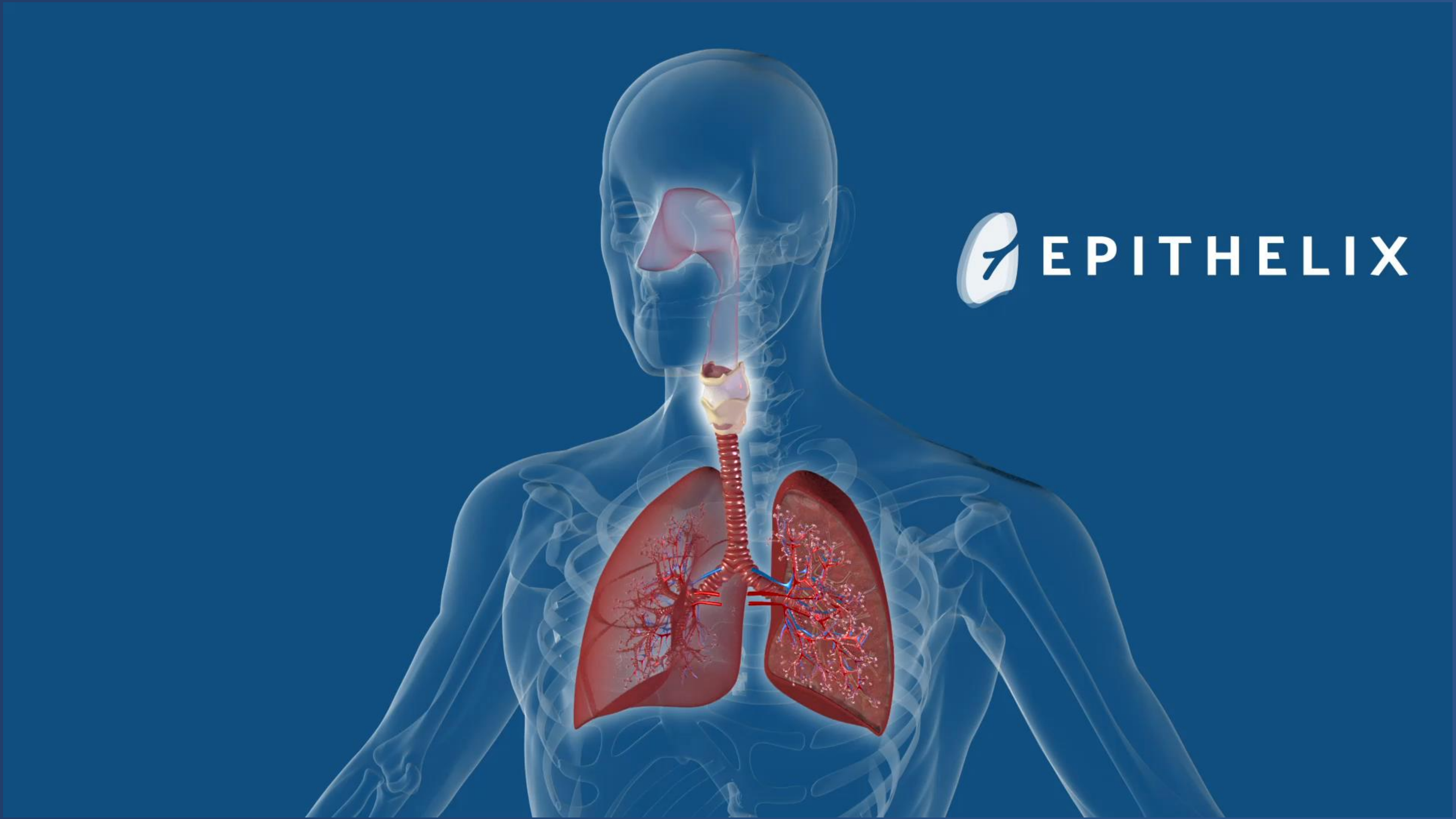
EPITHELIX
IN VITRO RESPIRATORY SOLUTIONS

Nasal, Bronchial, Small-Airways and Alveolar 3D human Models for Inhalation toxicity and Infectious Diseases Research

Samuel Constant, Ph.D., CEO

Adebiotech, 07/06/2023

3D ALI airway epithelia products | MucilAir™ | SmallAir™ | AlveolAir™



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Reconstitution process | MucilAir™

Reconstitution process SmallAir™ | AlveolAir™ Macrophages

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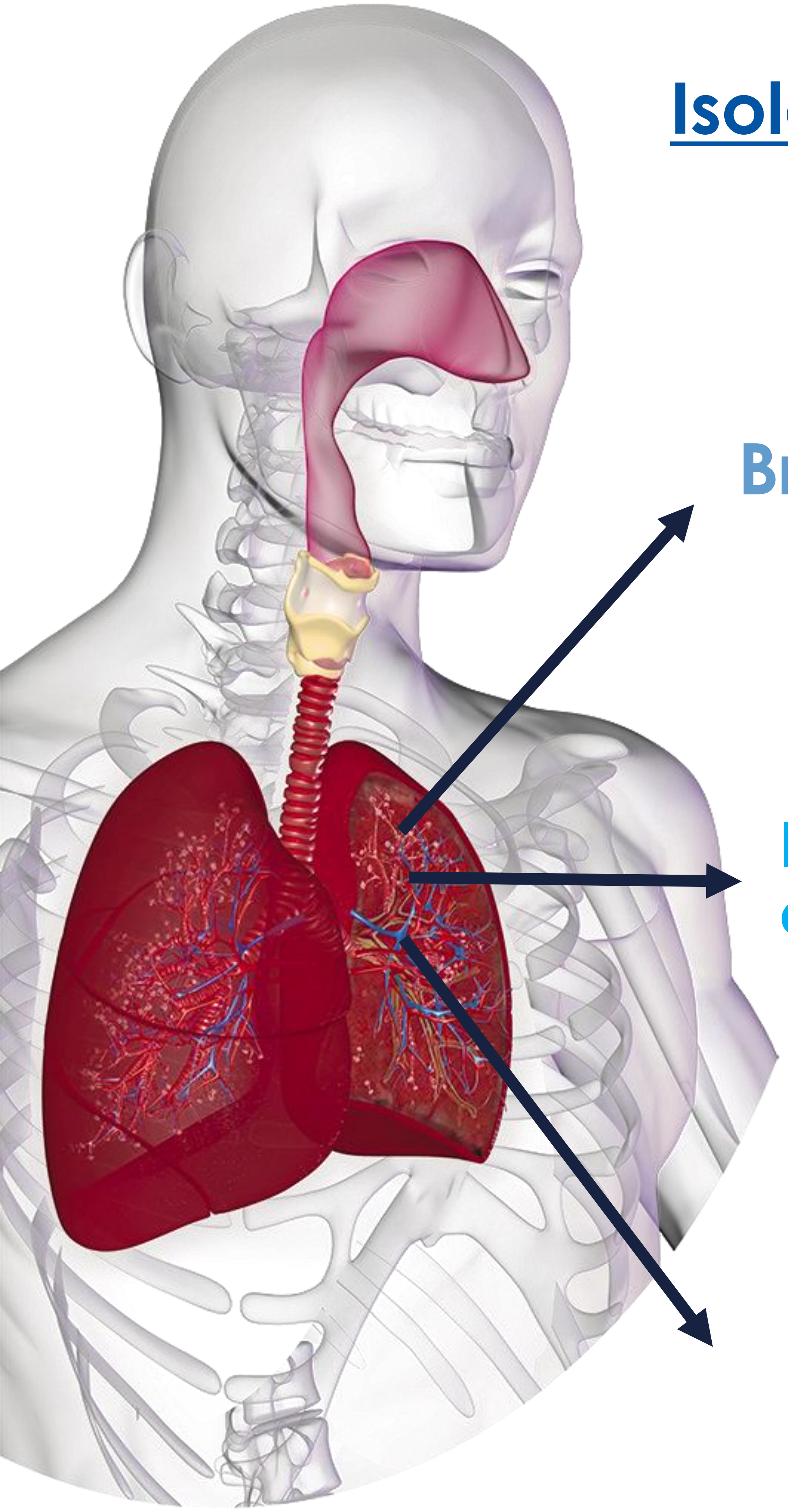
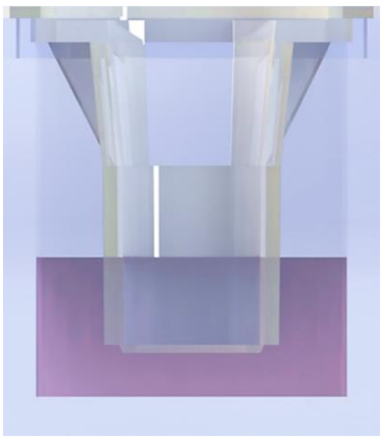


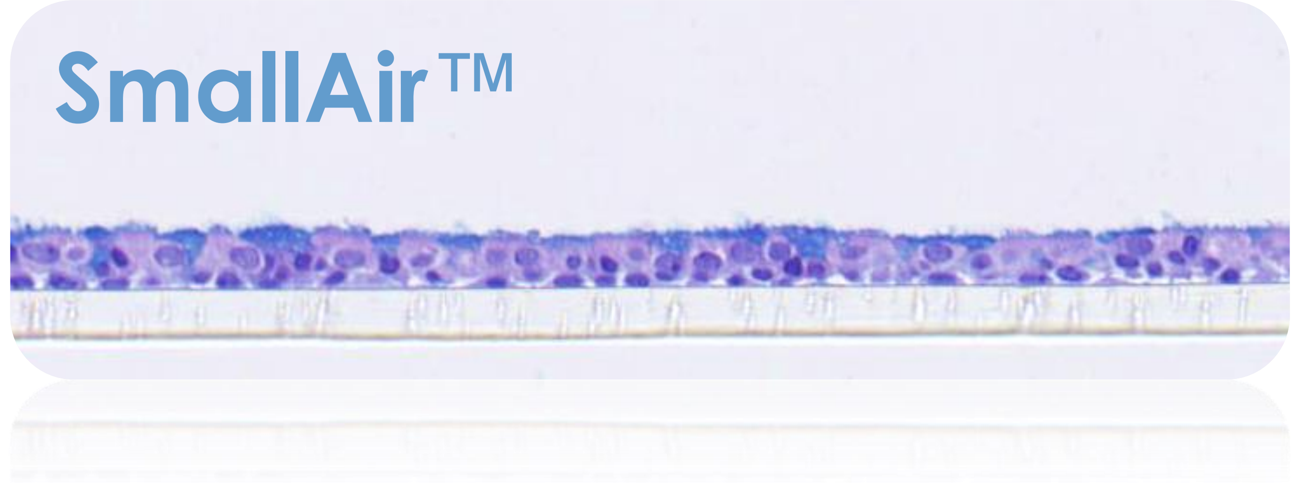
Isolation → **Cryopreservation** → **Seeding** → **ALI & differentiation**

