

NEW ASSESSMENT METHODS FOR ENDS INGREDIENTS

Arno GUTLEB

Luxembourg Institute of Science and Technology (LIST)

INVITROLIZE

Luxembourg

arno.gutleb@list.lu

arno.gutleb@invitrolize.com



Conflict of Interest Statement



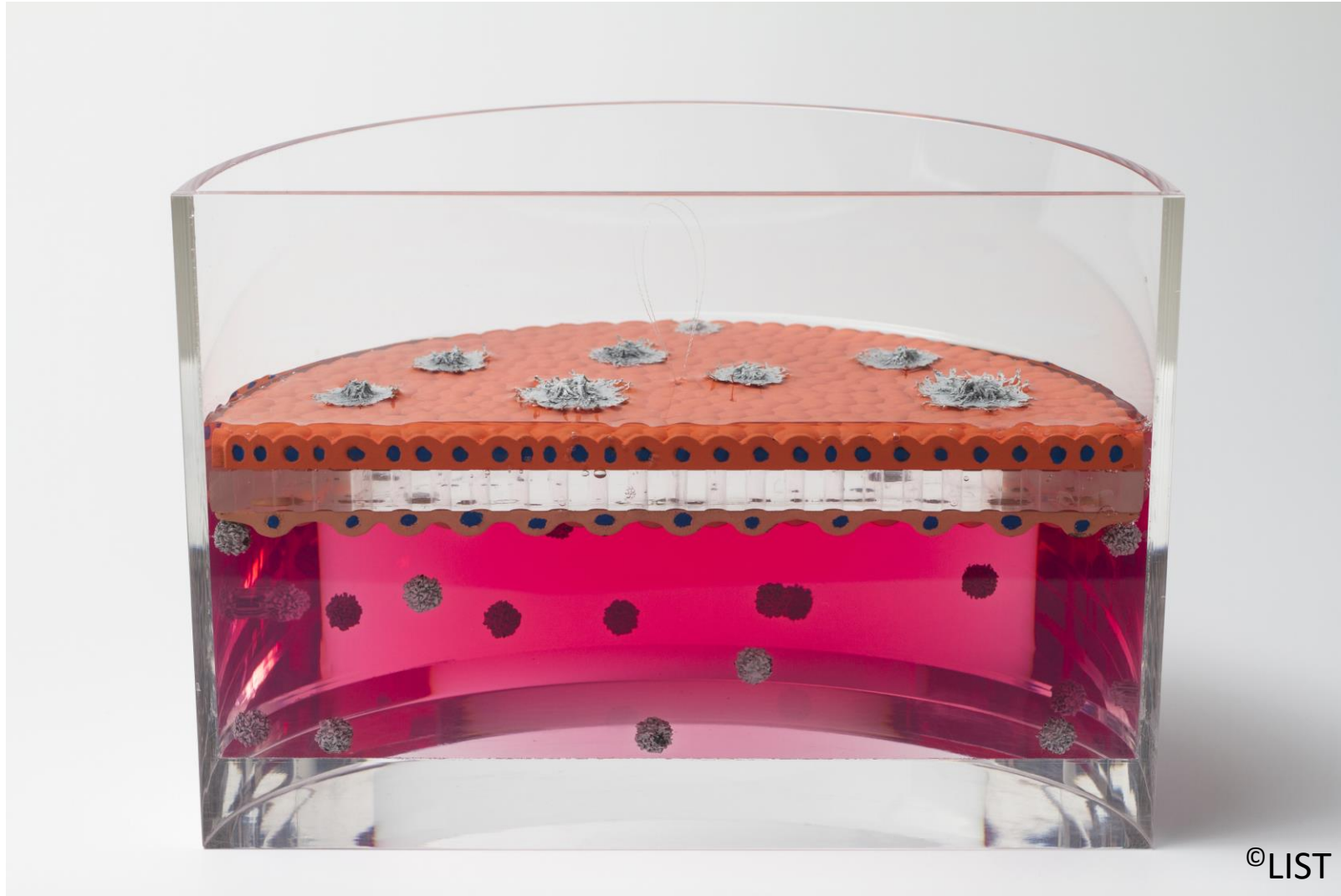
The author is the founder of *INVITROLIZE* sarl

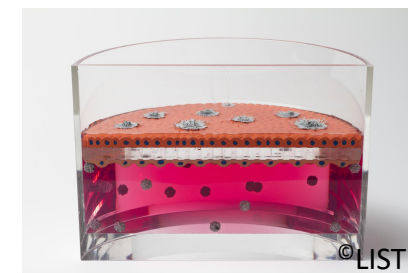
NOT a systematic review but will show some basic principles

Companies mentioned are examples but the list is for sure not complete and no endorsement is given

Some companies provided slides

How far has the community come ?





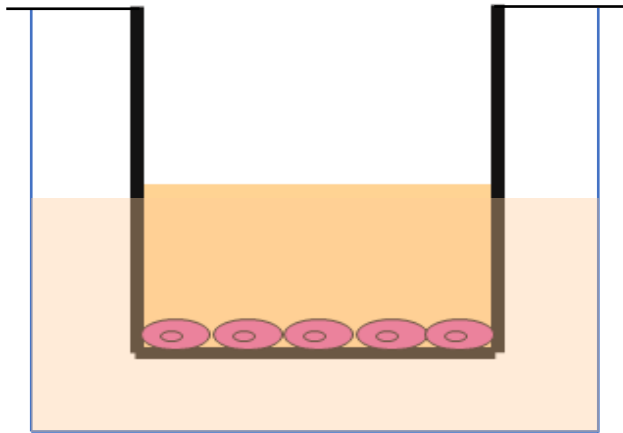
EURL ECVAM Review of non-animal models in biomedical research - Respiratory tract diseases 🧬

284 models have been identified

<https://data.jrc.ec.europa.eu/dataset/176d71e6-5082-4b29-8472-b719f6bda323>

Why Air - Liquid Interphase (ALI)

- ? *in vitro* – *in vivo* ?

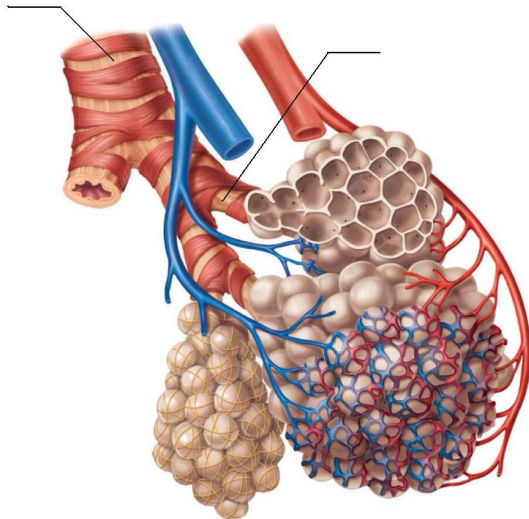


APPLIED IN VITRO TOXICOLOGY
Volume 4, Number 2, 2018
Mary Ann Liebert, Inc.
DOI: 10.1089/aivt.2017.0034

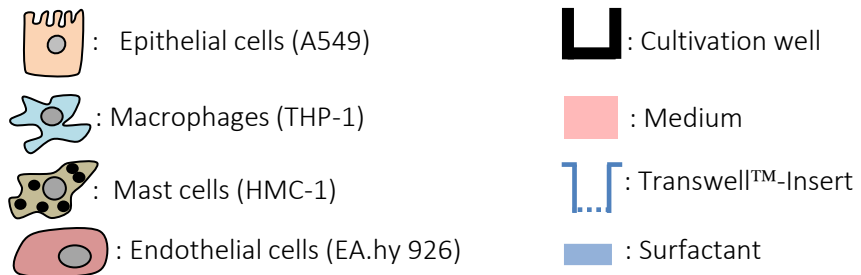
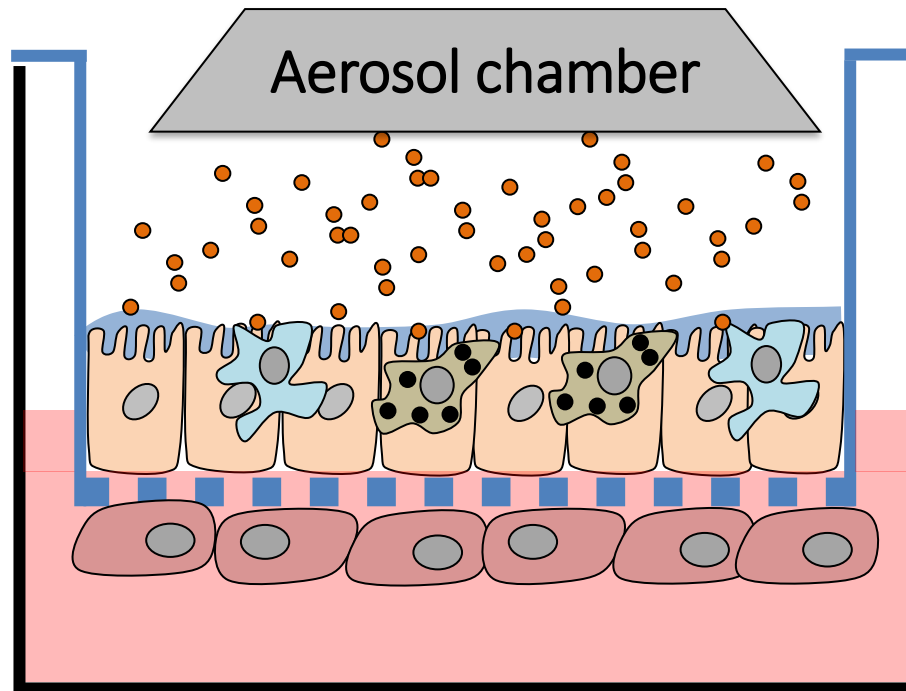
MEETING REPORT

