Air Liquid Interphase (ALI) exposure of *in vitro* models for alveolar toxicity

Arno C. Gutleb

arno.gutleb@list.lu

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DISCLAIMER

NO economical interest

NOT a systematic review but will show some basic principles

IN VIVO LIMITATIONS

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Costs – Ethics - Science

- 2005 10 billion euro were spent on animal experiments worldwide
- 100 million animals were used
- Many studies are required by legislation
- Value ? - transferability of the results to humans
  - Differences in particle deposition
  - Differences in the immune system
- Application of extreme doses
- False positive correlations of multiple endpoints
- Low toxicity of most of the tested chemicals
- Requested by Risk Assessors

- Alternative *in vitro* test methods
- Human cell-based *in vitro* cultures
- Directly relevant endpoints
- Dose determination at ALI is "easy"

Taylor et al., 2008; Rovinda and Hartung, 2009

WHY AIR - LIQUID INTERPHASE

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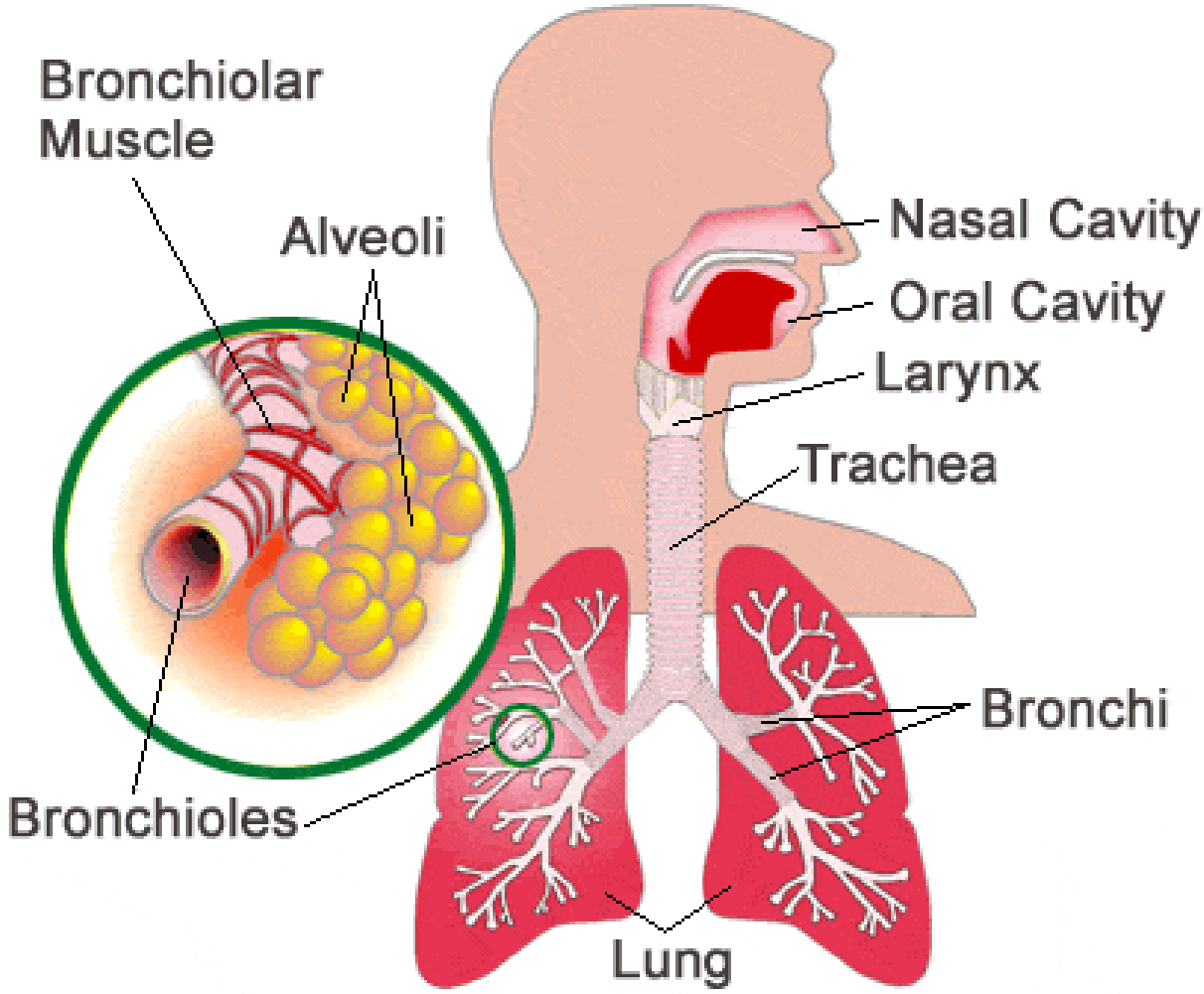
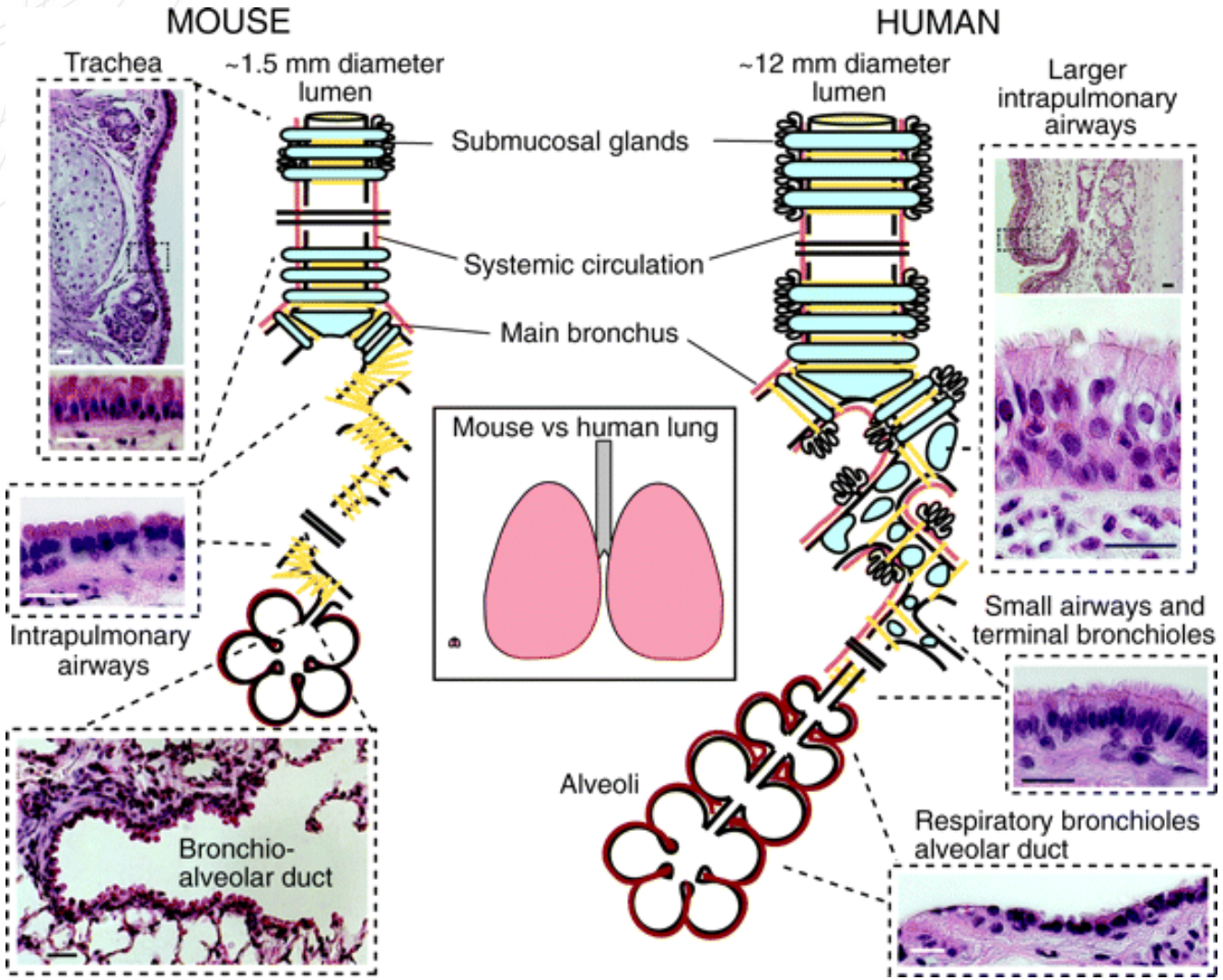
http://ericsnethkamp.zenfolio.com/blog/2017/1/drowning-a-wedding-dress