Bronchiolar cells →

→

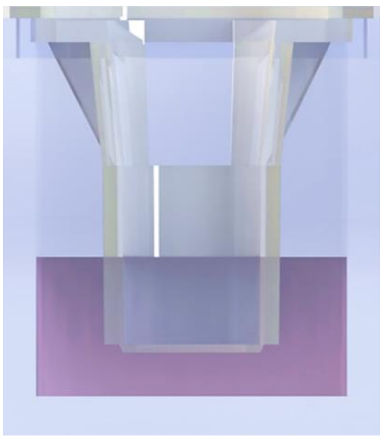


7 weeks →

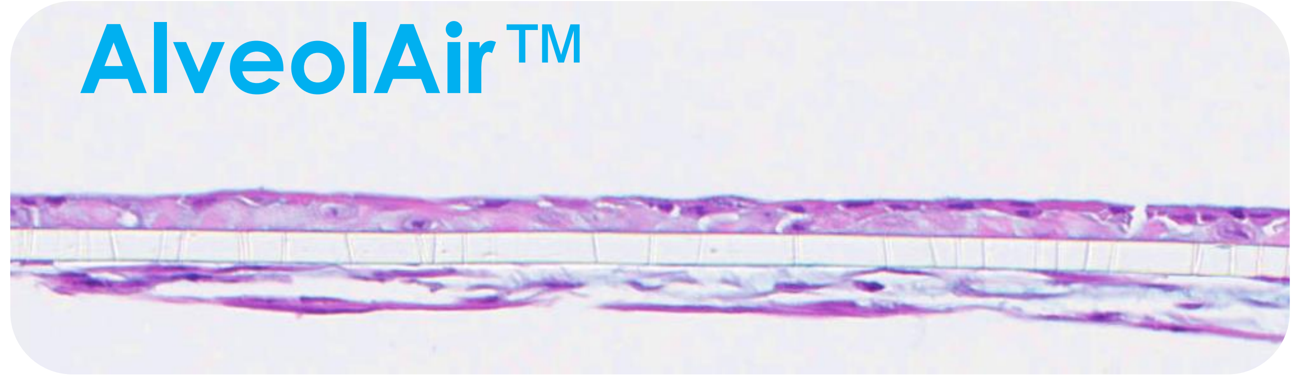


Pneumocytes & endothelial cells →

→



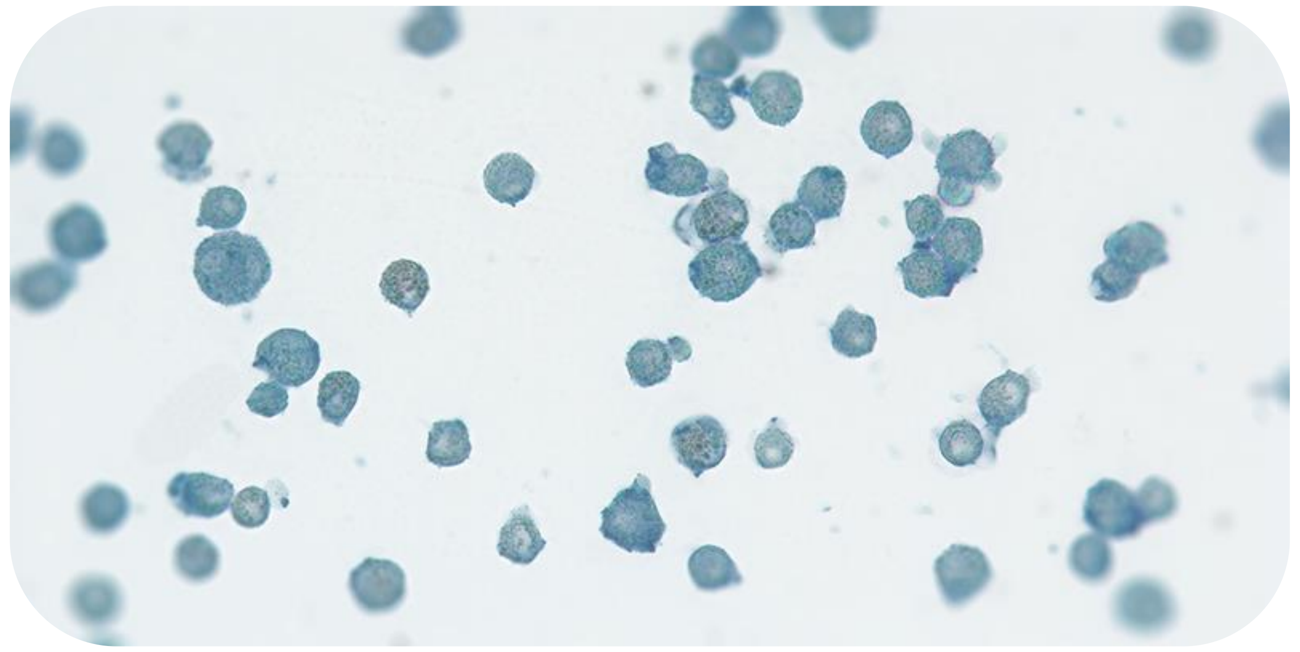
2 weeks →



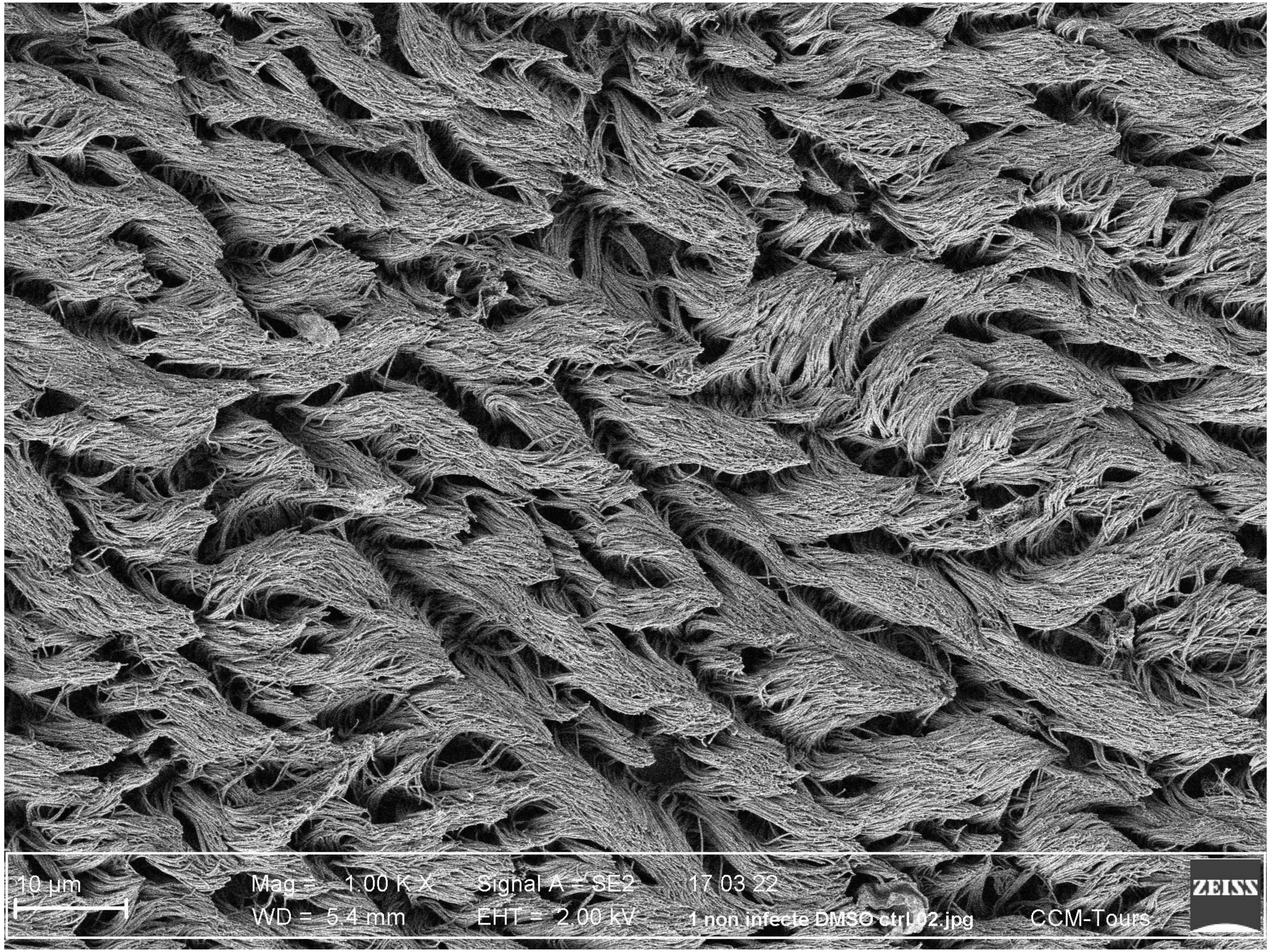
Isolation → **Cryopreservation**

Alveolar Macrophages →

→

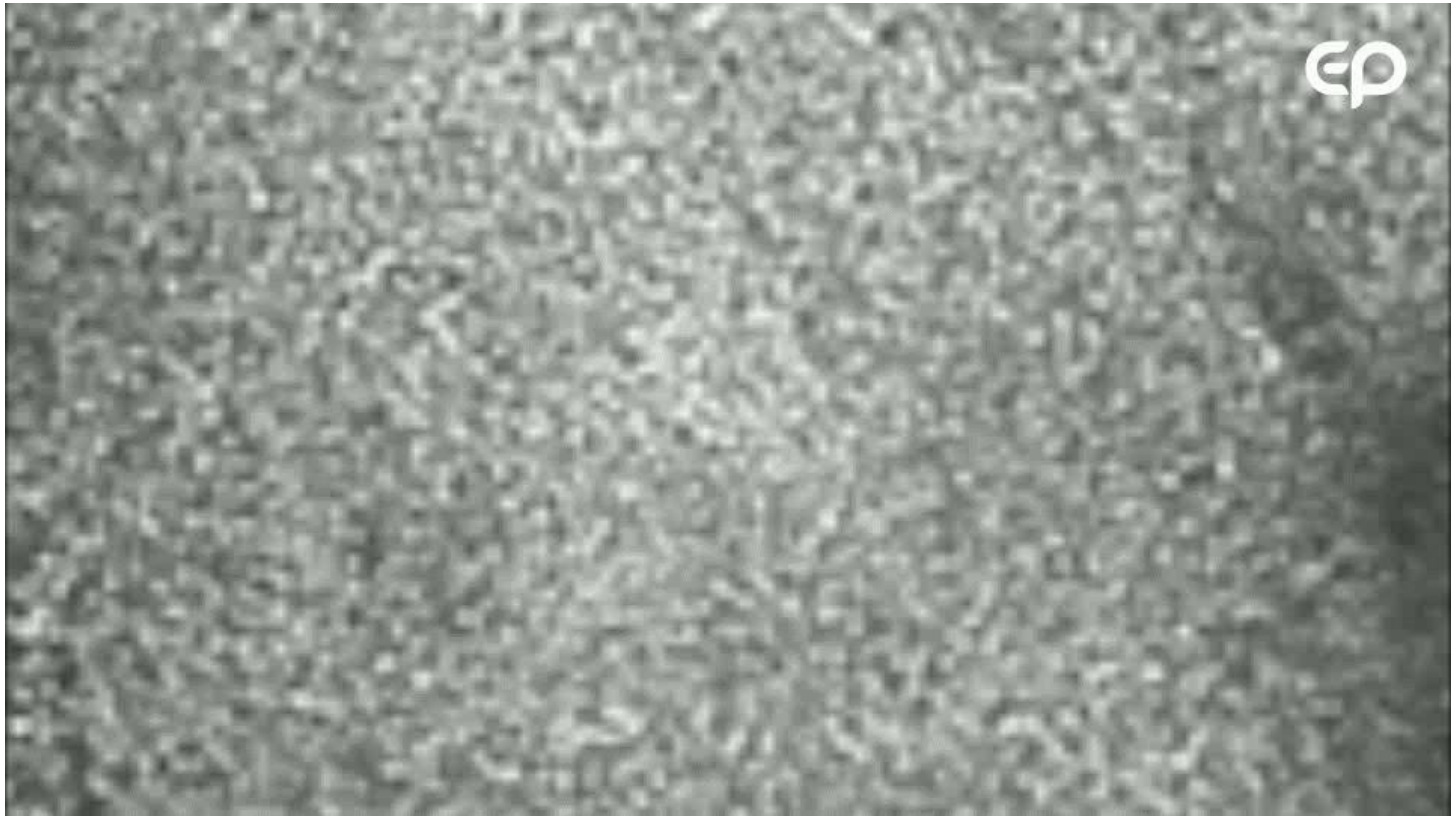


MucilAir™ | Electron Microscopy picture of the cilia



MucilAir™ healthy apical top view (SEM x10K)

Epithelix developed Cilia-X, a dedicated platform to measure cilia beating frequencies.



MucilAir™ healthy apical top view (phase contrast 5X, real time)

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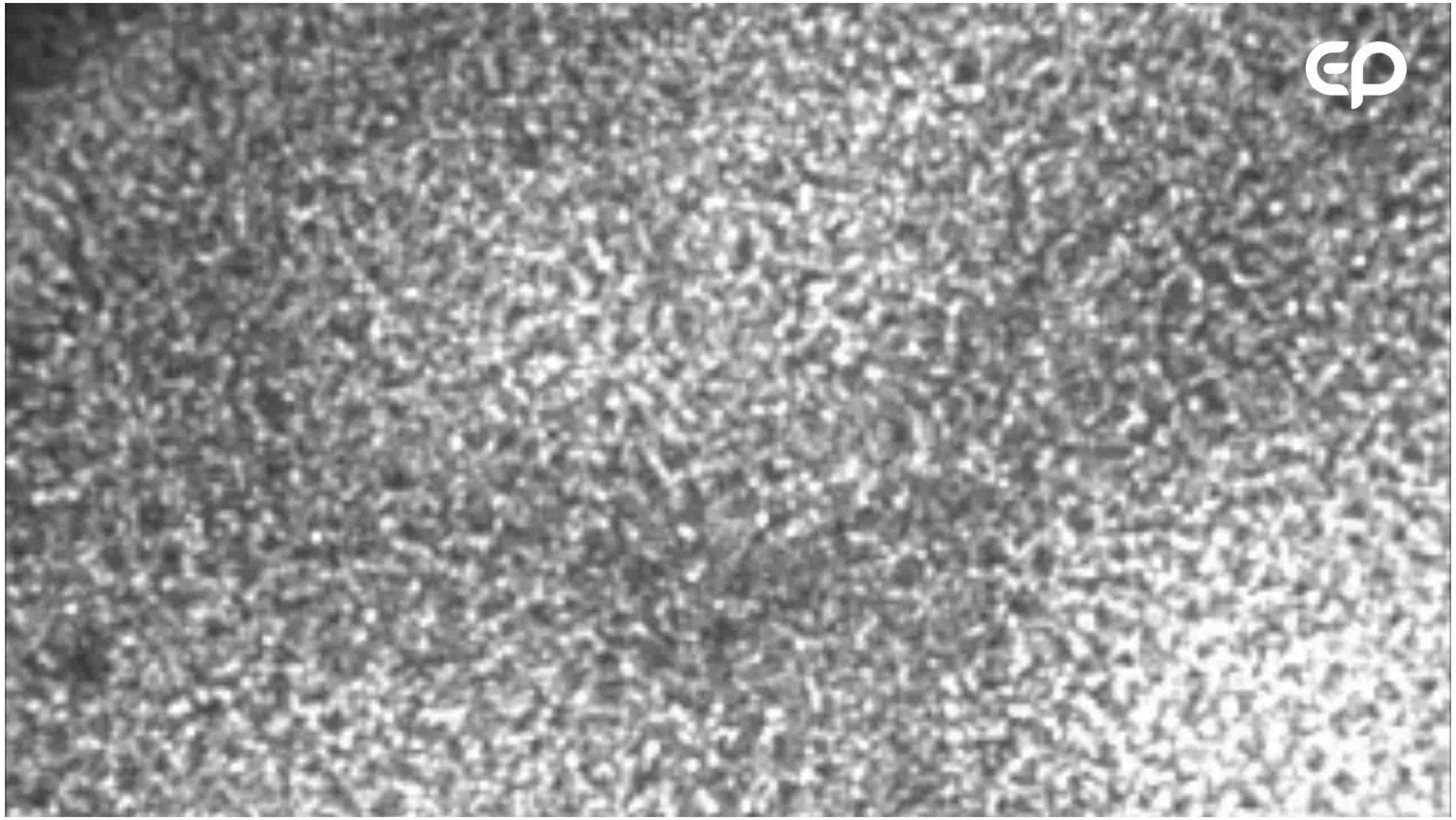
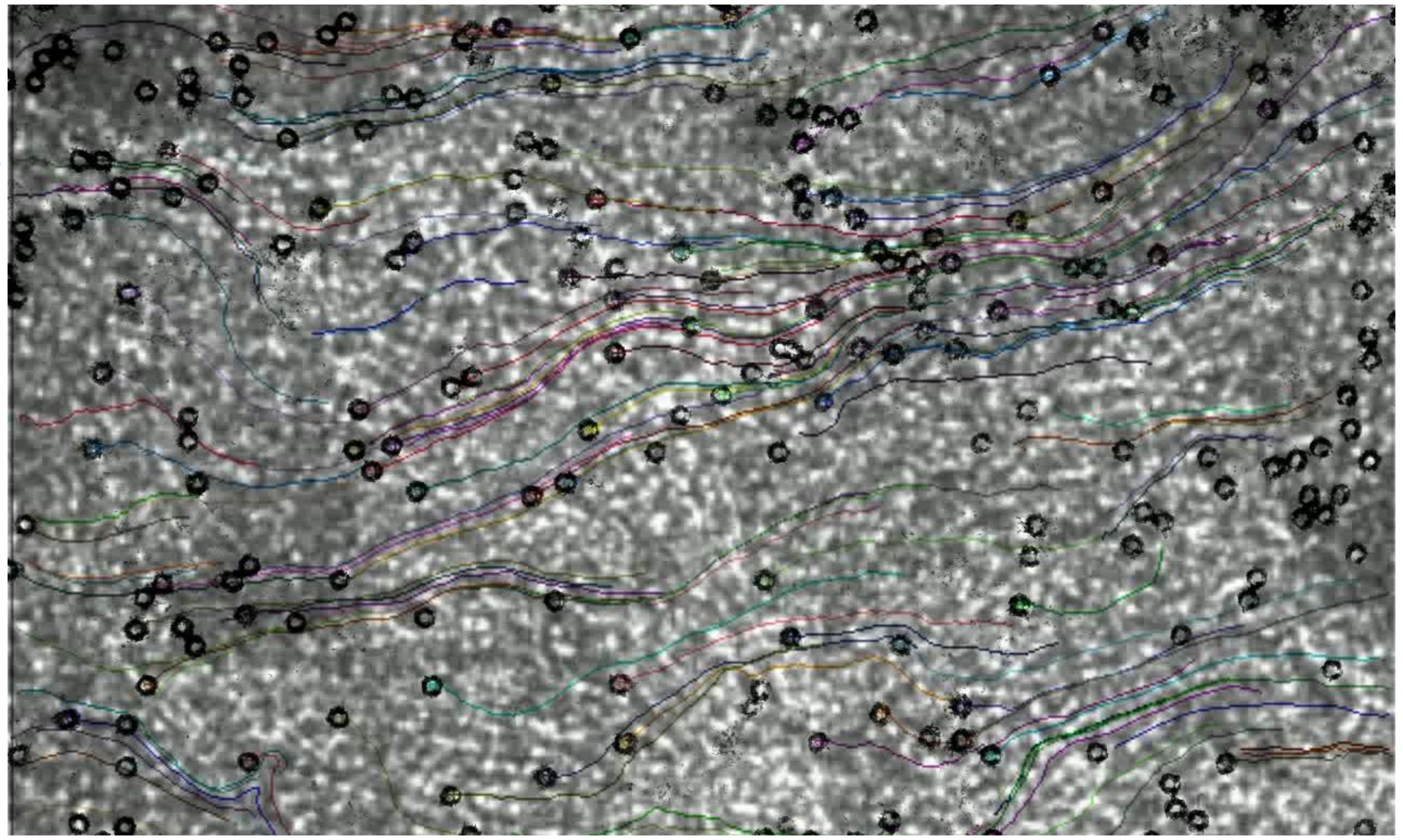
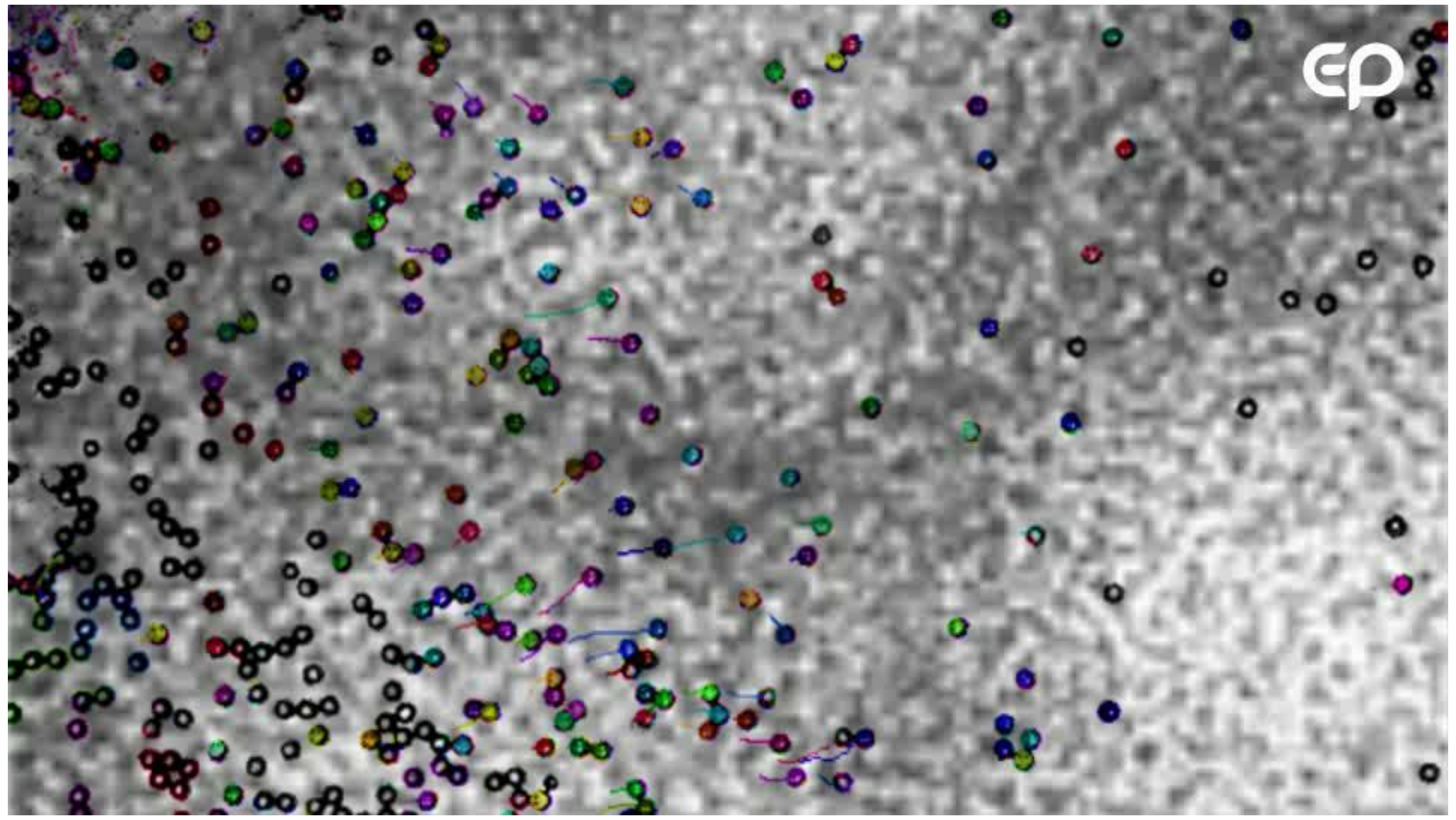


MucilAir™ healthy apical top view (phase contrast 5X, real time)

Mucociliary clearance (MCC) | pathologic example with MucilAir-CF (Cystic Fibrosis)