Air–Liquid Interface *In Vitro* Models for Respiratory Toxicology Research: Consensus Workshop and Recommendations

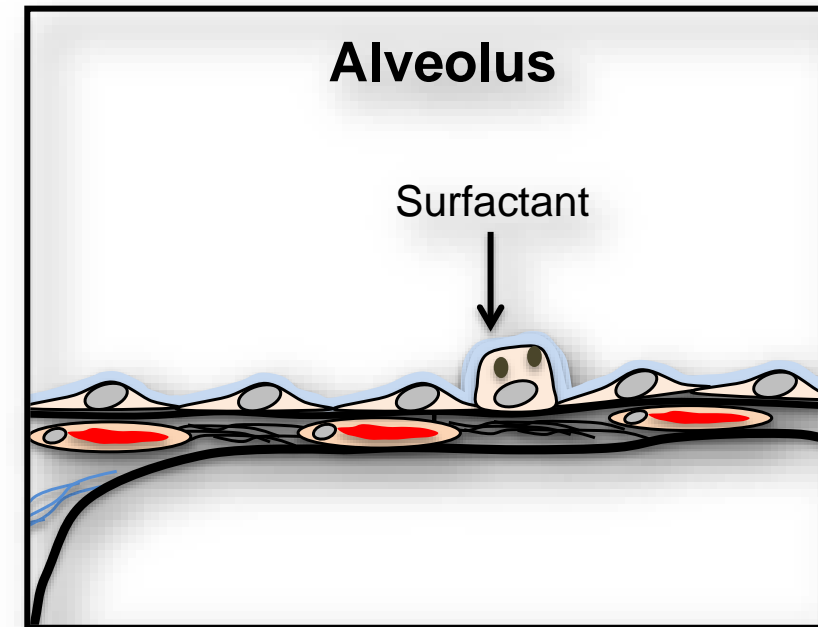
Ghislaine Lacroix,¹ Wolfgang Koch,² Detlef Ritter,² Arno C. Gutleb,³ Søren Thor Larsen,⁴ Thomas Loret,¹ Filippo Zanetti,⁵ Samuel Constant,⁶ Savvina Chortarea,^{7,8} Barbara Rothen-Rutishauser,⁷ Pieter S. Hiemstra,⁹ Emeric Frejafon,¹ Philippe Hubert,¹ Laura Gribaldo,¹⁰ Peter Kearns,¹¹ Jean-Marc Aublant,¹² Silvia Diabaté,¹³ Carsten Weiss,¹³ Antoinette de Groot,¹⁴ and Ingeborg Kooter¹⁵



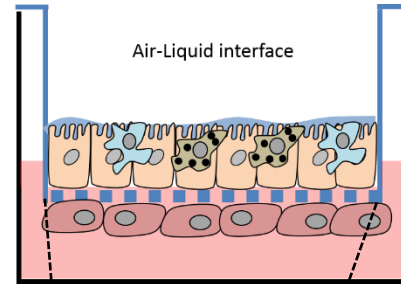
Current organisation of *in vitro* alveolar systems



in vivo anatomy of the alveoli

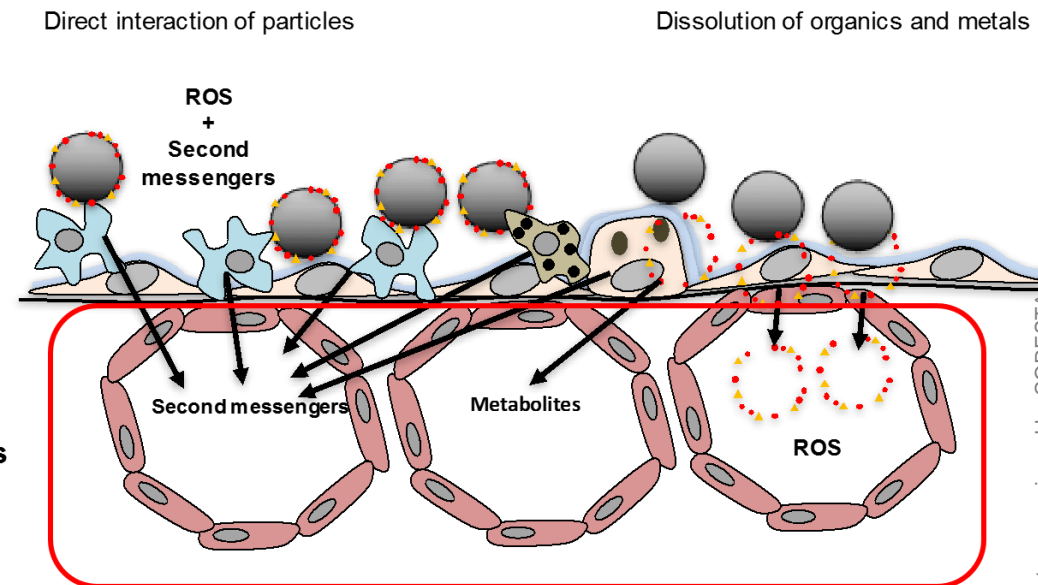


Model for Respiratory Irritation



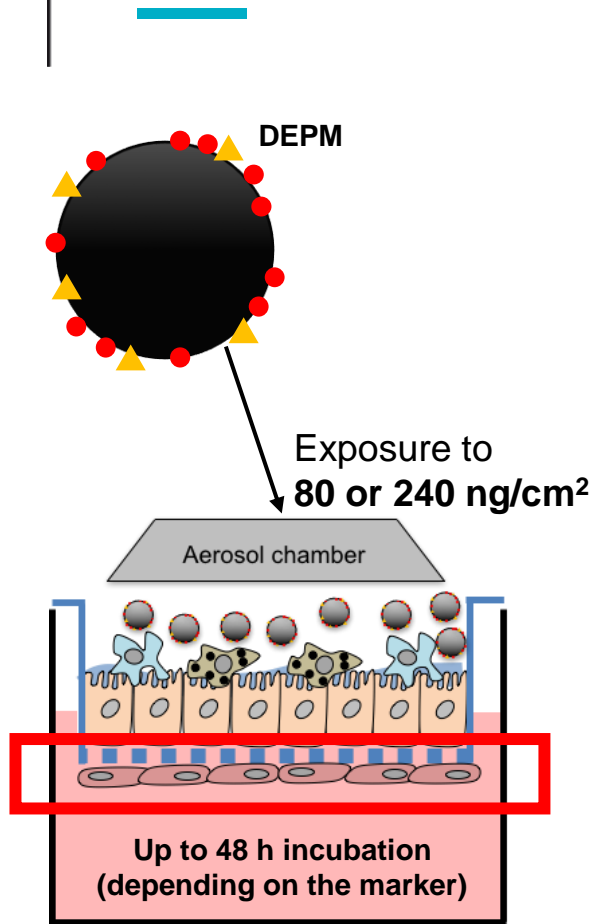
Alveolar surface

Alveolar capillaries

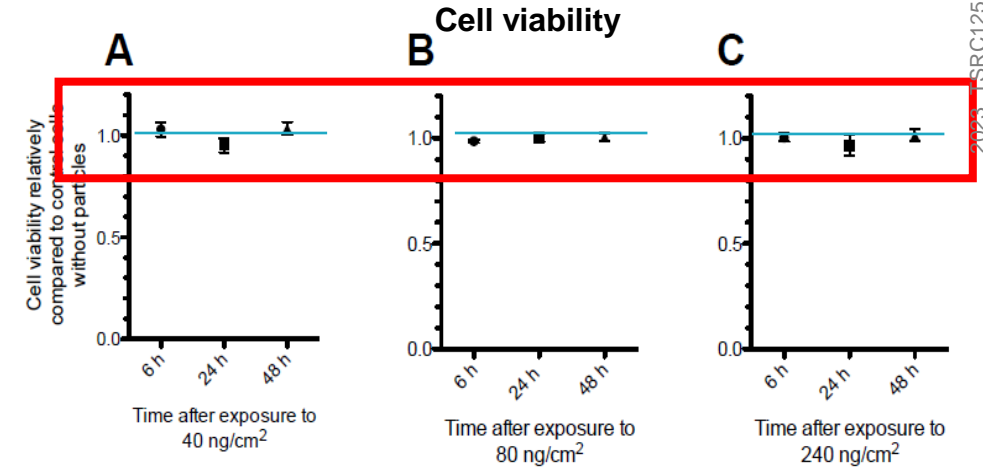


Case study: diesel exhaust particulate matter

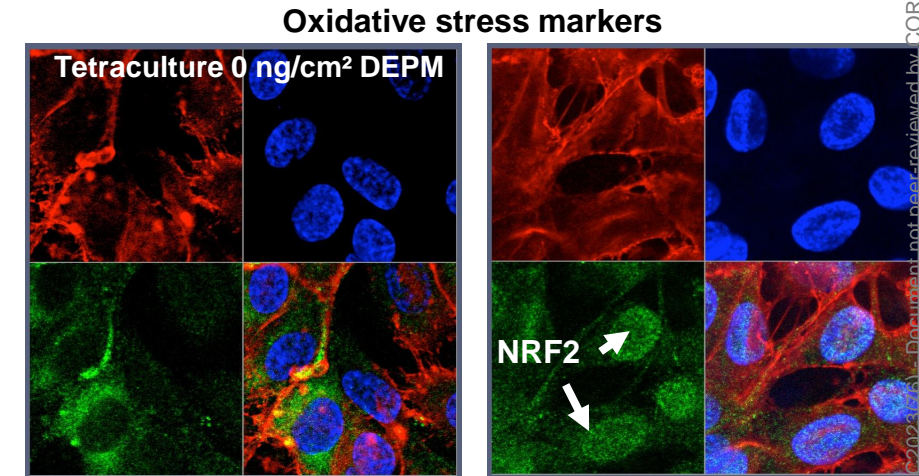
- ❖ Exposure concentrations selected on a realistic worst case scenario
- ❖ No effects on cell viability
- ❖ No significant expression of oxidative stress related genes
- ❖ Significant translocation of NRF2 as marker for early stress response (TIER1)



