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Advanced in vitro lung models in nanotoxicology research – advantages and limitations

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Why do we need *in vitro* studies?

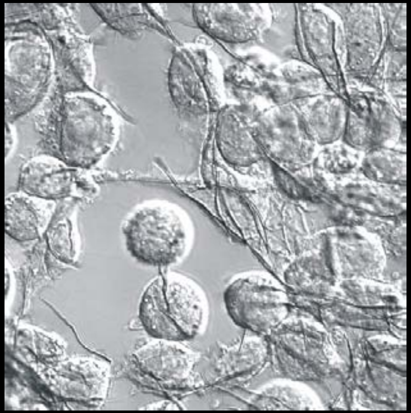
Toxicodynamic effects

- NP binding, interaction and induction of toxic effects at the cellular level

Research and Industry need **fast** and **low-cost screening** systems

- Versatile, simple as possible and reproducible
- Realistic in simulating the human organ of interest
- Able to reproduce findings of *in vivo* studies
- Diseased (human) cells

Alternative methods: **3R** (reduce, replace, refine)



Rothen-Rutishauser et al. Nanotoxicology (2010)

In vitro studies – Cell types

Primary cell cultures

- High phenotypic differentiation ☺
- Heterogeneous population of different cell types ☺ ☹
- Difficult to reproduce (donor variation) ☹
- Finite life span in culture ☹
- Lack of availability of normal human tissue ☹

Continous cell line

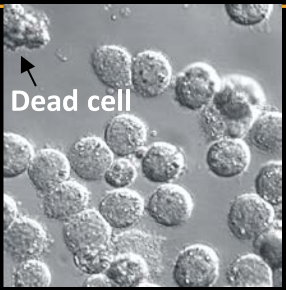
- Transformed cell lines
- Infinite life span in culture ☺
- Homogeneous ☺
- Better reproducible ☺
- Senesence! ☹
- Little phenotypic differentiation ☹

- ATCC (American Type Culture Collection)
- DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen)

After receiving the cells make aliquots from one passage, store them in liquid nitrogen. During one project go back after 20-50 passages to the original passage.

Use always the original cell lines

In vitro studies – Cell characterisation



Cell viability

- Observation under the light microscope every day

Cell growth

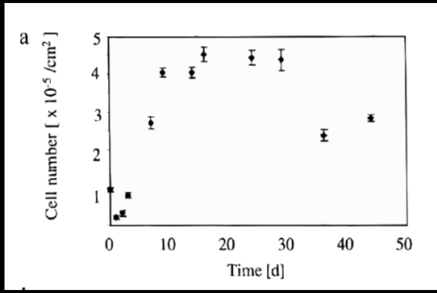
- Growth curve for dividing cells

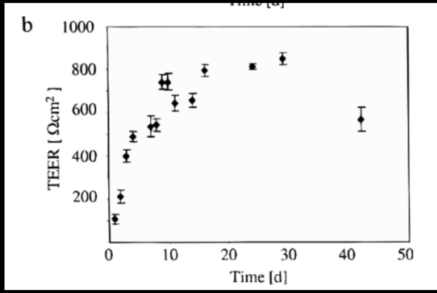
Cell morphology

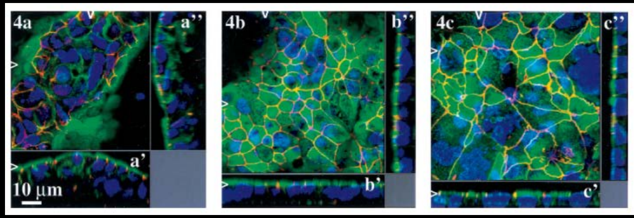
Cell differentiation

- Transepithelial electrical resistence, tightness (Epithelial cells)
- Expression of surface markers (Immune cells)

