Epithelix

in vitro Solutions for Respiratory Diseases and Chemical Testing

Epithelix' 3D *in vitro* models of the human airway epithelium for toxicity assessment

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PETA, 12/06/2018

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Part 1

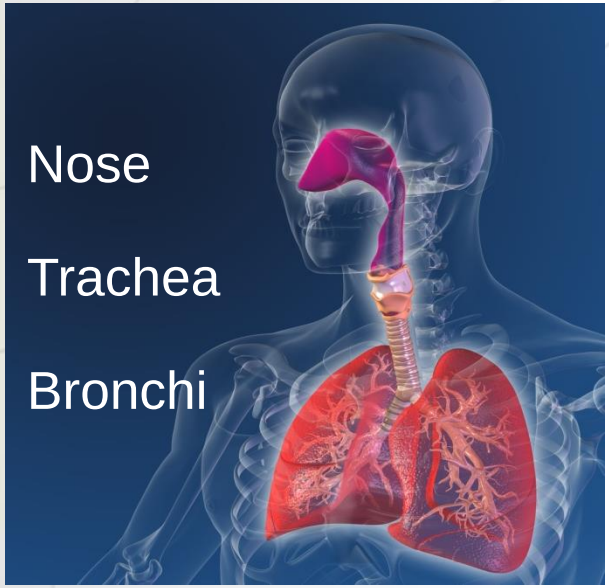
Currently Available *in vitro* 3D Airway Models and Characterization

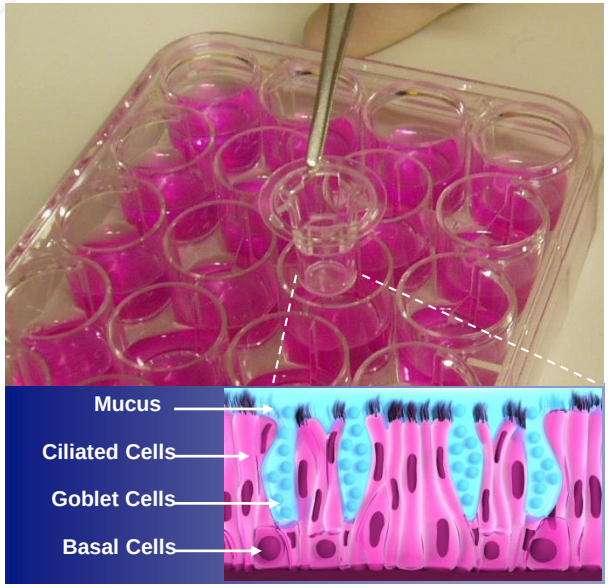
3D ALI airway epithelia

Epithelix

MucilAir™: Long shelf life *in vitro* Airway Tissues

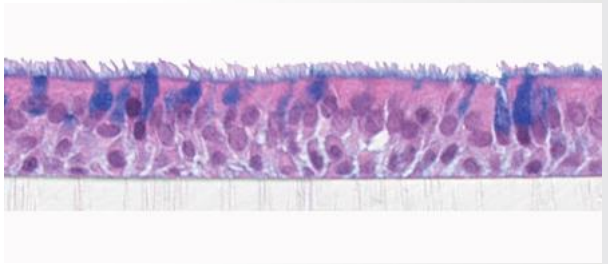
Primary Human Cells
Isolation, amplification & seeding





Air-Liquid interface, differentiation

Ready-to-use
Fully differentiated
Airway Epithelium

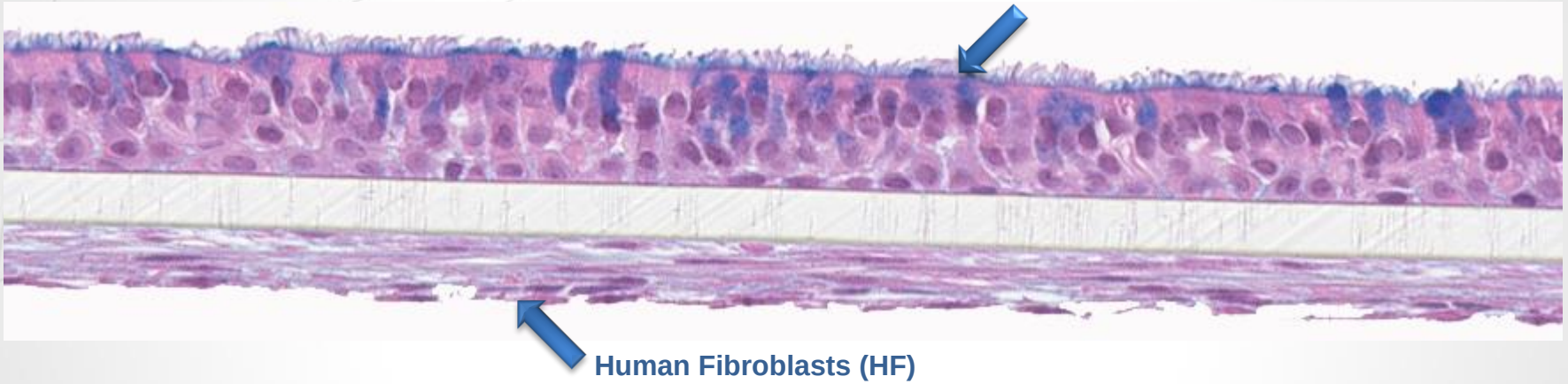


MucilAir™

Shelf-life of **1 year**

MucilAir™-HF

MucilAir™-HF: Long shelf-life co-culture model



Several versions are available:

- ✓ MucilAir™-HF-Normal
- ✓ MucilAir™-HF-Asthma
- ✓ MucilAir™-HF-COPD (Chronic Obstructive Pulmonary Disease)
- ✓ MucilAir™-HF-CF (Cystic Fibrosis)
- ✓ MucilAir™-HF-Allergic Rhinitis