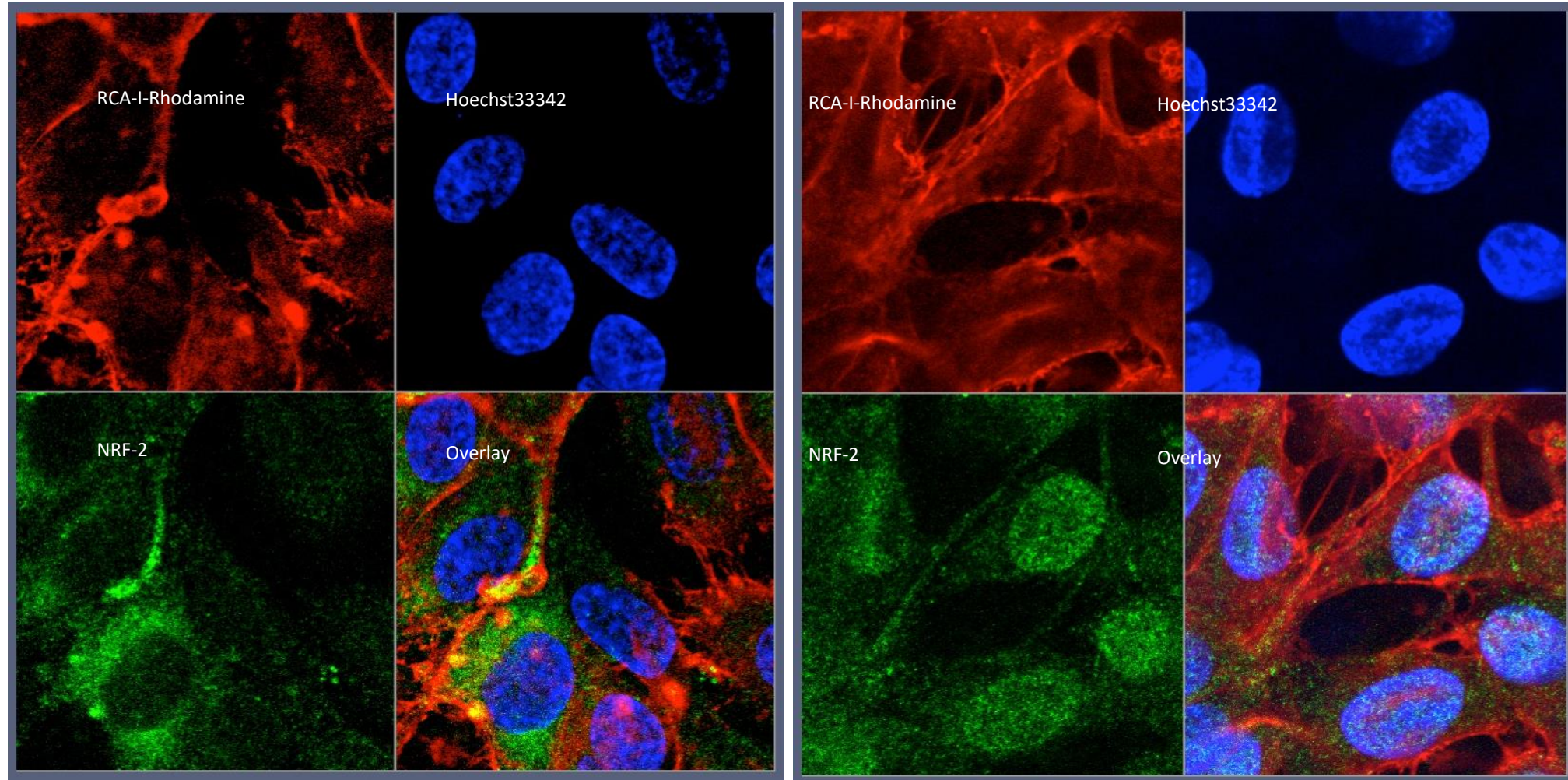
DEPM exposure induces detectable secondary effects on the endothelial side of the system



| Gene | Oxidative stress related genes | | | | | |
|--------------|--------------------------------|-------------|-------------|------------------------|-------------|-------------|
| | 80 ng/cm ² | | | 240 ng/cm ² | | |
| | 6 h | 24 h | 48 h | 6 h | 24 h | 48 h |
| <i>NEF2</i> | 0,99 ± 0,05 | 1,01 ± 0,08 | 1,05 ± 0,04 | 1,00 ± 0,03 | 0,95 ± 0,03 | 1,20 ± 0,16 |
| <i>NQO1</i> | 1,01 ± 0,05 | 0,98 ± 0,02 | 1,01 ± 0,01 | 1,03 ± 0,01 | 1,01 ± 0,03 | 1,08 ± 0,01 |
| <i>GST1</i> | 1,03 ± 0,04 | 1,00 ± 0,06 | 0,97 ± 0,03 | 0,97 ± 0,02 | 0,92 ± 0,06 | 1,10 ± 0,05 |
| <i>HMOX1</i> | 1,09 ± 0,07 | 0,95 ± 0,03 | 0,93 ± 0,03 | 0,97 ± 0,04 | 0,97 ± 0,07 | 1,13 ± 0,11 |
| <i>HMOX2</i> | 1,14 ± 0,15 | 1,09 ± 0,02 | 1,03 ± 0,01 | 1,01 ± 0,04 | 1,09 ± 0,07 | 1,16 ± 0,15 |
| <i>SOD1</i> | 0,91 ± 0,10 | 0,98 ± 0,07 | 1,05 ± 0,04 | 0,98 ± 0,03 | 1,01 ± 0,05 | 1,06 ± 0,04 |



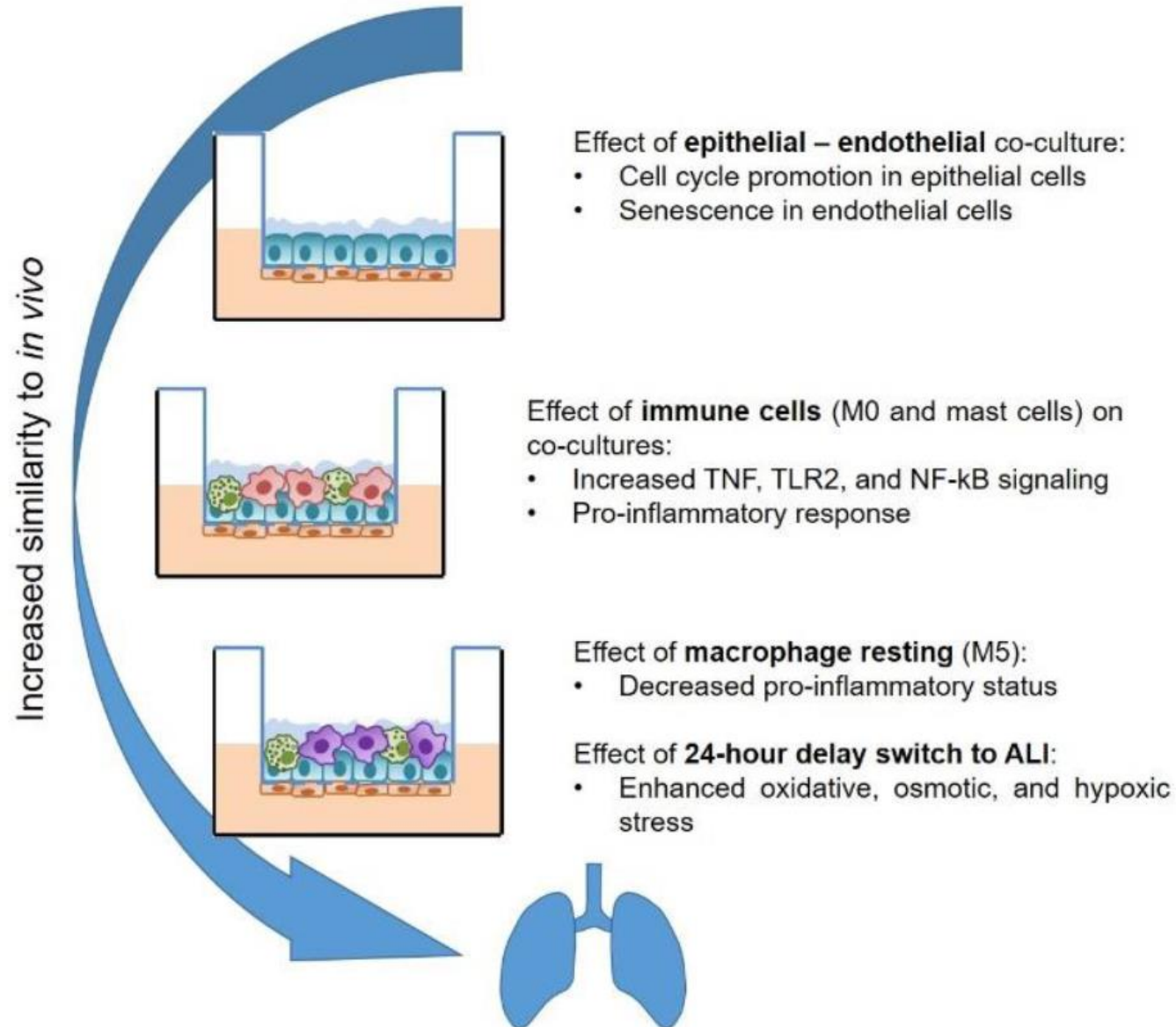
Nuclear translocation of NRF2 in the endothelial part of the tetraculture (4h)