Each time when a parameter is changed



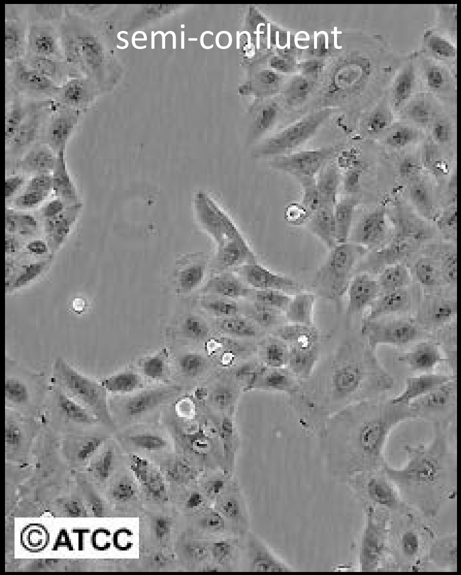


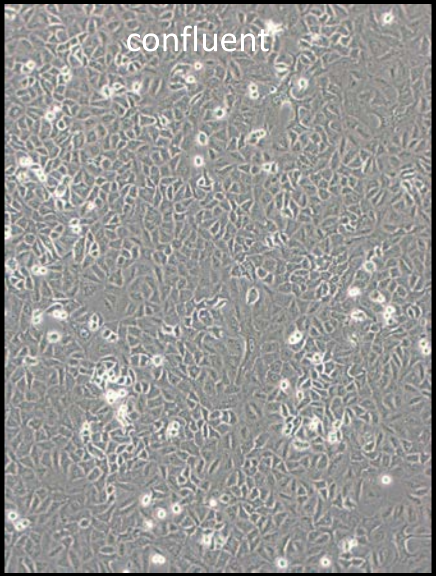


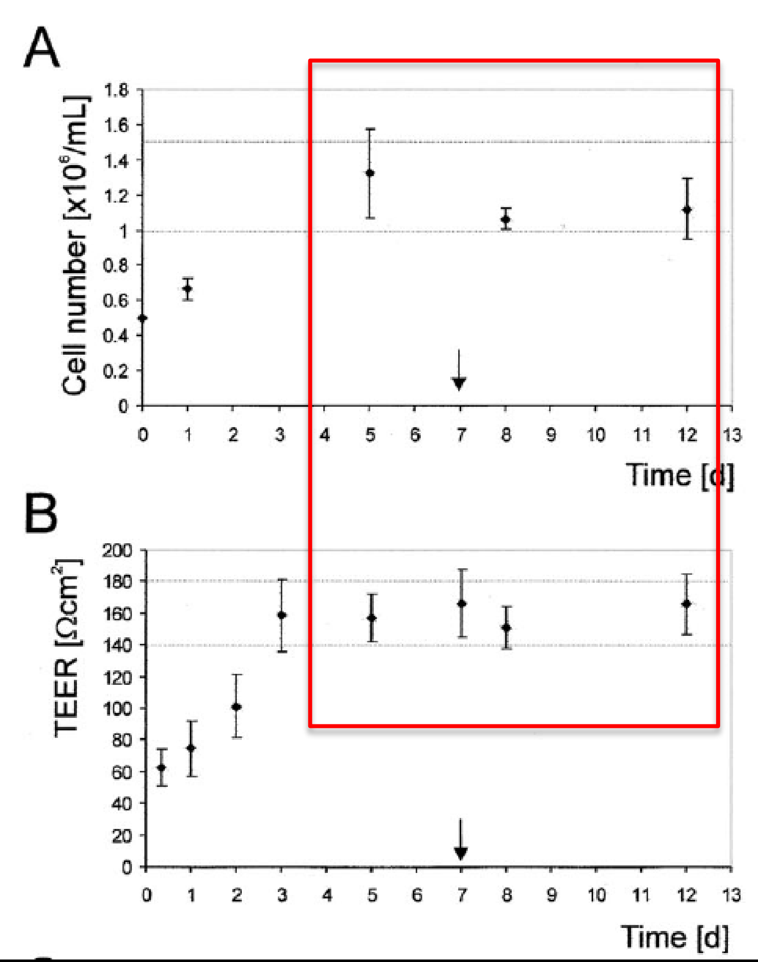
Characterization of Caco-2 cells
(Rothen-Rutishauser et al. Pharm Res 2000)

