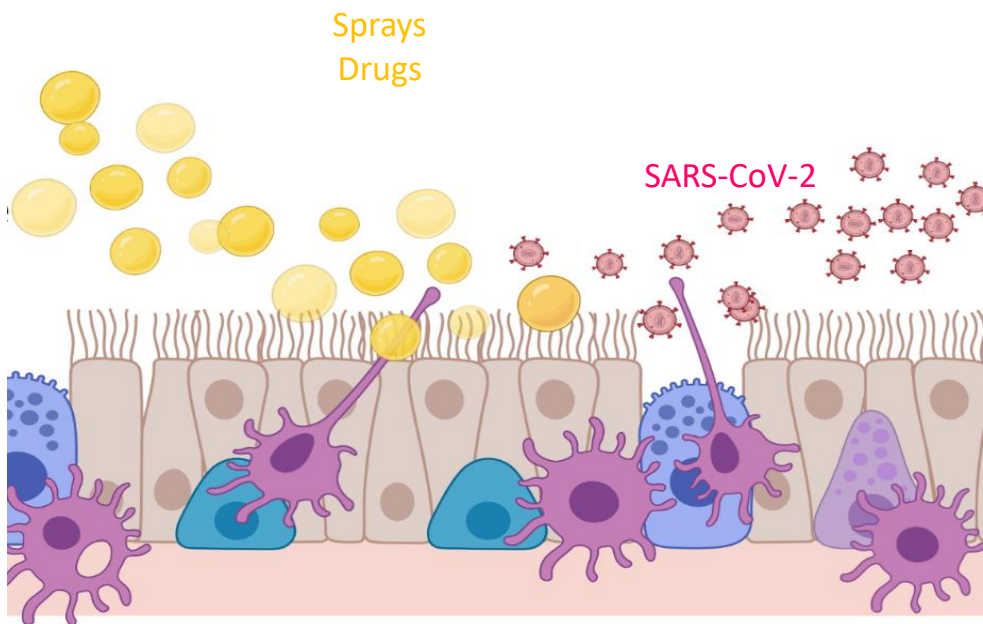
Stefanie Dichtl (Post Doc)

not depicted:

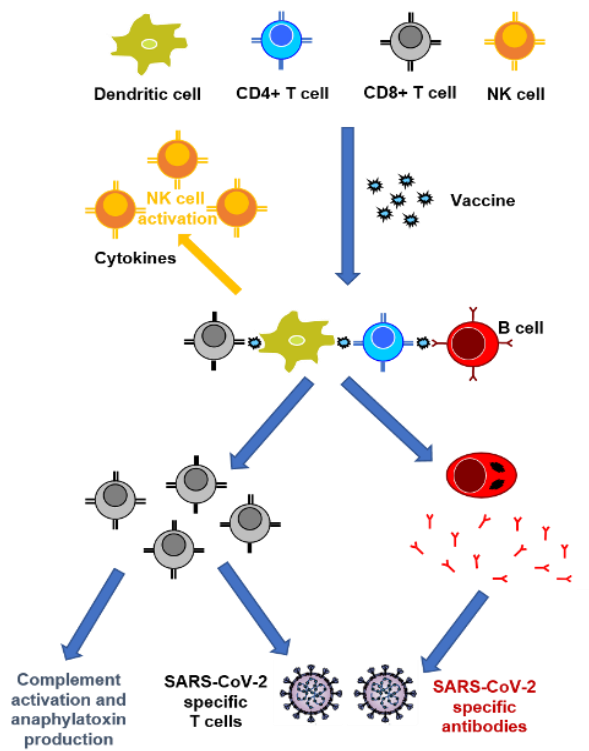
Giulia Bertacchi (PhD Student),
Anna-Lena Wyremblewski (MSc student)
Jasmin Mattiuz (MSc student)
Christina Witting (Technician)

How can infections and inflammatory reactions or alternative therapeutic options be mimicked using alternative approaches?

Cell culture



Patient material



MUI / Bullock

©M. Reindl

Upper respiratory tract:

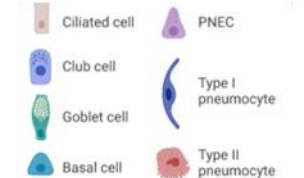
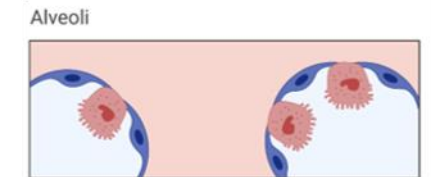
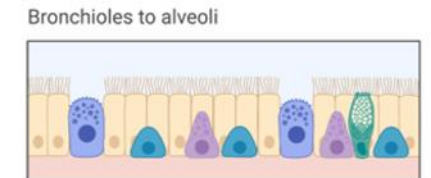
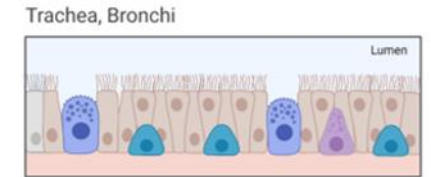
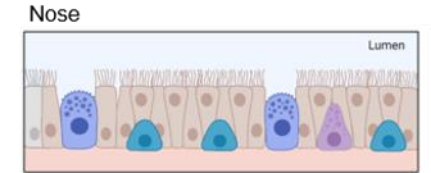
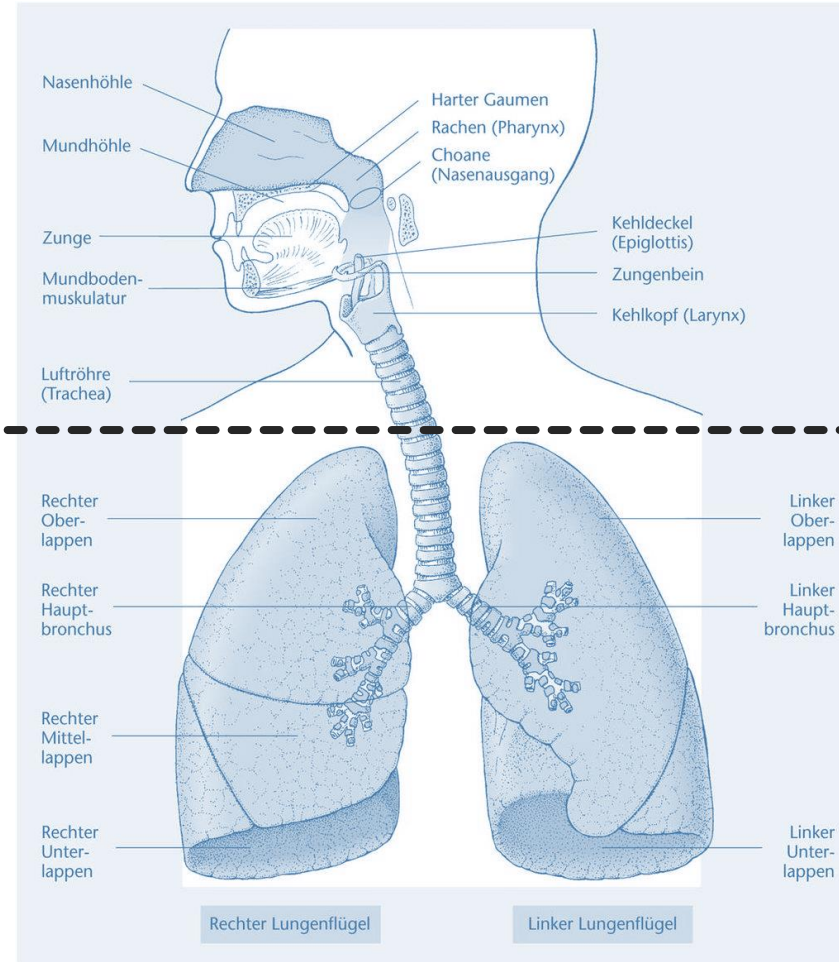
Human Coronaviruses

229E, NL63, OC43 und HKU1

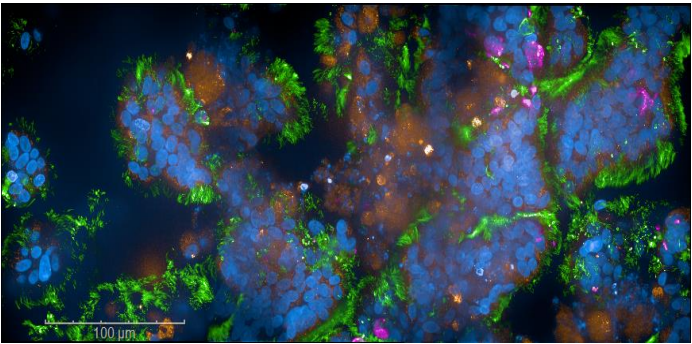
(Omicron variants)