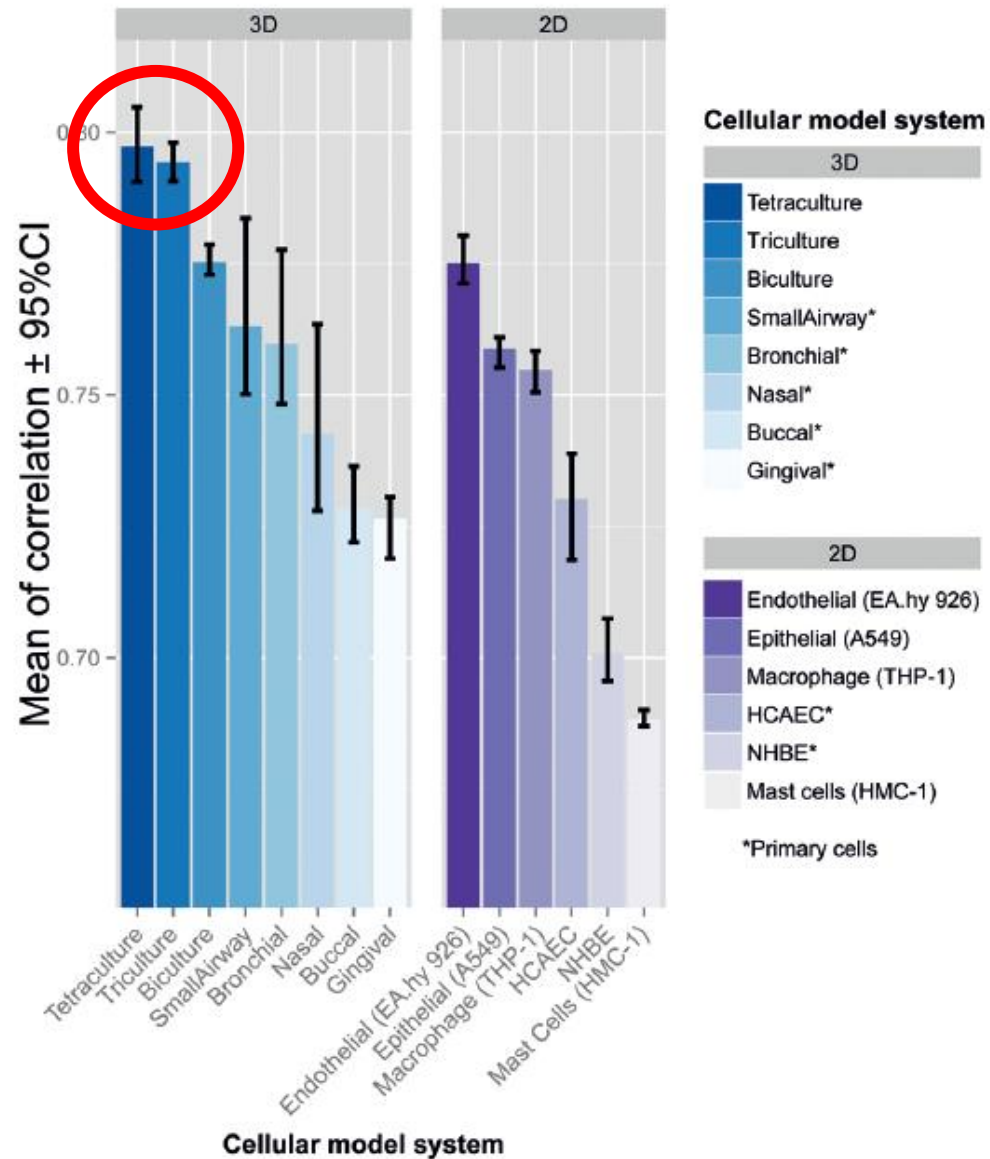
Tetraculture 0 ng/cm² DEP

Tetraculture 240 ng/cm² DEP

Complexity matters



Complexity matters



Research Article

How Complex Should an *In Vitro* Model Be? Evaluation of a Complex 3D Alveolar Model with Transcriptomic Data and Computational Biological Network Models

Diego Marescotti¹, Tommaso Serchi², Karsta Luettich¹, Yang Xiang¹, Elisa Moschini², Marja Talikka¹, Florian Martin¹, Karine Baumer¹, Remi Dulize¹, Dariusz Peric¹, David Bornand¹, Emmanuel Guedj¹, Alain Sewer¹, Sebastien Cambier², Servane Contal², Aline Chary², Arno C. Gutleb², Stefan Frentzel¹, Nikolai V. Ivanov¹, Manuel C. Peitsch¹ and Julia Hoeng¹

¹PMI R&D, Philip Morris Products S.A., Neuchâtel, Switzerland; ²Luxembourg Institute of Science and Technology (LIST), Esch-sur-Alzette, Luxembourg

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journal homepage: www.elsevier.com/locate/toxlet

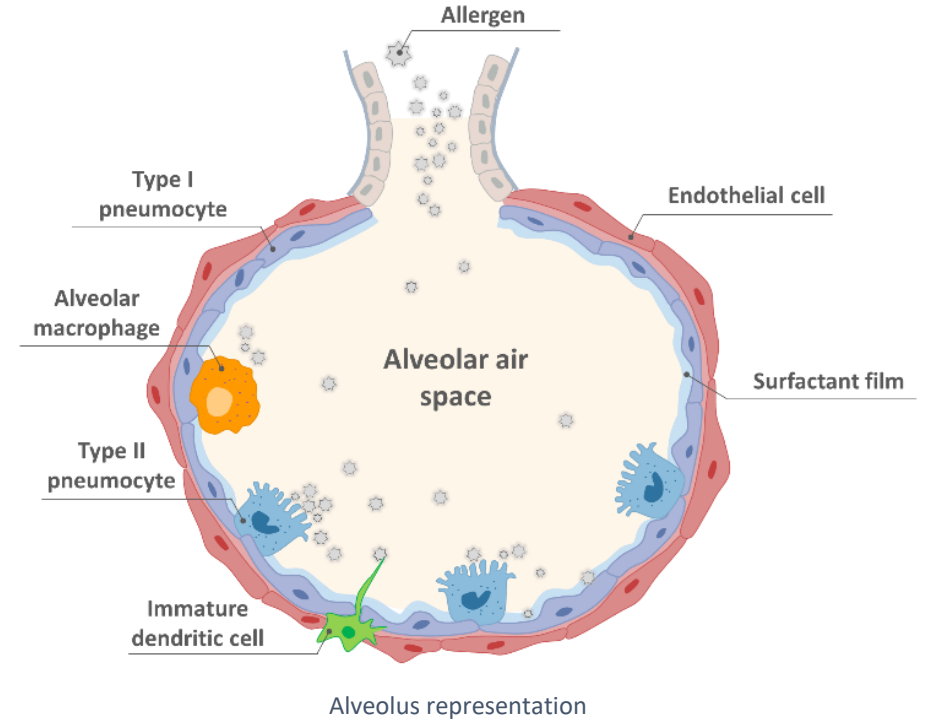
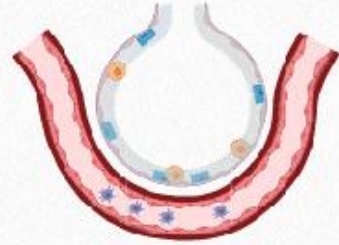


Comparison of the biological impact of aerosol of e-vapor device with *MESH*[®] technology and cigarette smoke on human bronchial and alveolar cultures