Cell characerization – structure and function

A549 epithelial cells



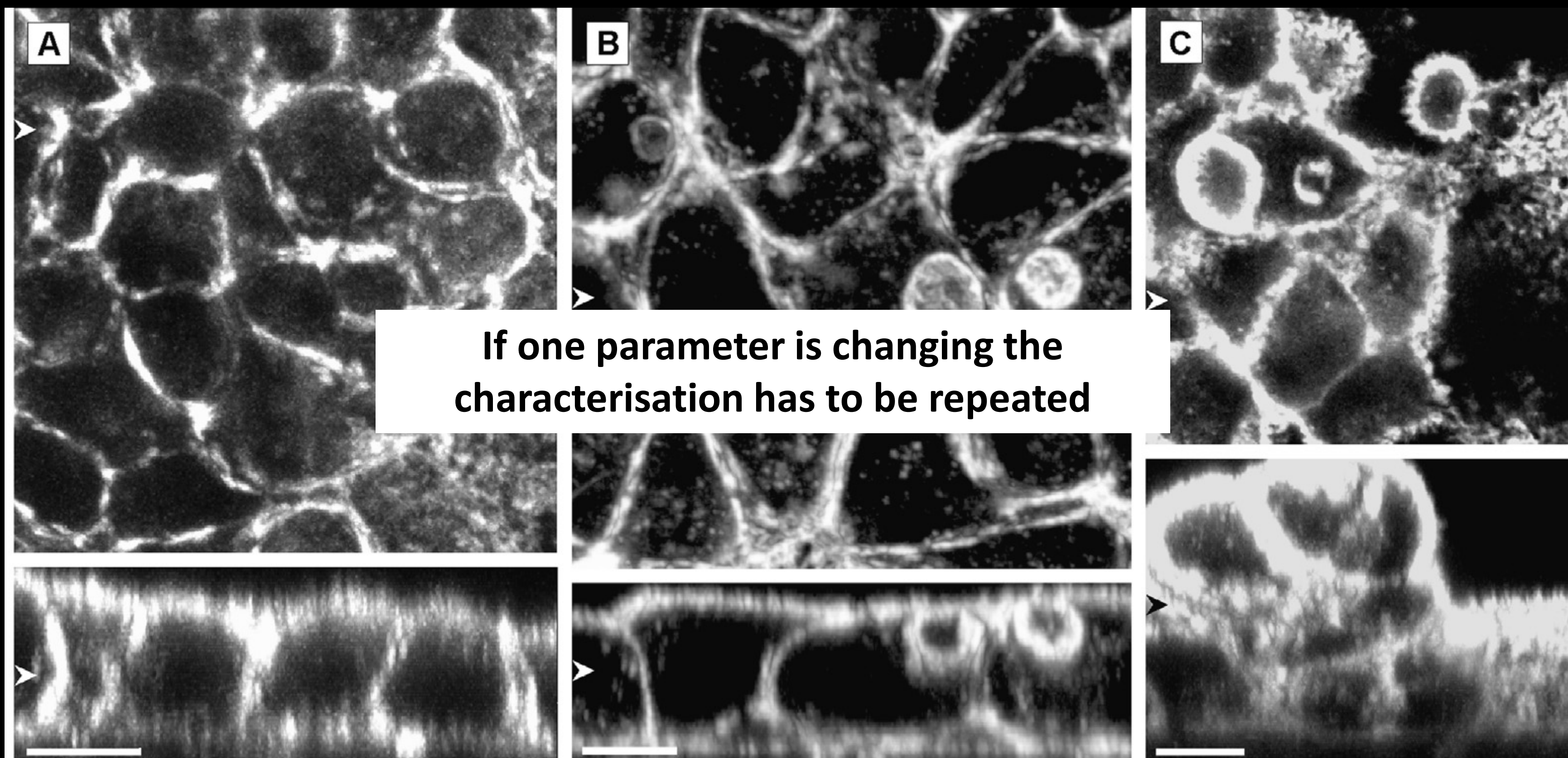




Rothen-Rutishauser et al. Am J Respir Cell Mol Biol 2005

Cell characerization – structure and function

Suspension | 2d at air | 4d at air



If one parameter is changing the characterisation has to be repeated

Blank et al. J Aerosol Med 2006

Which cell culture model?

1) What is the **basic question**?

   Cell-particle interactions

   Risk assessment of nanoparticles

   Cellular interplay of different cell types upon particle exposure

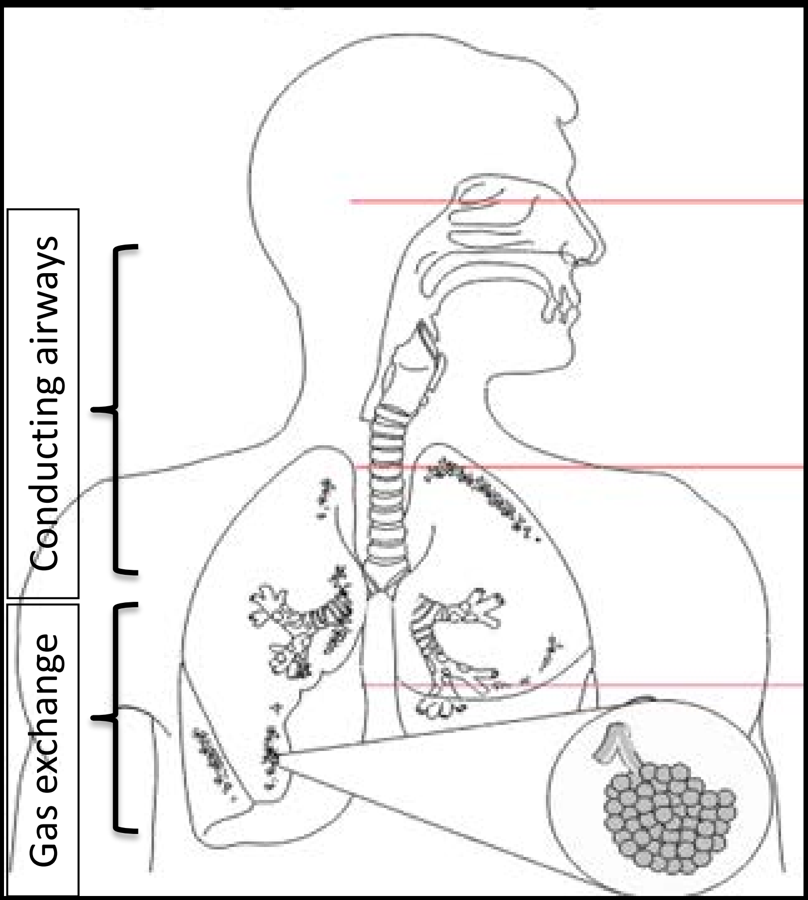
2) Which **cell types**?

   Epithelial and endothelial cells, macrophages, dendritic cells, fibroblasts….

3) Which **equipment** is availabale?