Electron Microscopy

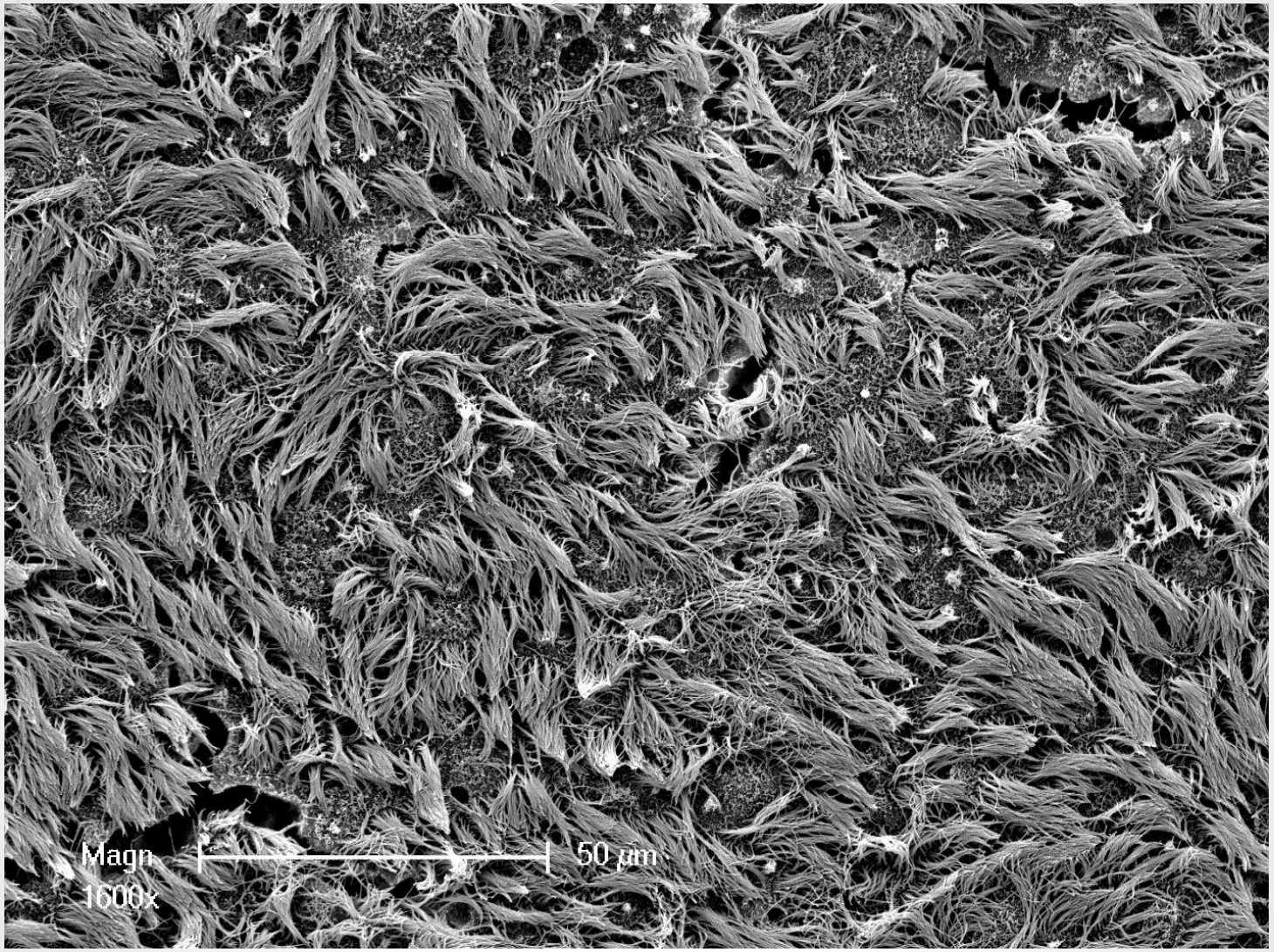
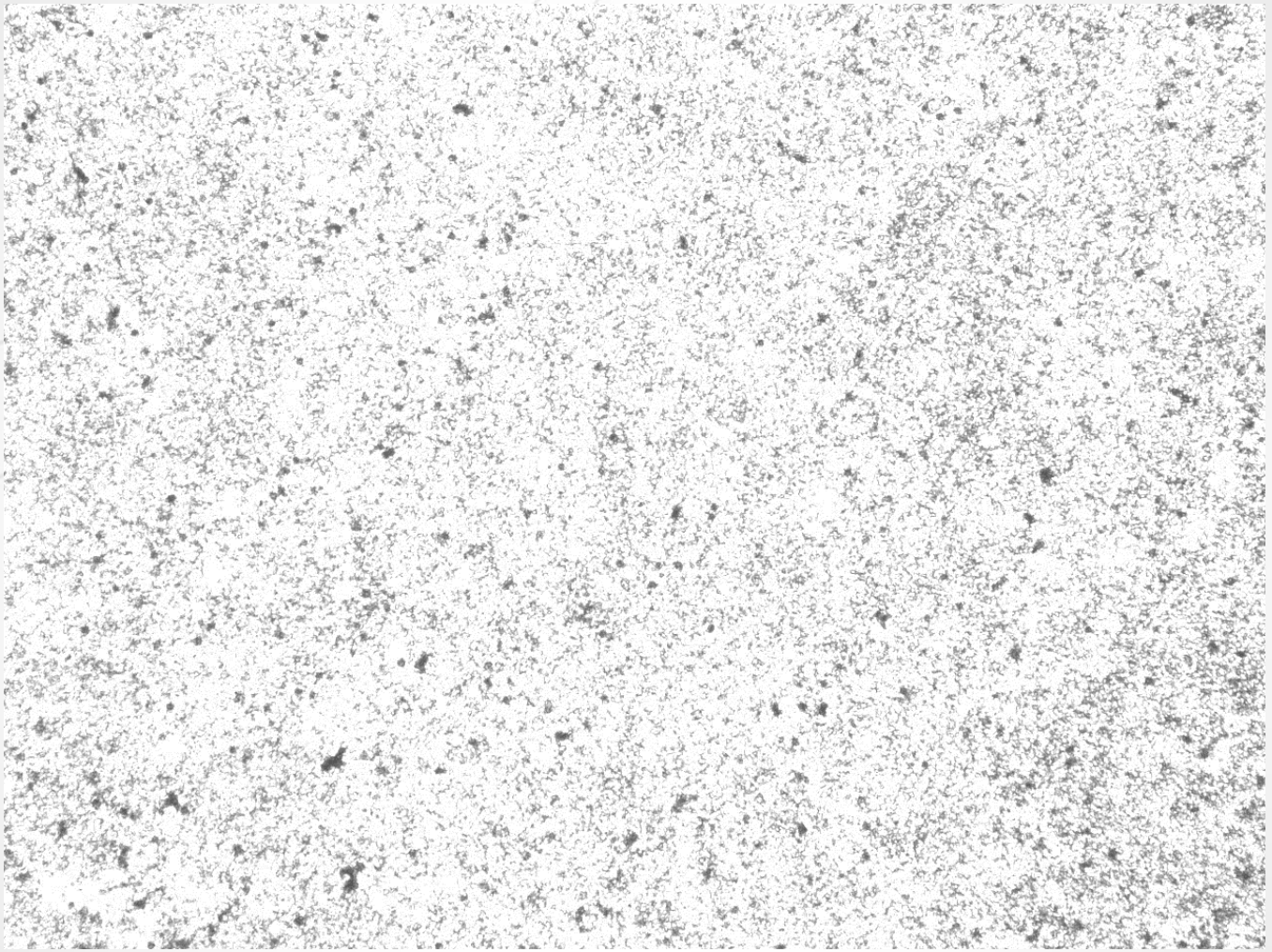


Photo Courtesy of Charles River Laboratories (www.criver.com)