Lower respiratory tract:

SARS-CoV / MERS-CoV / SARS-CoV-2

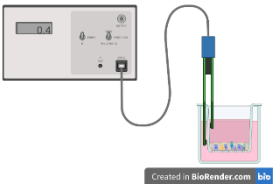


in vitro modelling of respiratory challenges in human-centered systems: ALI model

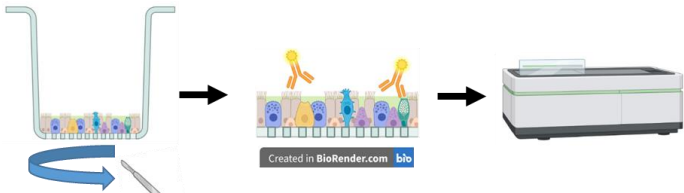


Read-out parameters (1 filter):

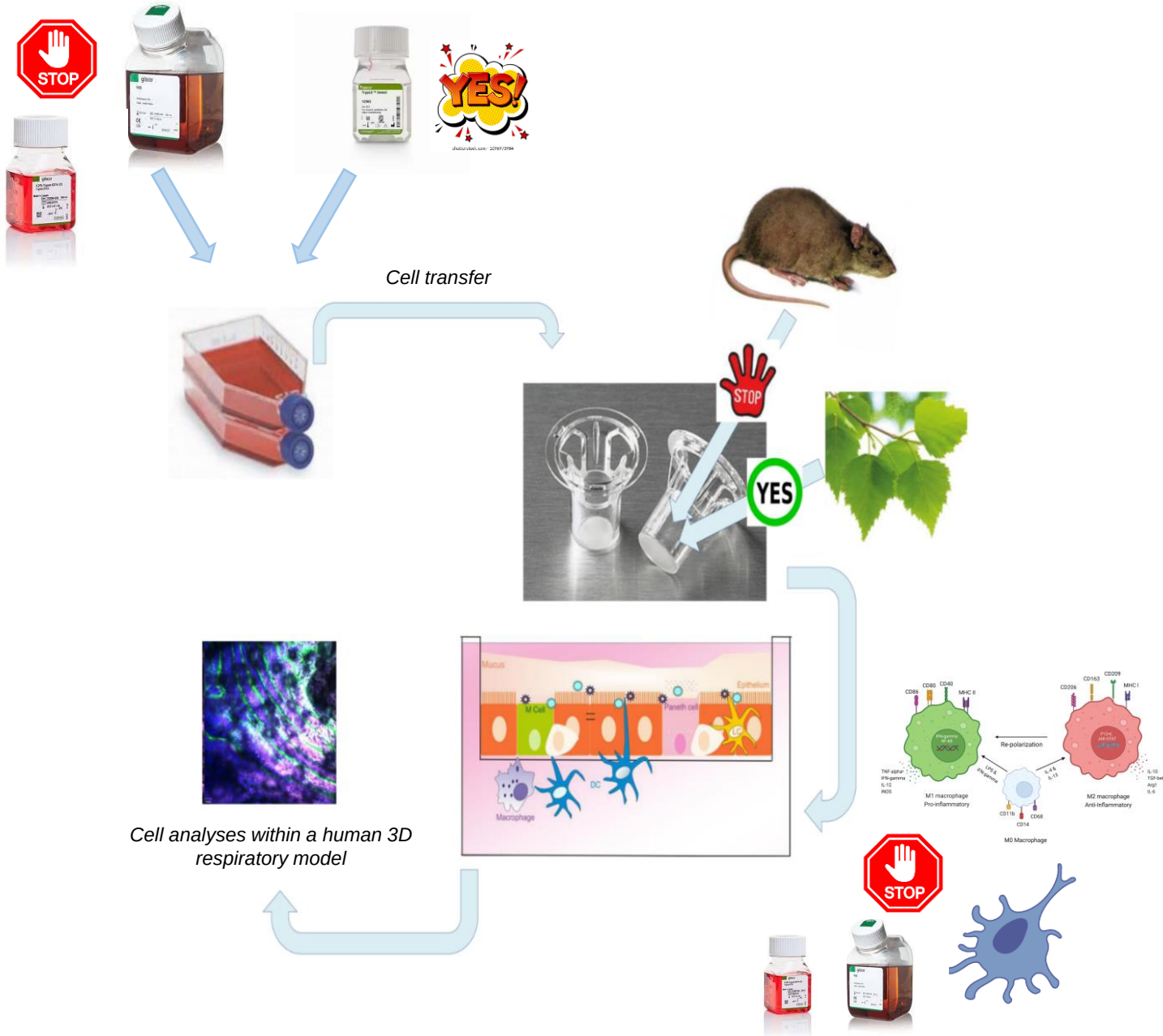
TEER



HC/HT screening
Live Cell imaging
Confocal imaging



Cytokines
Viral load
Anaphylatoxins



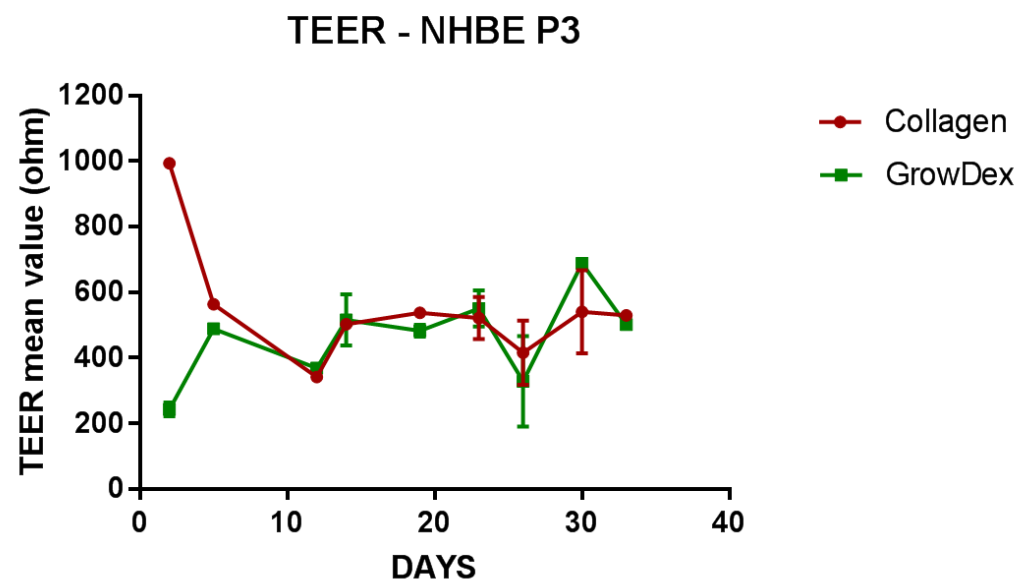
GrowDex

- Is a birch derived hydrogel
- Works as extracellular matrix → 3D growth of NHBE cells
- Used instead of rat tail collagen on Transwells
- Cellulose based hydrogel can be digested using cellulase → no harming of cells

Meanwhile there are different GrowDex hydrogels on the market



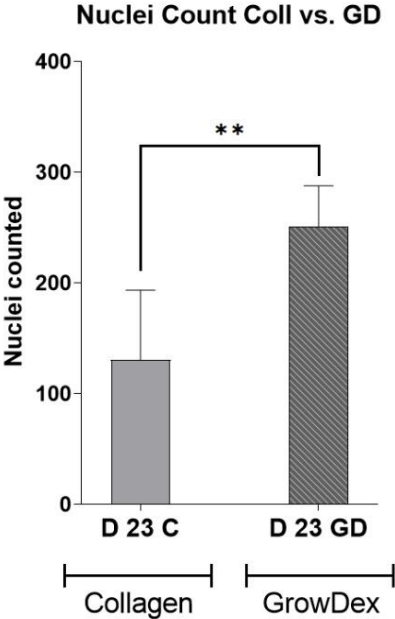
GrowDex vs. rat tail collagen::