   Fully equipped cell culture lab (Biosafety hazard level 2)

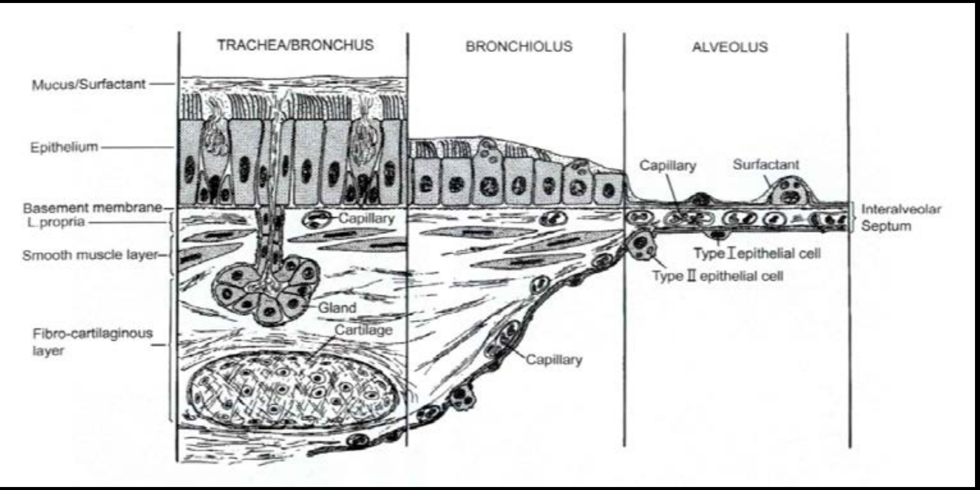
The human lung: airway – alveolar wall



Dr. P. Straehl, BAFU, Abt. Luft-reinhaltung

Ciliated, columnar/cuboidal epithelial cells

Thin, outspreaded epithelial cells



Ochs and Weibel. Functional design of the human lung for gas exchange. In Fishman´s Pulmonary Diseases and Disorders, New York, 2008

Lung cell models

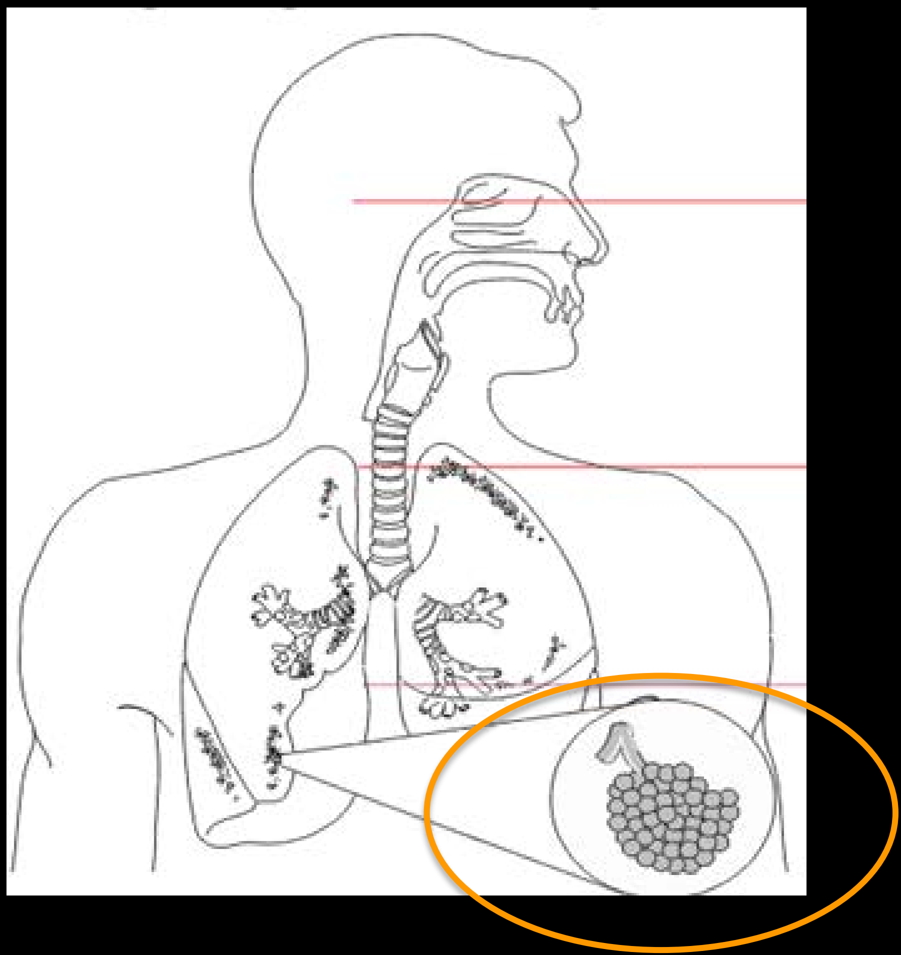
Table 1: Human cell culture models mimicking the epithelial barriers found in the human lung