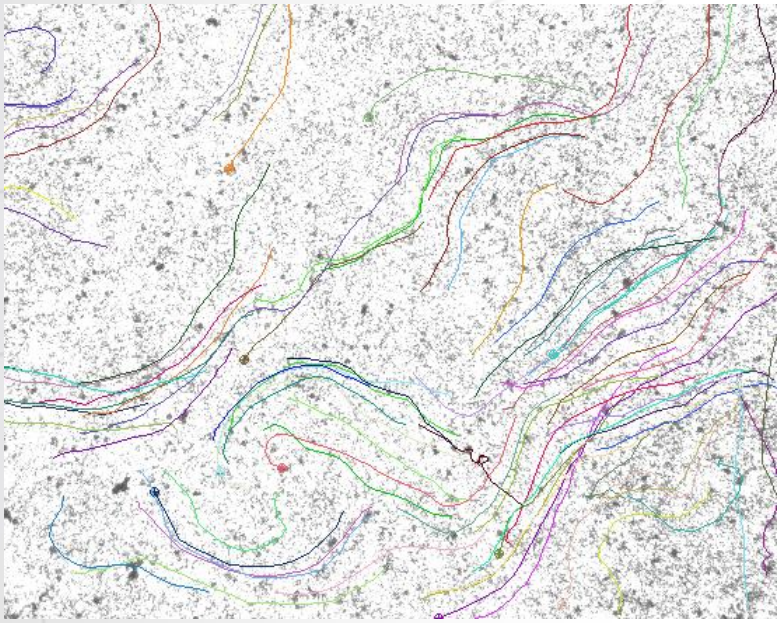
Mucociliary clearance analysis - MucilAir



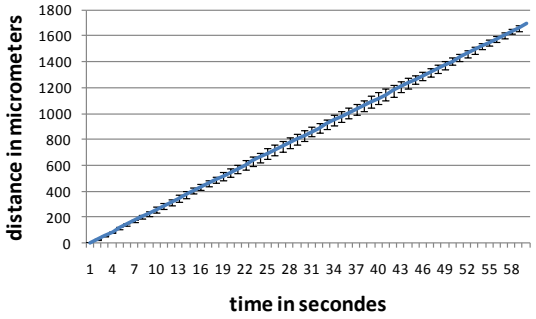
Microbeads of 30 microns are seeded onto the apical surface of MucilAir. The particle movement is then tracked using dedicated software for calculating velocities of beads' movement.

Mucociliary Clearance Analysis

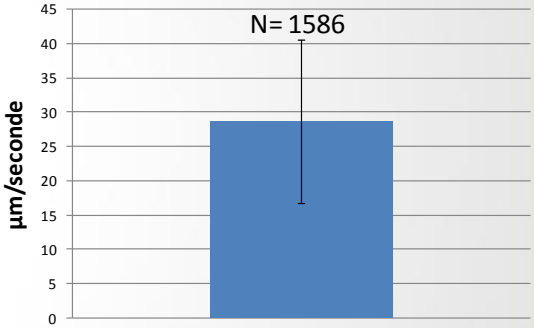
Particle tracking on MucilAir



(a)

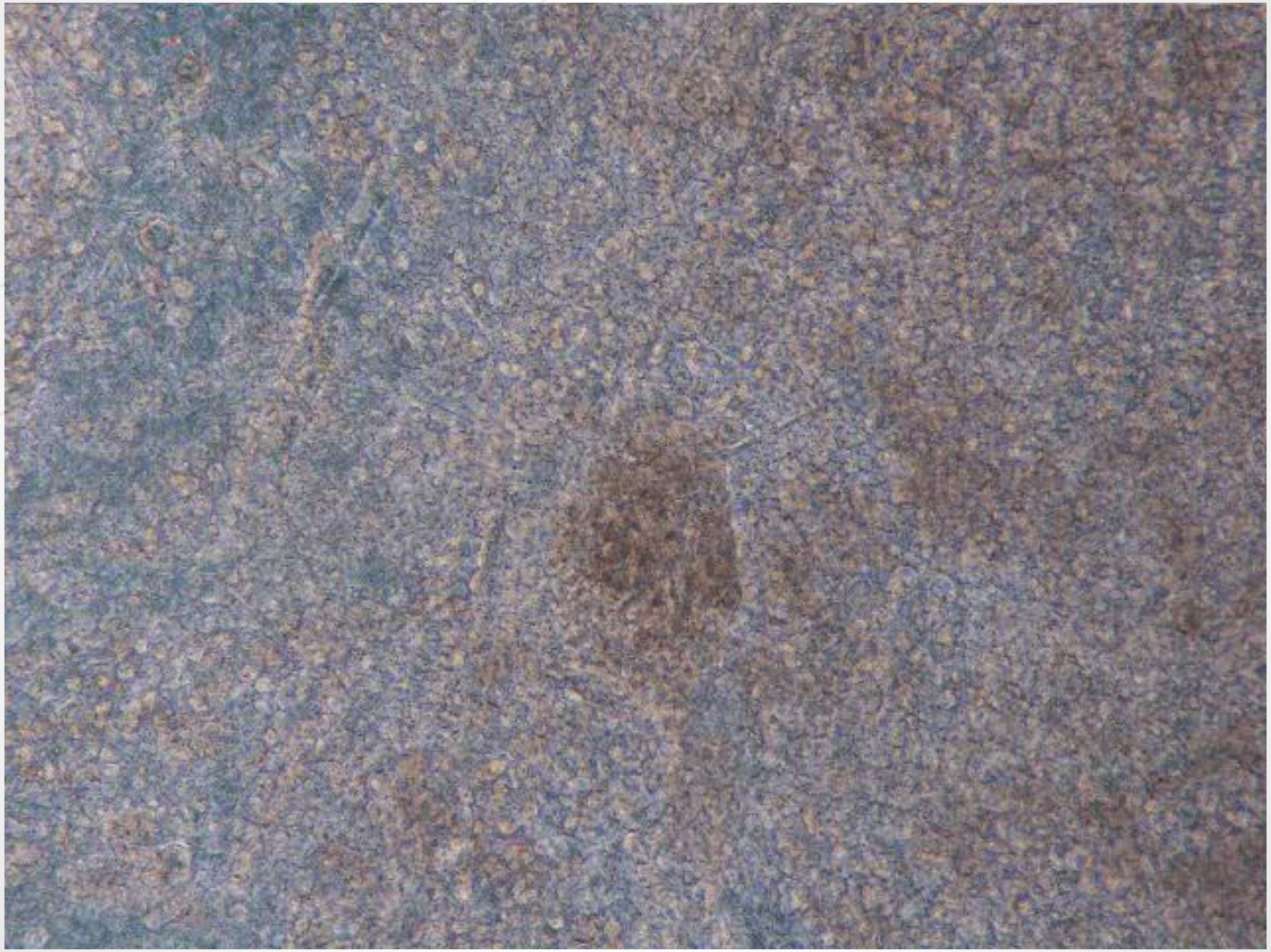


(b)