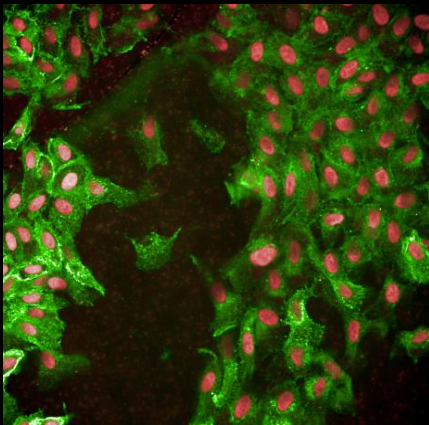
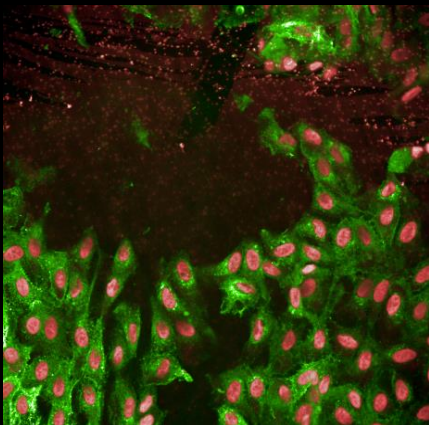
- Trans-Epithelial Electrical Resistance (TEER) as general indicator for epithelial Integrity
- Intact Tight Junctions: ↑ TEER levels
- Completely differentiated epithelium: ↑ TEER levels



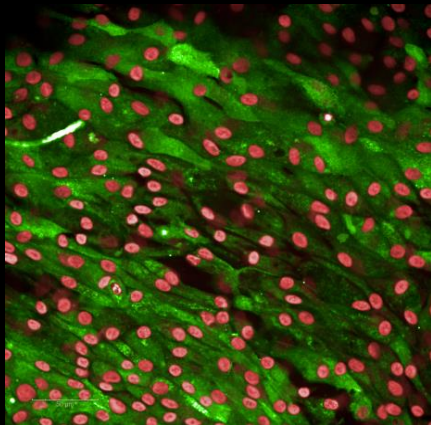
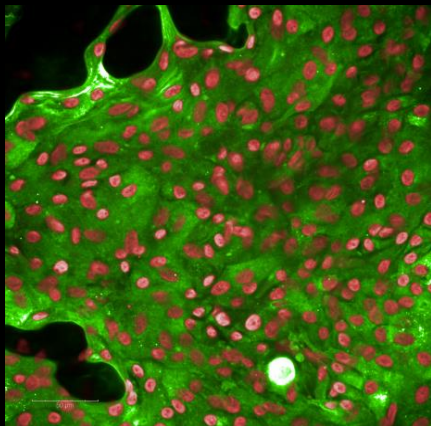
GrowDex vs. rat tail collagen



Collagen



GrowDex

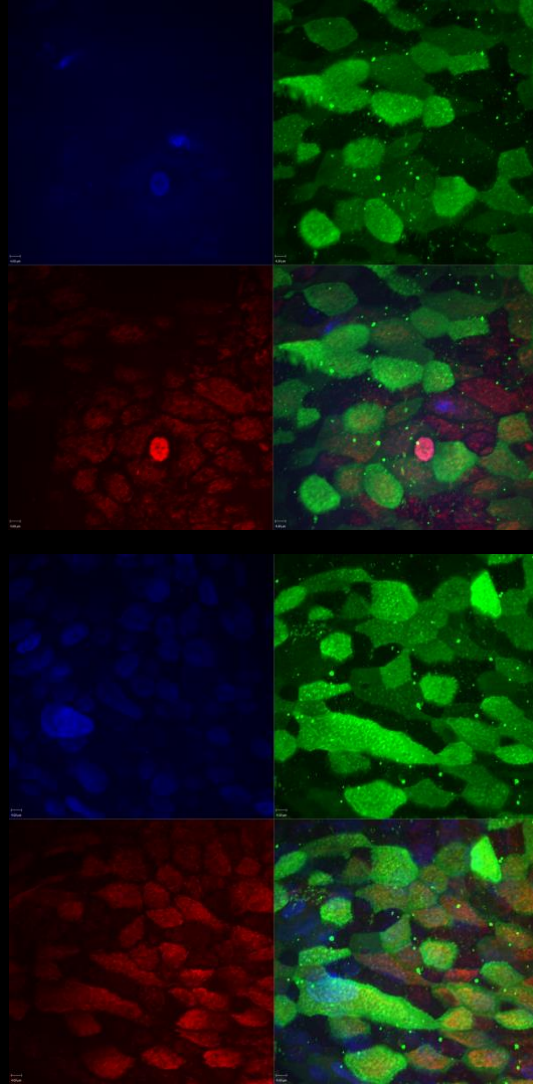


NHBE / P3 / Day 23 in ALI

Draq5-Nuclei
WGA- cell surface

GrowDex vs. rat tail collagen

Collagen



NHBE 15 Days in ALI

On **GrowDex**:

Higher proliferation rate of
cells and faster
differentiation

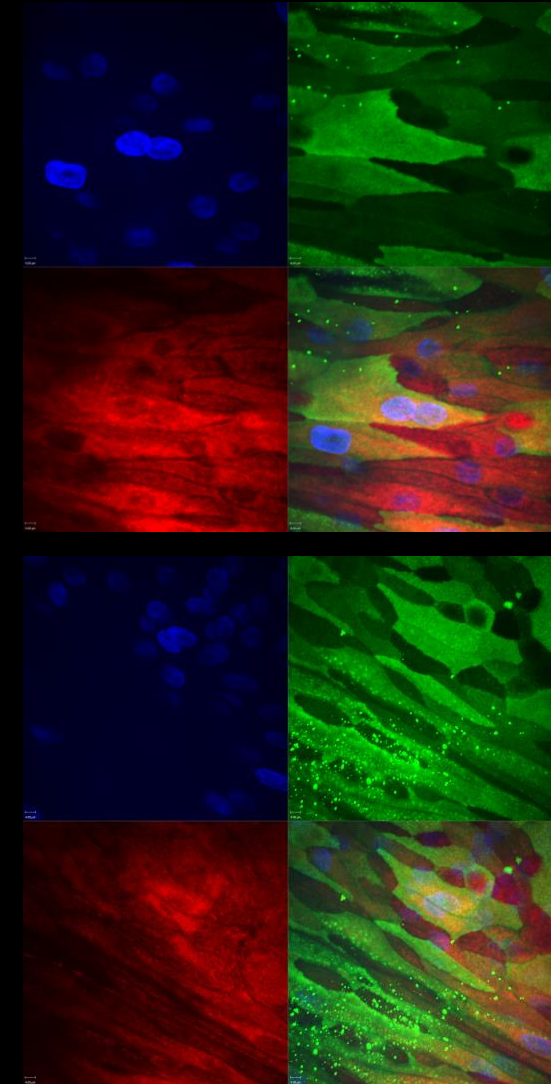
→ More cell layers
→ Faster cilia development