WHY AIR - LIQUID INTERPHASE



http://www.medicinenet.com/smokers_lung_pathology_photo_essay/article.htm

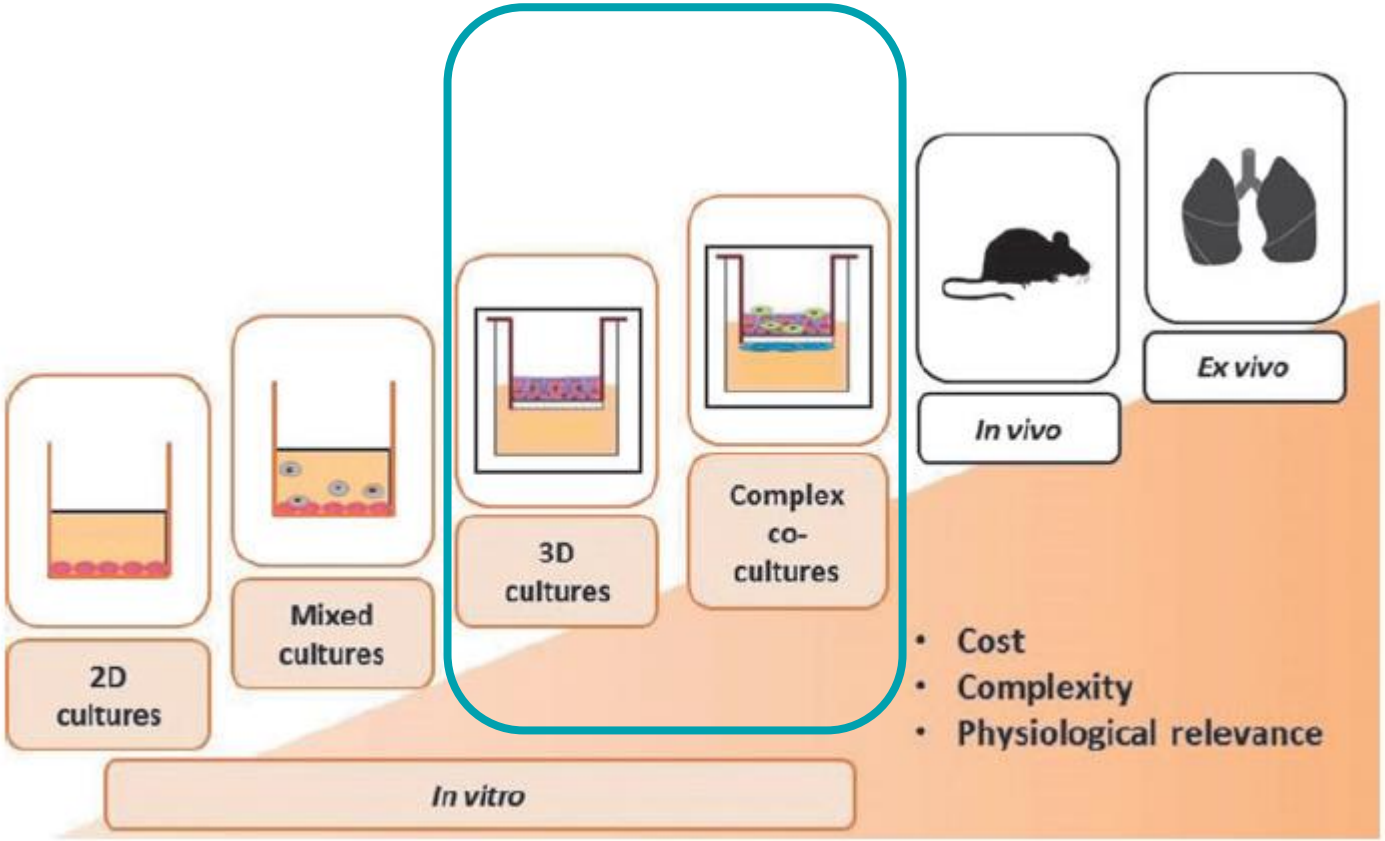
MOUSE VERSUS HUMAN AIRWAYS



Rock et al., 2010

PULMONARY MODELS

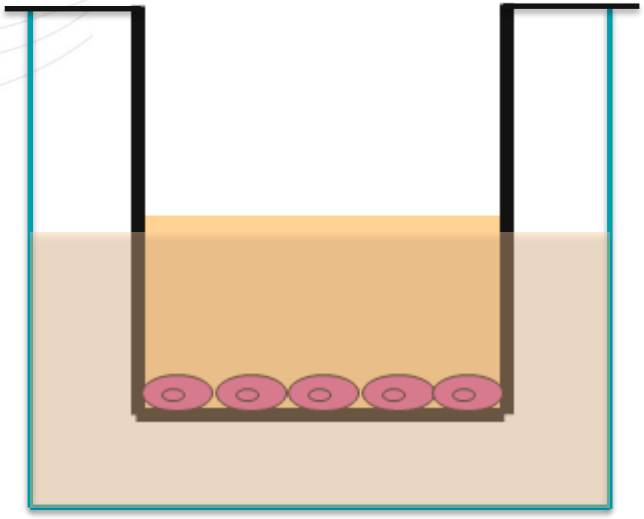
- Available models
  - *in vivo*
  - *ex vivo*
  - *in vitro*