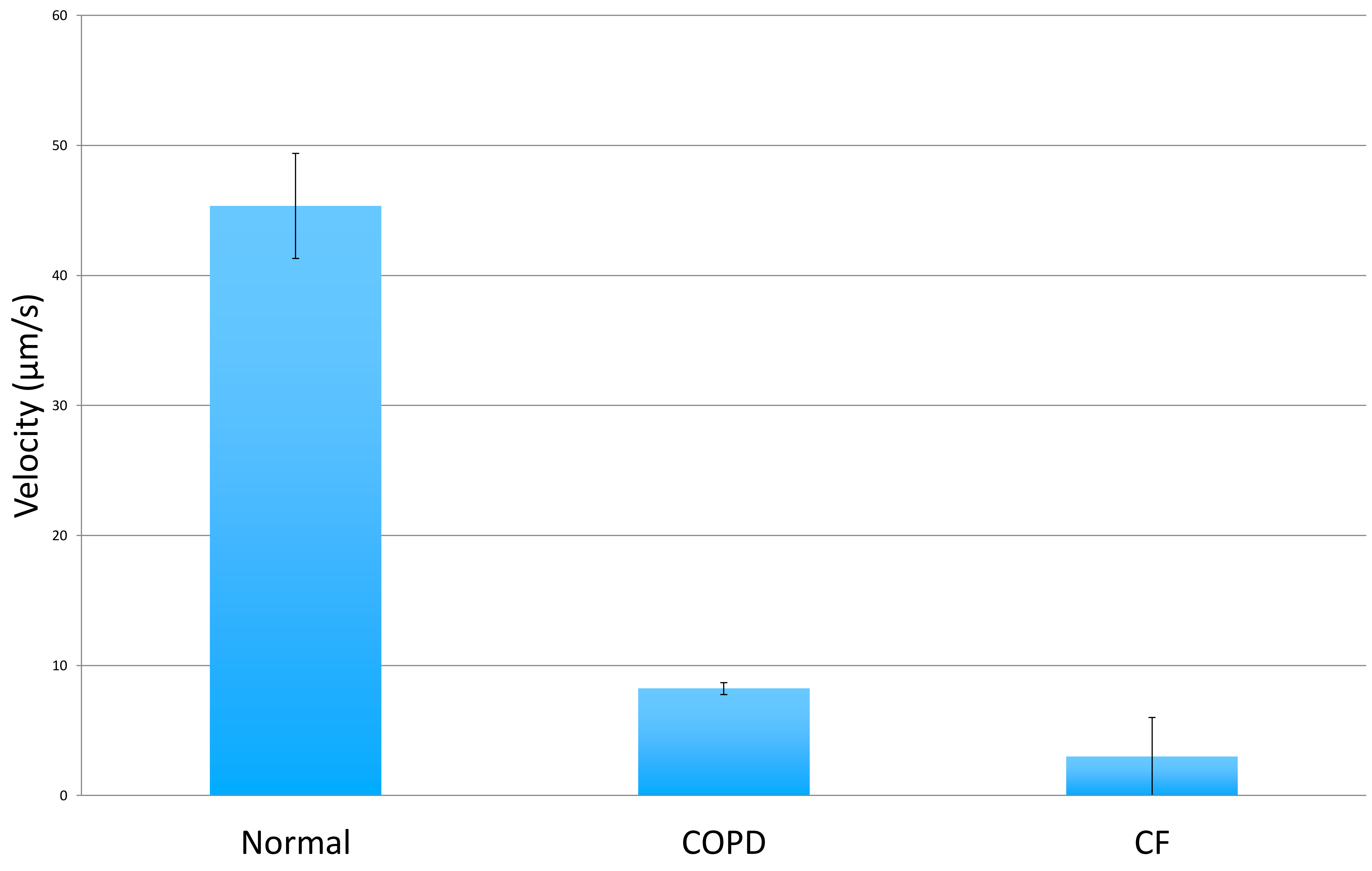


MucilAir™-Healthy apical top view (phase contrast 5X, real time)



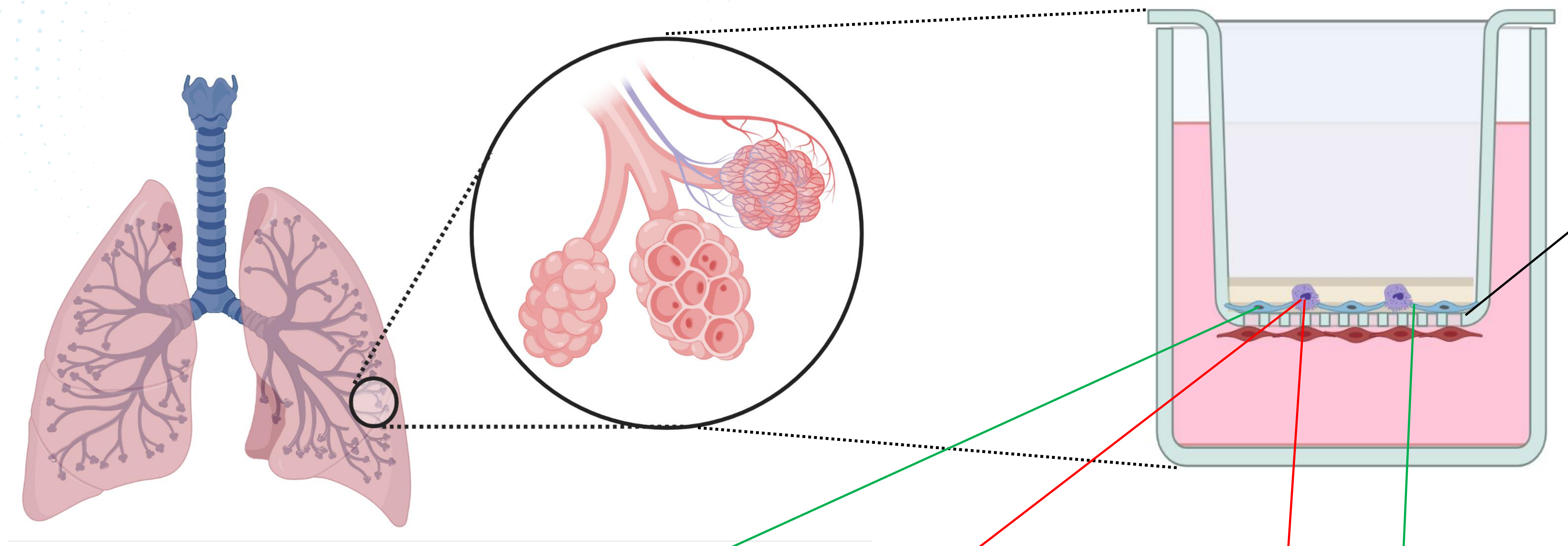
MucilAir™-Cystic Fibrosis apical top view (phase contrast 5X, real time)

Comparison of particles velocities between MucilAir™-Normal, MucilAir™-COPD and MucilAir™-CF



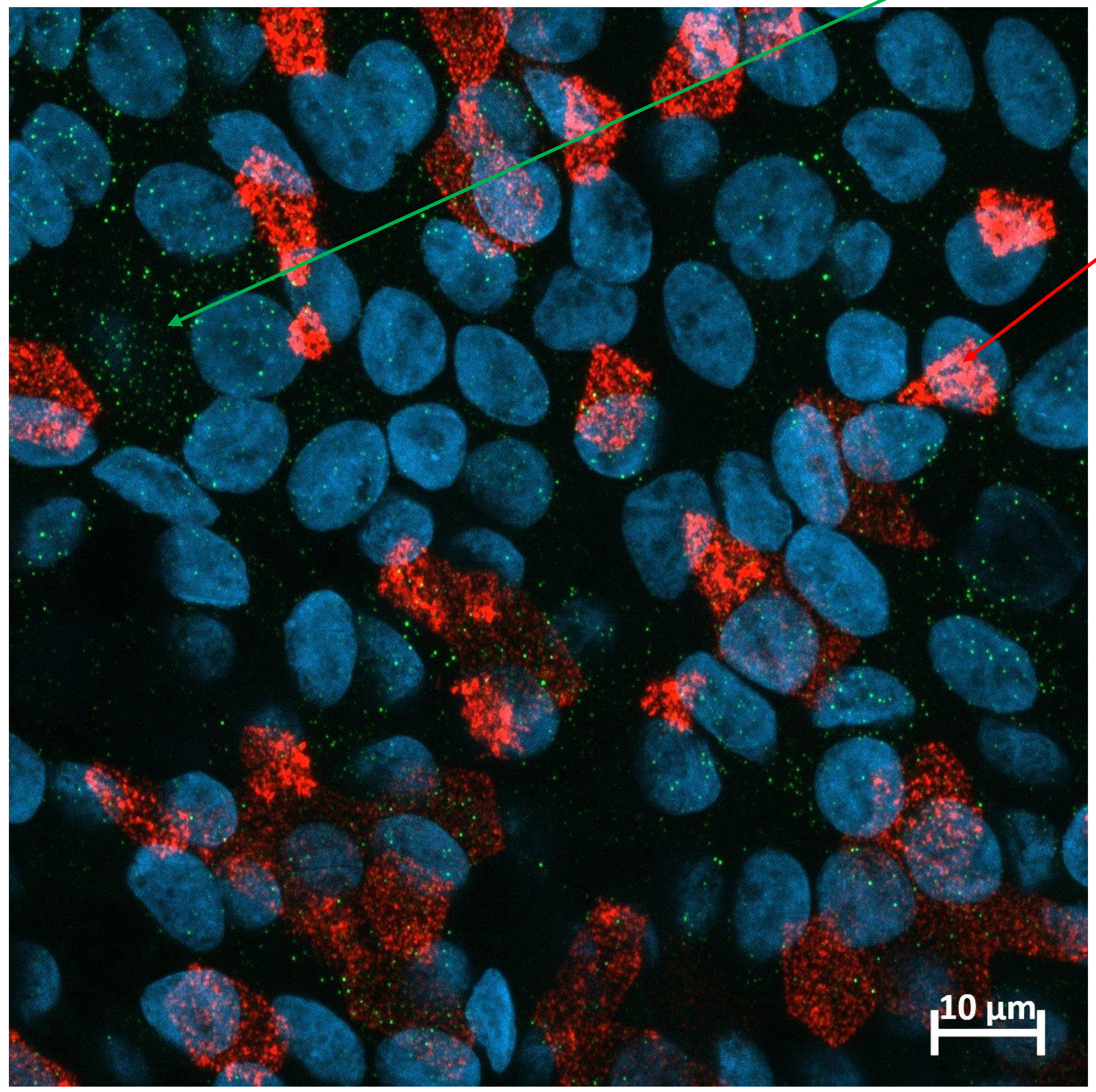
Epithelix internal study

AlveolAir™ Characterisation



SPC = 650 ± 0.09 pg/mL (n=3)

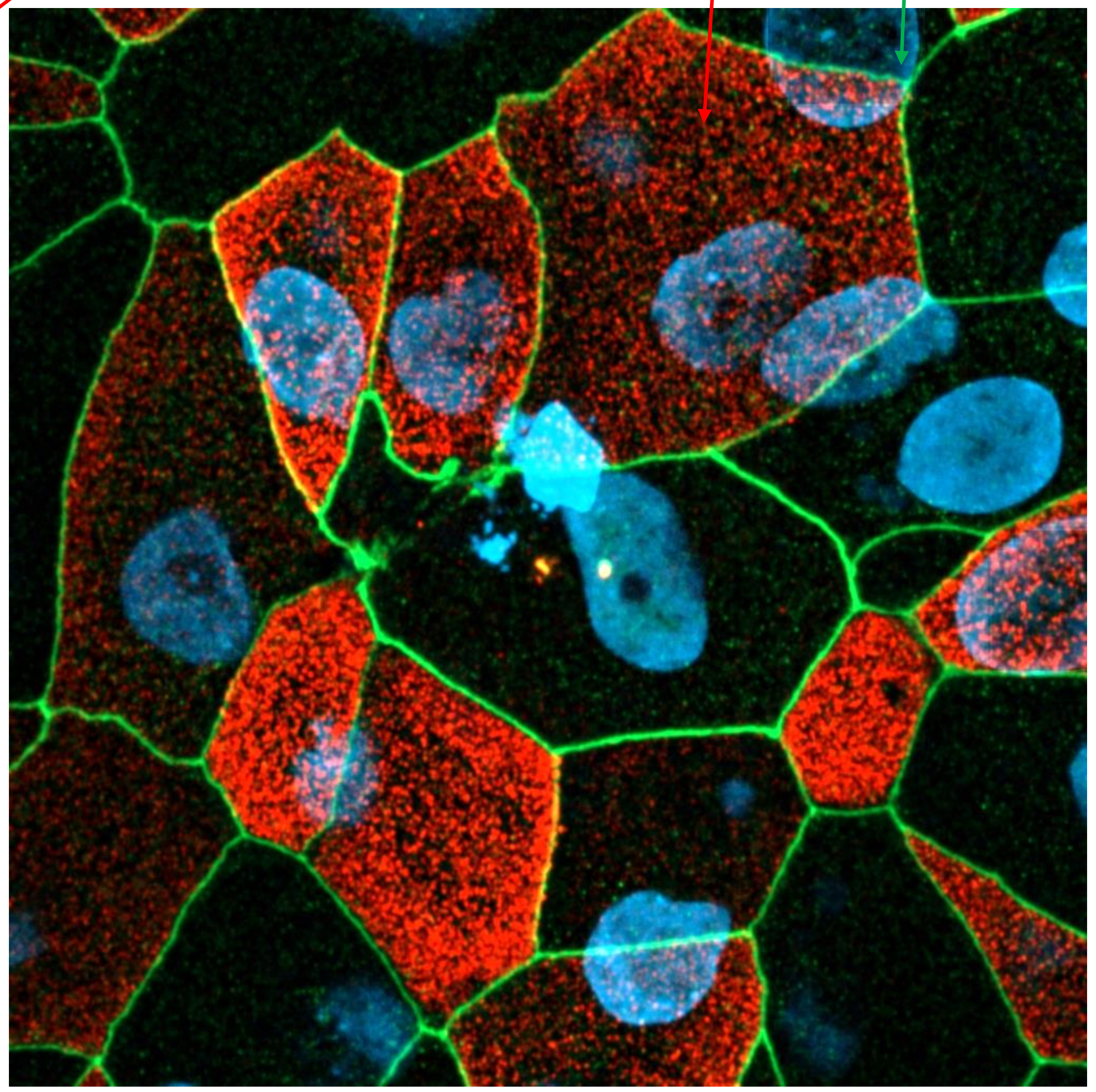
Pneumocyte type I ⟶	**CAV-1**
Pneumocyte type II ⟶	**HTII-280**
Endothelial cell ⟶	**CD34 & vWF**
Surfactant ⟶	**SPC**