Hoechst - Nuclei

Mitotracker

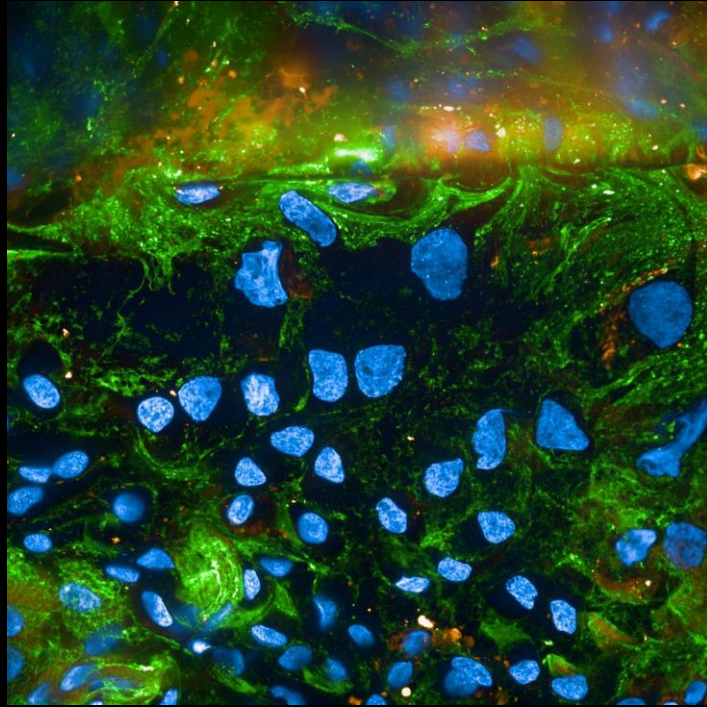
WGA- cell surface

GrowDex

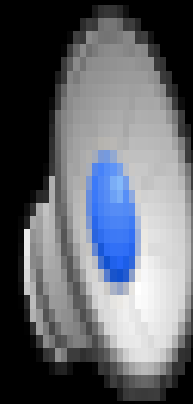
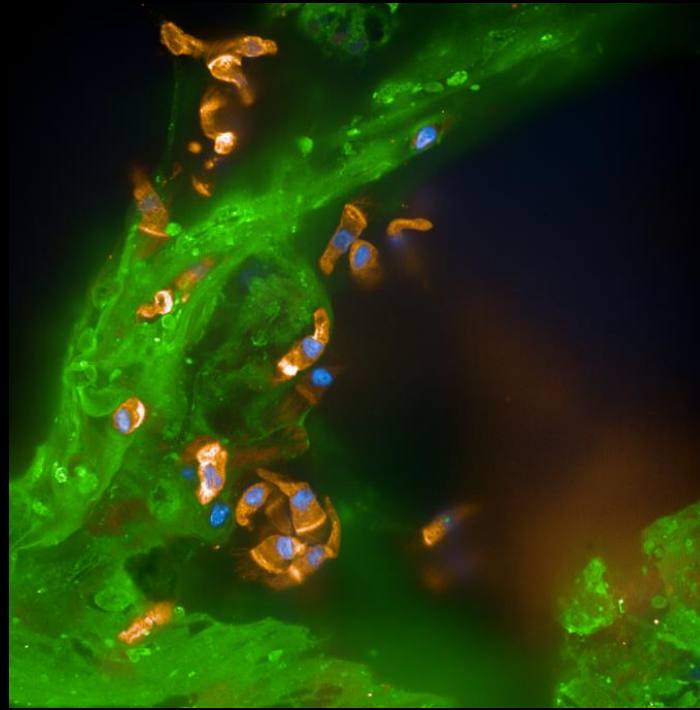


NHBE 15 Days in ALI

in vitro modelling of respiratory challenges in human-centered systems: ALI model

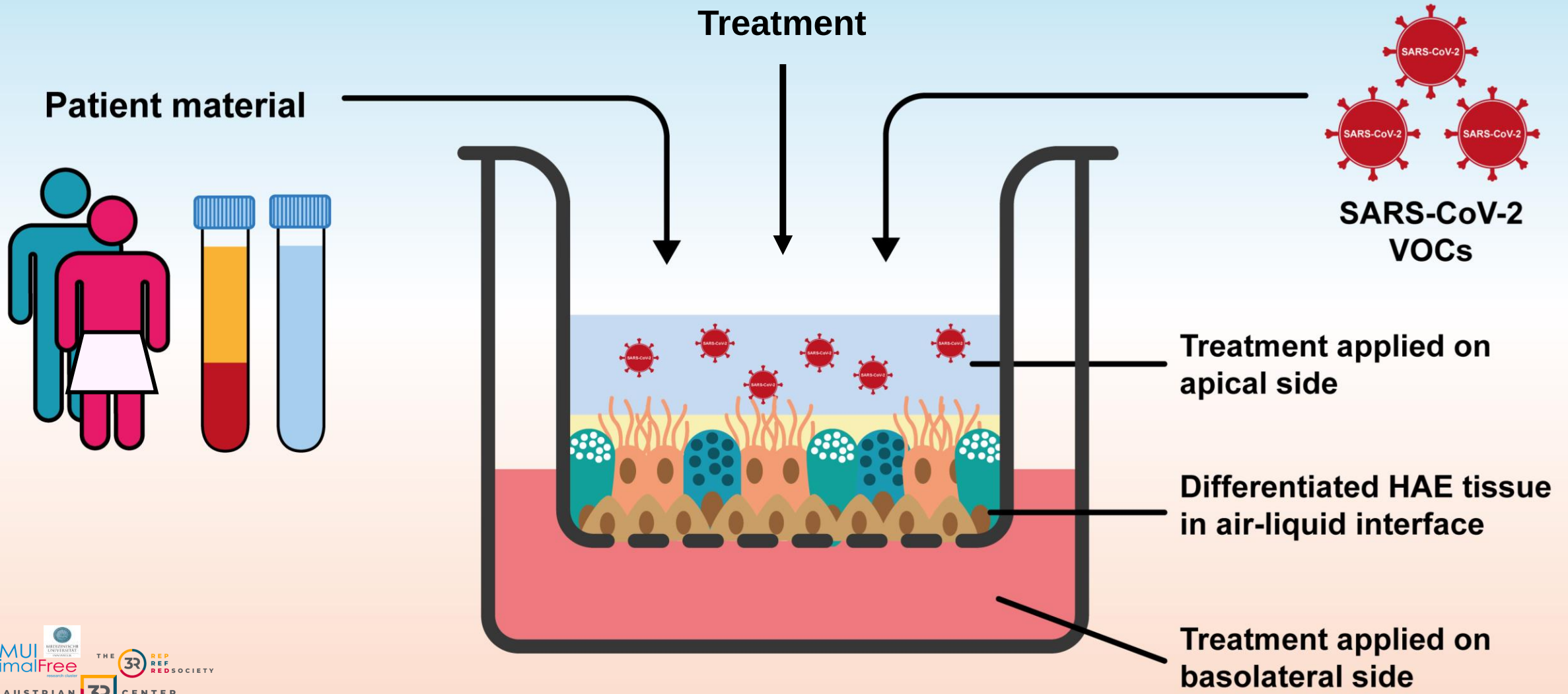


Cilia
Mucus-producing cells | Mucus
Nuclei



in vitro modelling of respiratory challenges in human-centered systems: ALI model

Dichtl et al., PMID: 36250084 PMCID: PMC9556863 DOI: 10.3389/fmed.2022.1005589
Zaderer et al., PMID: 36949547 PMCID: PMC10032620 DOI: 10.1186/s12931-023-02397-3
Zaderer et al., PMID: 36316674 PMCID: PMC9624019 DOI: 10.1186/s12931-022-02223-2

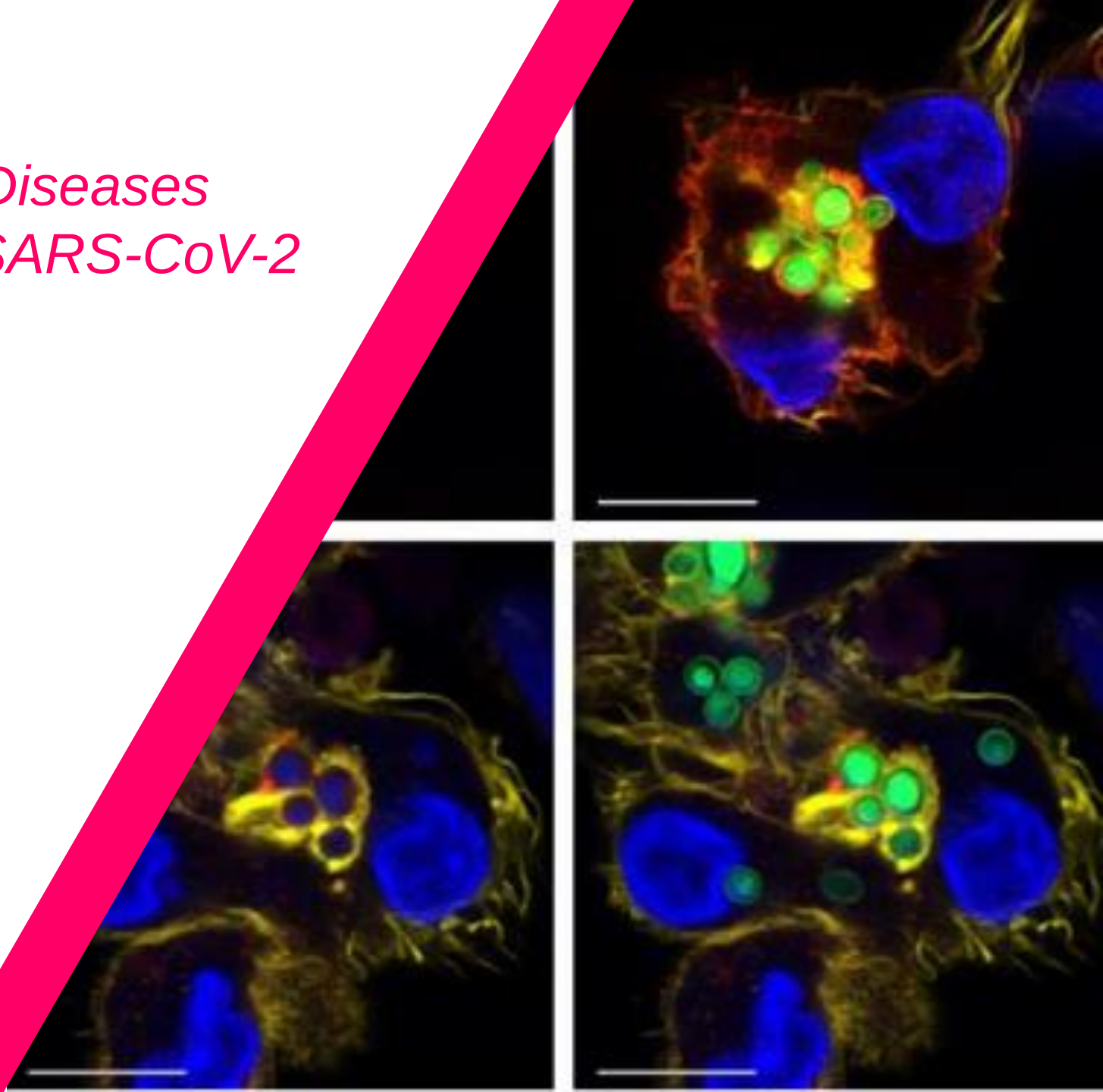
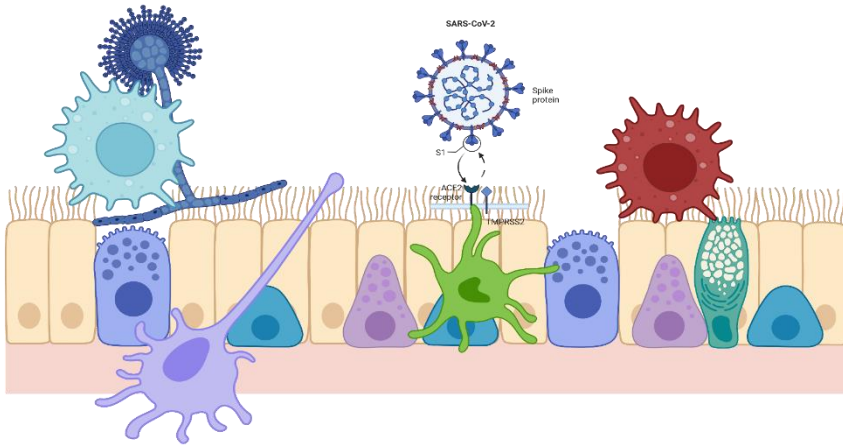