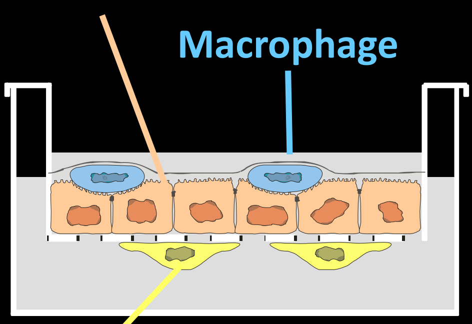
Airway epithelial cells	**References**
Calu-3 (ATCC HTB-55)	[172-179]
16HBE14o- (can be obtained from D.C. Gruenert)	[117, 155, 162, 180, 181]
BEAS-2B (ATCC CRL-9609)	[114, 115, 182-185]
NuLi-1 (ATCC CRL-4011)	[116]
Primary airway epithelial cells	
hBEpC	[98, 99, 186, 187]
Alveolar epithelial cell lines	
A549 (ATCC CL-185): ATII phenotype	[105, 119, 143, 145, 183, 187-189]
Immortalized human ATII cells with ATI phenotype	[128]
NCI-H441 (ATCC HTB-174): ATII and Clara cell phenotype	[122-127]
Primary alveolar epithelial cells	
hAEpC: ATII cells that differentiate *in vitro* into ATI-like morphology	[102-104]
3D cultures	
3D aggregates of A549 cells	[131]
Bilayer co-culture model: epithelial & endothelial cells	[190-193]
Triple cell co-culture model: epithelial cells, macrophages, dendritic cells	[82, 137, 194, 195]
Double, Triple and quadruple cell co-culture models: epithelial cells, endothelial cells, mast cells, macrophages	[140]
Biomimetic microsystems	
Breathing lung-on-a-chip: epithelial & endothelial cells	[142]
Perfused chip: epithelial & endothelial cells	[141]

AT = alveolar type

Jud et al. Swiss Med Wkly 2013

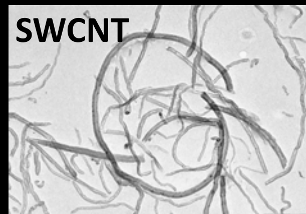
3D human lung epithelial barrier model

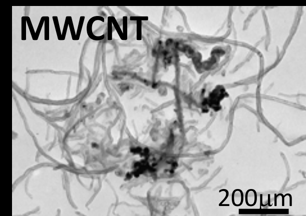




Rothen-Rutishauser et al. Am J Respir Cell Mol Biol 2005;
Blank et al. Am J Respir Cell Mol Biol 2007
Rothen-Rutishauser et al. Review, Exp Opin Drug Metab Toxicol 2008
Lehmann et al. Eur J Pharm Biopharm 2010

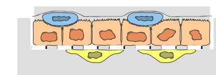
Advantages of 3D models





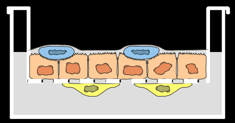
20µg/mL

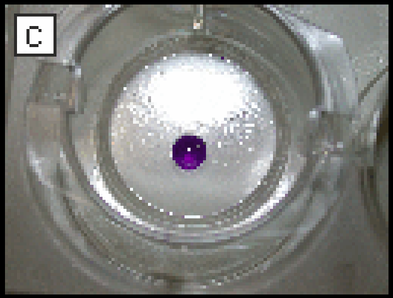
Wick et al. Tox Letters 2007

In vitro system								
Nanofibre	SWCNTs	MWCNTs	SWCNTs	MWCNTs	SWCNTs	MWCNTs	SWCNTs	MWCNTs
Cytotoxicity (LDH release)	-	-	-	-	-	-	-	-
TNF-α ELISA	++	++	++	++	N/A	N/A	++ (Upper+Lower)	++ (Upper+Lower)
IL-8 ELISA	N/A	N/A	N/A	N/A	++	++	++ (Upper) - (Lower)	++ (Upper) - (Lower)
GSH content	-	++	-	++	++	++	++ (Upper+Lower)	++ (Upper+Lower)

Clift et al. Tox Sci 2013

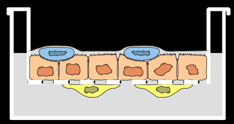
Air-liquid exposures of nanomaterials

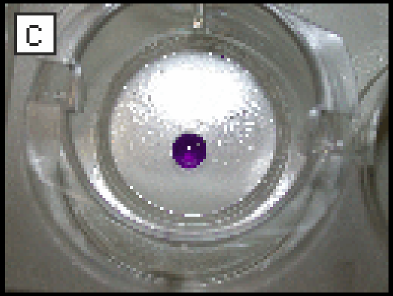




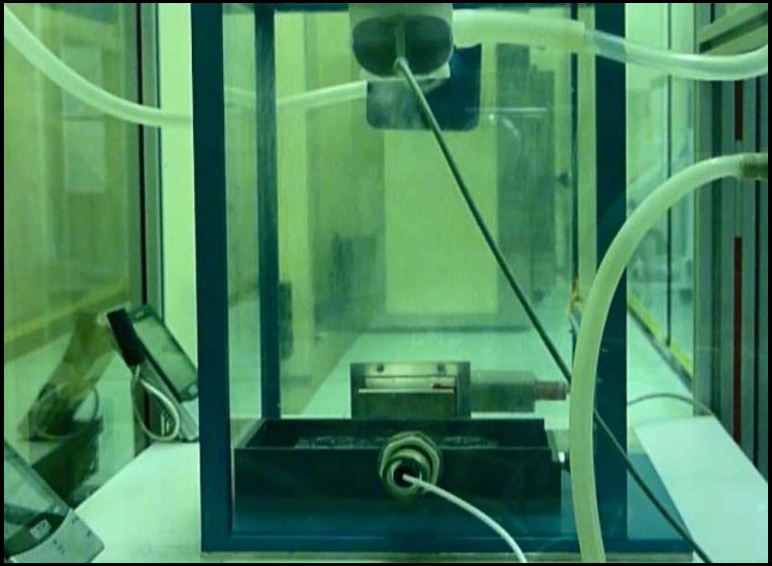
Blank et al. J Aerosol Med 2006;
Blank et al. Am J Respir Cell Mol Biol 2007