CAV-1

DAPI

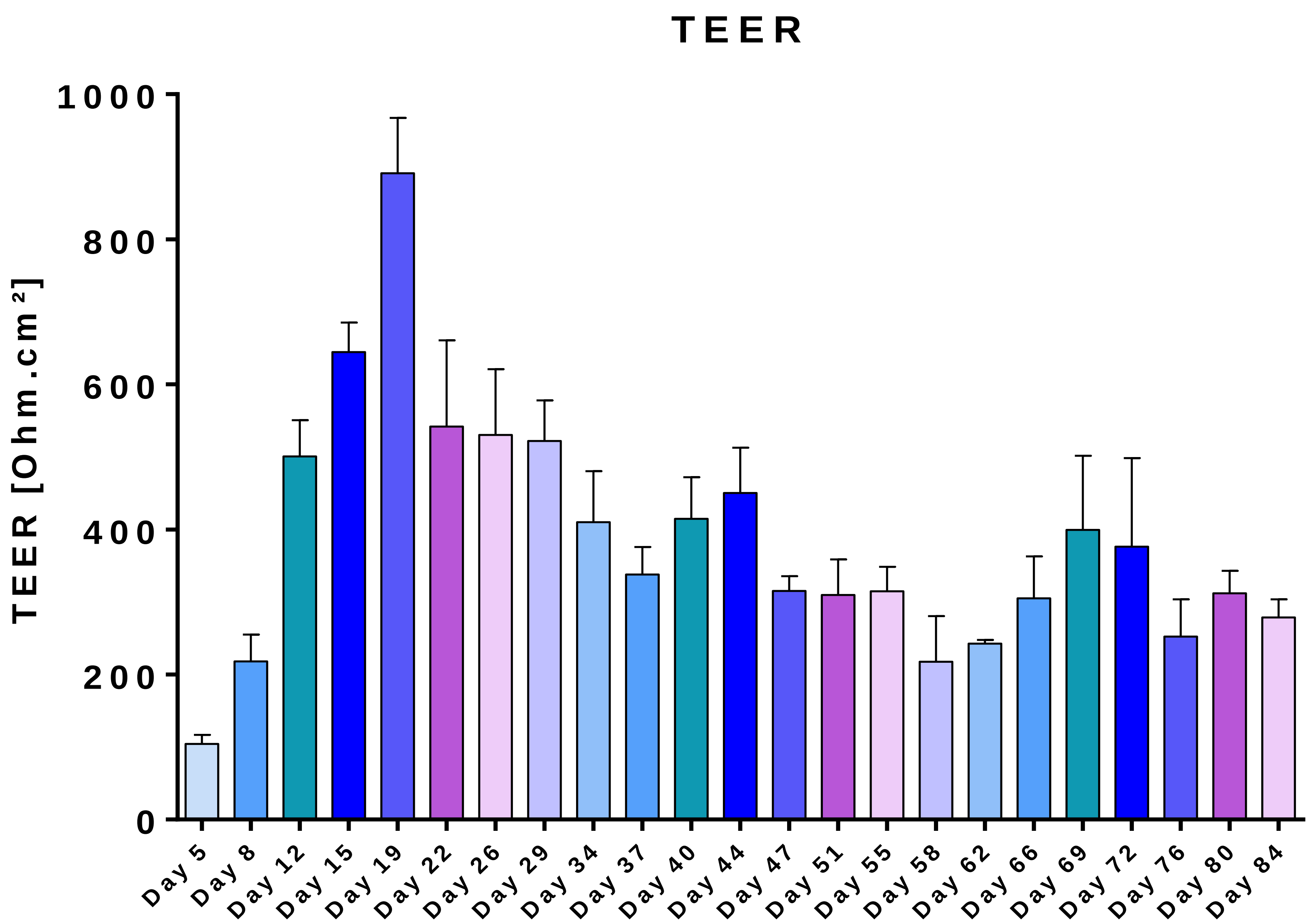
HTII-280



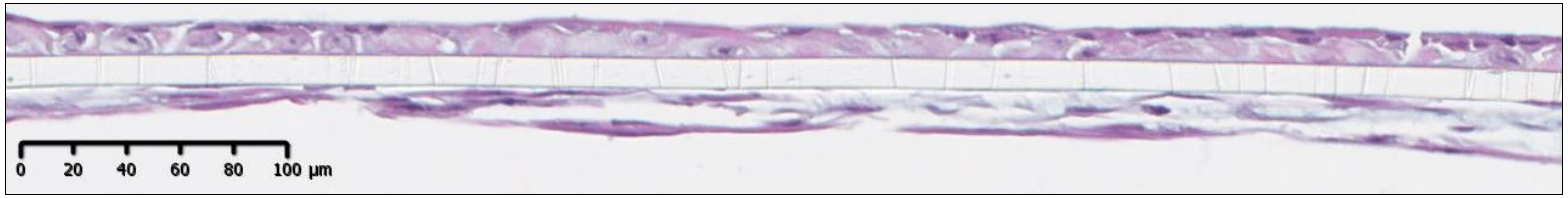
ZO-1

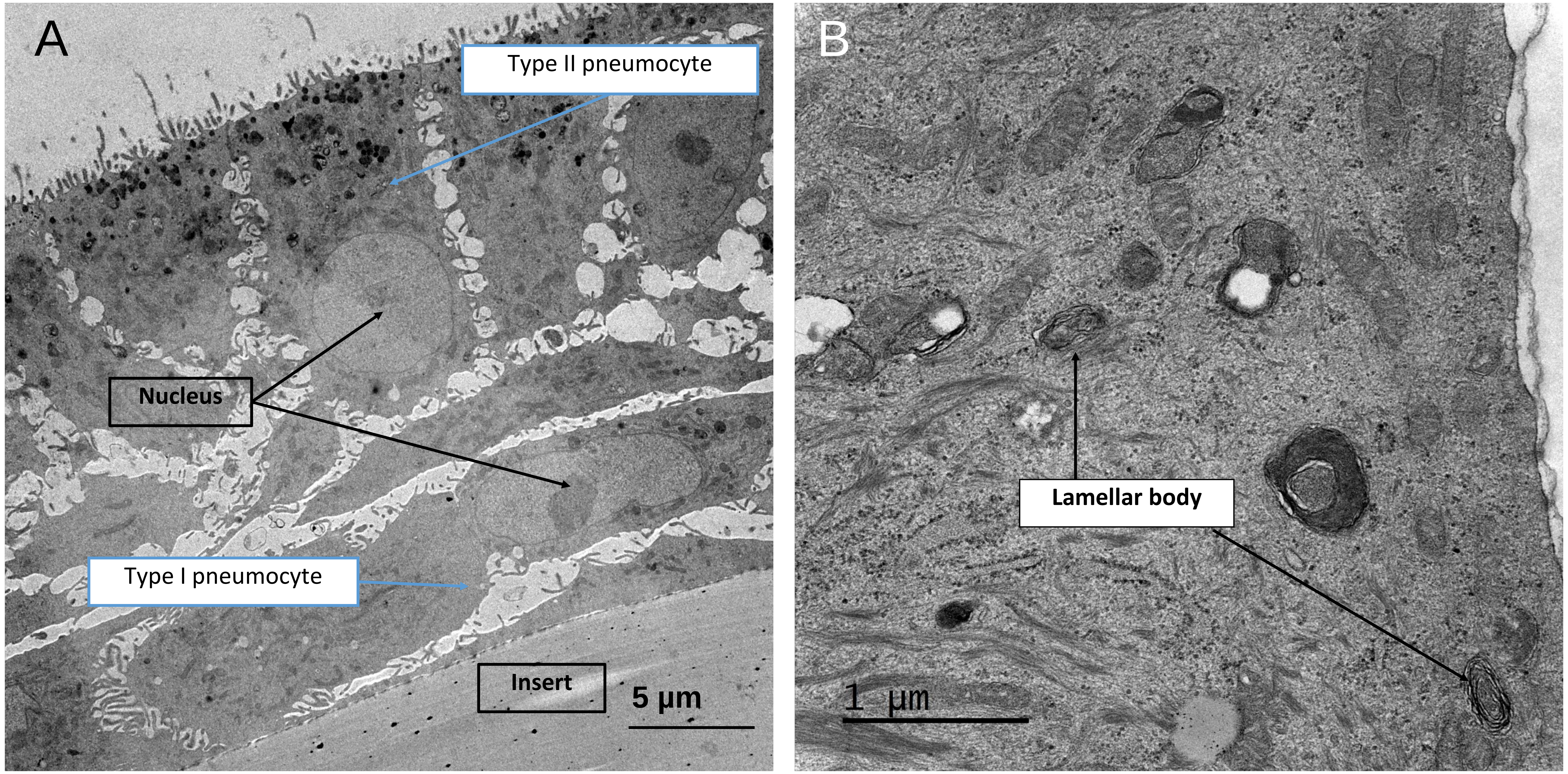
DAPI

HTII-280



AlveolAir™ Characterisation – Histology – Electron microscopy



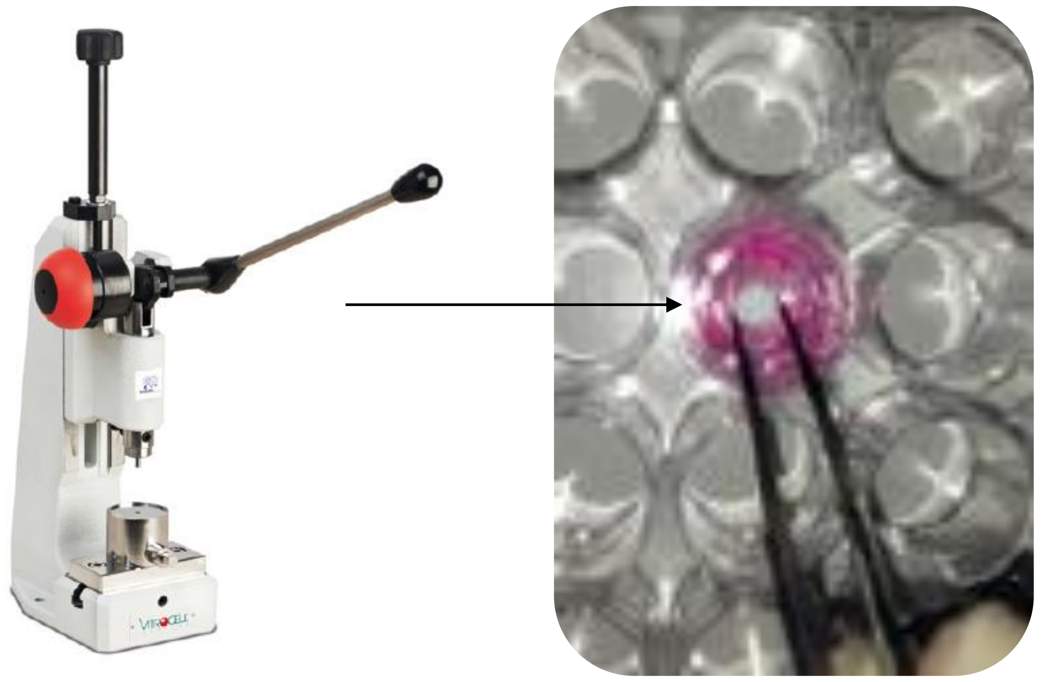


Exposure systems | Examples

Static Exposure

Liquids - Solutions

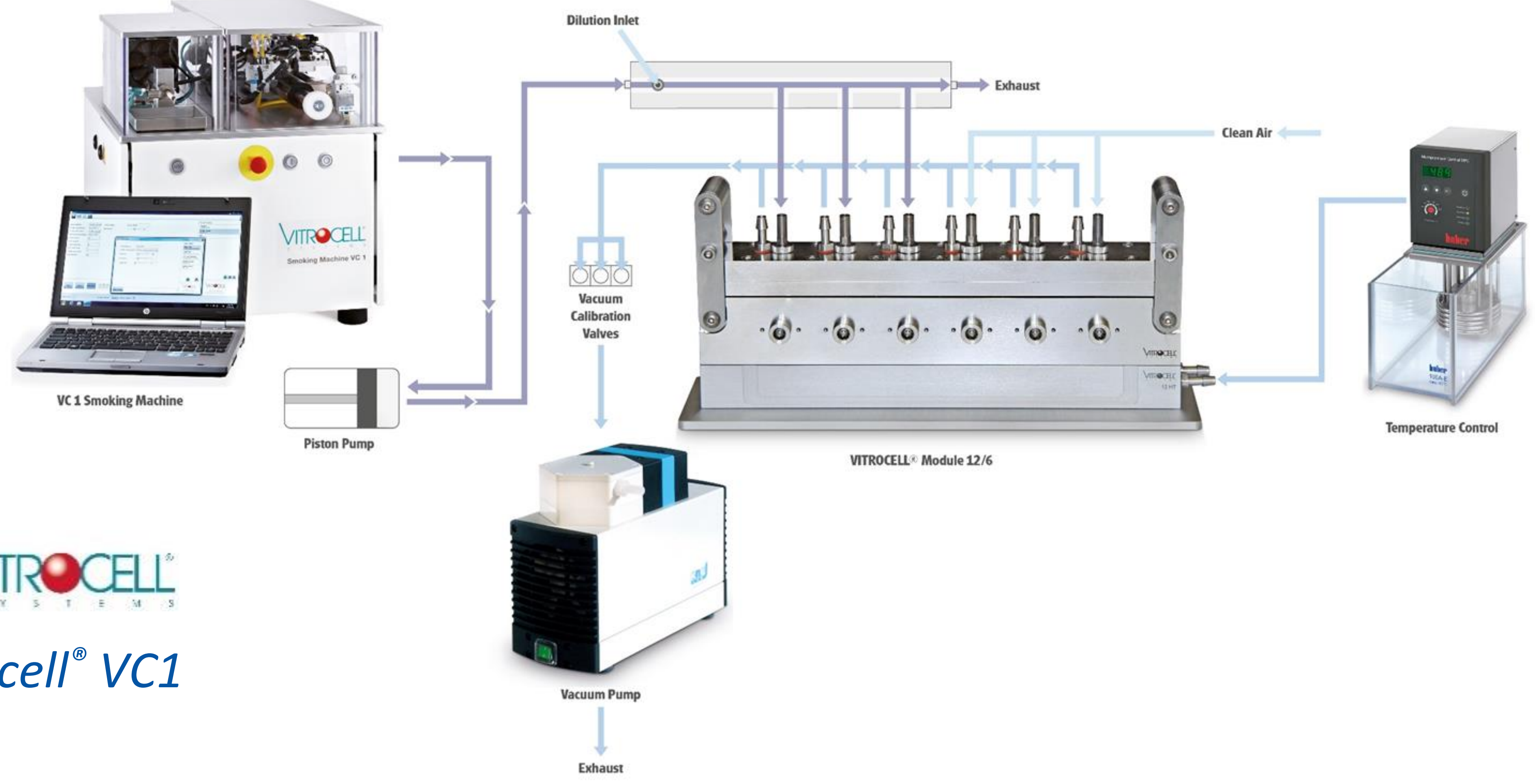
Solids (tablets)