Key messages

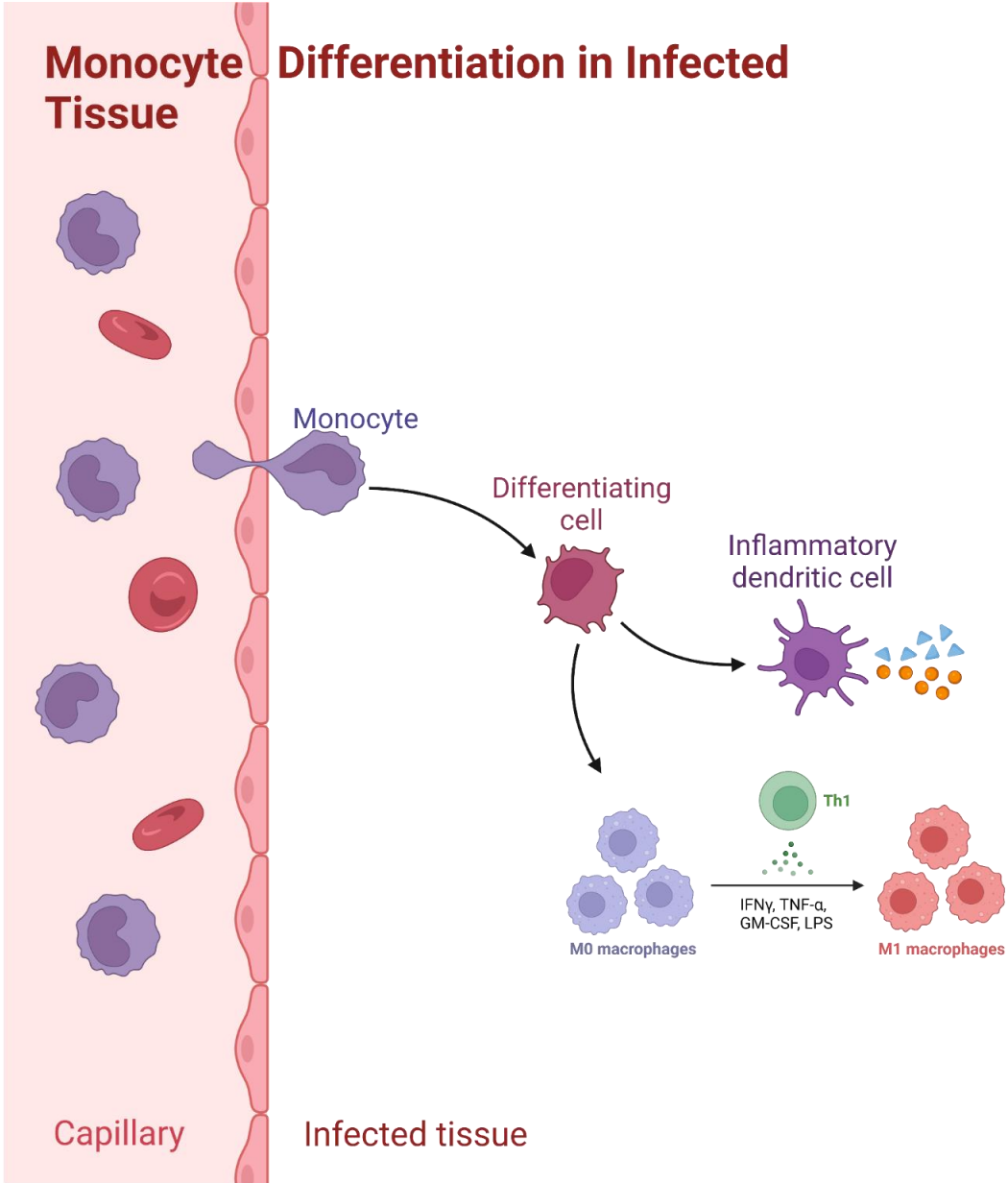
- xeno-free GrowDex shows no differences in epithelial integrity of NHBE cells compared to rat tail collagen
- GrowDex leads to faster proliferation and differentiation of NHBE cells, when seeded on Transwells
- Our animal-free lung model shows fully differentiated, pseudostratified epithelial growth.
The system is very close to the *in vivo* situation and offers a very good base to study respiratory infections.
It is even possible to add patient material as serum and saliva to the model and allowing personalized testing approaches.