Air-liquid exposures of nanomaterials





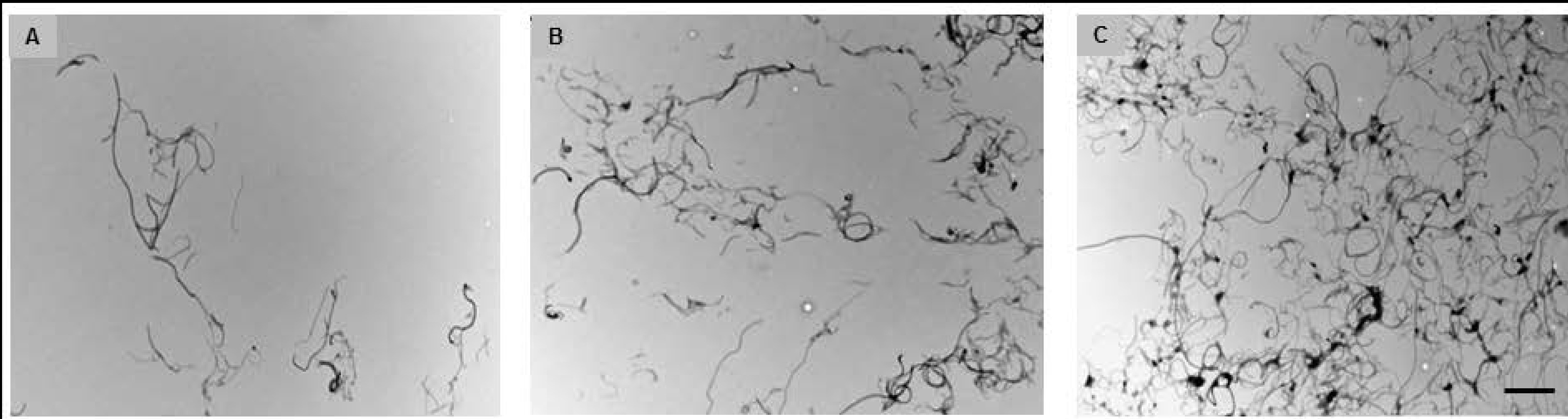
Blank et al. J Aerosol Med 2006; Blank et al. Am J Respir Cell Mol Biol 2007

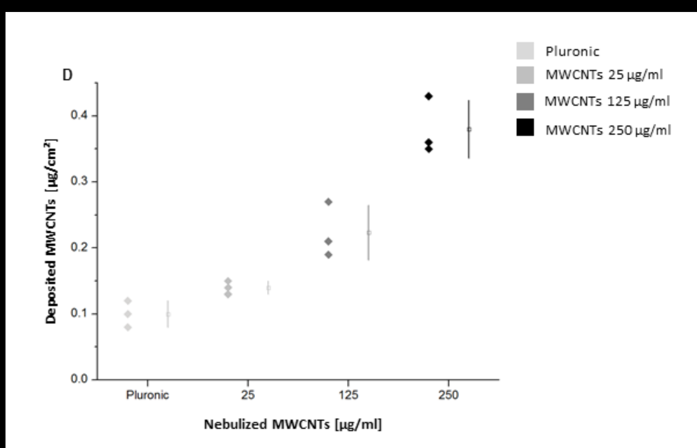


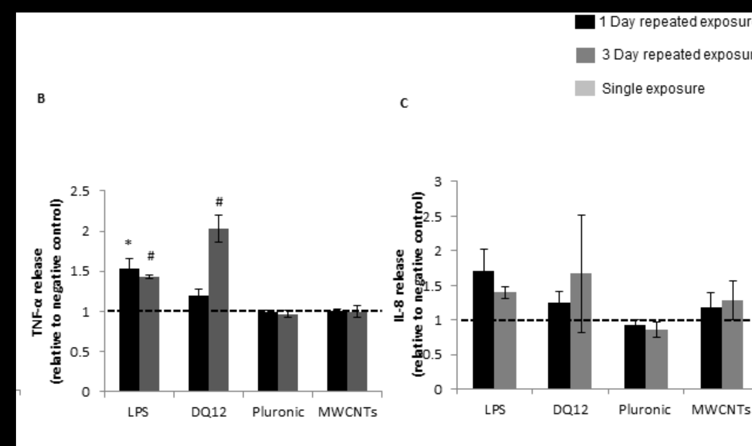
Lenz et al. Part Fibre Tox 2007

Exposure to carbon nanotube-based aerosols

Multi-walled carbon nanotubes







Chortarea et al. Nanotoxicology 2015 (in press)

Nanomaterials – realistic dose exposures?