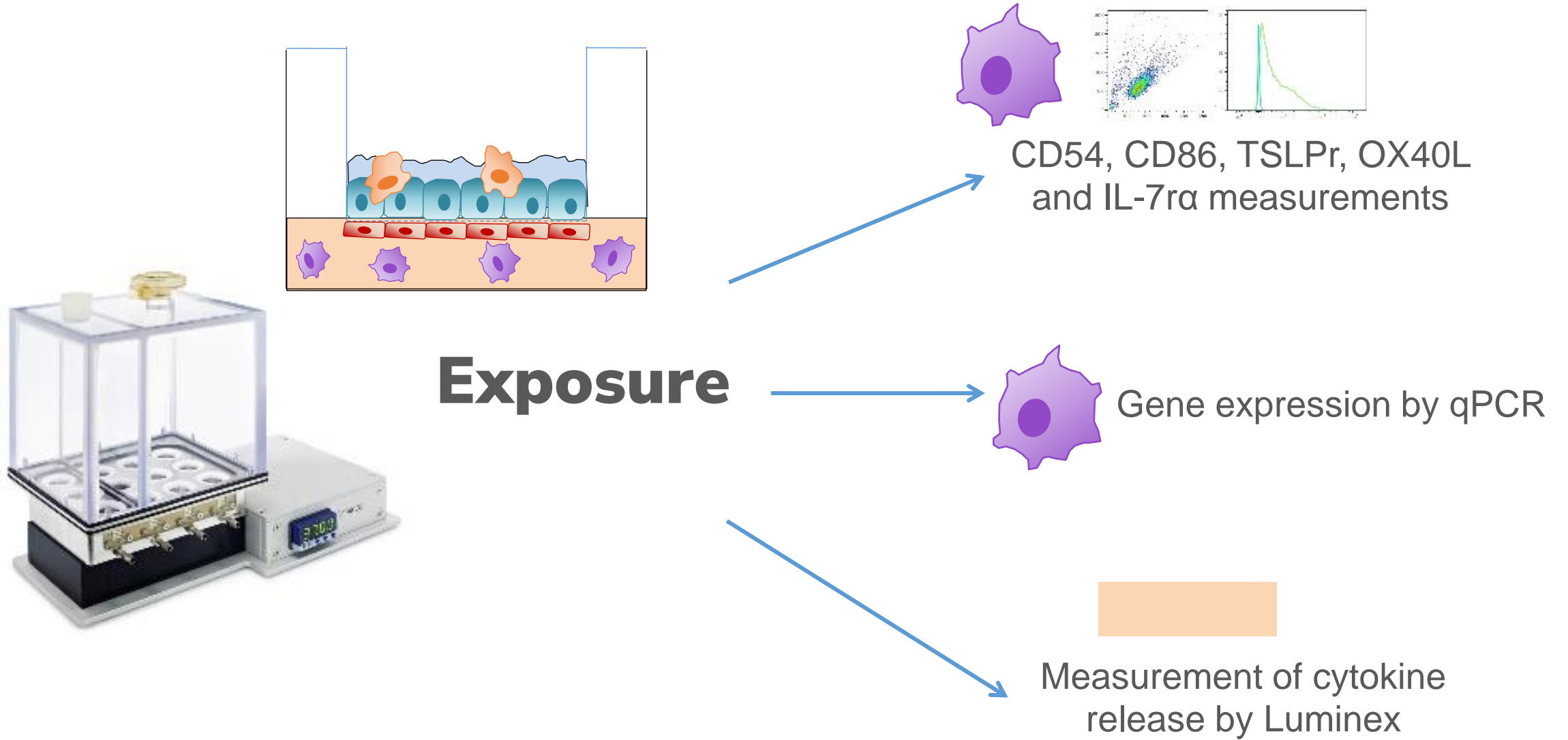
Albert Giralt^a, Anita R Iskandar^{a,*}, Florian Martin^a, Elisa Moschini^b, Tommaso Serchi^b, Athanasios Kondylis^a, Diego Marescotti^a, Patrice Leroy^a, Laura Ortega-Torres^a, Shoab Majeed^a, Celine Merg^a, Keyur Trivedi^a, Emmanuel Guedj^a, Stefan Frentzel^a, Nikolai V. Ivanov^a, Manuel C Peitsch^a, Arno C Gutleb^b, Julia Hoeng^a



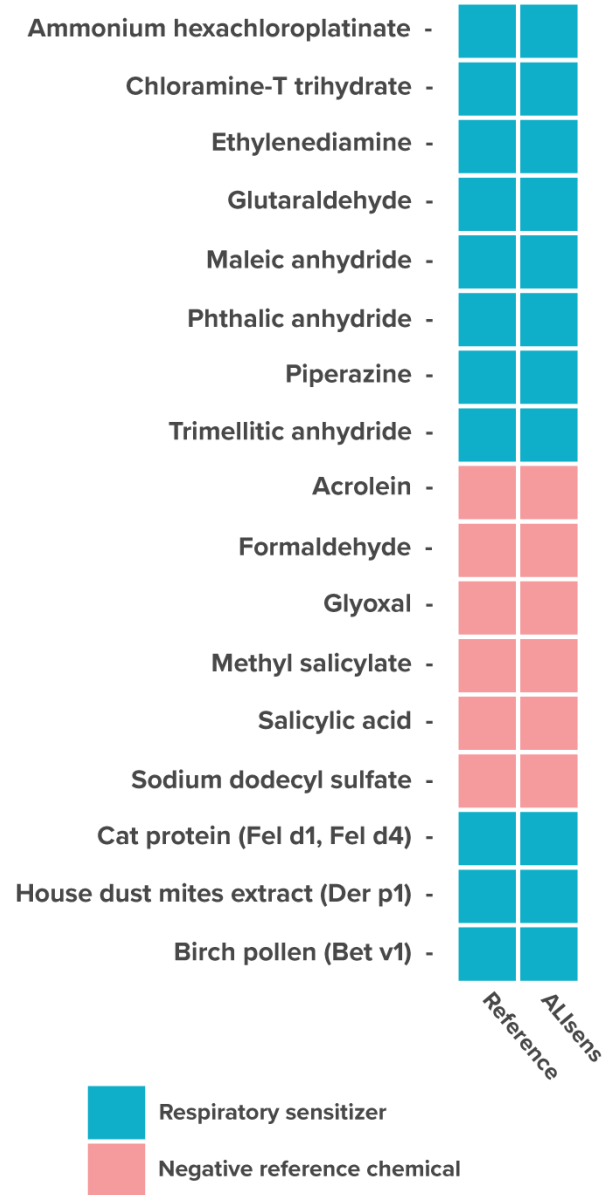
One solution: the ALIsens[®] model.



Identification is based on three groups of endpoints.



ALIsens[®] vs human *in vivo* data.



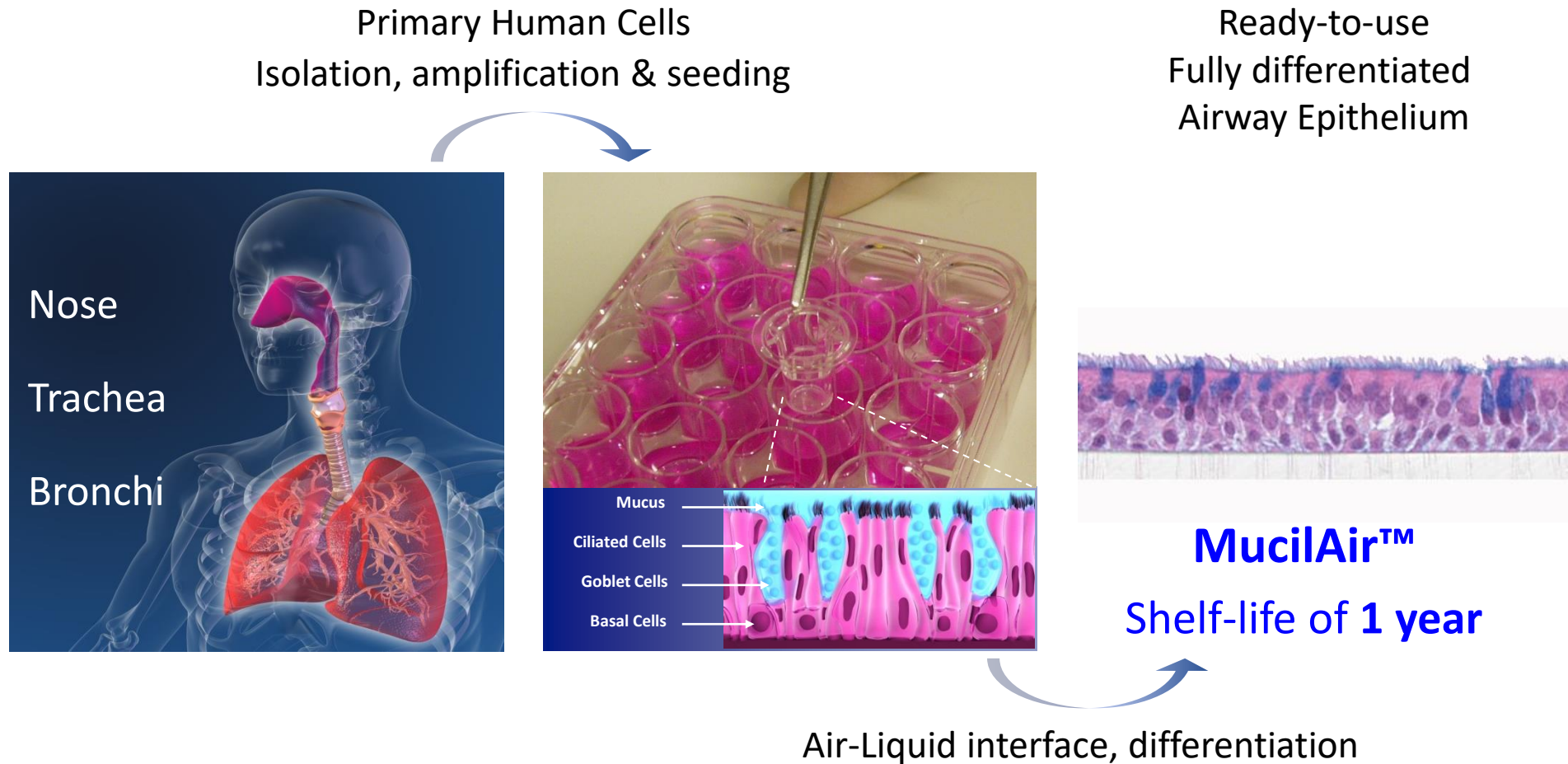
~ 20 known LMW respiratory sensitizers
 6 known skin sensitizers
 4 known HMW (= protein) sensitizers
 ~ 10 «innocent» chemicals