NAMs in context of Infectious Diseases based on Aspergillus spp. Or SARS-CoV-2

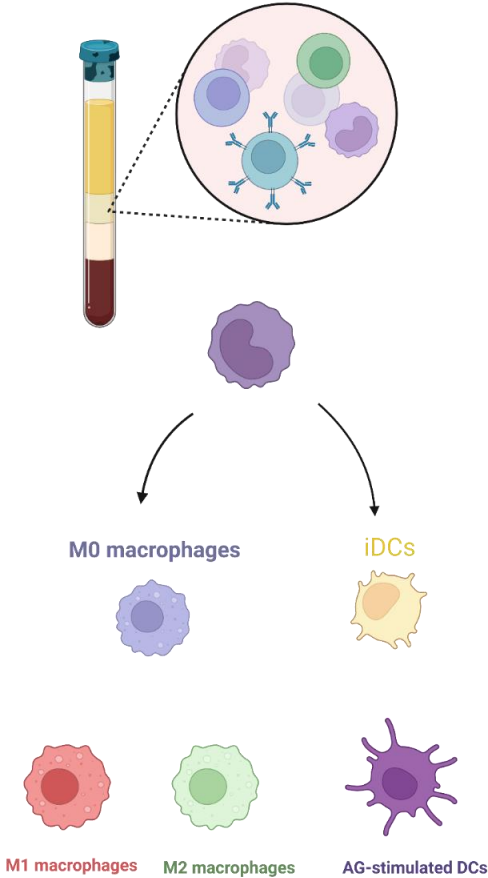
Adding IMMUNE CELLS and COMPONENTS



Dendritic cell differentiation and macrophage polarization



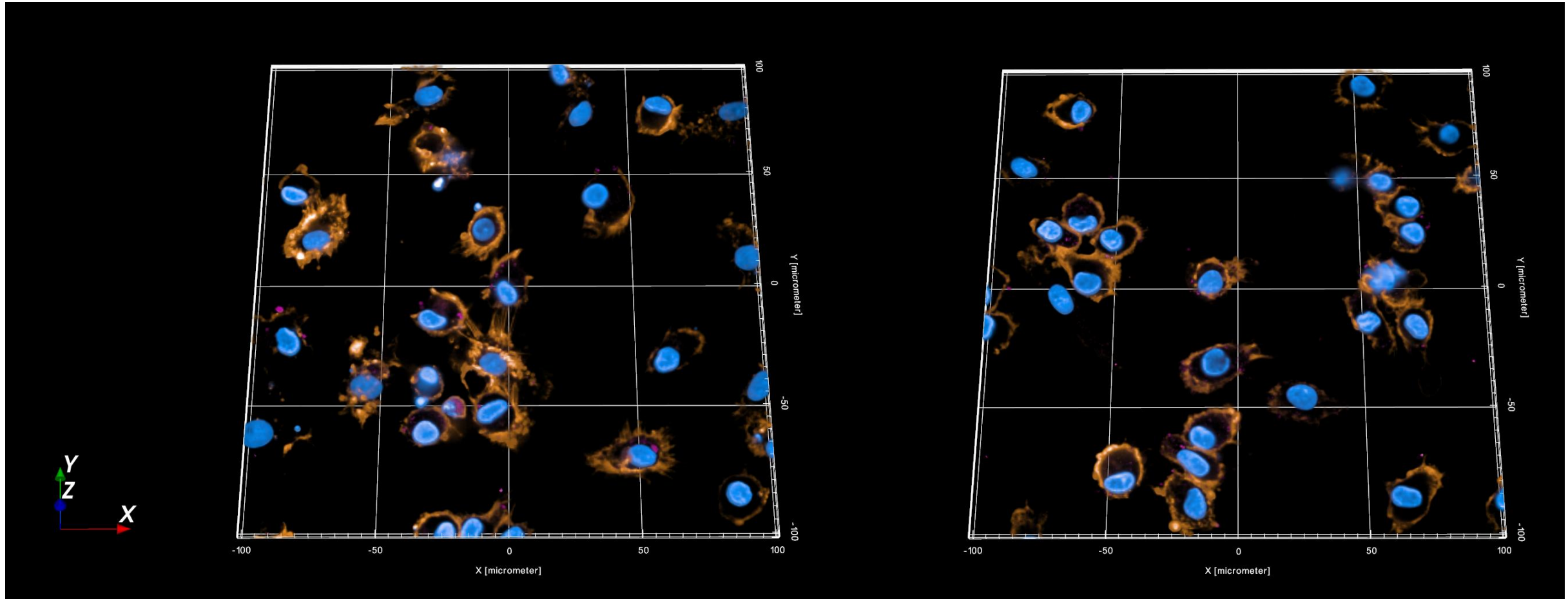
In vitro simulation of monocyte differentiation



Dr. Paul Hörtnagl

Assoc.-Prof. Beate Rinner

Dendritic cell differentiation and macrophage polarization



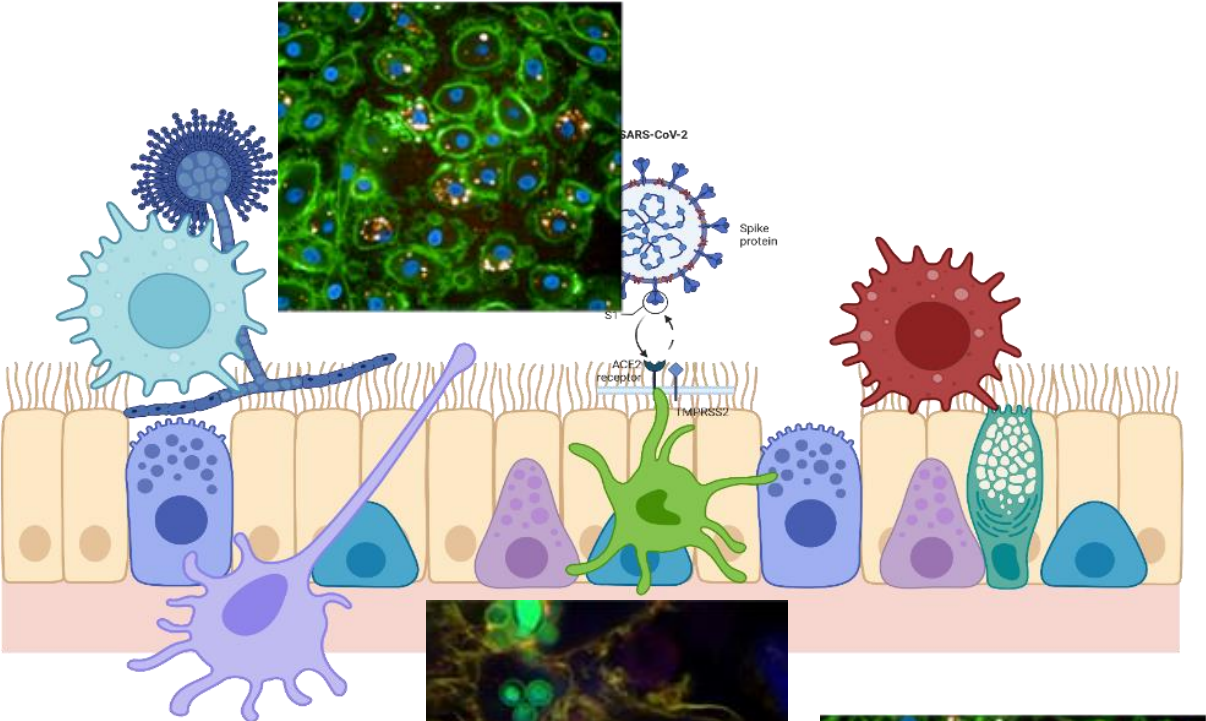
HIV-exposed DCs generated in:

10% FCS

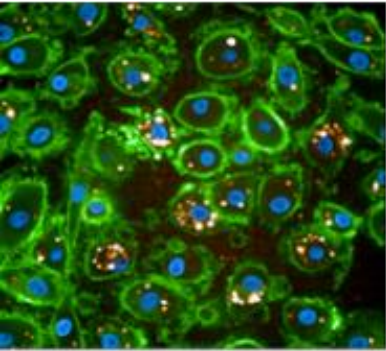
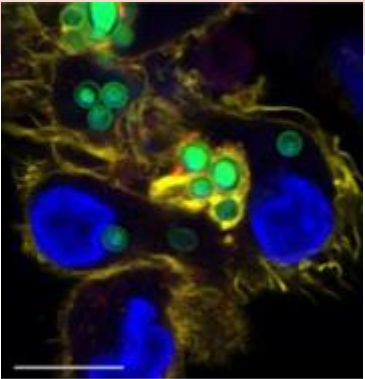
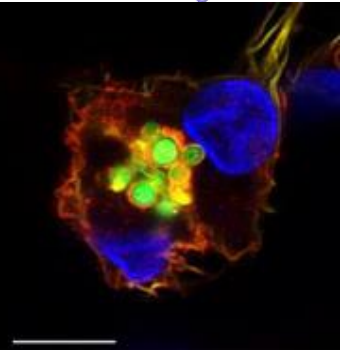
5% hPL