Inhalation to MWCNT (5 mg/m^3, 5 hours/day, 5 days/week) for 15 days

⇒ 31.2 µg MWCNT/ mouse lung

Sargent et al. Part Fibre Toxicol 2014

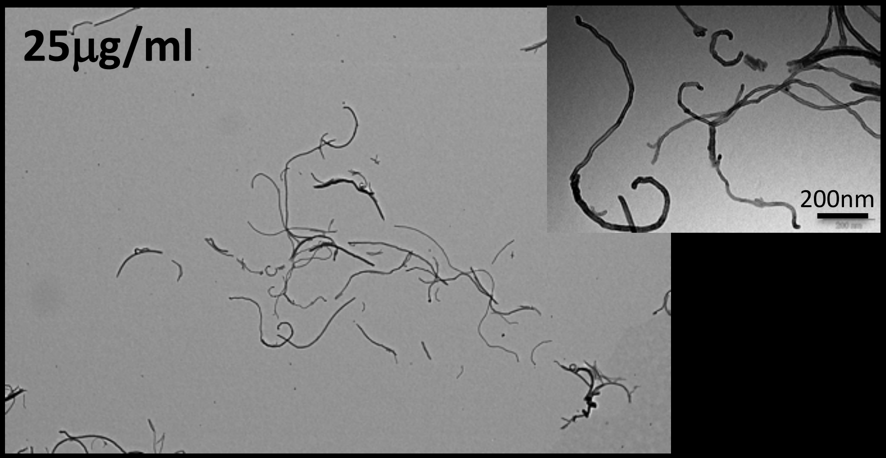
Surface of mouse lung 500 cm^2

Stone et al. Am J Respir Cell Mol Biol 1992

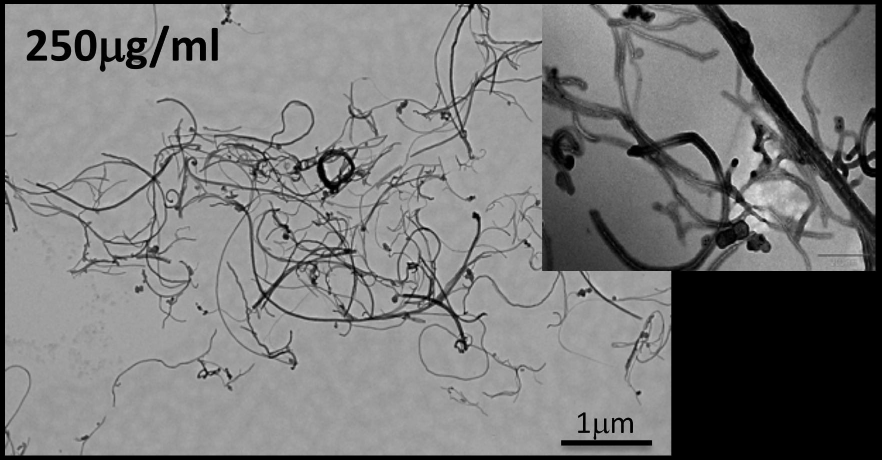
⇒ **0.06 µg/cm^2**
(human occupational exposures)

Full working lifetime exposure to 1 mg/m^3 aerosol concentration of CNT ranged from **12.4 to 46.5 µg/cm^2** alveolar mass retention