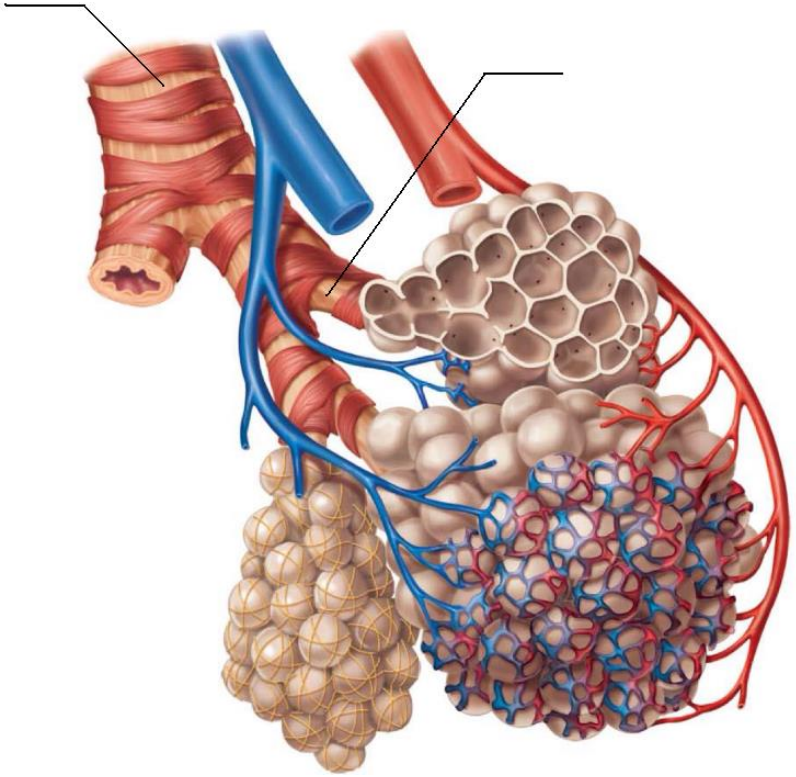


Lacroix et al., 2018

WHY AIR - LIQUID INTERPHASE

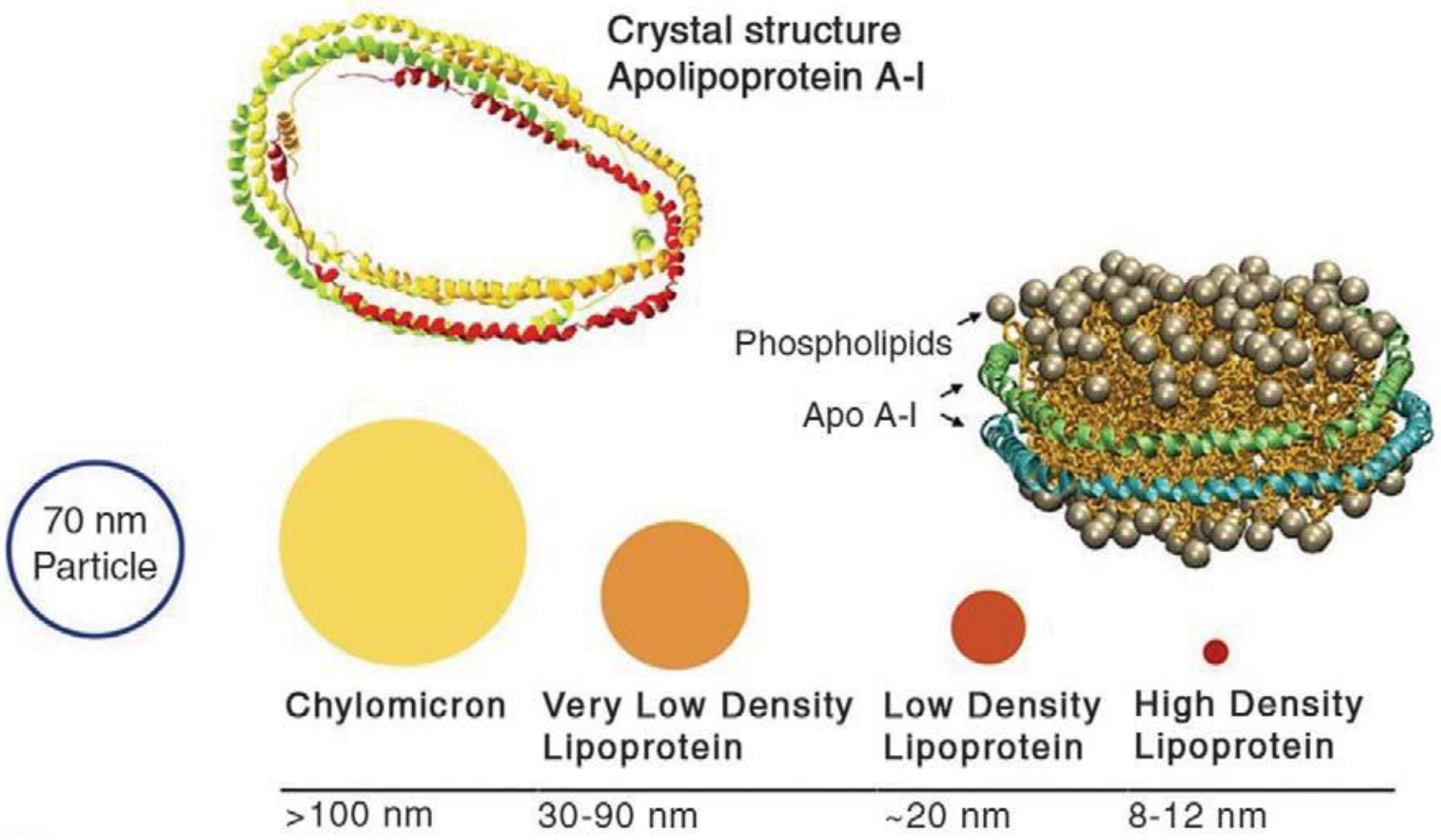
? in vitro – in vivo ?





http://socratic.org/questions/how-does-the-structure-of-the-alveoli-relate-to-its-function-in-the-lungs