Dendritic cell differentiation and macrophage polarization

Apical: Macrophages



Basolateral: DCs

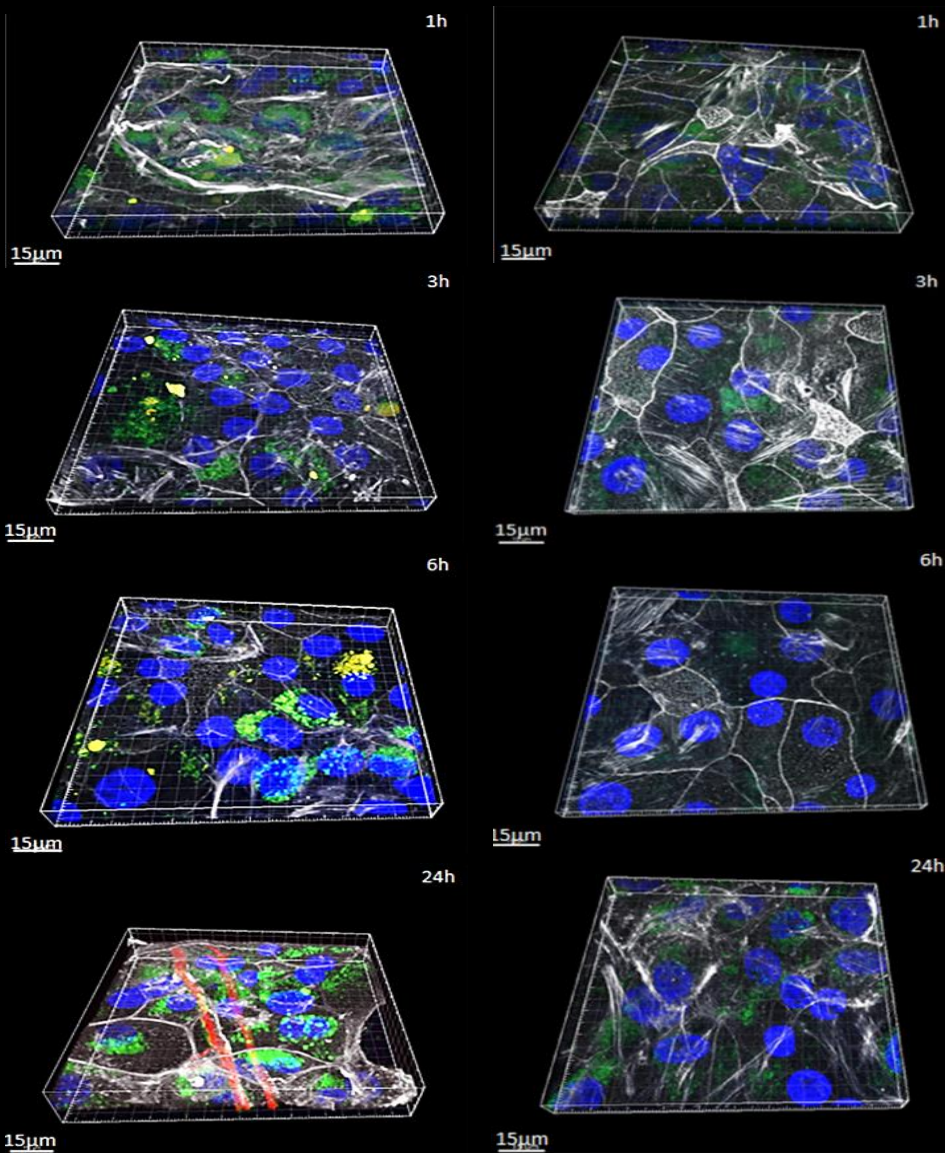


Macrophages
Neutrophils
T and B cells

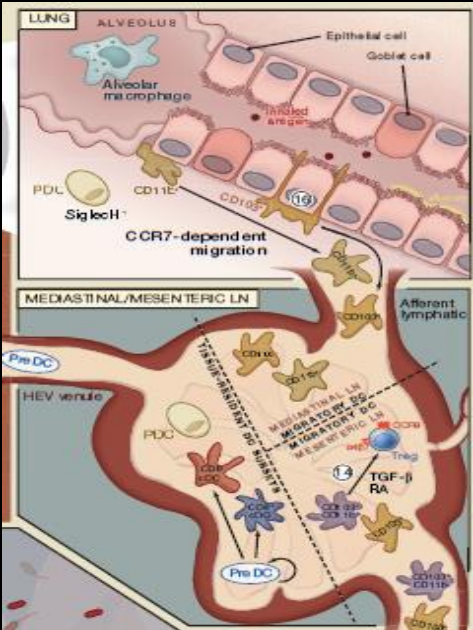
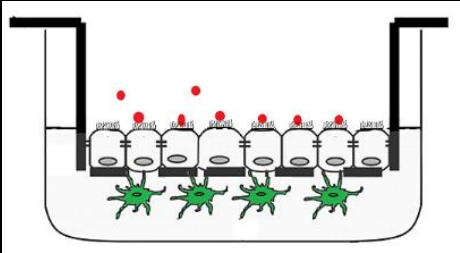
Epithelial / Immune cell Co-Culture: Dendritic Cells (DCs) Sense Fungal Conidia



Conidia/ Hyphae
DCs
Actin
DC-grabbed
conidia



Infected Non-infected

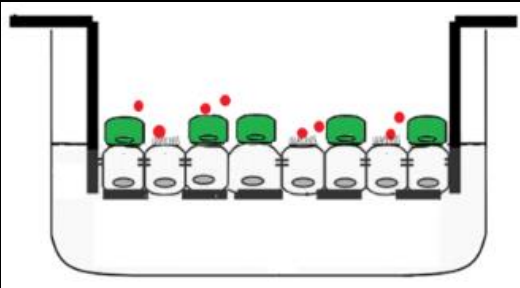
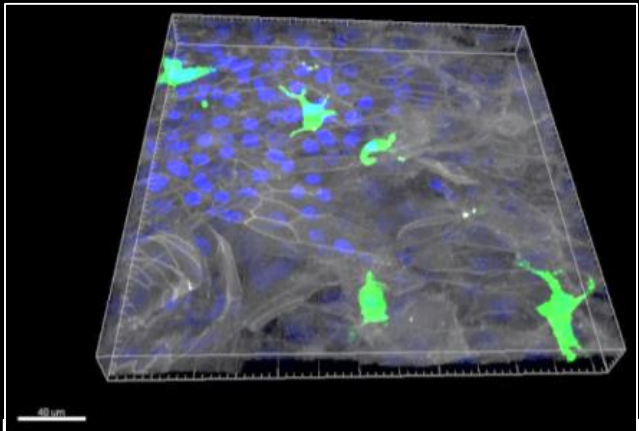


From: Idayaga J, Steinman RM:
Snapshot – Dendritic Cell
doi: 10.1016/j.cell.2011.08.010

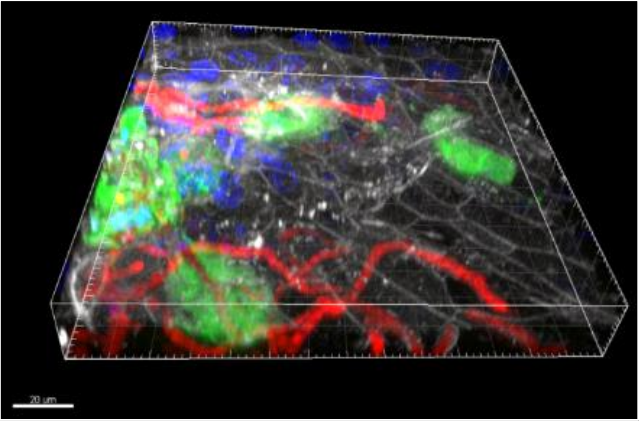
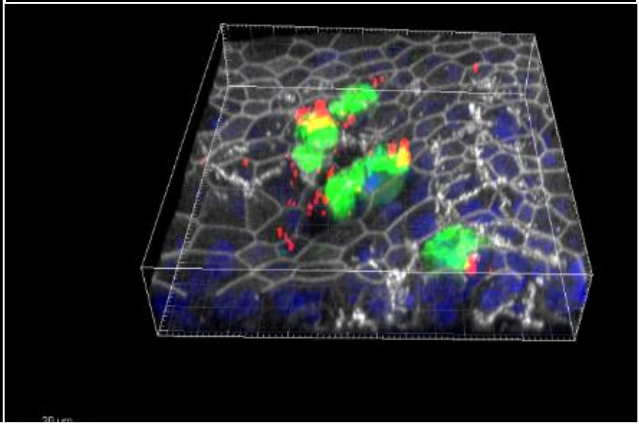
Epithelial / Immune cell Co-Culture: Macrophages Sense Fungal Conidia