All correct identified

Client samples of unknown expected status

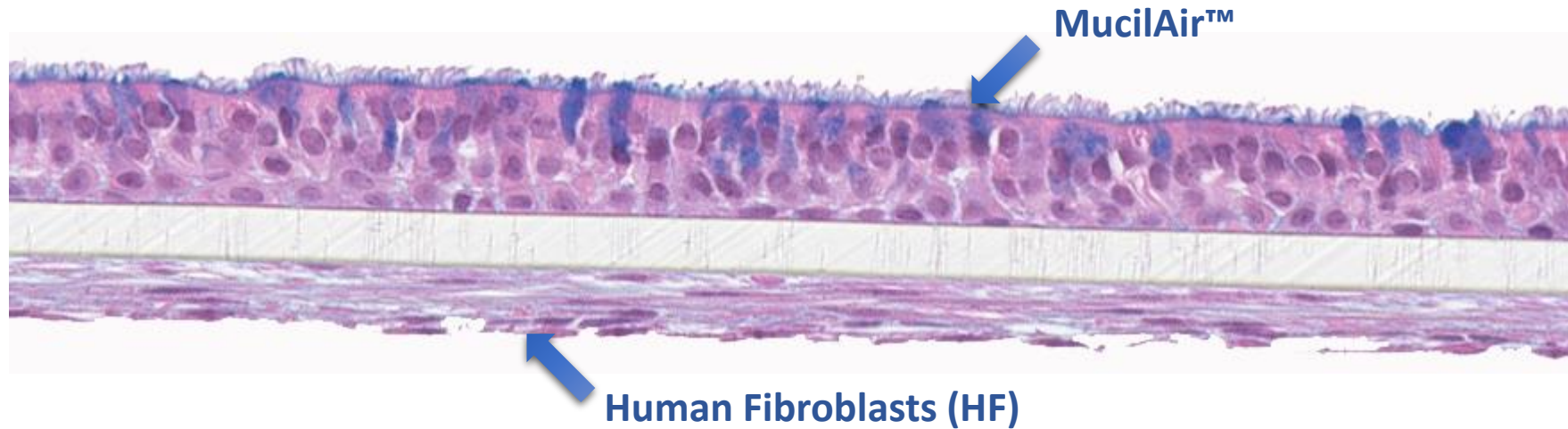
Commercial Primary Cell-based Systems

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



MucilAir™-HF

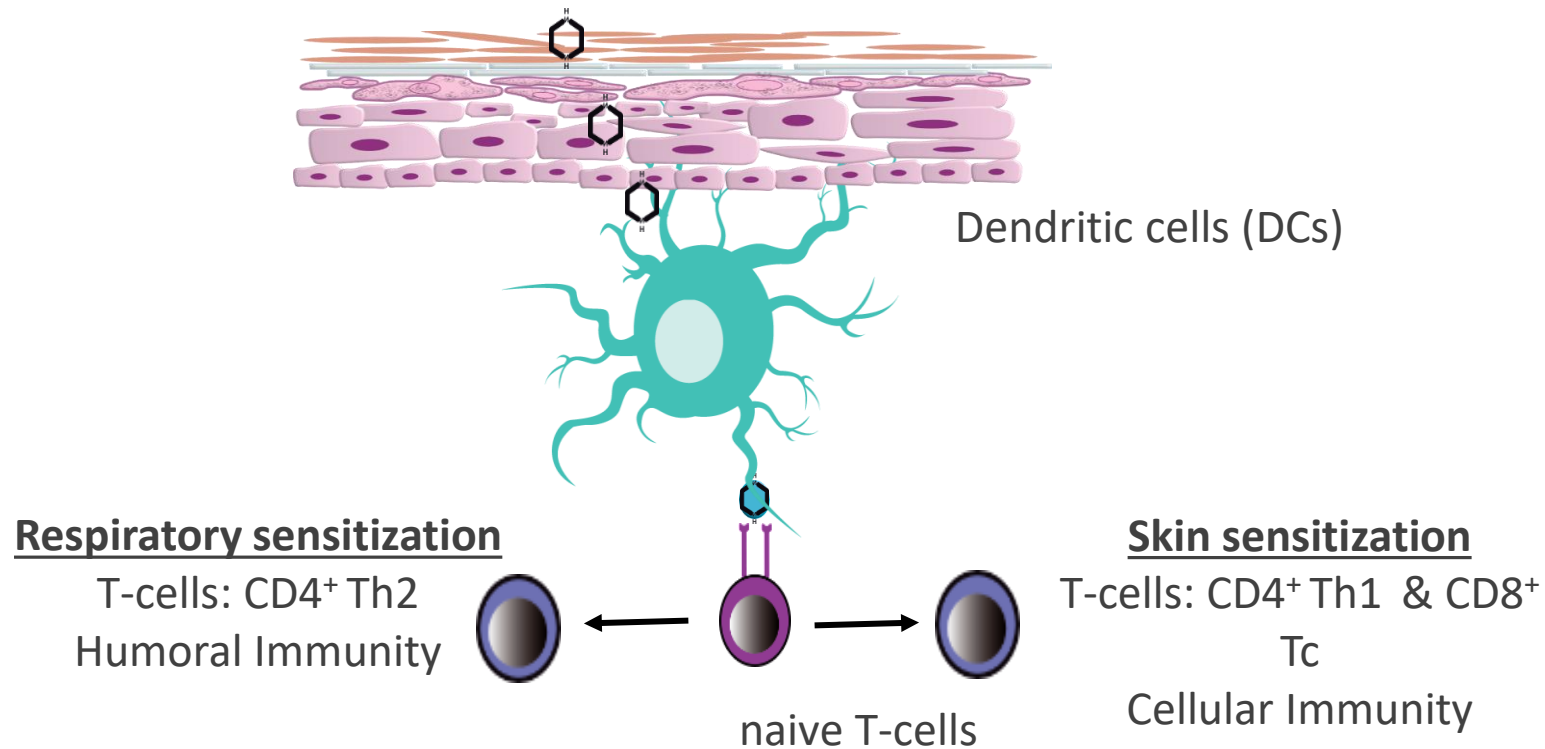
MucilAir™-HF: Long shelf-life co-culture model



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

The GARD platform – biological system



GARD = Genomic Allergen Rapid Detection™

Human dendritic-like cell line (SenzaCells™) able to recognize allergens

Genomics-based approach with machine-learning technology

<https://senzagen.com>

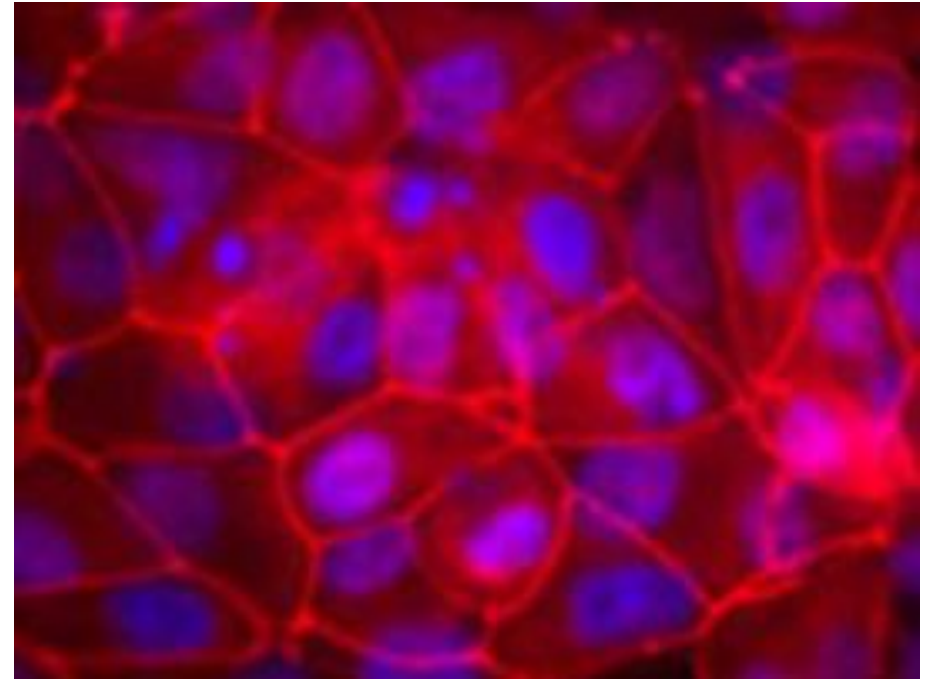
Alternative type I cell line

CI-hAELVi = alveolar type I cell line

CI-huAEC = Lung epithelial cell line

Specifications of CI-hAELVi

- ⊙ Characteristic expression of epithelial cell markers (e.g. Occludin, ZO-1)
- ⊙ Type-I-like properties (expression of Caveolin-1, absence of surfactant Prot C)
- ⊙ Growth in liquid-liquid as well as air-liquid conditions
- ⊙ Tight intercellular junctions and formation of desmosomes
- ⊙ Low permeability
- ⊙ Transepithelial Electrical Resistance (TEER)-formation