WHAT A CELL "SEES" FROM NANO



Lynch and Dawson, 2008

ALI APPLICATIONS

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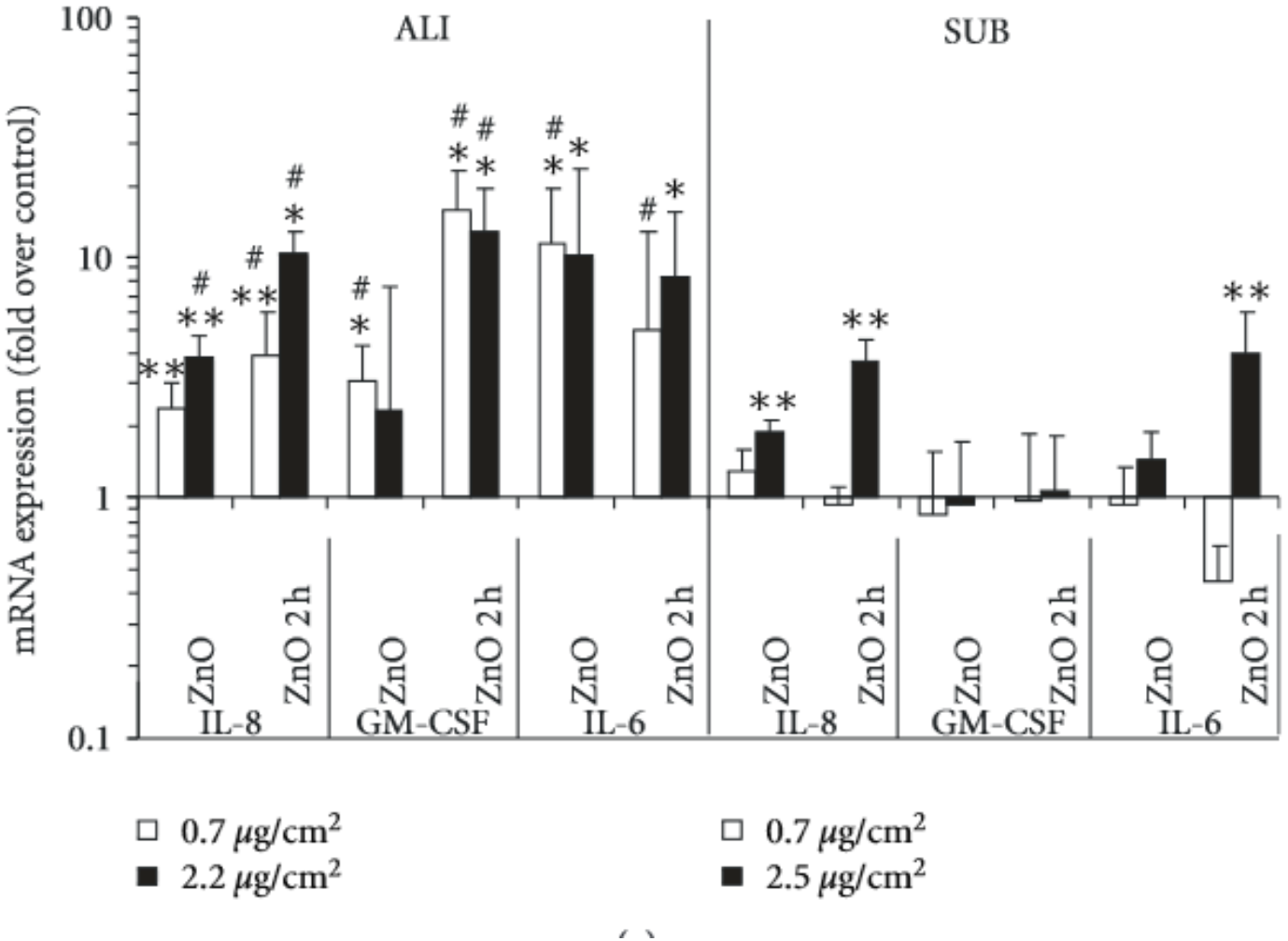
- Skin cells
- Cornea cells
- Nasal cells
- Tracheal cells
- Bronchial cells
- Alveolar cells
- Melanocytes
- Immune cells

- Human
- Chicken
- Rat

- (Nano)Particles
- Virus / Bacteria
- Chemicals / Pharmaceuticals / Tobacco products

ALI – SUBMERGED CULTURE



Lenz et al., 2013

HUMAN CELL CULTURE

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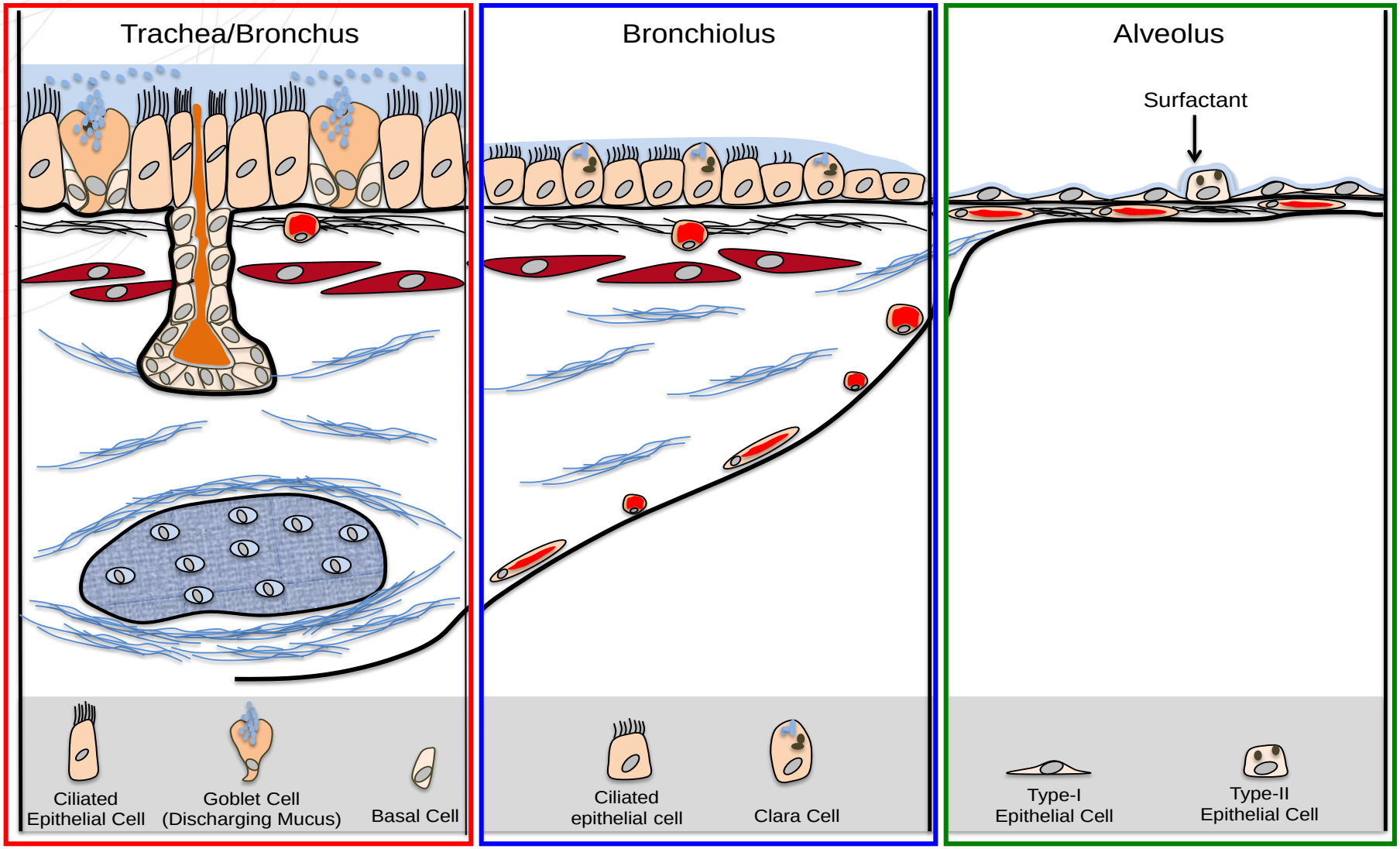
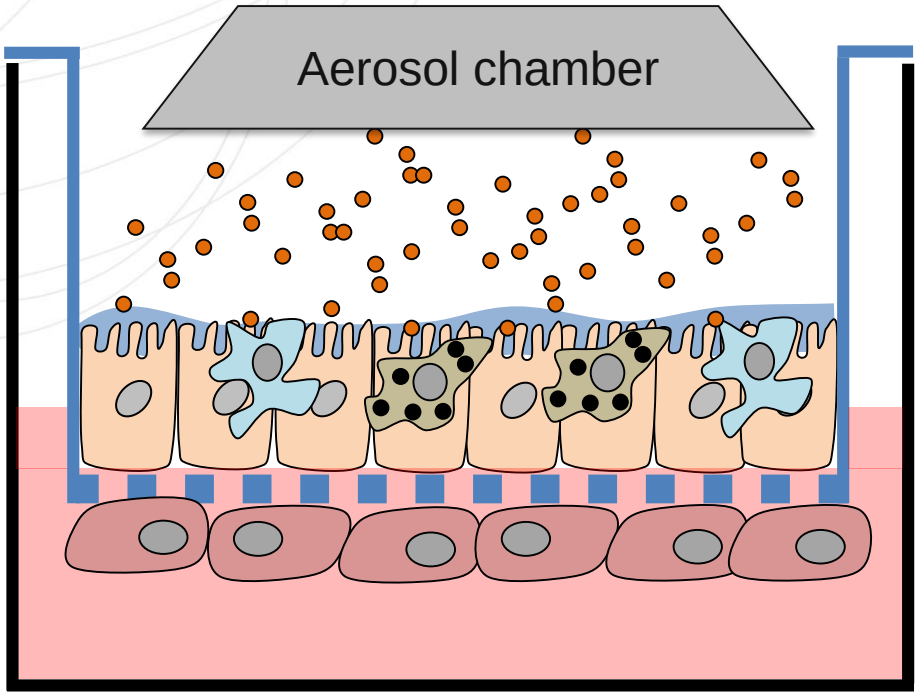
- Airway epithelial cells
  - Calu-3 (Bivas-Benita et al., 2004; Grenha et al., 2007; Rotoli et al., 2008)
  - 16HBE14o (Cozens et al., 1994; Brzoska et al., 2004; Holder et al., 2008)
  - BEAS-2B (Herzog et al., 2007, Jang et al., 2006; Park et al., 2007; Veranth et al., 2007)
- Alveolar epithelial cells
  - A549 (Duffin et al., 2007; Park et al., 2007; Stearns et al., 2001)
  - Immortalized human alveolar type 2 cells with alveolar type 1 phenotype (Kemp et al., 2008)
- 3D cultures
  - Primary endothelial and epithelial cells (Hermanns et al., 2009, 2010)
  - Triple cell co-culture model (epithelial cells, primary macrophages and dendritic cells) (Rothen-Rutishauser et al., 2007, 2008a, b, 2011)
  - Pentaculture model (epithelial cells, endothelial cells, macrophages, mastcells) (Alvaro-Moreno et al., 2008; Klein et al., 2013)
- 3D human reconstructed airways (bronchial)
  - Epithelix, MatTEK
- Organ-on-a-chip (Huh et al., 2009, 2011, 2012)