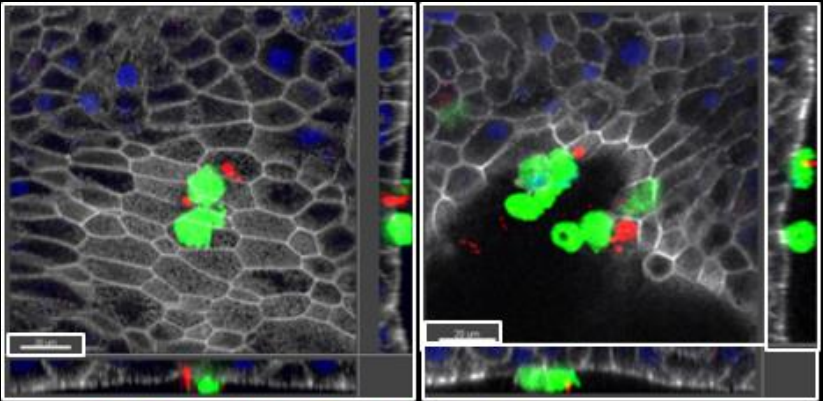
Non-infected



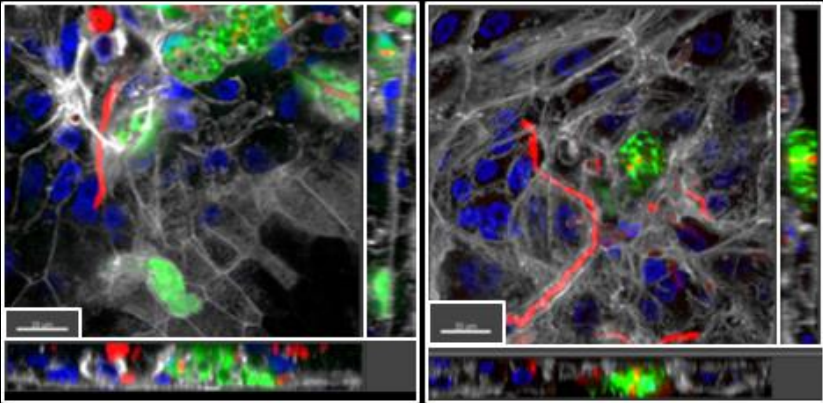
Infected



6h



24h

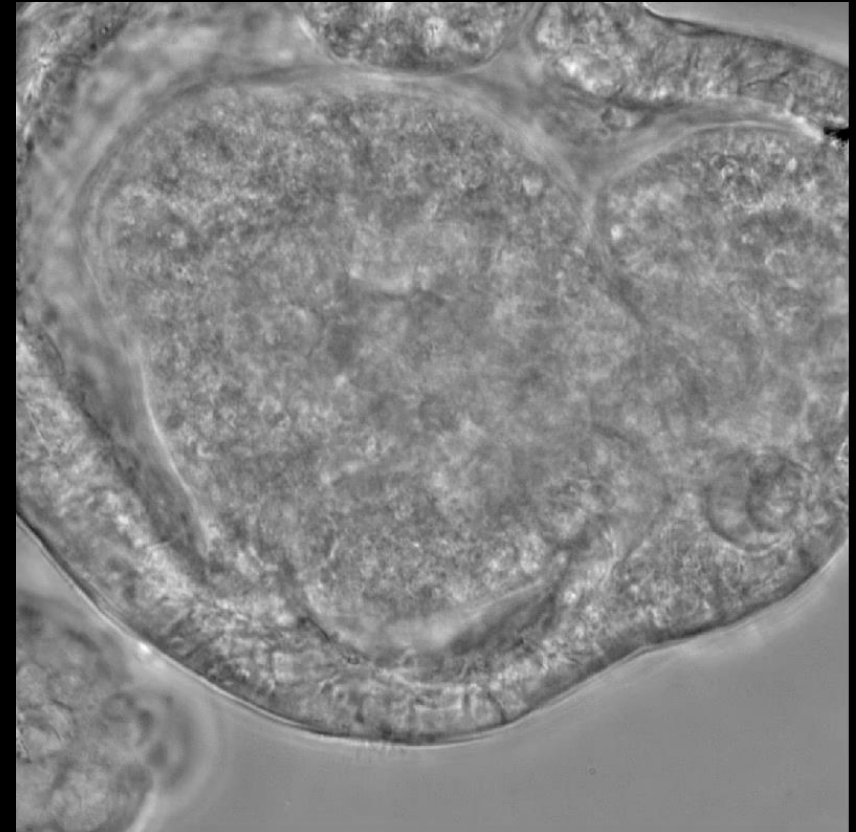
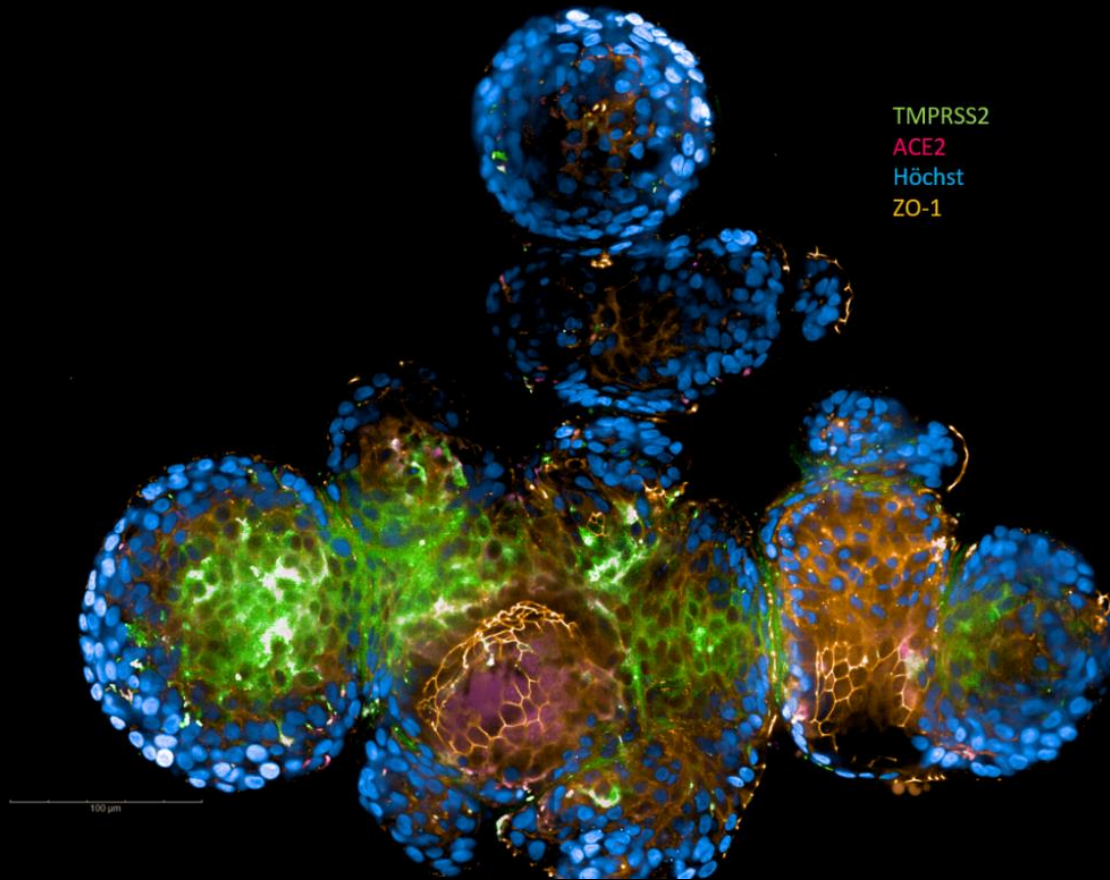


Conidia/ Hyphae
DCs
Actin
DC-grabbed
conidia

Key messages

- Spatio-temporal imaging allows to visualize initial steps of microbe-epithelial-immune cell interaction and entry via these barriers
- Immune cells are completely functional within the immune-competent barrier model and multiple parameters can be measured using such complex models (evaluation of (anti-)inflammatory events, pathogen-induced gene signatures, mucociliary clearance, -omics...)
- *Our immune-competent barrier models are highly flexible, translatable to other species and thus allow comparative infectiology independent on the pathogen used (viruses, fungi, bacteria, co-infections)*

in vitro modelling of respiratory challenges in human-centered models: organoid model

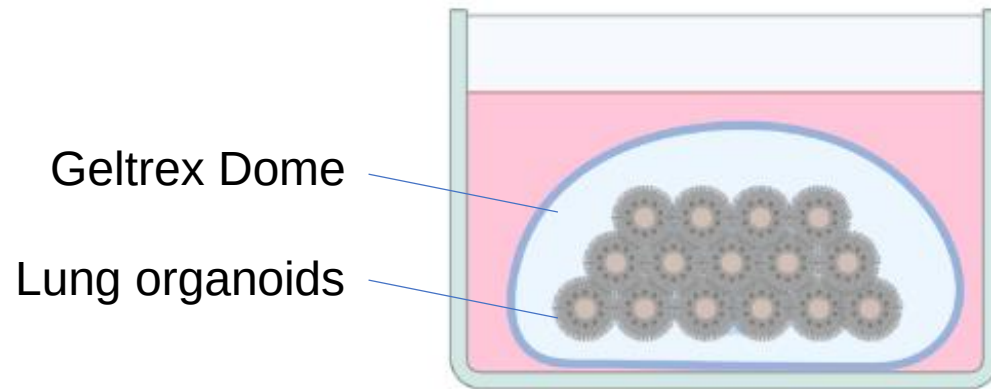


Apical/out Organoids:
Optimal for apical treatment options, high throughput

Lung organoids

- 3D structures, which self-organize into airway-like tissue structures
- Lung organoids extracted from adult tissue present basal, club, goblet and ciliated cells
- Coexistence of proximal and distal cell types
- Cultivation:

Geltrex/Matrigel



Celvivo



Geltrex/Matrigel

- Extracted from murine Engelbreth-Holm-Swarm tumors
- Mouse - human
- Undefined composition

Celvivo



- In vivo like situation (blood flow)
- No artificial matrix needed
- Individual speed dependent on the size of the organoids

Key messages

- Partially apical out in Celvivo basal out culture
- Highly induced ciliated cells during apical out and proximal differentiated lung organoids in Celvivo system
- Difficulties to generate apical out and proximal differentiated lung organoids can be avoided by using the Celvivo system. This system allows a cultivation of lung organoids without an artificial murine matrix and therefore facilitates the polarity reversal more easily.