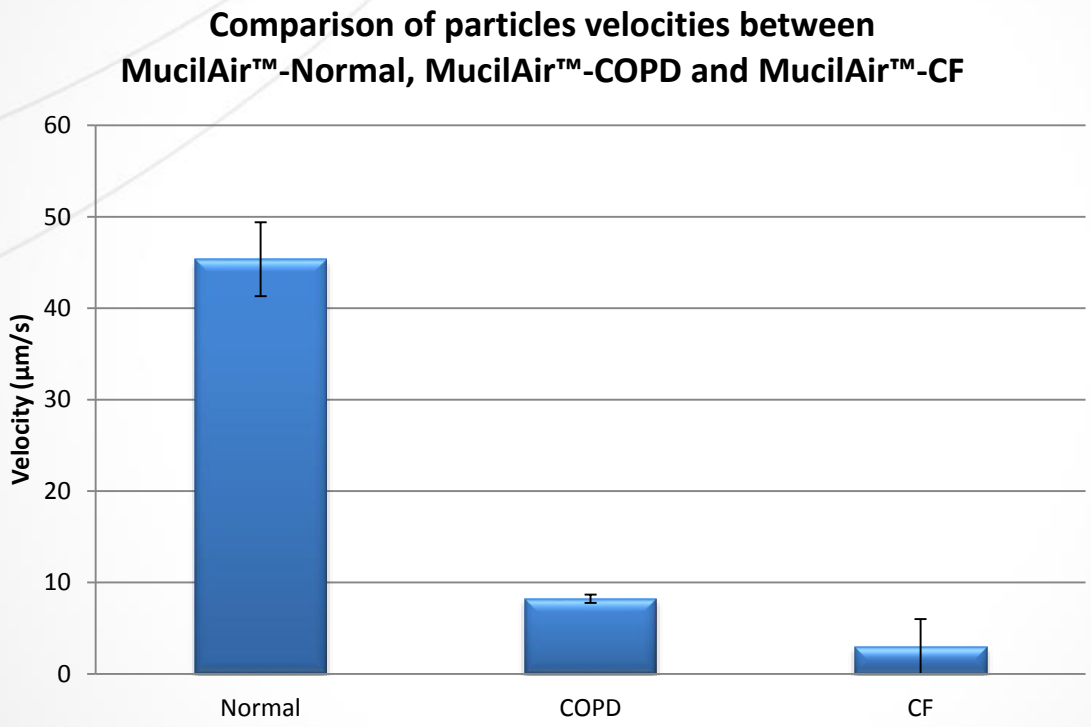


(c)

Mucociliary clearance analysis - MucilAir-CF



Mucociliary Clearance Analysis

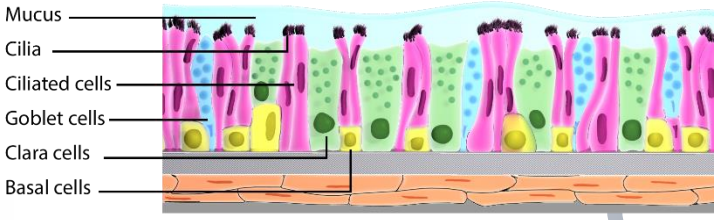


The Main Characteristics of MucilAir™

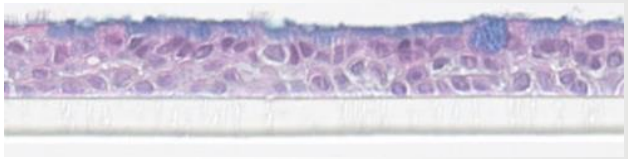
- It has a unique shelf-life of **one year**
- It **mimics** the morphology and functions of the normal human airway epithelium.
  - ✓ Active Ion transport
  - ✓ Metabolic activity
- Epithelia from several **different pathologies** are available (Asthma, COPD, Cystic Fibrosis, Allergic Rhinitis, etc.)
- It is **easy** to handle and maintain
- **Serum free** medium
- **Worldwide Shipping**

SmallAir™ : Long shelf life *in vitro* Airway Tissues

Primary Human Cells
Isolation, amplification & seeding



Ready-to-use
Fully differentiated
Airway Epithelium

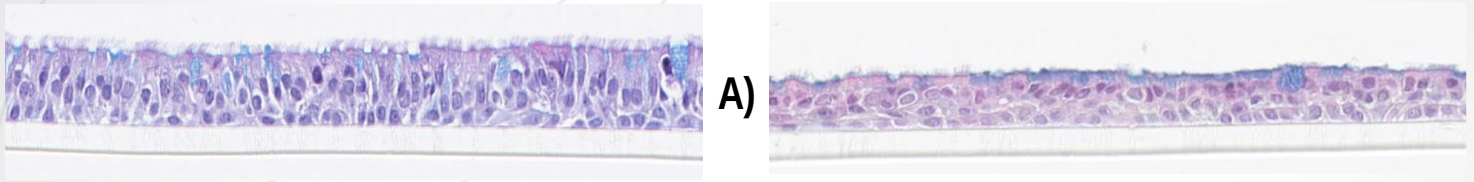


SmallAir™

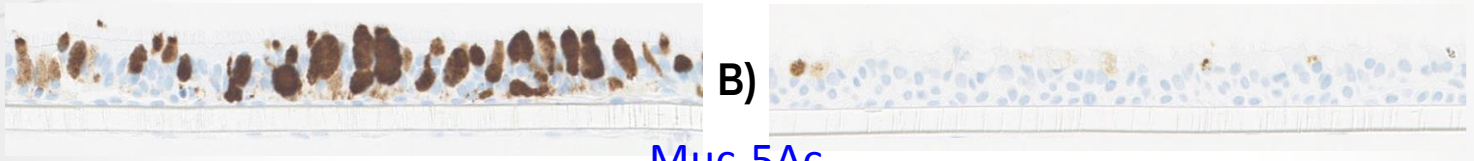
Air-Liquid interface, differentiation

SmallAir™ - Histology - Immunohistochemistry

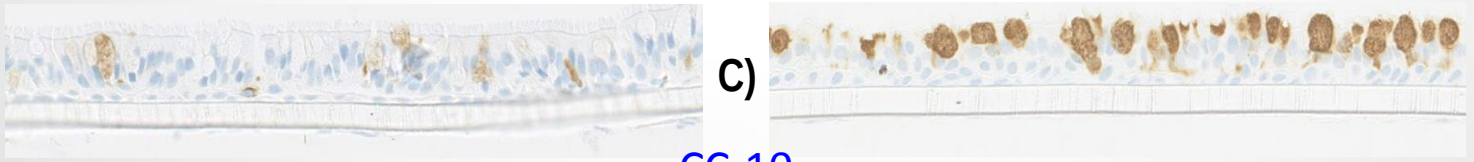
MucilAir™ (Bronchial) **SmallAir™ (Small-Airways)**



H/E-Alcian Blue



Muc-5Ac
(Goblet Cells marker)



CC-10
(Club Cells marker)

Huang et al. **Establishment and Characterization of an in vitro Human Small Airway Model (SmallAir™).** *Eur. J. Pharm. Biopharm.* 2017