Trachea/Bronchus
Ciliated Epithelial Cell
Goblet Cell (Discharging Mucus)
Basal Cell
Bronchiolus
Ciliated epithelial cell
Clara Cell
Alveolus
Surfactant
Type-I Epithelial Cell
Type-II Epithelial Cell

Current organisation of the *in vitro* system



: Epithelial cells (A549)

: Macrophages (THP-1)

: Mast cells (HMC-1)

: Endothelial cells (EA.hy 926)

: Cultivation well

: Medium

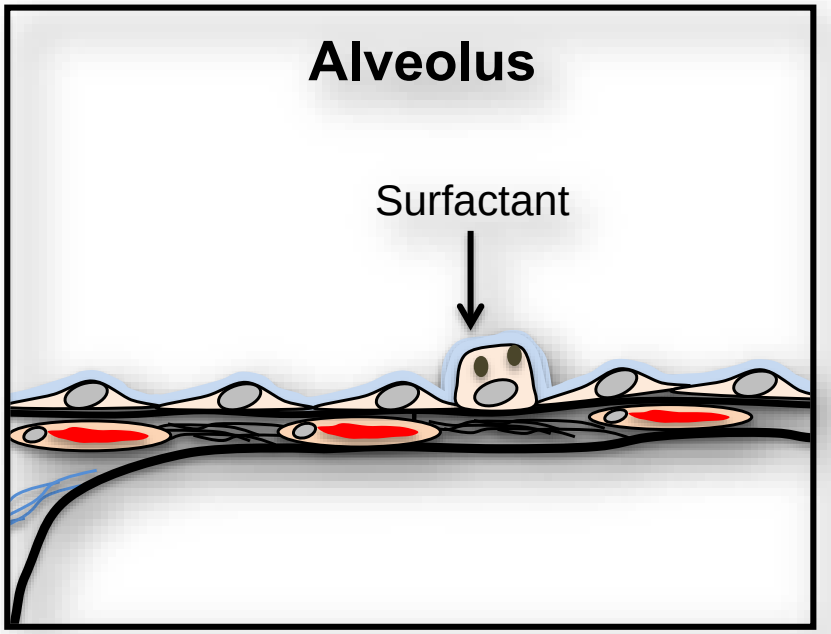
: Transwell™-Insert

: Surfactant

LIST.lu

Universität Trier

In vivo anatomy of the alveoli



Typ-I Epithelial cell

Typ-II Epithelial cell

Capillary

Klein et al., 2013

SURFACTANT PRODUCTION IN A549 CELLS

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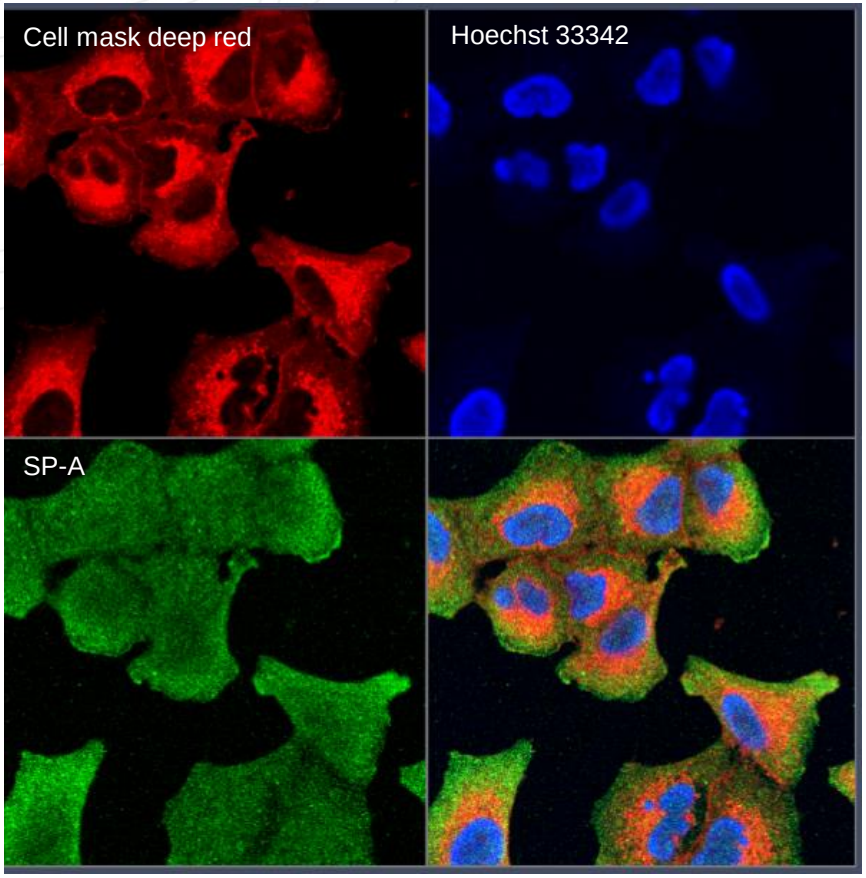
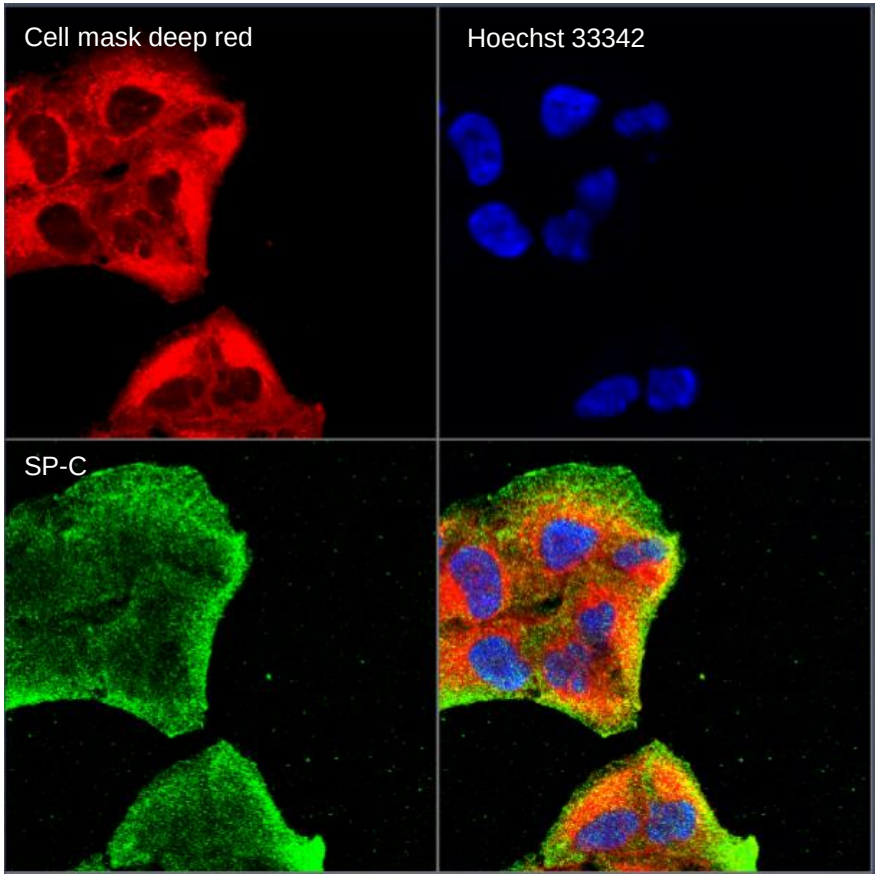
Surfactant protein A



Surfactant protein C



SECRETION OF SURFACTANT AND DETERMINATION OF SURFACE TENSION

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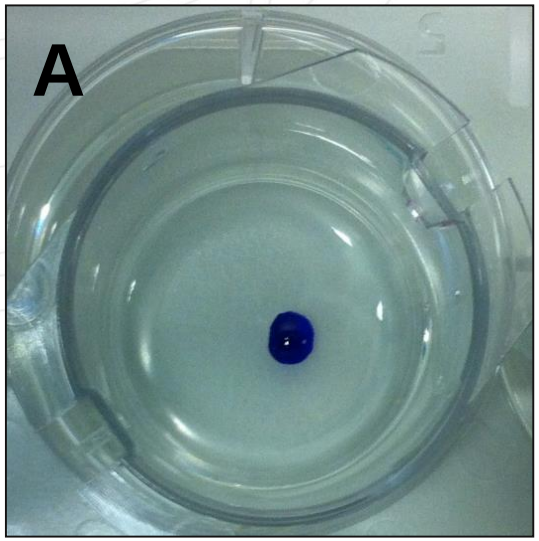
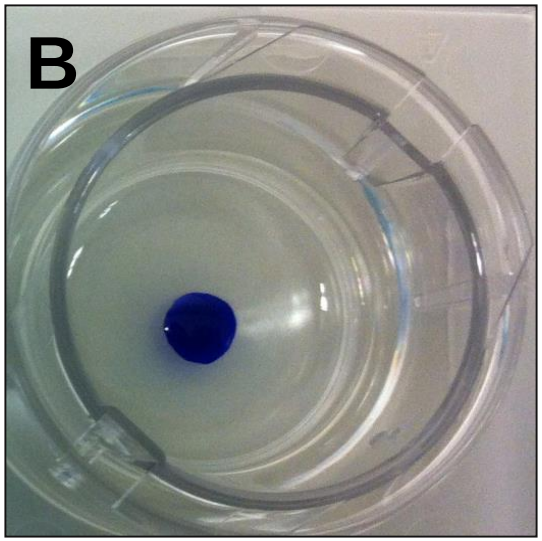
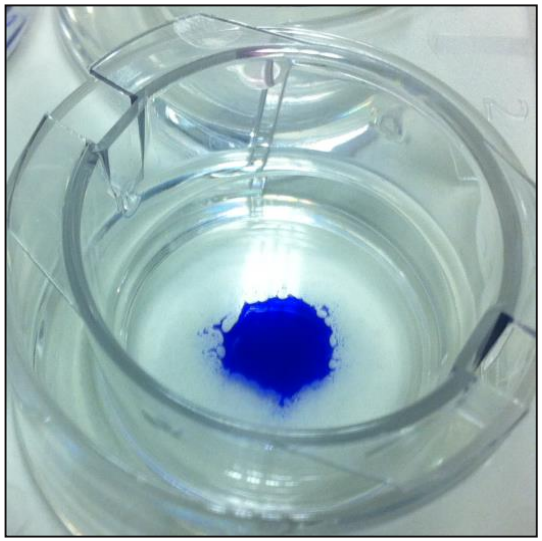