Vitrocell® Epithelix Nanopress

Dynamic Exposure

Gas or smoke



Vitrocell® VC1

Nebulized liquid

Vitrocell® Cloud Alpha

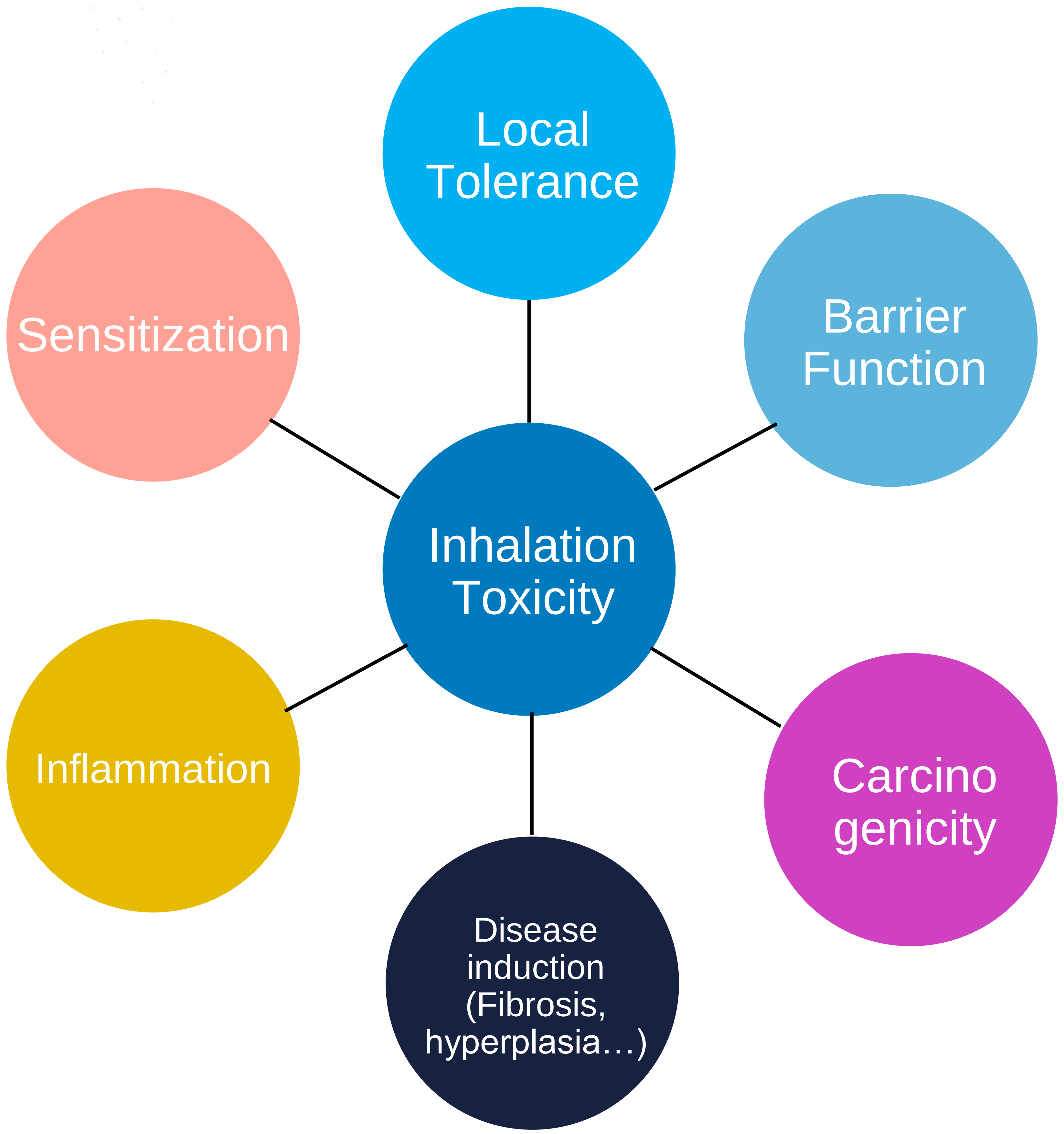
Dry Powder

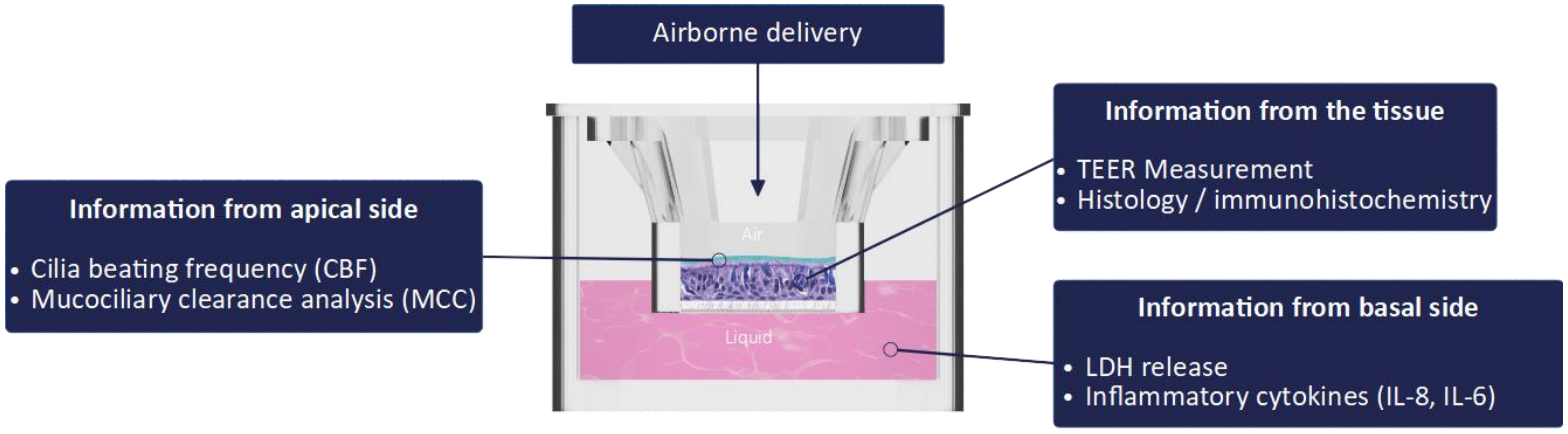
Vitrocell® Powder chamber

3D *in vitro* ALI airway epithelia for

Inhalation toxicity assessment

Evaluation of inhalation toxicity of inhaled xenobiotics/drugs





Evaluation of inhalation toxicity of inhaled xenobiotics/drugs

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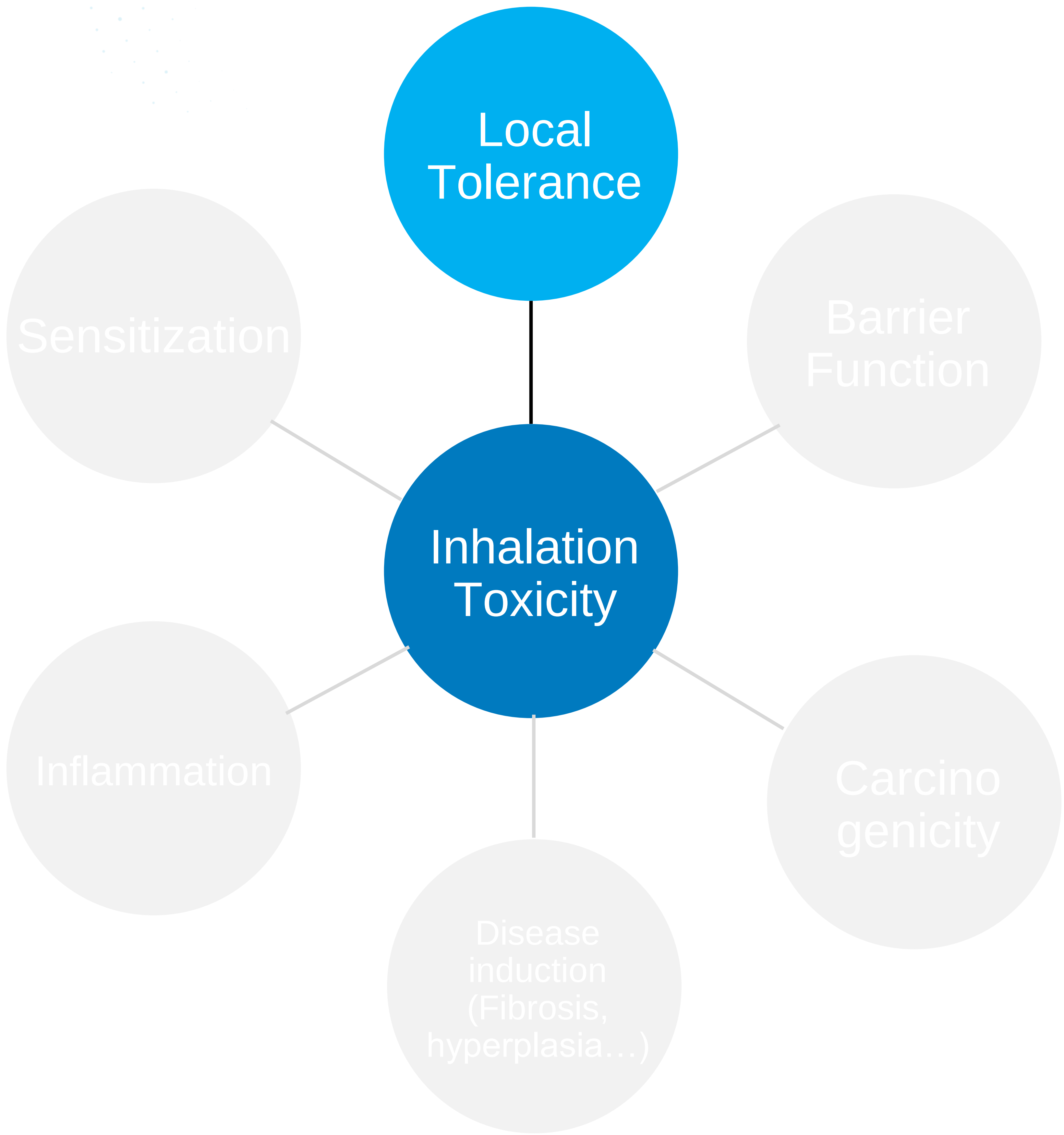
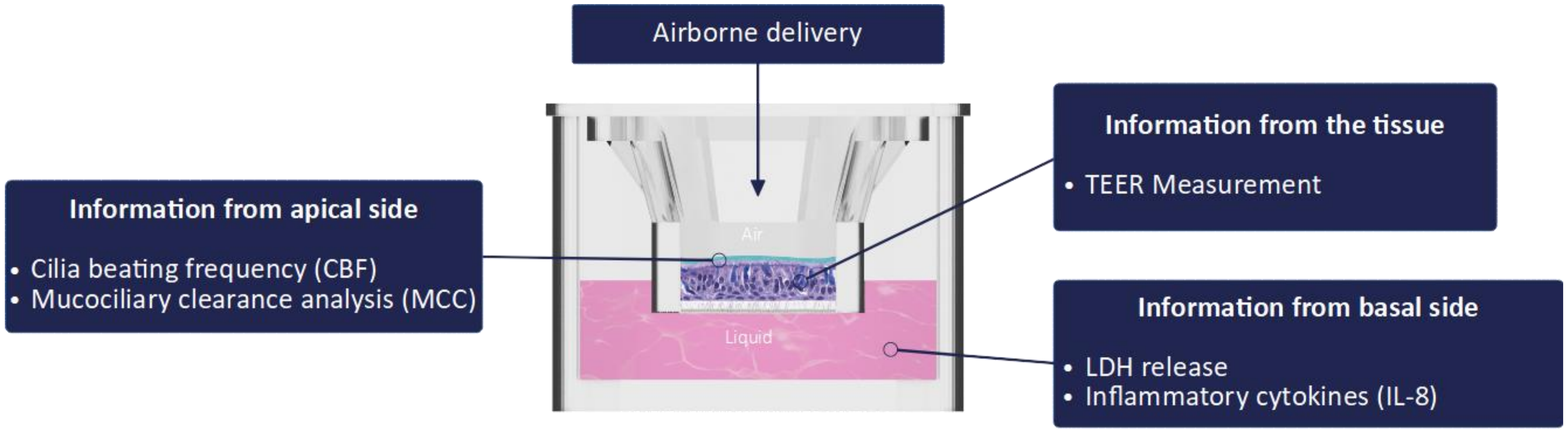
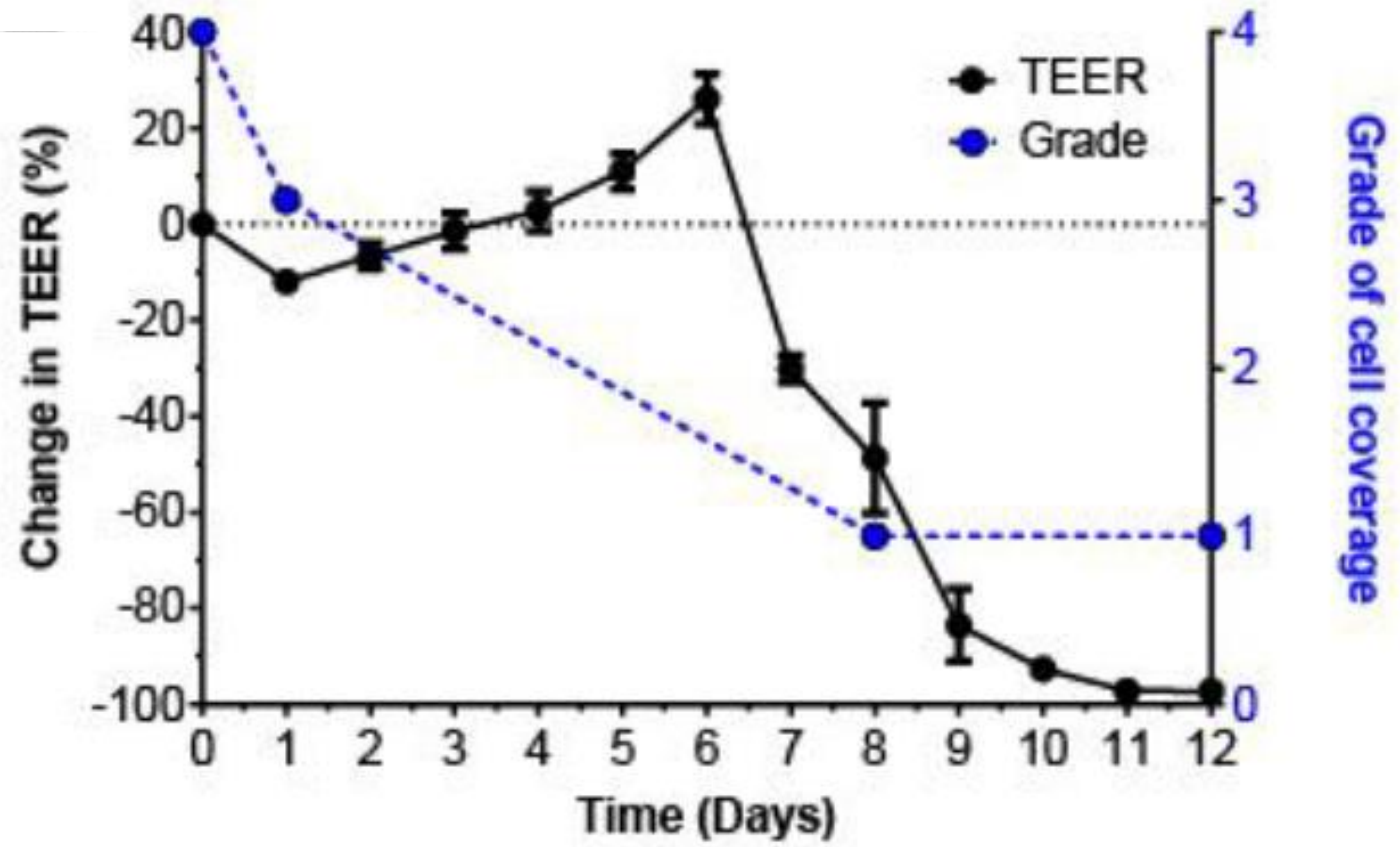


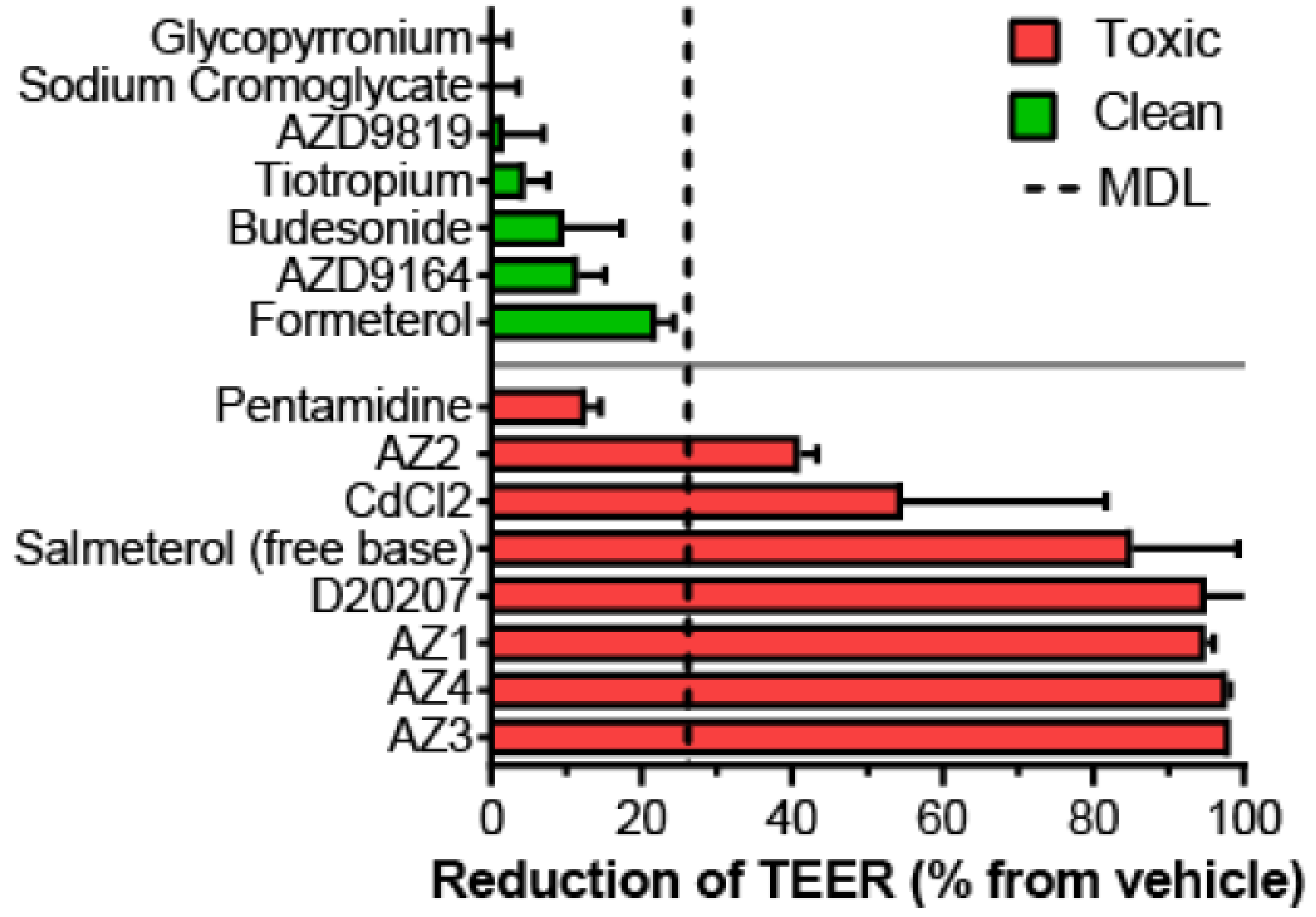


Prediction of respiratory Toxicity

A 3D human airway model enables prediction of respiratory toxicity of inhaled drugs *in vitro*

Kinga Balogh Sivars[1†]*, Ulf Sivars[4†], Ellinor Hornberg[4†], Hui Zhang[3†], Lena Brändén[3†], Rosy Bonfante[5], Song Huang[5], Samuel Constant[5], Ian Robinson[2††], Catherine J Betts[3††] and Per Åberg[2†]





15 compounds tested

88% sensitivity

100% specificity

Toxicol Sci. 2018 Mar 1;162(1):301-308.

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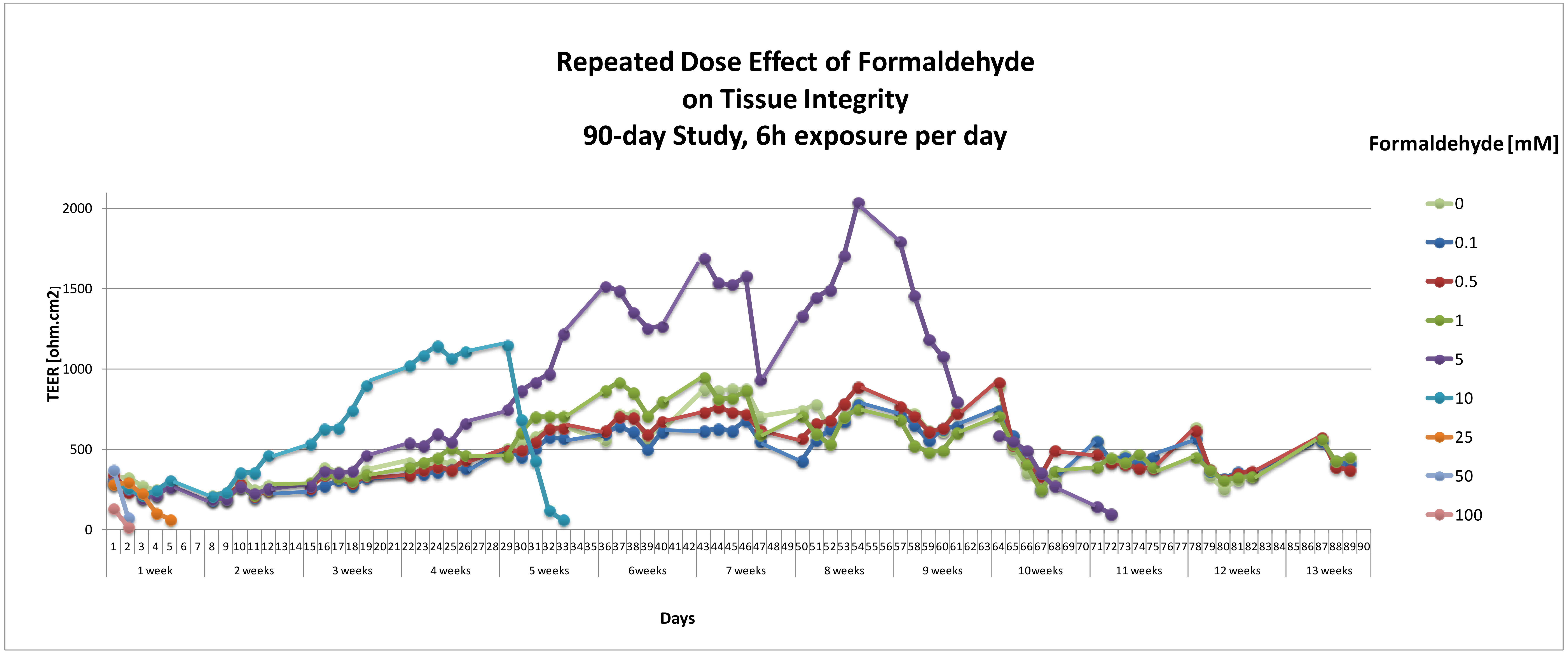
First transposition of the OECD 413 guideline in vitro



Example of a 90 days repeated dose exposure study on MucilAir™. 6 hours per day exposure to Formaldehyde for a period of 90 days. Every day, tissue Integrity (TEER) were measured (N=3) then epithelia were reused for the next exposure. (Epithelix internal study)

Prediction of respiratory Toxicity using MucilAir™

EPITHELIX

OECD
Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2022)31

Unclassified — **English - Or. English**

1 September 2022

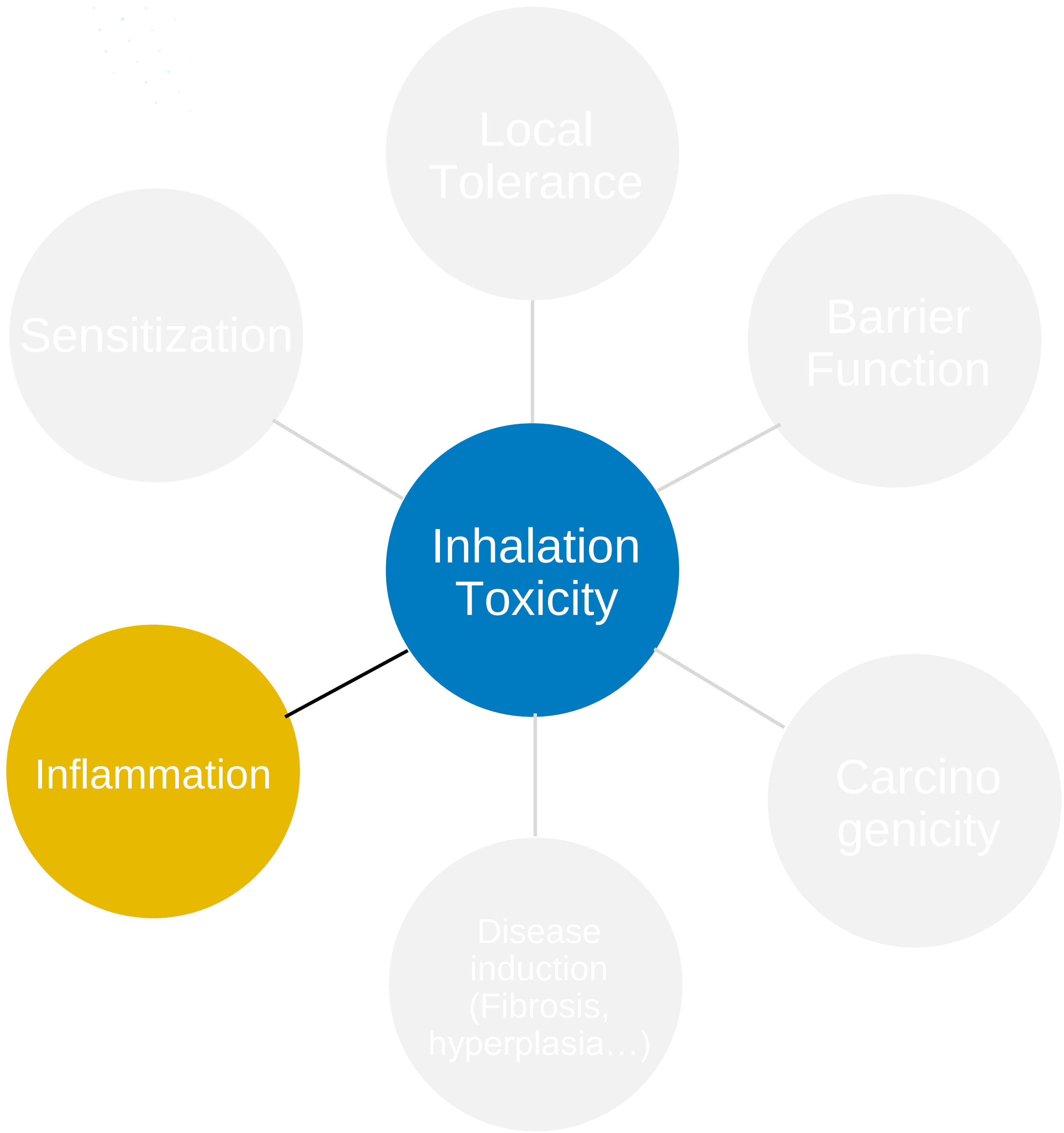
ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