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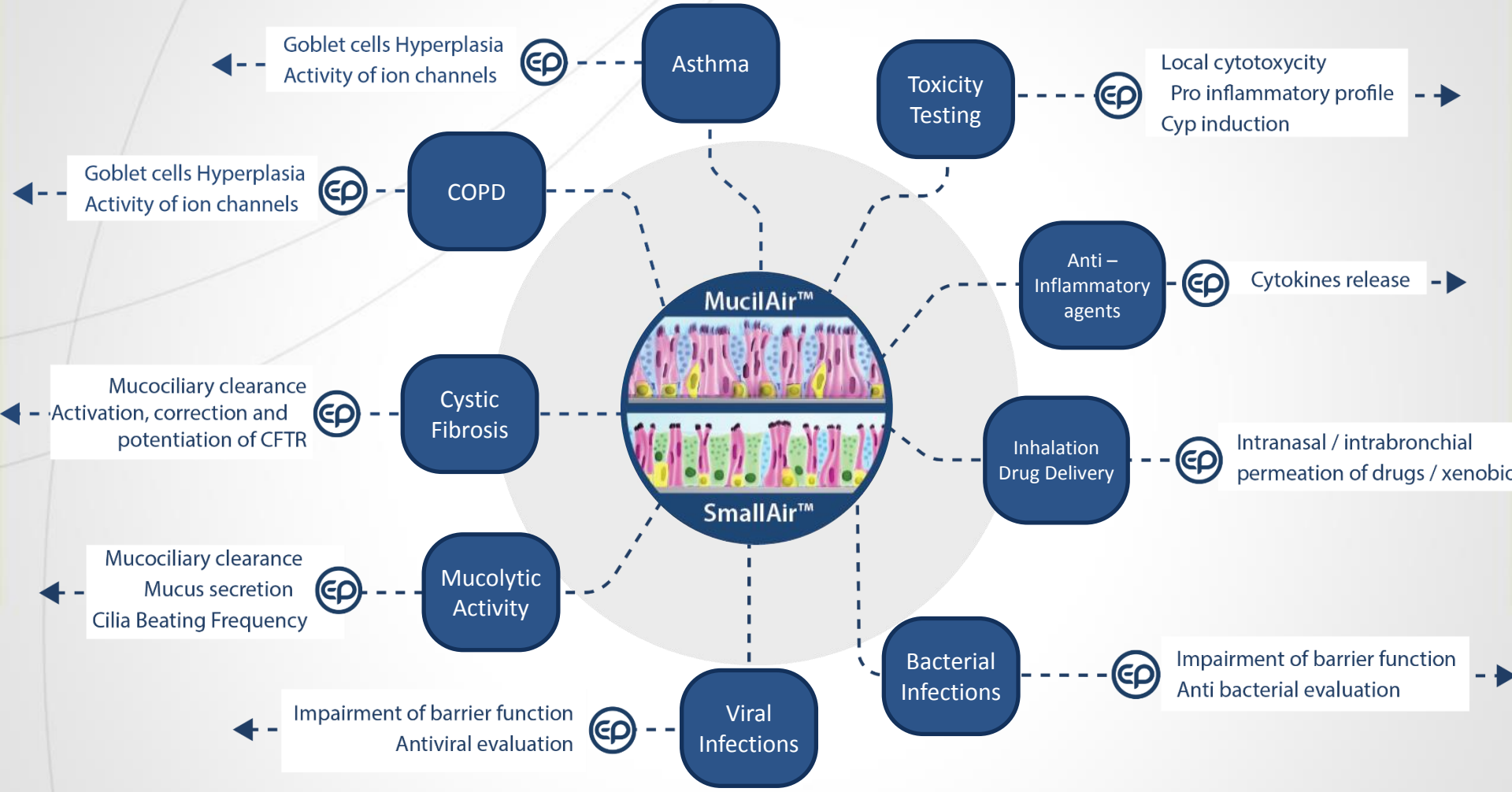
Part 2

Functionnal Assays using Epithelix' 3D *in vitro* models of the human airway epithelium

Functional *In Vitro* Assays using ALI culturess



Exposure systems

Static Exposure Systems :

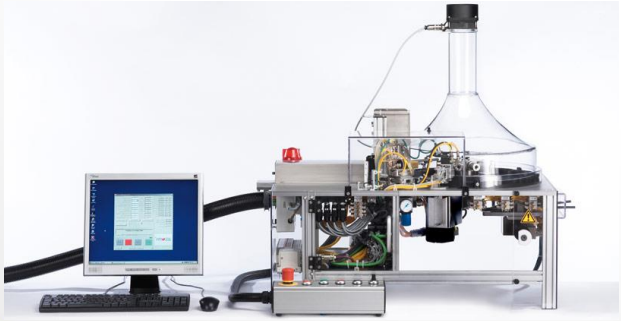
Liquids - Solutions

Solids (Tablets)

Dynamic Exposure Systems :