A549 cells grown at the ALI



A549 cells grown under submerged conditions



EA.hy 926

HISTAMINE IN HMC-1 HUMAN MAST CELLS

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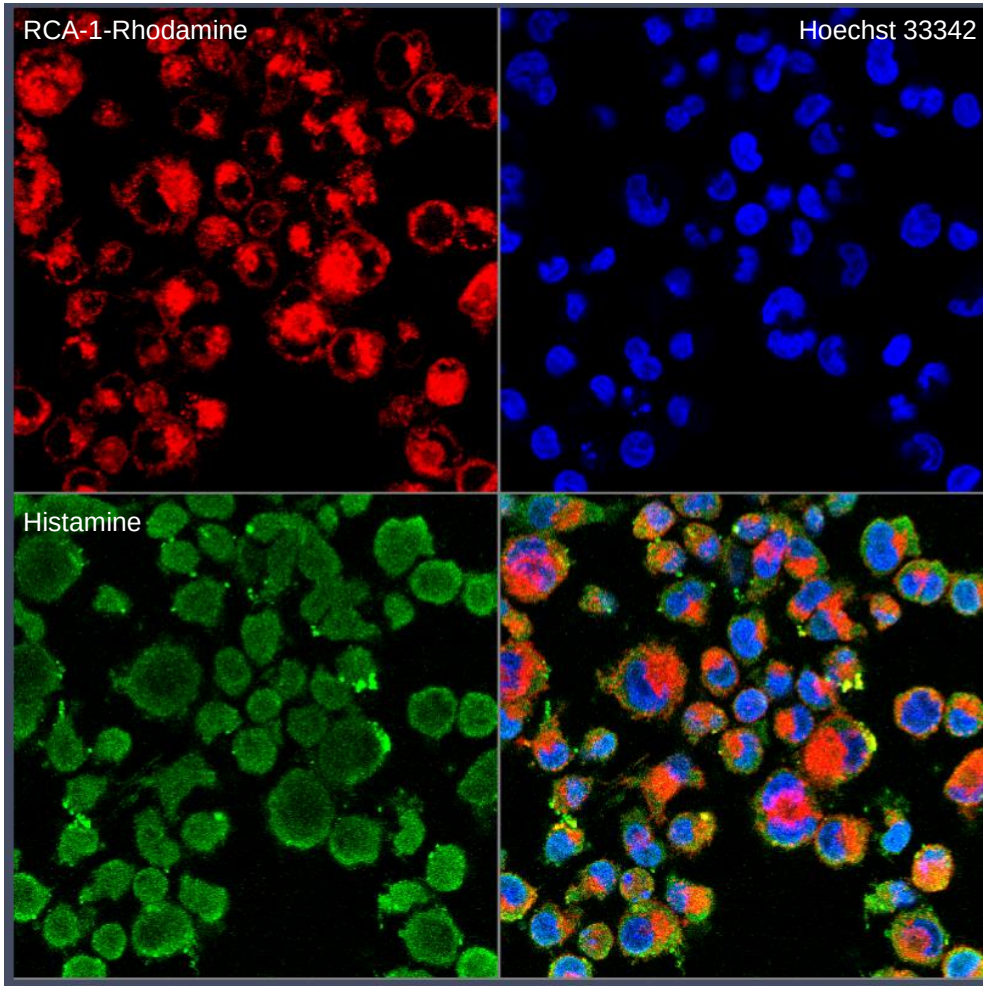

3D STRUCTURE OF TETRACULTURE

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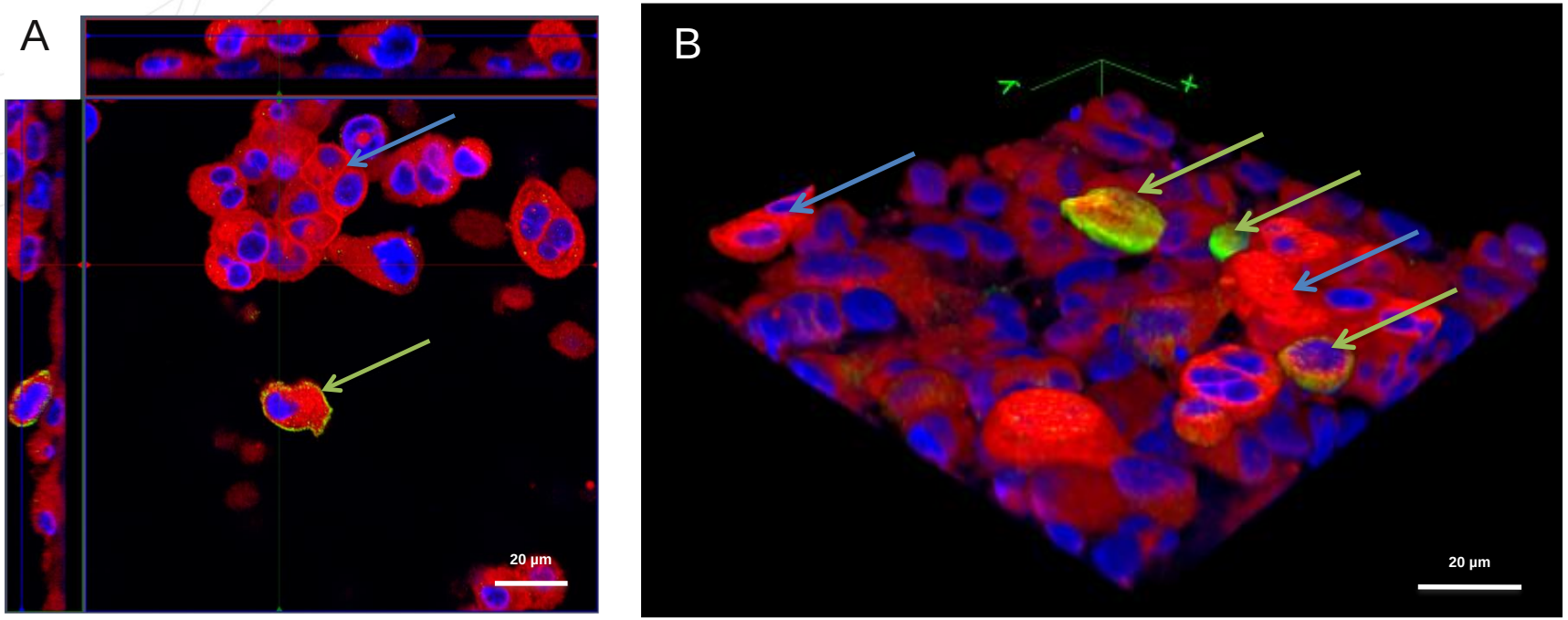

Z-stack image series to analyze the distribution of THP-1 macrophages and HMC-1 in the tetraculture system present in the apical compartment of the insert.

PHAGOCYTOSIS

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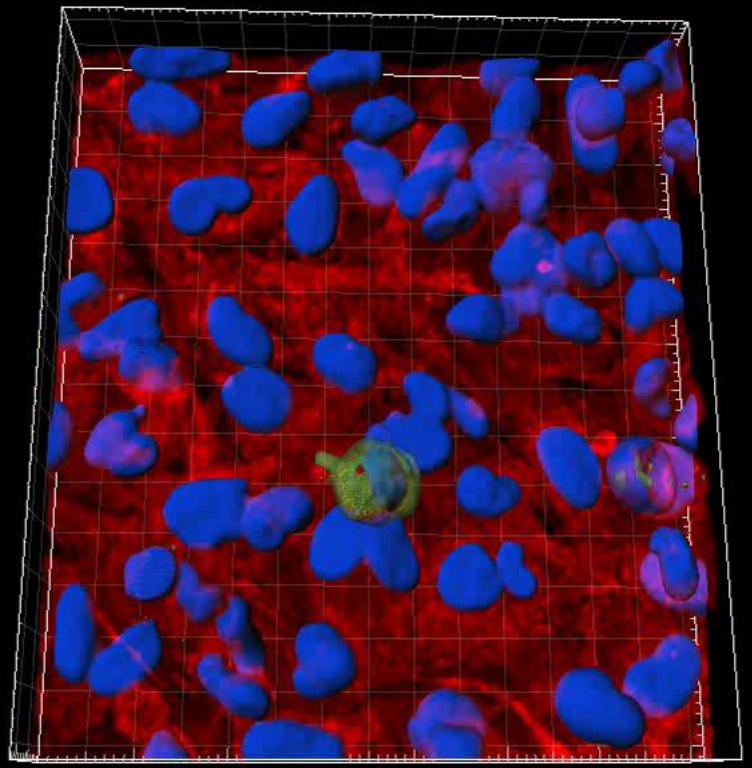