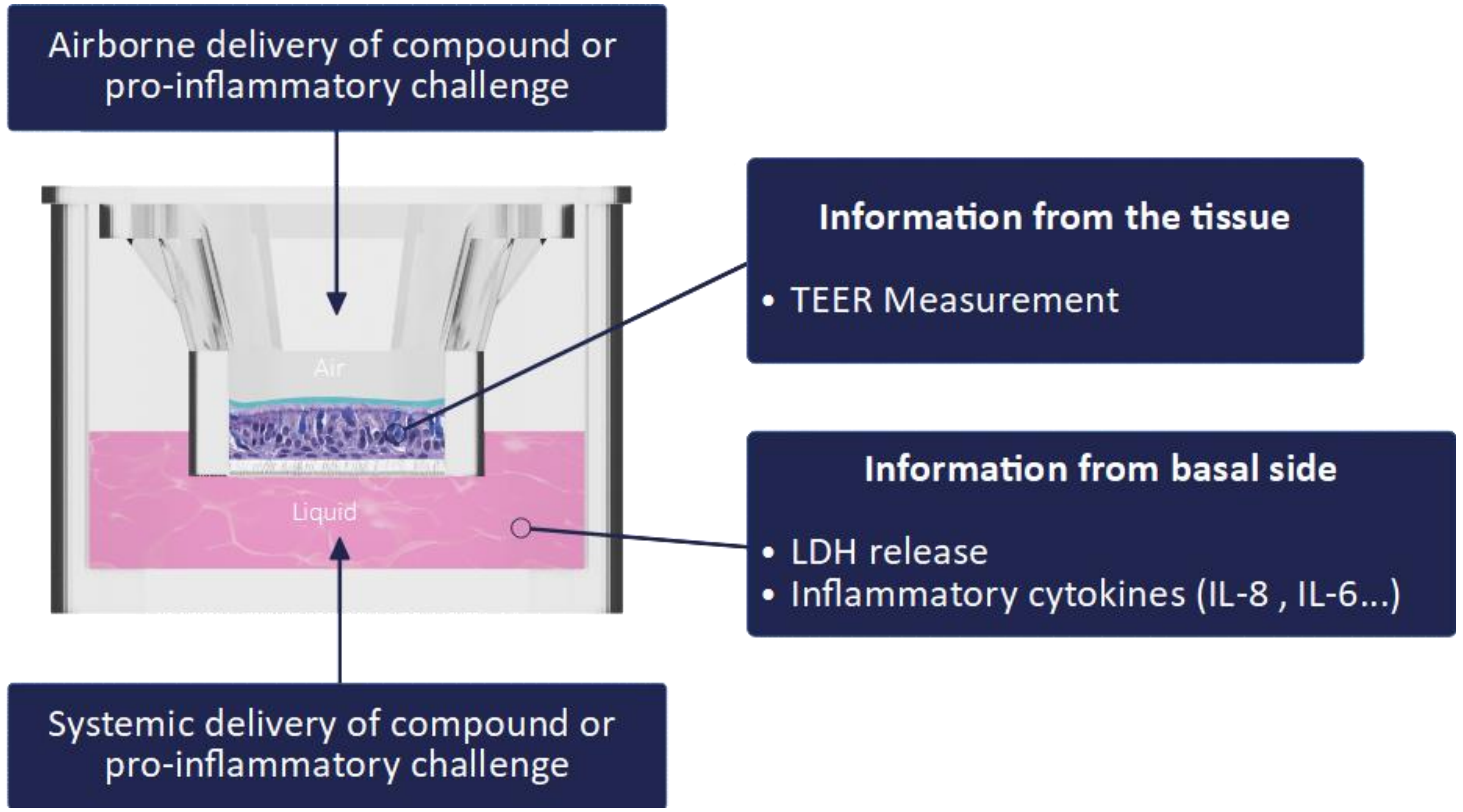
Case Study on the use of an Integrated Approach for Testing and Assessment (IATA) for New Approach Methodology (NAM) for Refining Inhalation Risk Assessment from Point of Contact Toxicity of the Pesticide, Chlorothalonil.

Series on Testing and Assessment
No. 367

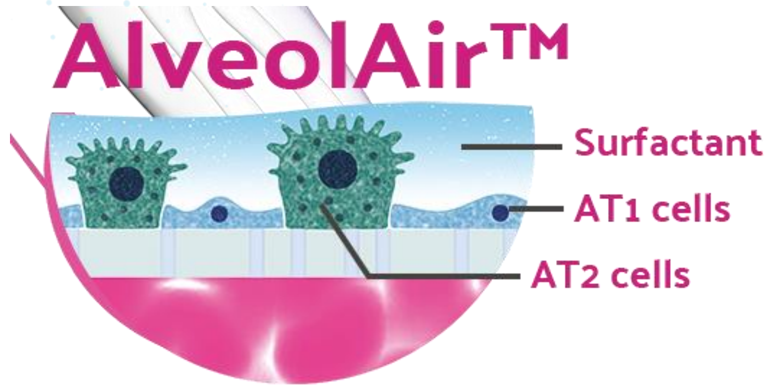
Replacement of a 90 Days rat inhalation study (OECD TG413) using MucilAir™

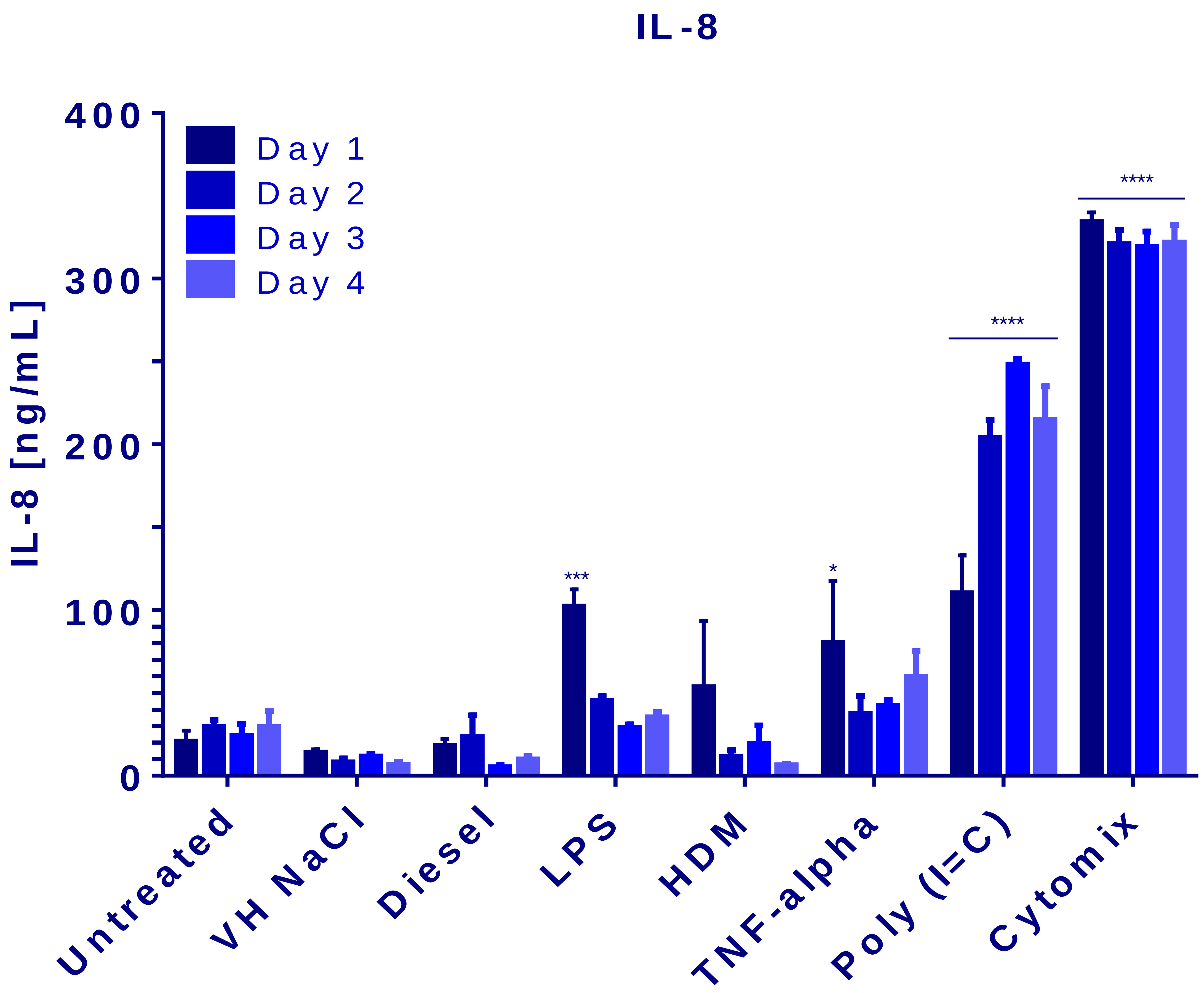
Evaluation of inhalation toxicity of inhaled xenobiotics/drugs





Pro-inflammatory monitoring using AlveolAir™





Repeated daily exposure of pro-inflammatory compounds on AlveolAir™

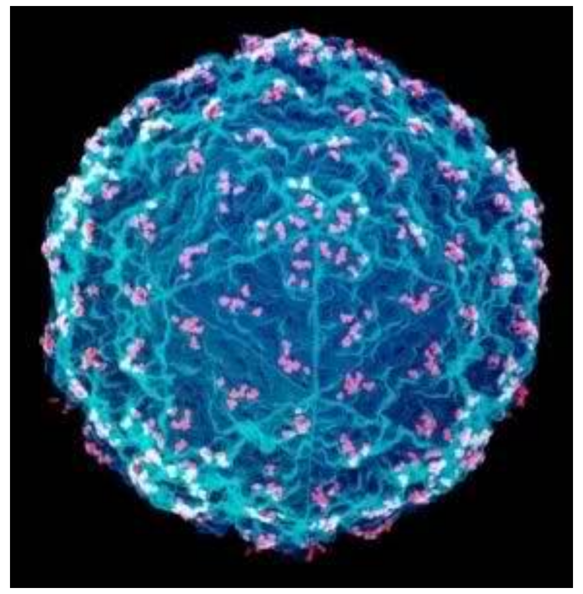
Stimulus	Diesel	LPS	HDM	TNF-alpha	Poly(I=C)	Cytomix
Concentration [mg/mL]	1.67	0.2	20	$5 \cdot 10^{-4}$	10	0.2 LPS + $5 \cdot 10^{-4}$ TNF-alpha + 1 % FBS
Exposure	Apical (10 µL)	Apical (10 µL)	Apical (10 µL)	Apical (10 µL)	Apical (10 µL)	Basal (700µL)

EPITHELIX
IN VITRO RESPIRATORY SOLUTIONS

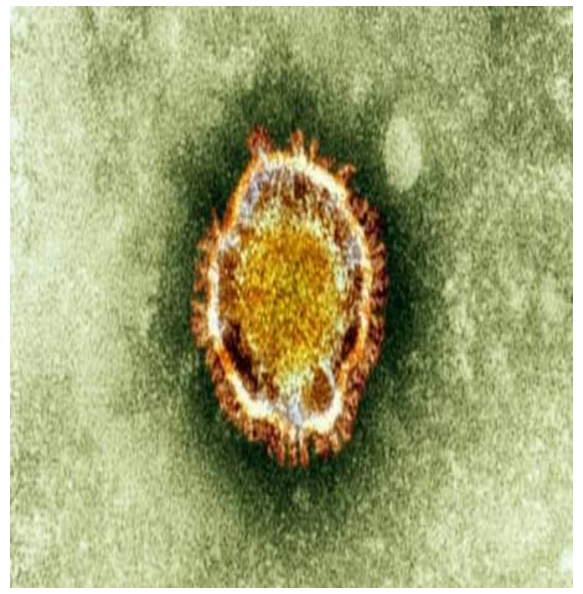
3D *in vitro* ALI airway epithelia for

Antiviral evaluation

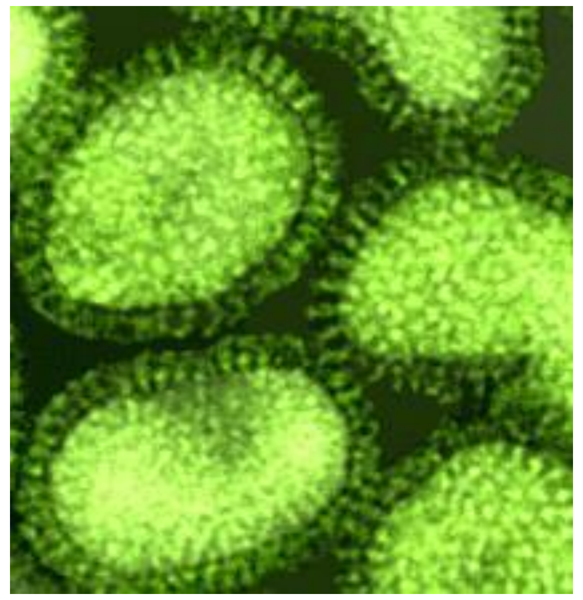
In vitro antiviral testing using MucilAir™/ SmallAir™/ AlveolAir™



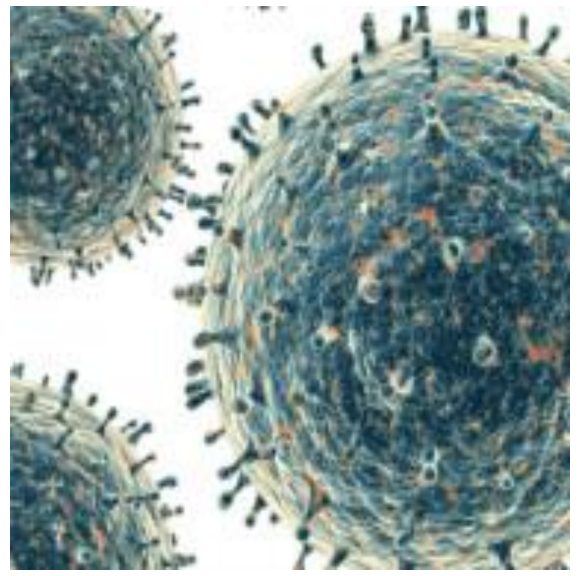
Rhinovirus
HRV-A16
HRV-B14
HRV-C15



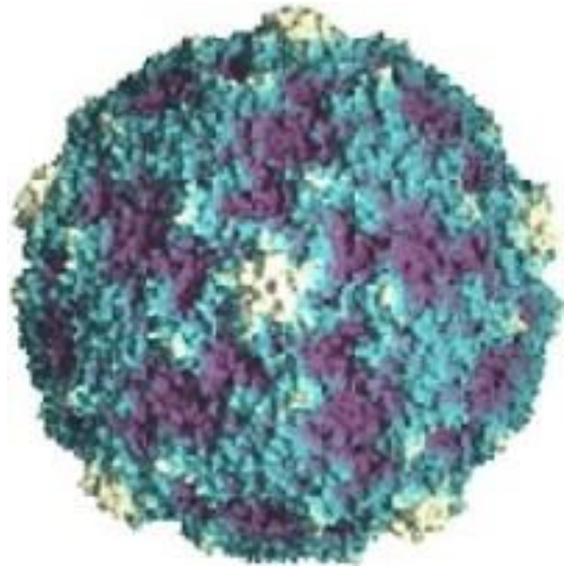
Coronavirus
OC 43
SARS-CoV-2



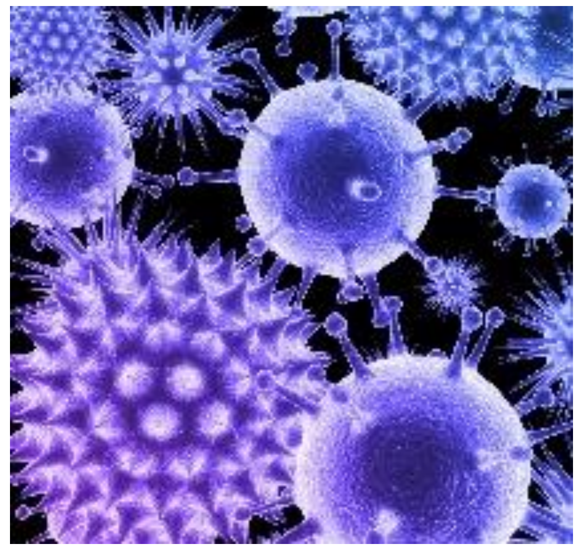
Influenza
Influenza A
(H1N1 and H3N2)
Influenza B