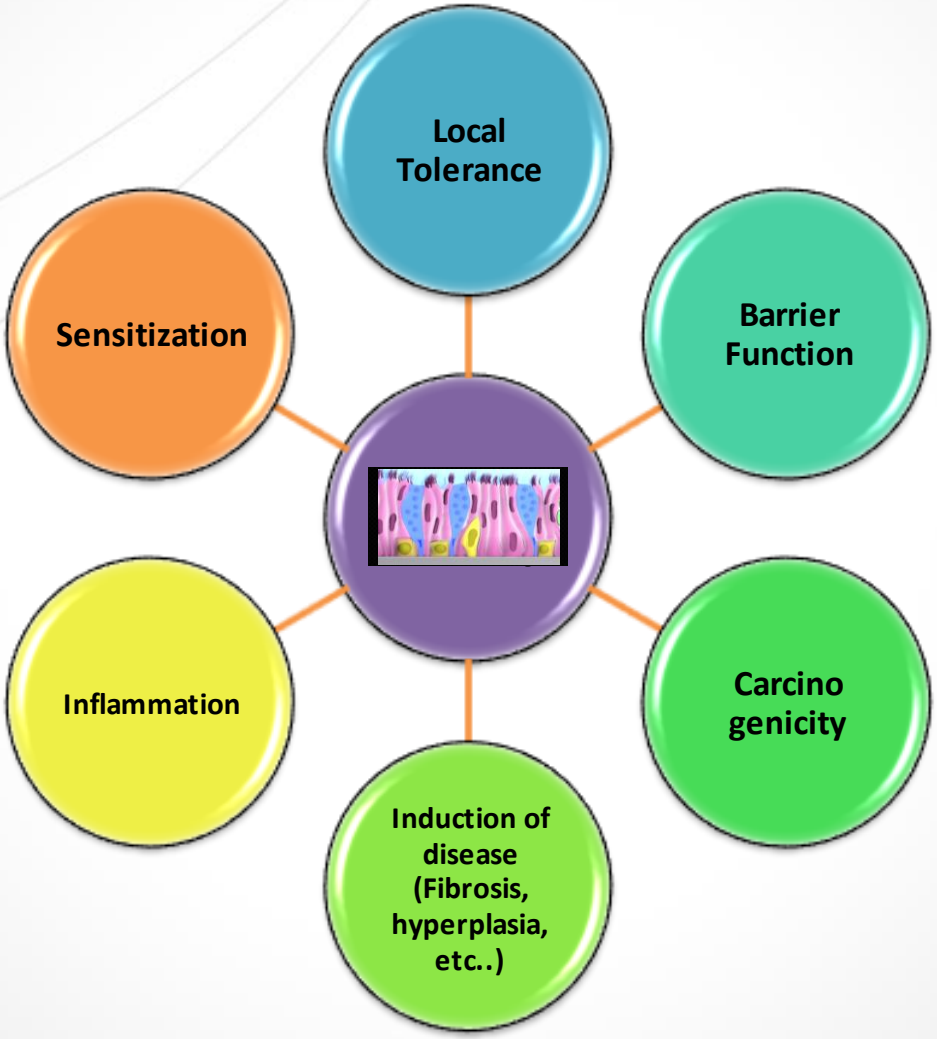
Gas or smoke

VITROCELL SYSTEMS

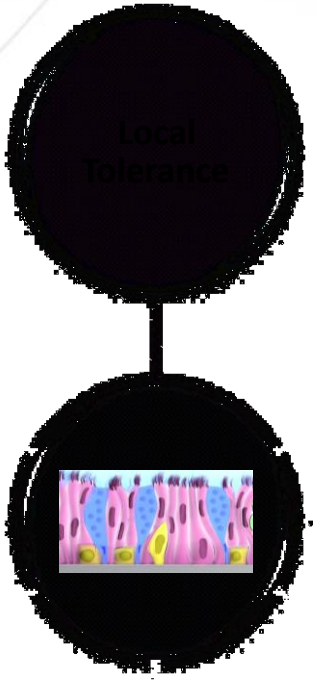


Nebulizing chambers

Evaluation of inhalation toxicity of inhaled drugs



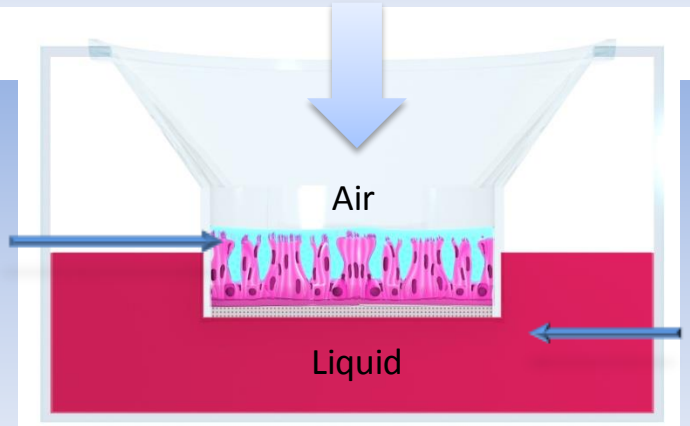
Evaluation of local tolerance



Toxicity Testing

Multiple end-points testing strategy for monitoring acute, chronic and long term effect of molecules/mixtures

Apical Exposure
(e.g. liquids, solids, nanoparticles, gas, smoke, etc.)



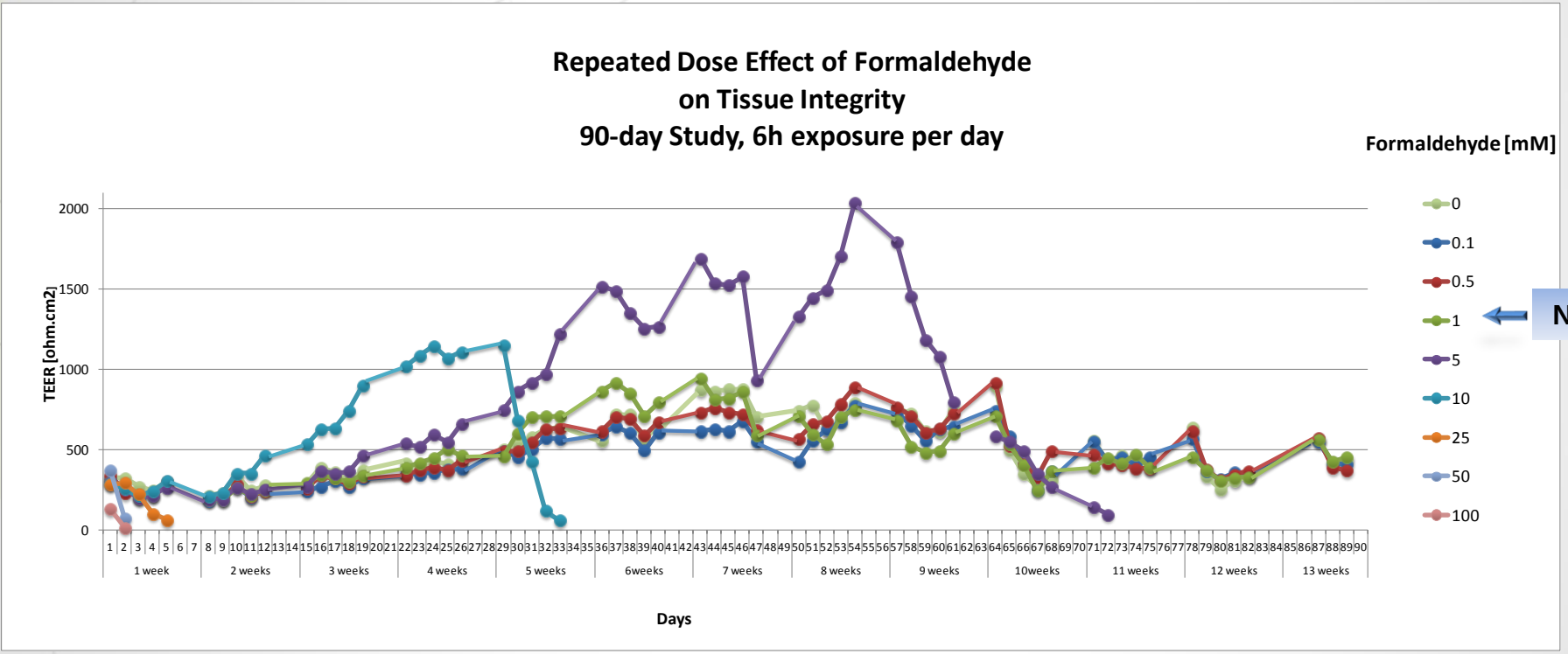
Information from Apical Side
- TEER measurement
- Resazurin test
- Cilia beating Monitoring
- Morphology
- Mucin secretion

Information from Culture Medium
- Secretion of soluble factors (cytokines/chemokines/ metalloproteinases)
- LDH release