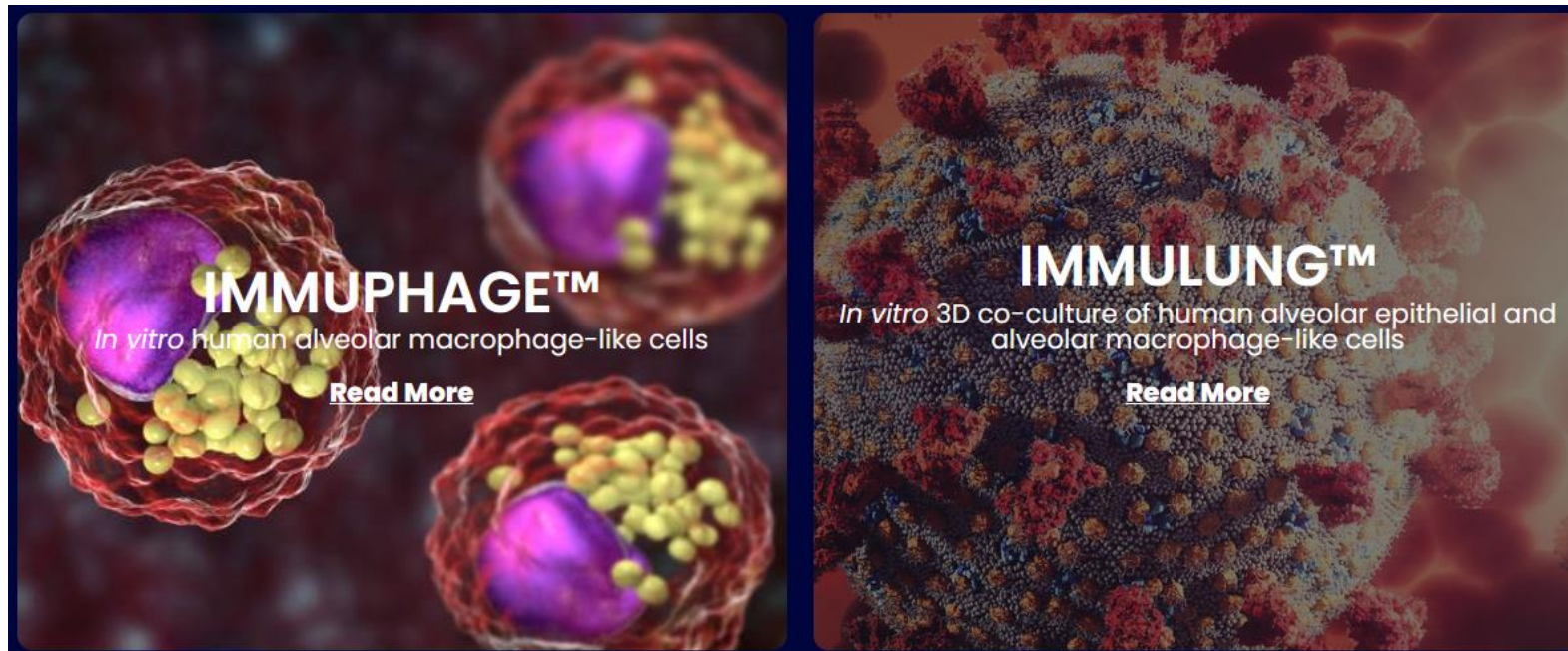
<https://www.inscreenex.de/>

Immortalized human cells

Immortalized alveolar cell line

Immortalized alveolar macrophage-like cells

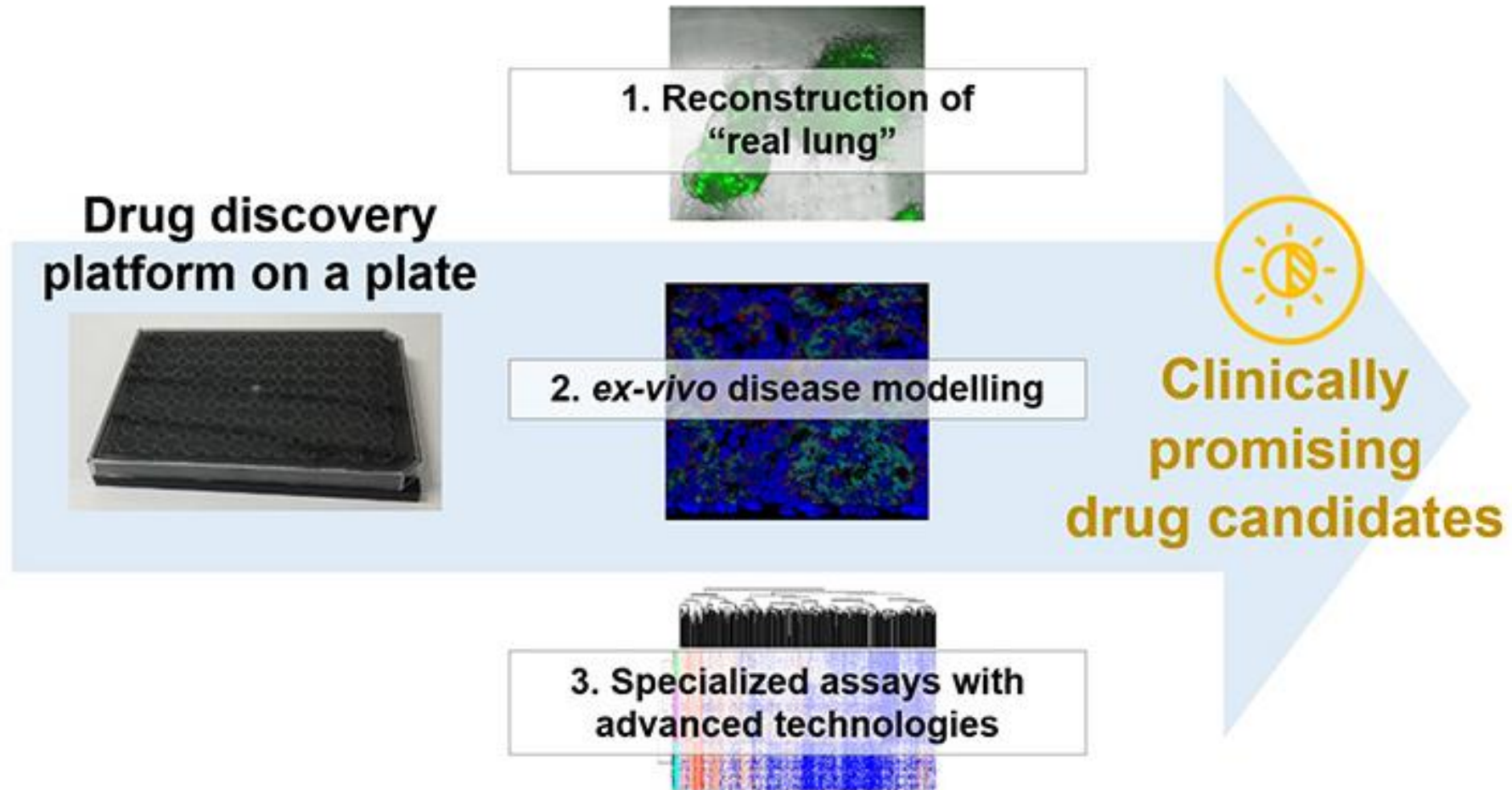
ALI - and liquid-liquid versions (24 well format)