Respiratory Syncytial Virus
RSV-A
RSV-B

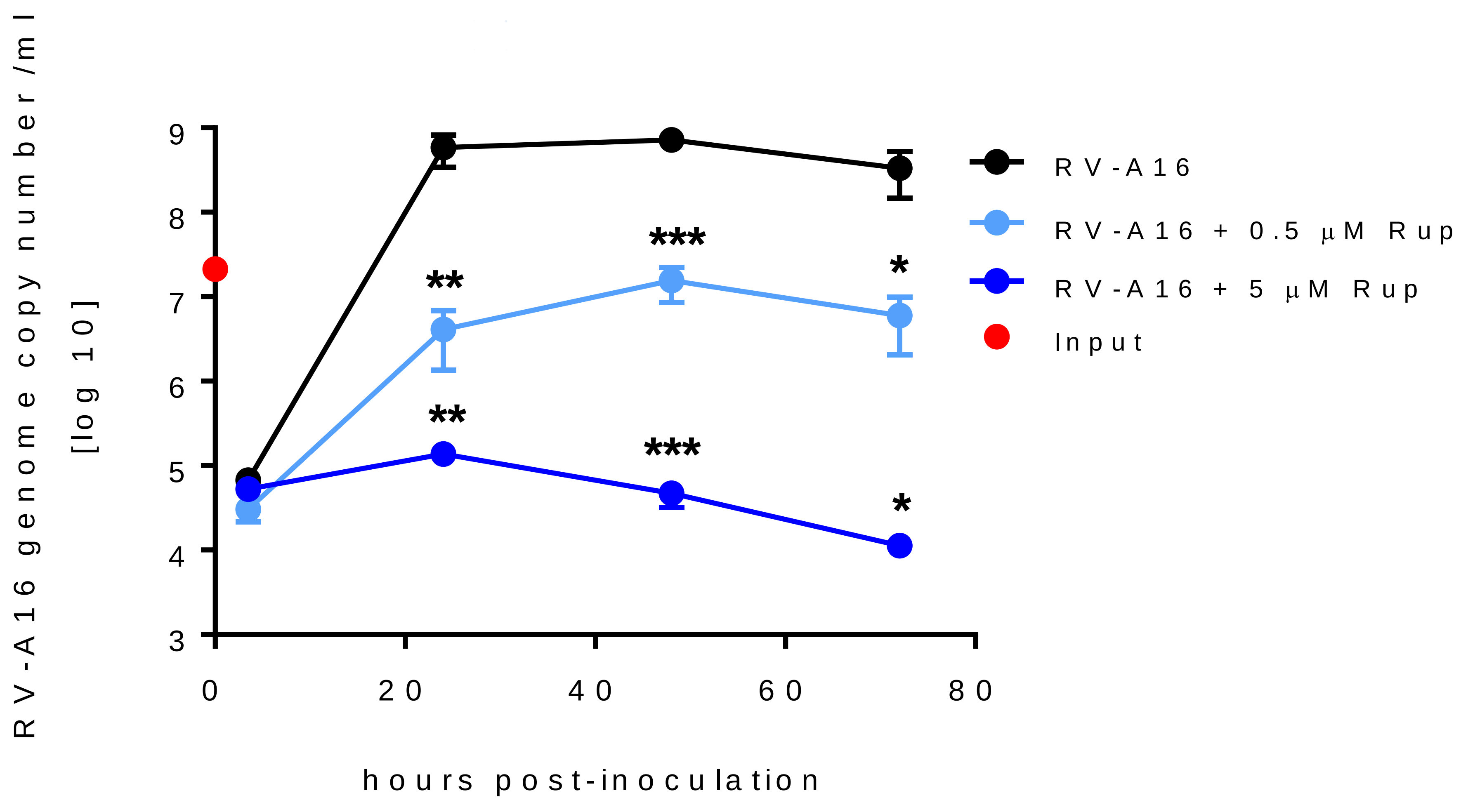


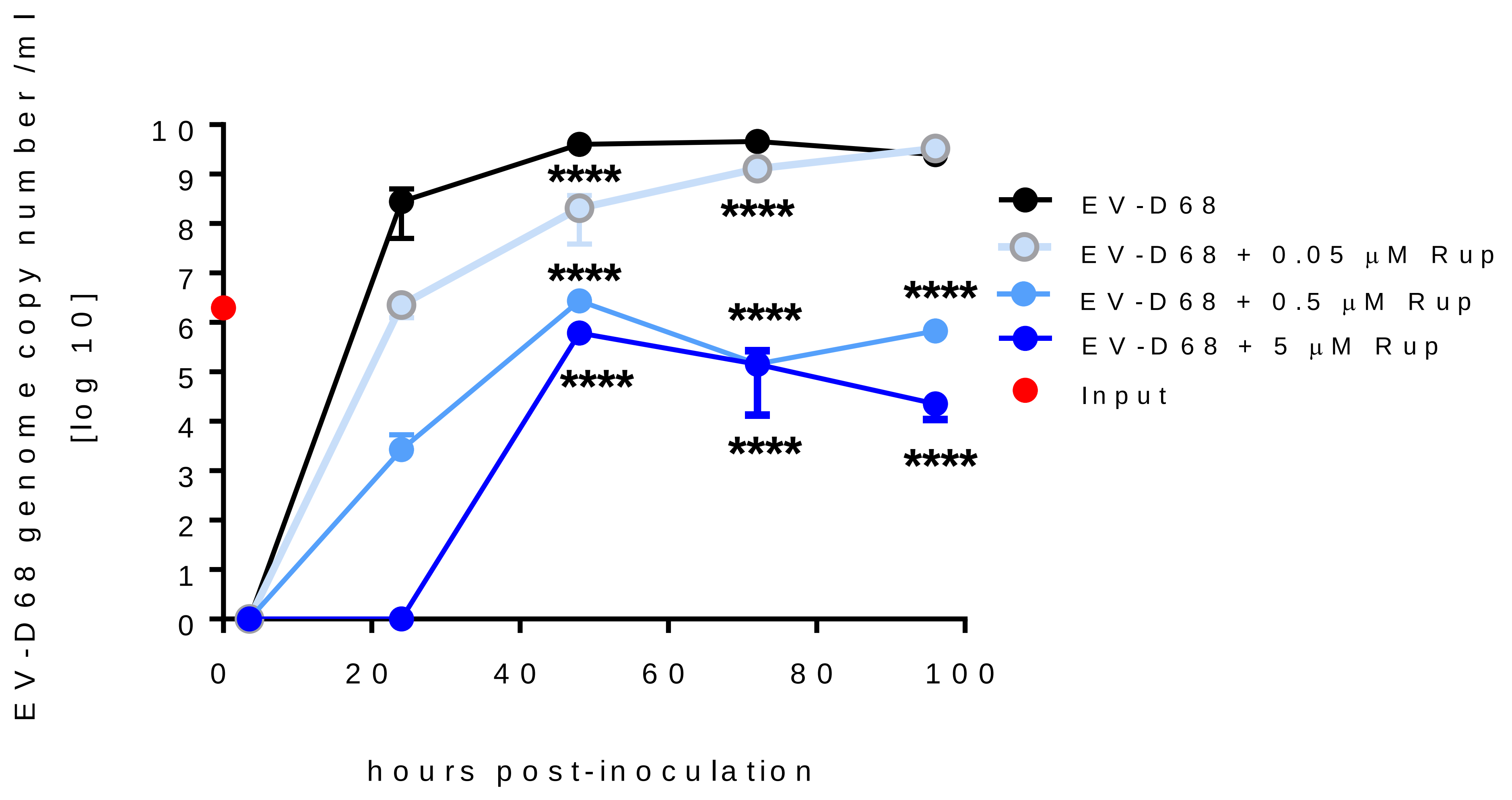
Respiratory Enterovirus
EV-68

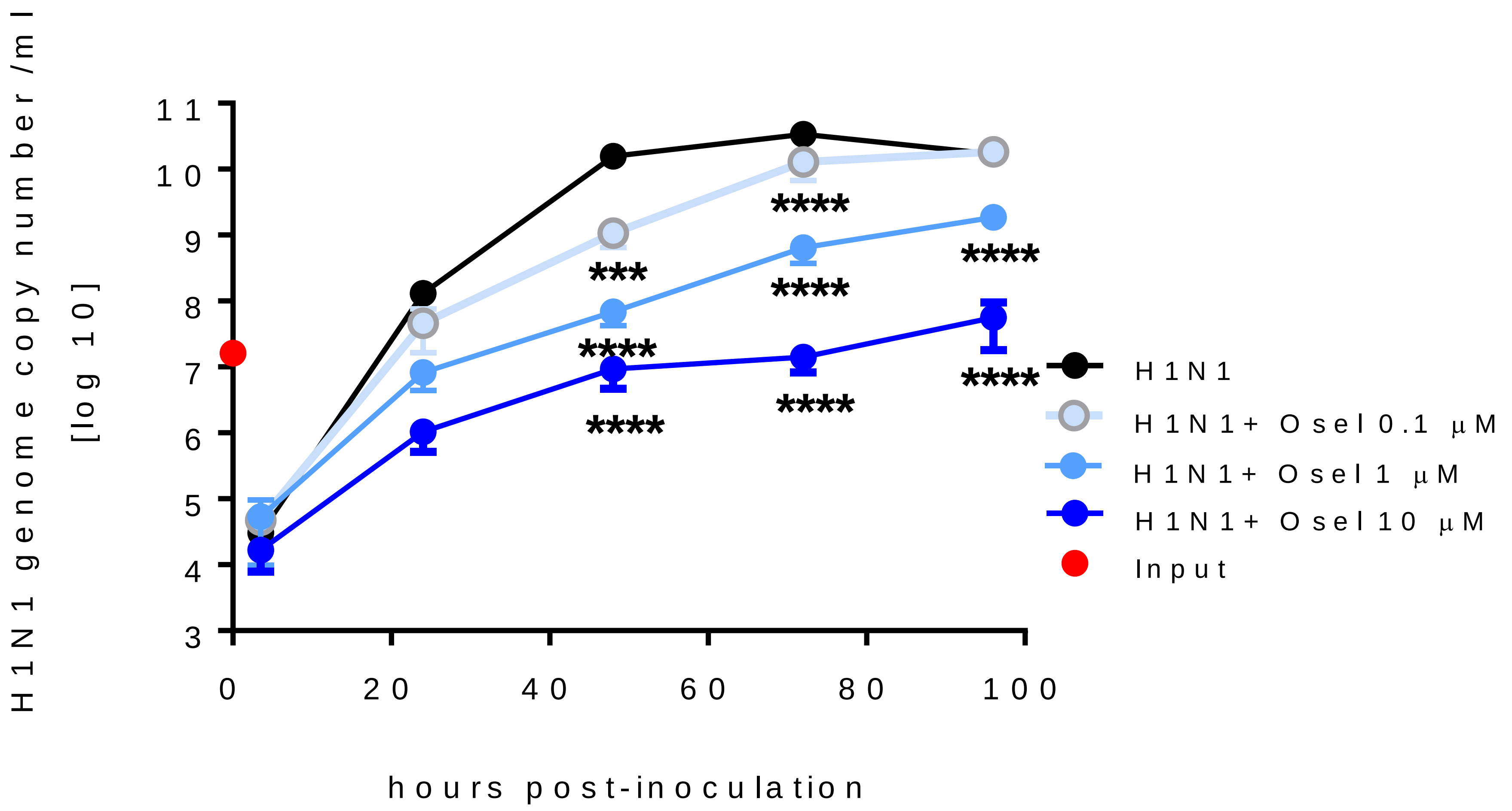


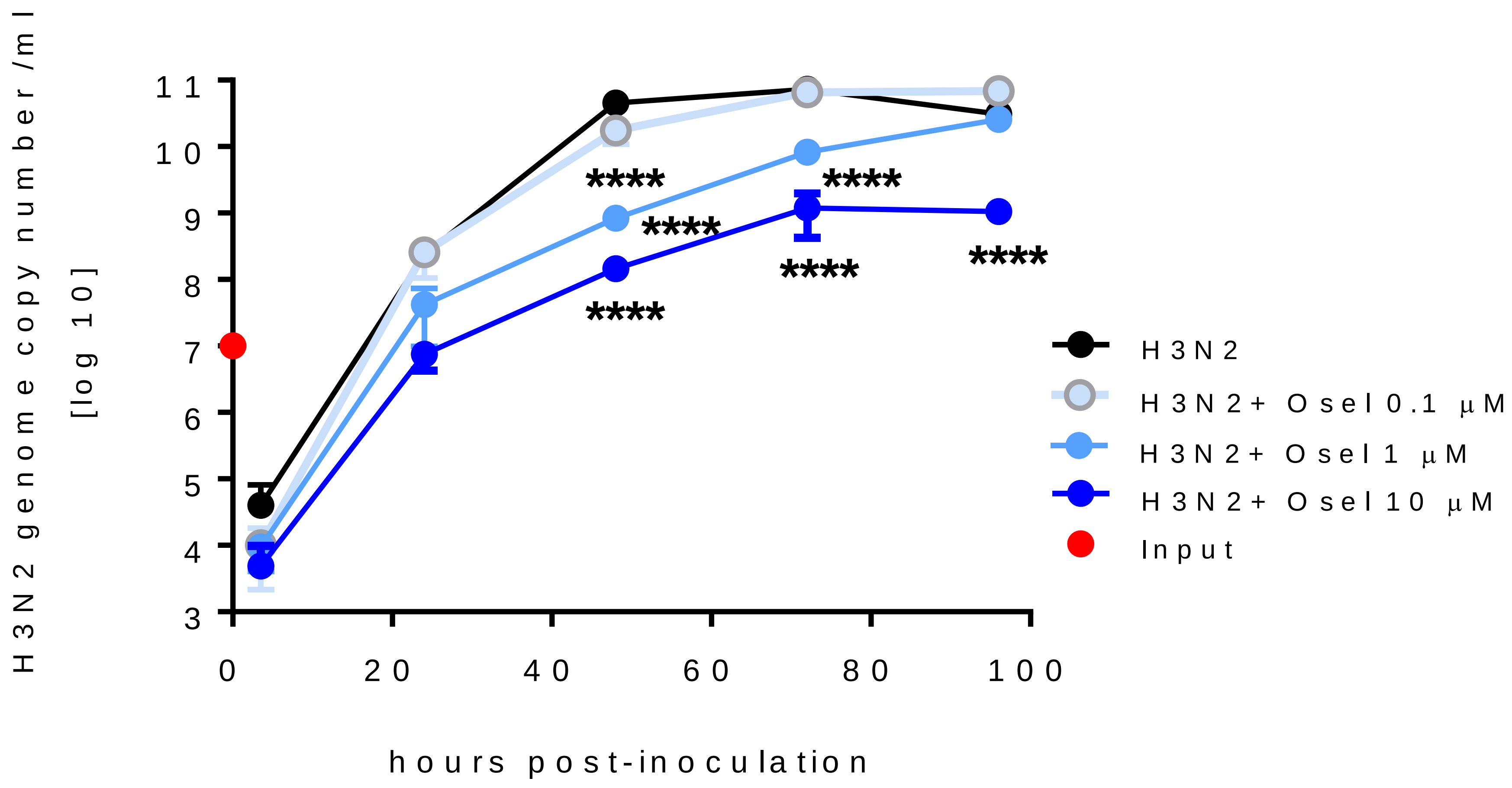
Others
Metapneumovirus
Parainfluenza virus 3

Inhibition of virus replication using antivirals



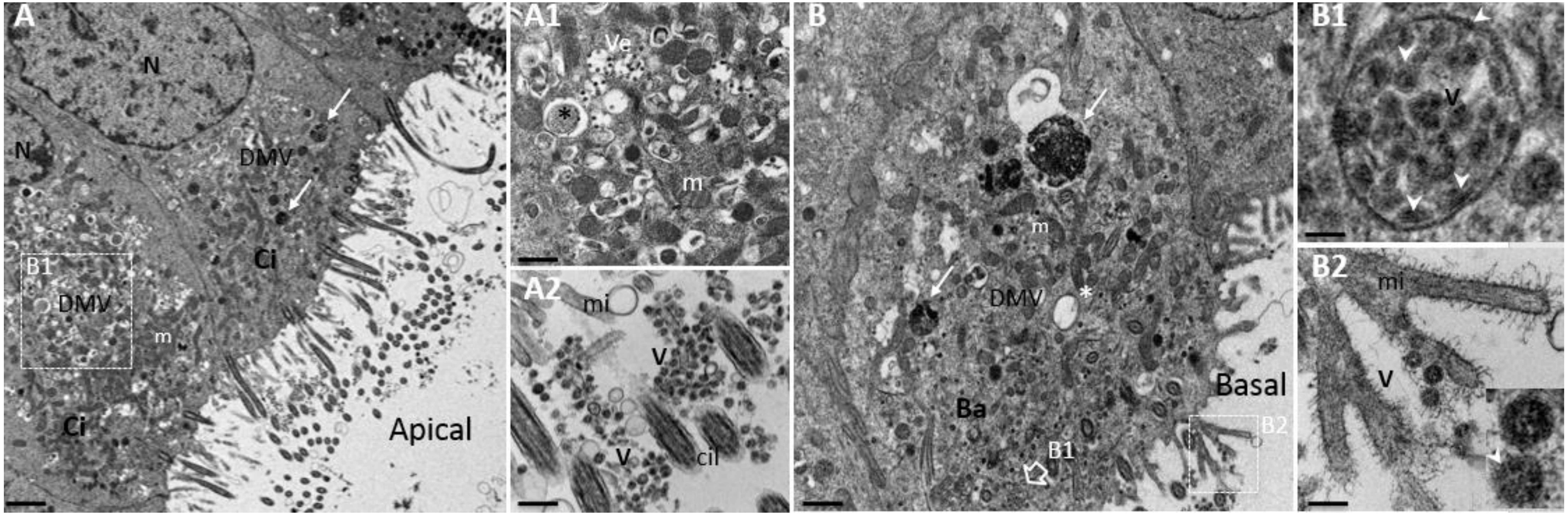






Reference antivirals inhibit viral production in a dose dependent manner in MucilAir™