+ Cells information RNA/DNA/Proteins

Repeated Dose Toxicity Testing

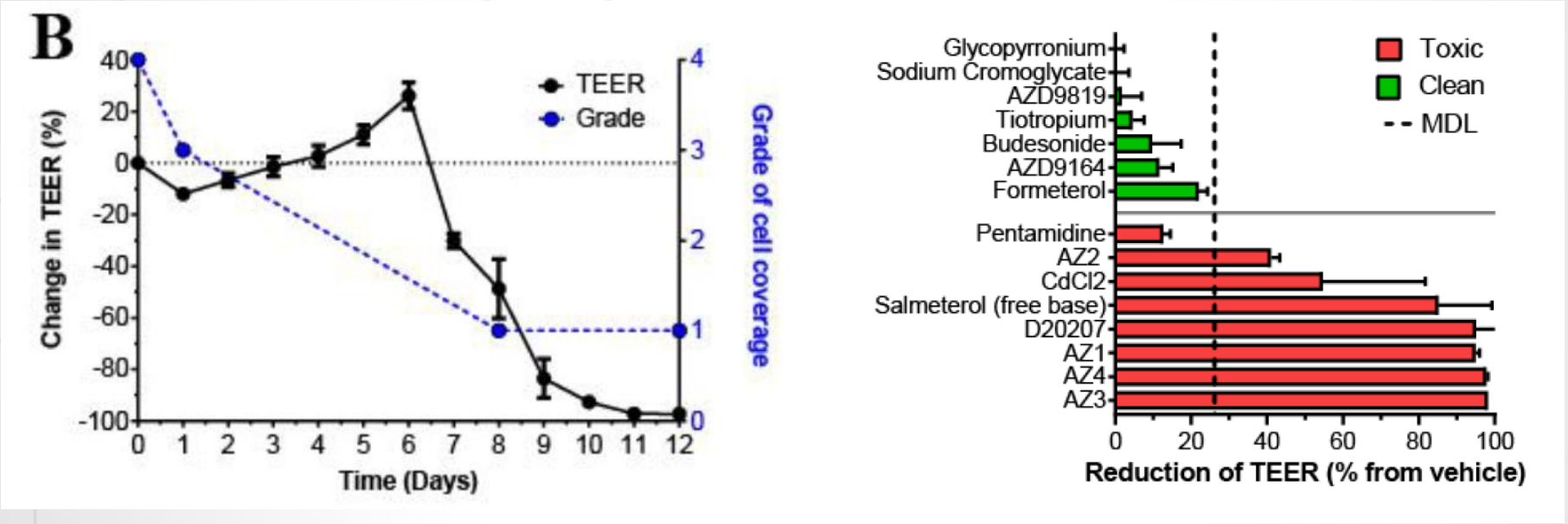
First transposition of the OECD 413 guideline *in vitro*



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.

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

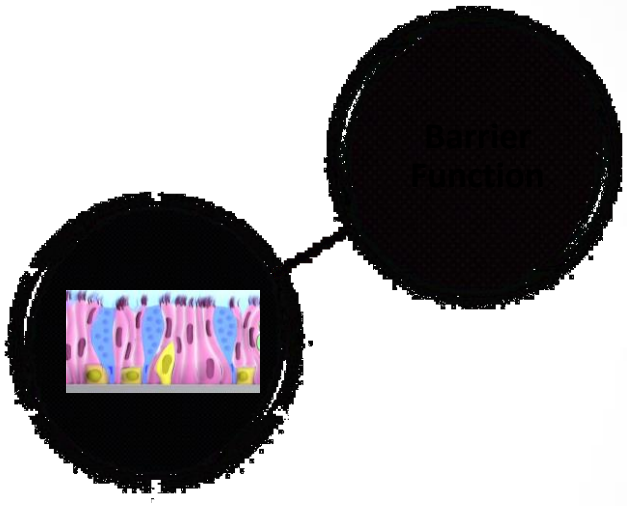
Kinga Balogh Sivars[1†*], Ulf Sivars[4†], Ellinor Hornberg[4†], Hui Zhang[3†], Lena Brändén[3†], Rosy Bonfante[5], Song Huang[5], Samuel Constant[5], Ian Robinson[2††], Catherine J Betts[3††] and Per Åberg[2†]



15 compounds tested
88% sensitivity
100% specificity

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

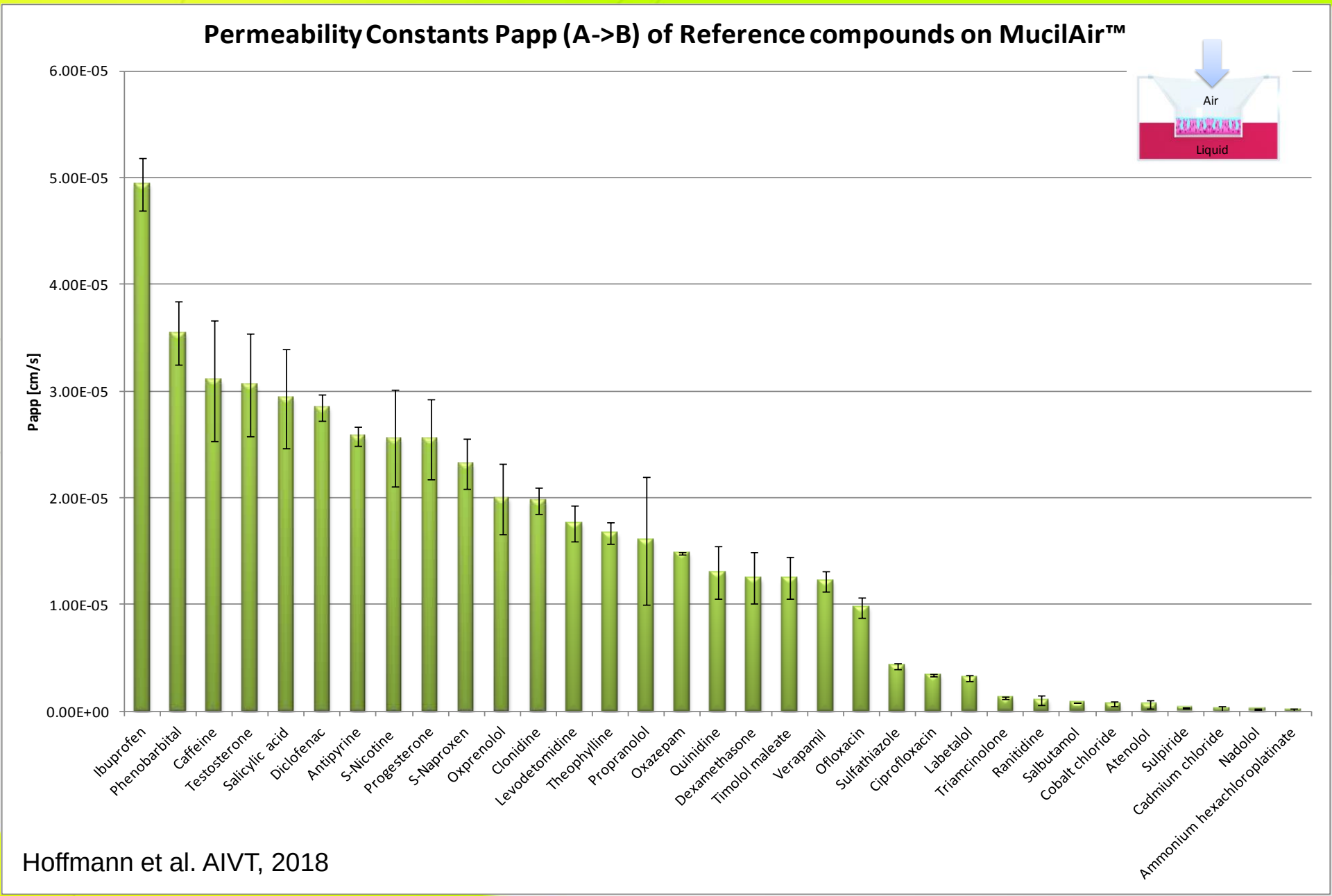
Evaluation of barrier function



End points:

- ✓ Amount of Xenobiotic trapped by mucus
- ✓ Trans-epithelial permeability (Papp)
- ✓ Epithelial uptake
- ✓ Input parameters for PBTK modelling

Trans epithelial transport of API or Xenobiotics

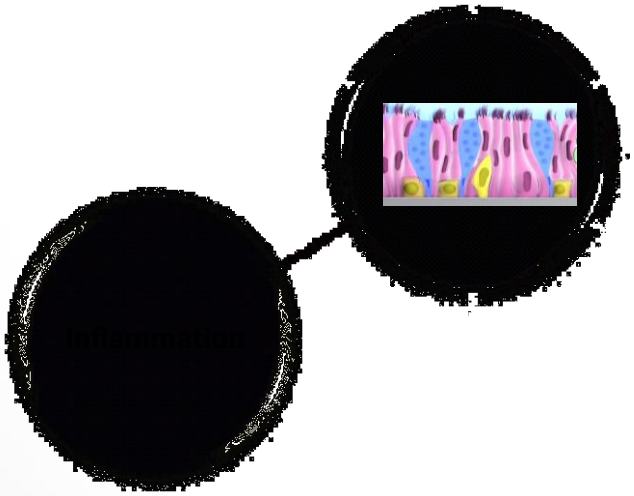


Hoffmann et al. AIVT, 2018

Evaluation of mucosal inflammation

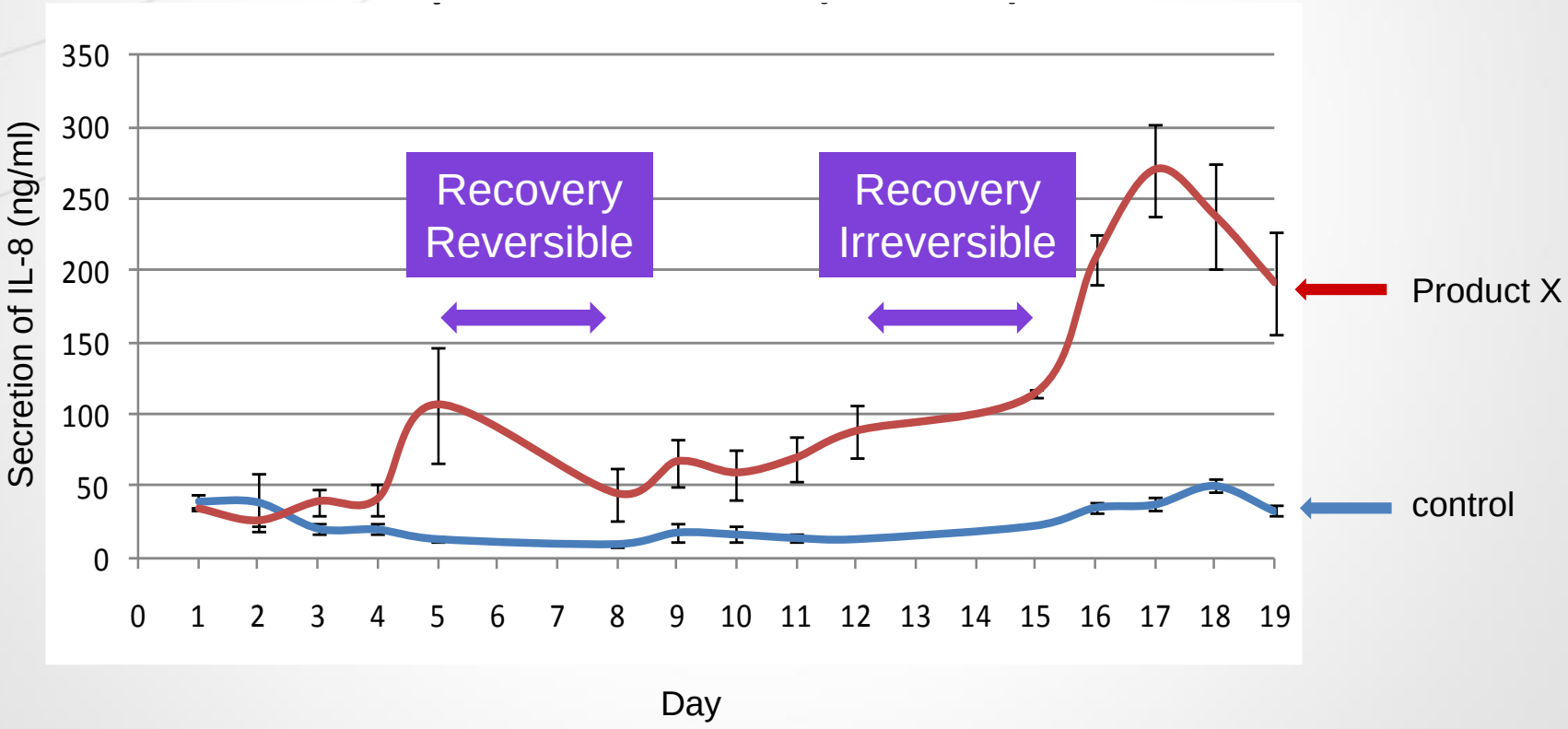
End points:

- ✓ Inflammatory cytokines release (Il-8; Il-6, etc..)



Pro-inflammatory monitoring using MucilAir™

Example of Repeated Dose Exposure Study
20 Days study – 2 Doses per day



Conclusion

- ALI 3D Human Airway Models are useful tools to evaluate the effect of inhaled xenobiotics on :
  - ✓ Local toxicity
  - ✓ Respiratory absorption
  - ✓ Mucociliary clearance
  - ✓ Mucosal inflammation

- Needs to define an integrated testing strategy for *in vitro* inhalation testing of xenobiotics based on efficiency of each cellular model and assay

- Assays need to be developed on emergent Small Airway models

- More physiologically relevant models are needed for the alveolar region

Thanks for your attention

French Lab

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

Swiss Lab

- Paul Alouani
- Sacha Benaoudia
- Dr. Bernadett Boda
- Rosy Bonfante
- Emilie Ferreira
- Ina Fureraj
- Matia Gojun
- Dr. Song Huang
- Xiao-Yann Huang
- Faten Hussein
- Marc Lanzillo
- Melany Monachino
- Rebecca Pimenta
- Jimmy Vernaz
- Karin Weber

18 Prizes :

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