<https://immuone.com/>

iPS based model

Stem cell differentiation induction technology



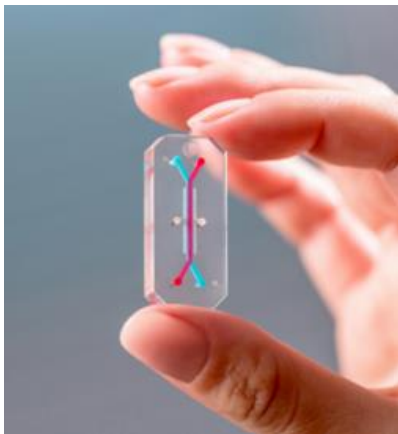
Lung-on-a-chip

Advantages

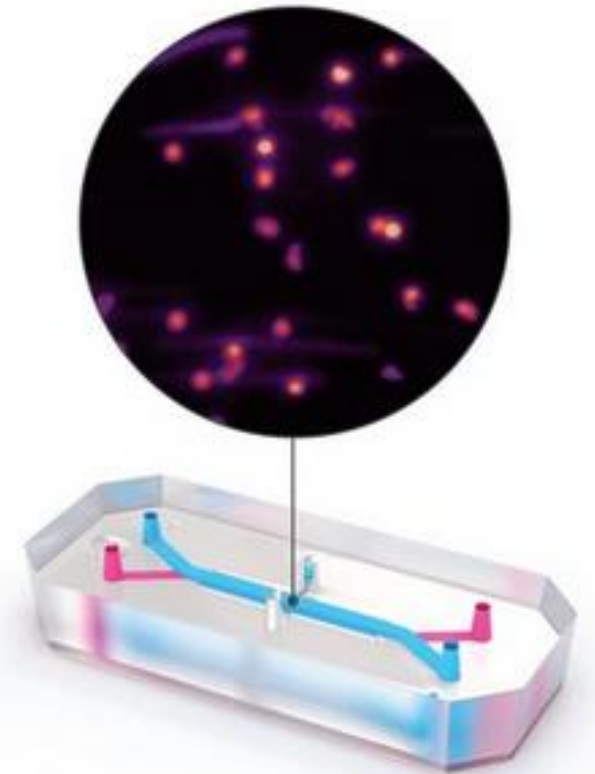
Air-liquid-interphase culture

Primary human cells (alveolar and endothelial)

Relevant physiological stress (stretching)



Alveolus
Lung-Chip



<https://www.emulatebio.com>

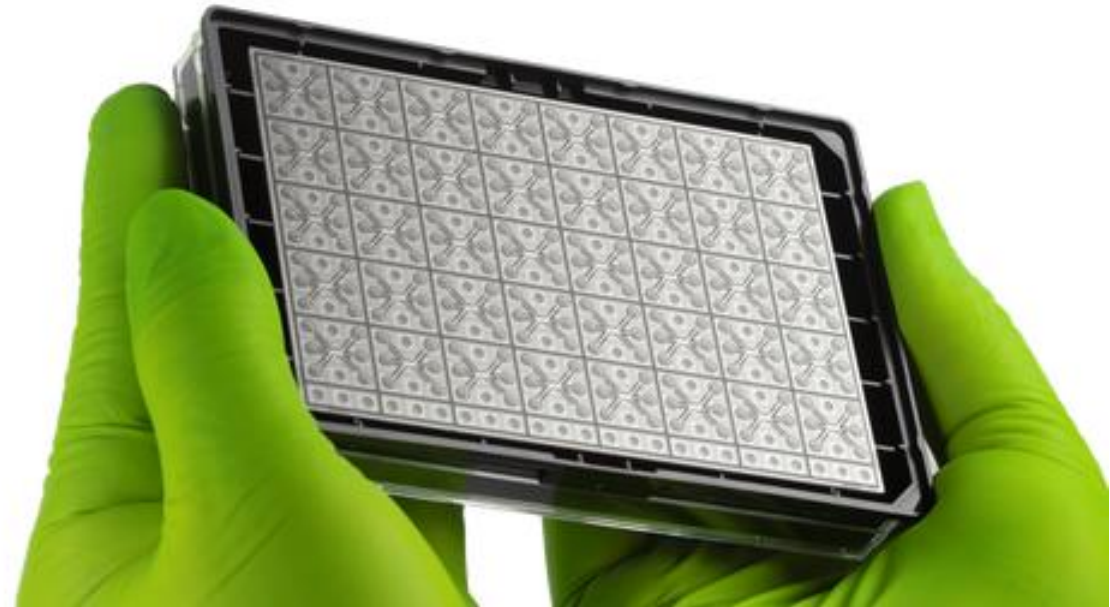
Lung-on-a-chip

Advantages

Air-liquid-interphase culture

Primary human cells (alveolar and endothelial)

Relevant physiological stress (stretching)



Lung-on-a-chip

Advantages

Air-liquid-interphase culture

Used for many cell types

(Relevant physiological stress stretching)?

Combination with inHALES possible

Combination with other tissues possible



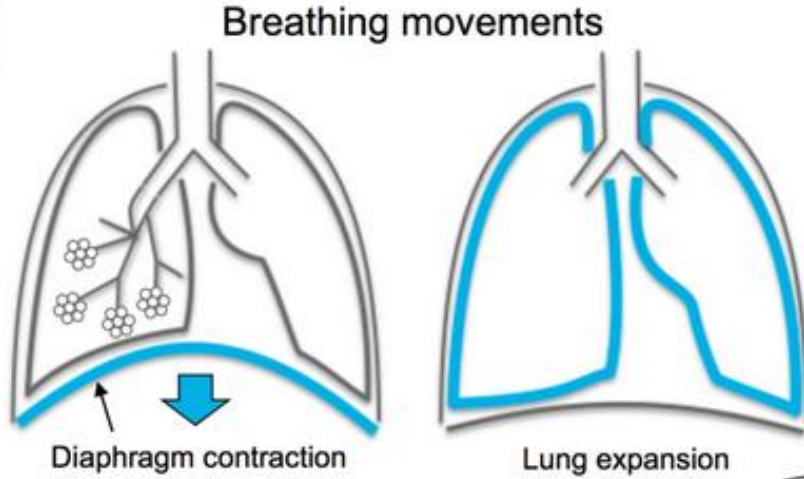
What is missing??



Pressure in the lung

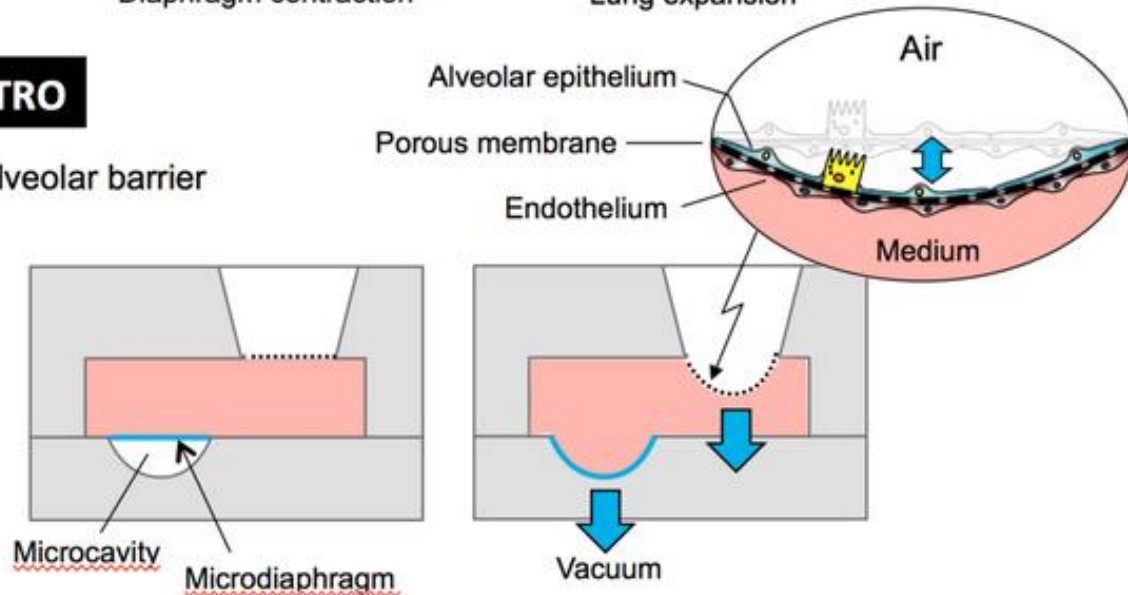
ALVEOLIX LUNG-ON-CHIP

IN-VIVO

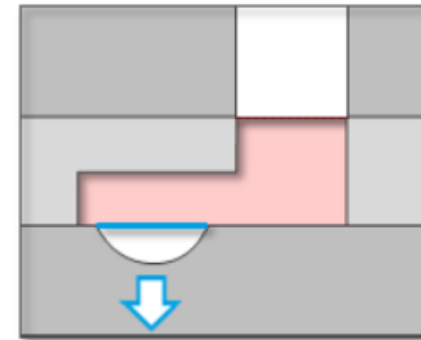


IN-VITRO

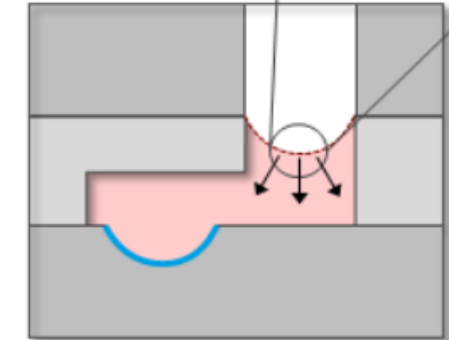
Lung alveolar barrier



- In-vivo-like features:
- 3D deformation
 - 10% mechanical strain
 - 10 cycles/min



Microdiaphragm actuation



Alveolar membrane deflection

Summary and Conclusion

- Complexity adds to relevance but needs to be benchmarked
- Cell-culture models for any region of the lung available
- Such models need to be cultured at the ALI
- Dosimetry can be difficult especially in OoC systems
- Pressurized systems start to become available
- All systems have advantages and limitations

Your research question will guide your choices

LIVE 2024

LUNG IN VITRO event 20-21
for innovative & predictive models JUNE 

Nice, France

<https://www.epithelix.com/events/live2024>

Merci villmols



For all questions contact me at: arno.gutleb@invitrolize.com

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