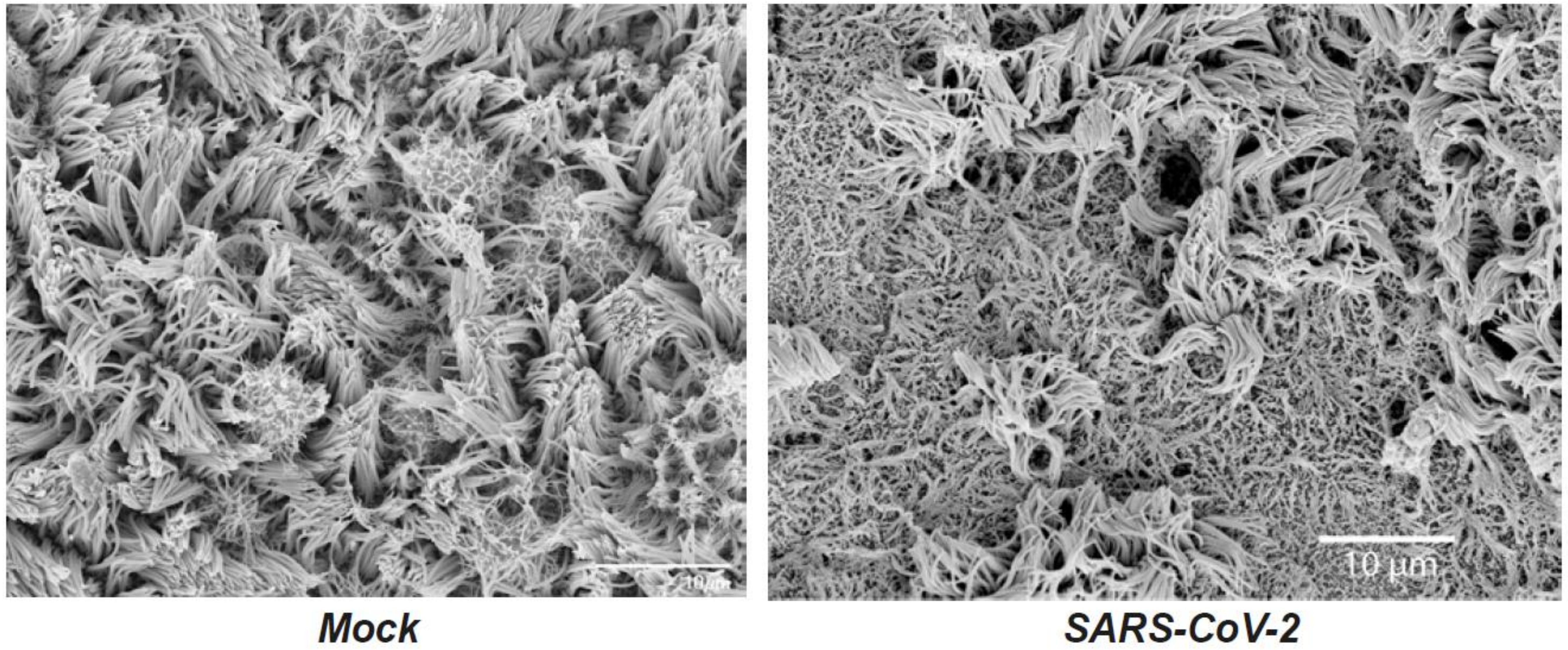
Boda et al. **Antiviral drug screening by assessing epithelial functions and innate immune responses in human 3D airway epithelium model** Antiviral research. 2018

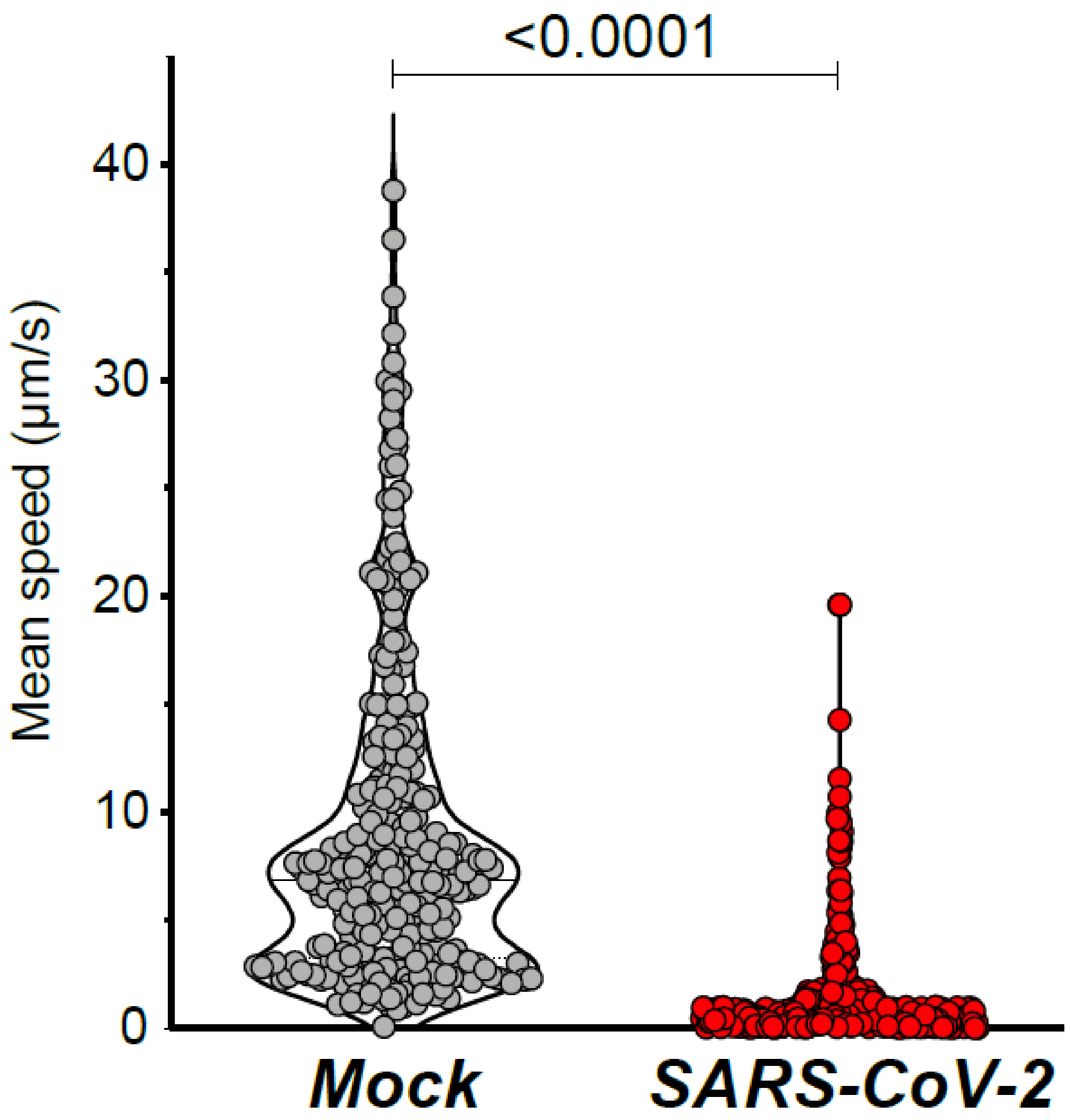


SARS-CoV-2 infects mainly ciliated and goblet cells

Pizzorno et al., **Characterization and treatment of SARS-CoV-2 in nasal and bronchial human airway epithelium** *Cell Reports Medicine, 2020*

SARS-CoV-2 impairs cilia function

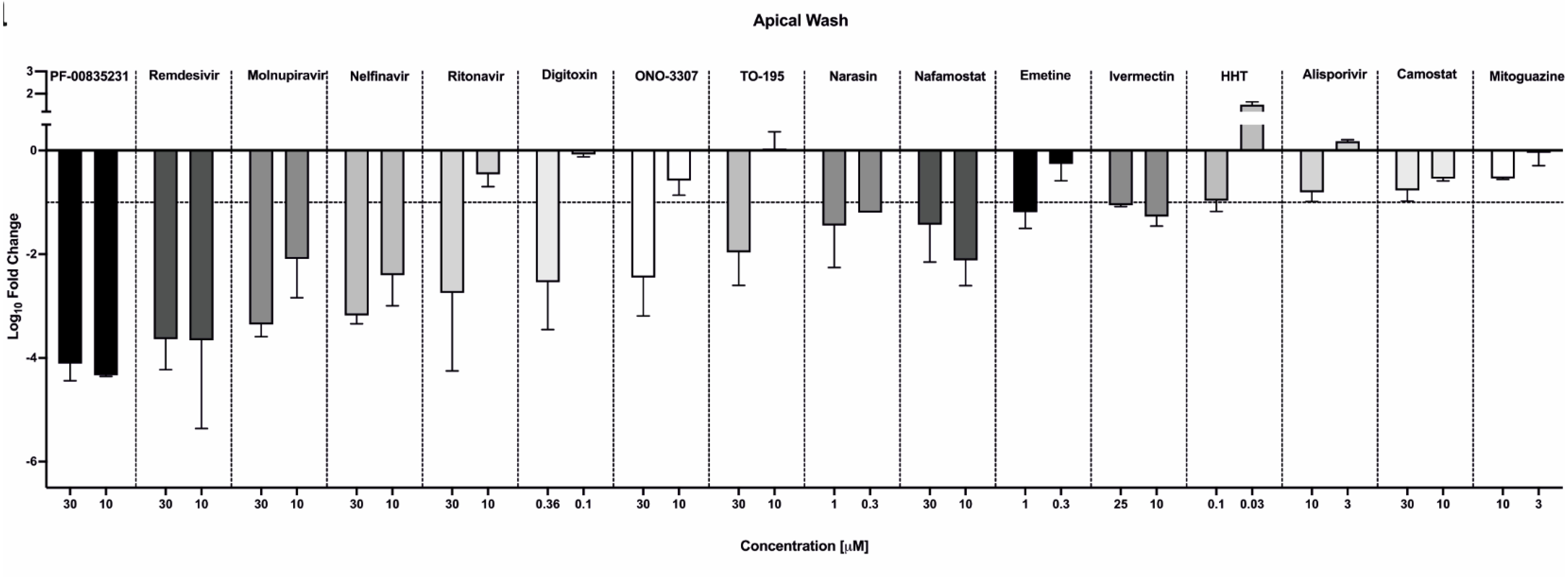




SARS-CoV-2 impairs mucociliary clearance on MucilAir™

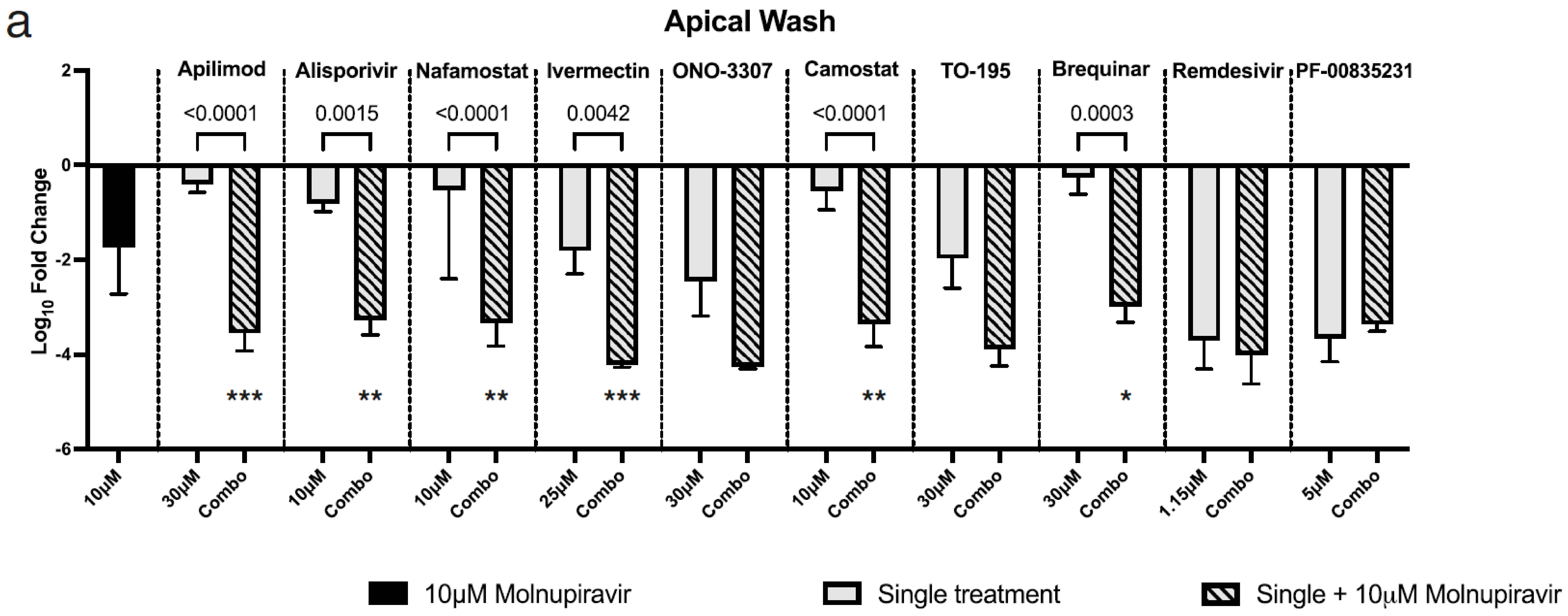
Robinot et al., **SARS-CoV-2 infection damages airway motile cilia and impairs mucociliary clearance**, *Nature, 12, 4354, 2021*

Antiviral evaluation of novel therapies against SARS-CoV-2 on MucilAir™



Jonsdottir et al., **Molnupiravir combined with different repurposed drugs further inhibits SARS-CoV-2 infection in human nasal epithelium in vitro** *Biomed Pharmacother.*, 2022

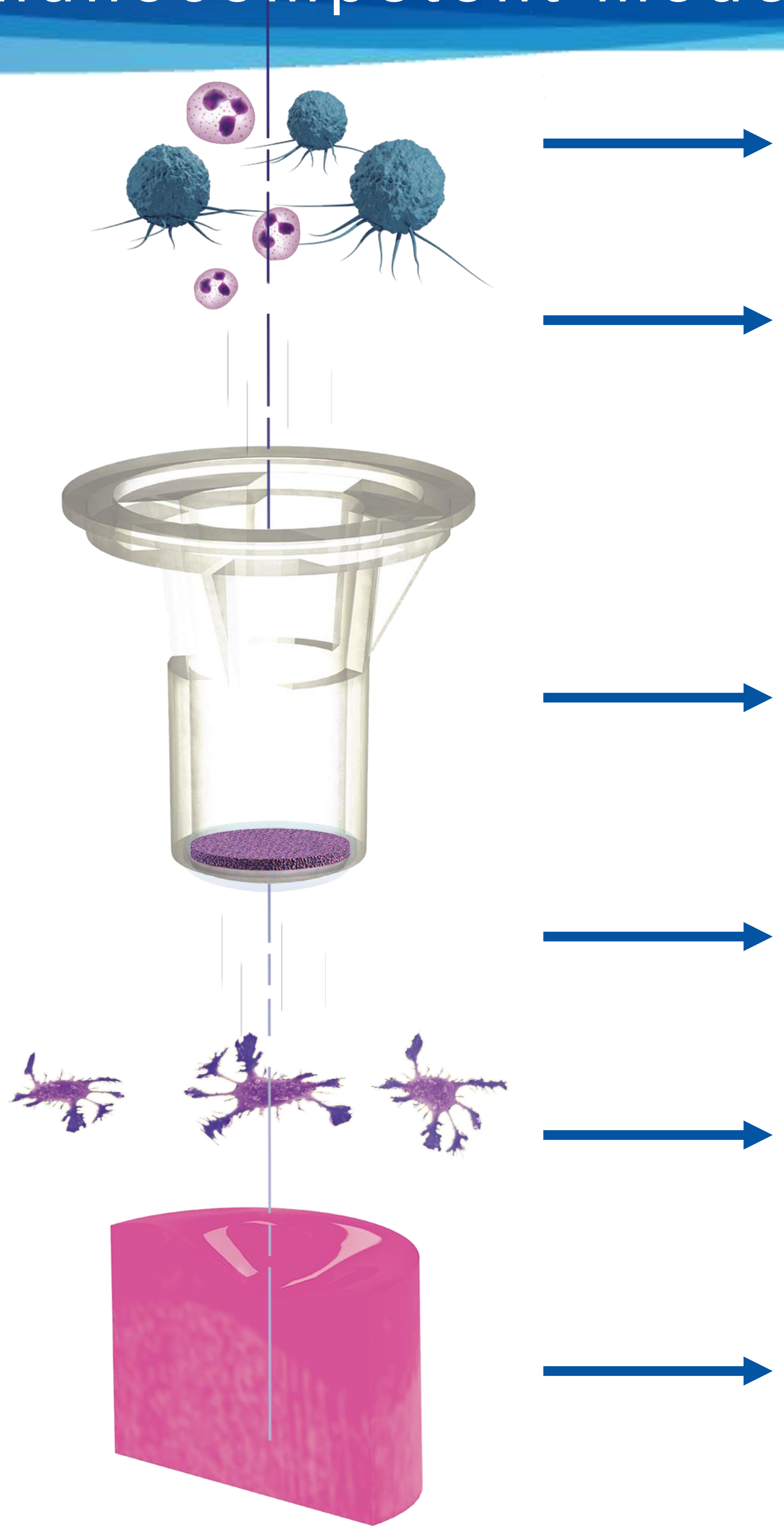
Antiviral evaluation of combos against SARS-CoV-2 on MucilAir™



Combination therapy can enhance the antiviral activity of Molnupiravir

Jonsdottir et al., **Molnupiravir combined with different repurposed drugs further inhibits SARS-CoV-2 infection in human nasal epithelium in vitro** *Biomed Pharmacother.*, 2022

What's next: Novel advanced fully primary human immunocompetent models



Primary lung Macrophages / Neutrophiles

Fully primary airway tissues

- ✓MucilAir™
- ✓SmallAir™
- ✓AlveolAir™

Standard Transwell culture support

Fibroblasts or endothelial cells layer

Dendritic cells

Serum free and chemically defined culture medium

Conclusion

EPITHELIX

- Nasal, Bronchial, Small-Airways and Alveolar 3D human Models are useful tools to evaluate effect of inhaled xenobiotics on:
  - ✓Local tolerance
  - ✓Respiratory absorption
  - ✓Mucociliary clearance
  - ✓Mucosal inflammation
  - ✓Respiratory disease induction (lung fibrosis, metaplasia)

- Airway epithelia are efficient platforms to evaluate antiviral and antibiotics strategies.

- Assays need to be developed on emergent immunocompetent models integrating alveolar macrophages, dendritic cells and neutrophils.

EPITHELIX
IN VITRO RESPIRATORY SOLUTIONS

Thank you for your attention

French Lab

- Carole Bertinetti
- Mendy Bouveret
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- Mireille CaulFuty
- Ophélie Verbeke
- Laurent Wiszniewski
- Dr. Ludovic Wiszniewski

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- Sacha Benaoudia
- Dr. Bernadett Boda
- Rosy Bonfante
- Caroline Chojnacki
- Charlène Constant
- Guillaume Dechanet
- Anaïs Horckmans

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- Emilie Ferreira
- Ina Fureraj
- Matia Gojun
- Dr. Song Huang
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