30.0 um

NUCLEAR TRANSLOCATION OF NRF2 IN THE ENDOTHELIAL PART OF THE TETRACULTURE (4H)

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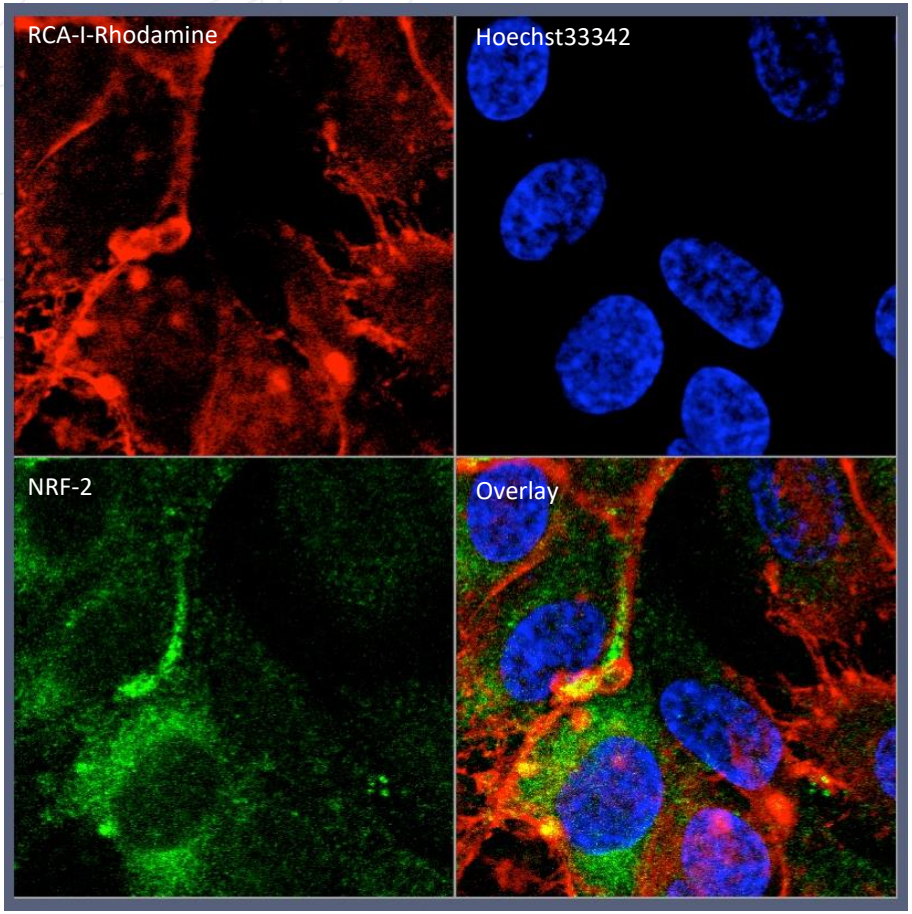

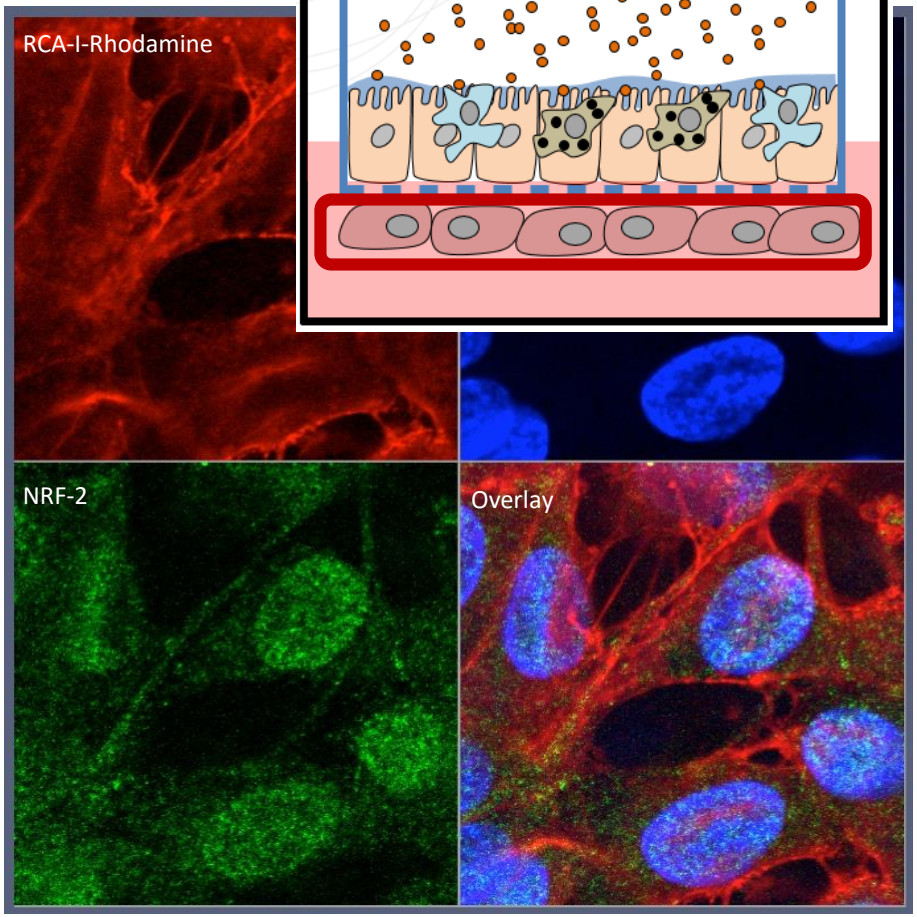

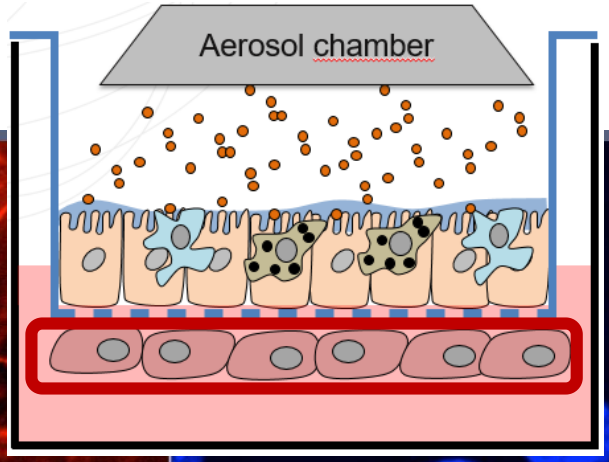

Tetraculture 0 ng/cm² DEPM

Tetraculture 240 ng/cm² DEPM

WHY ALI??

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- (1) ALI exposure systems resemble in-vivo exposure conditions
- (2) Cell systems (single- or multi-cell cultures, tissue slices) at ALI (may) produce surfactant
- (3) Clear differences in observed effects between ALI and submerged culture
- (4) Interaction of nano(particles) with the cell culture medium may alter the physico- chemical and hence the toxicological properties of the (nano)particles
- (5) Dose is better controllable at ALI cell systems (incl. real-time)

MORE RELEVANT

REFERENCES

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Rovinda and Hartung, 2009. ALTEX https://doi.org/10.14573/altex.2009.3.187
Taylor et al., 2008. Altern. Lab. Anim. 36, 327-342.

Merci villmols