Gangwal et al. Env Health Persp 2011



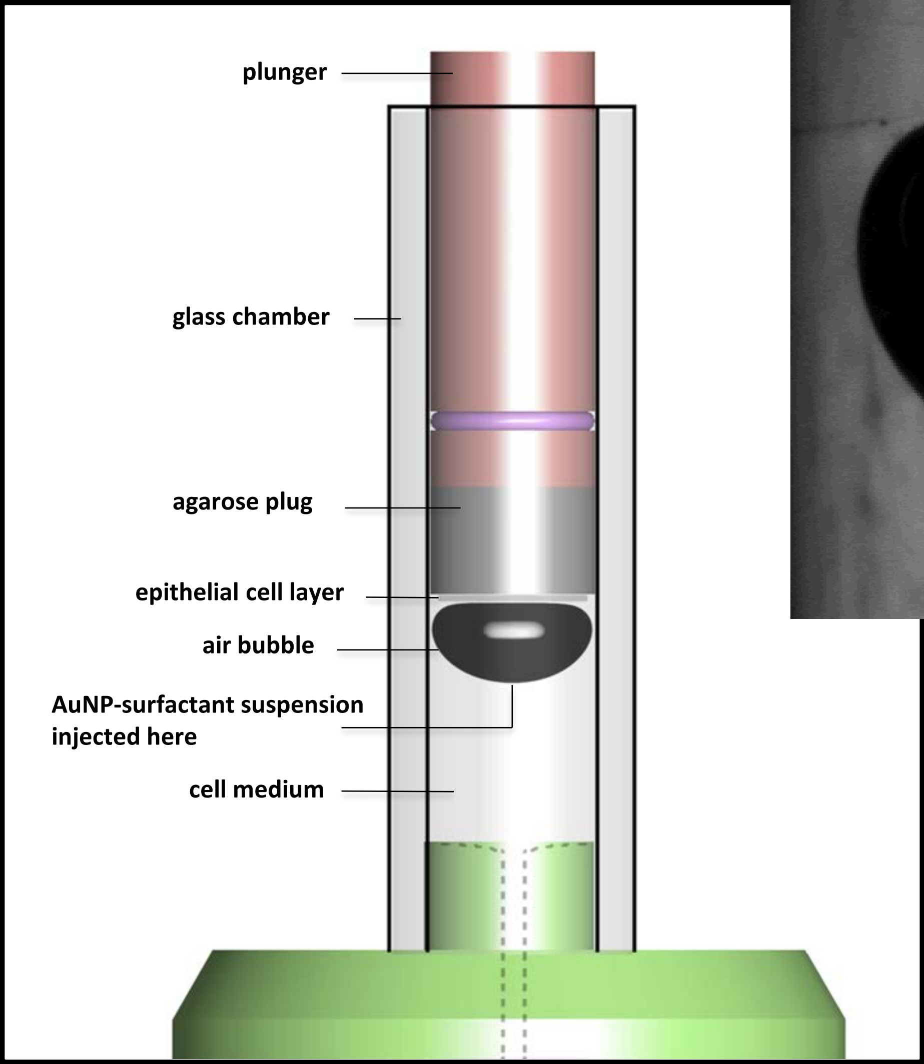
Deposition of 0.14 µg/cm^2

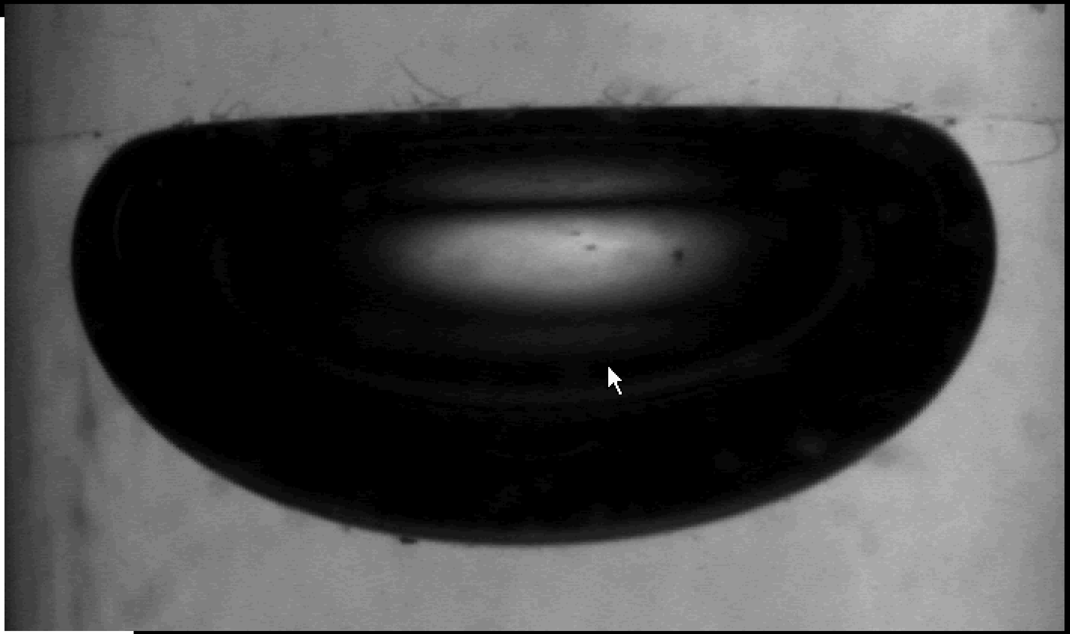


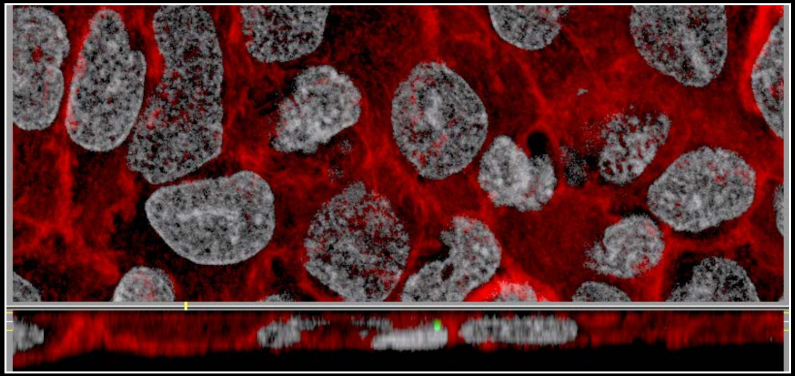
Deposition of 0.35 µg/cm^2

S. Chortarea, S. Beyeler

Captive bubble system – Breathing conditions

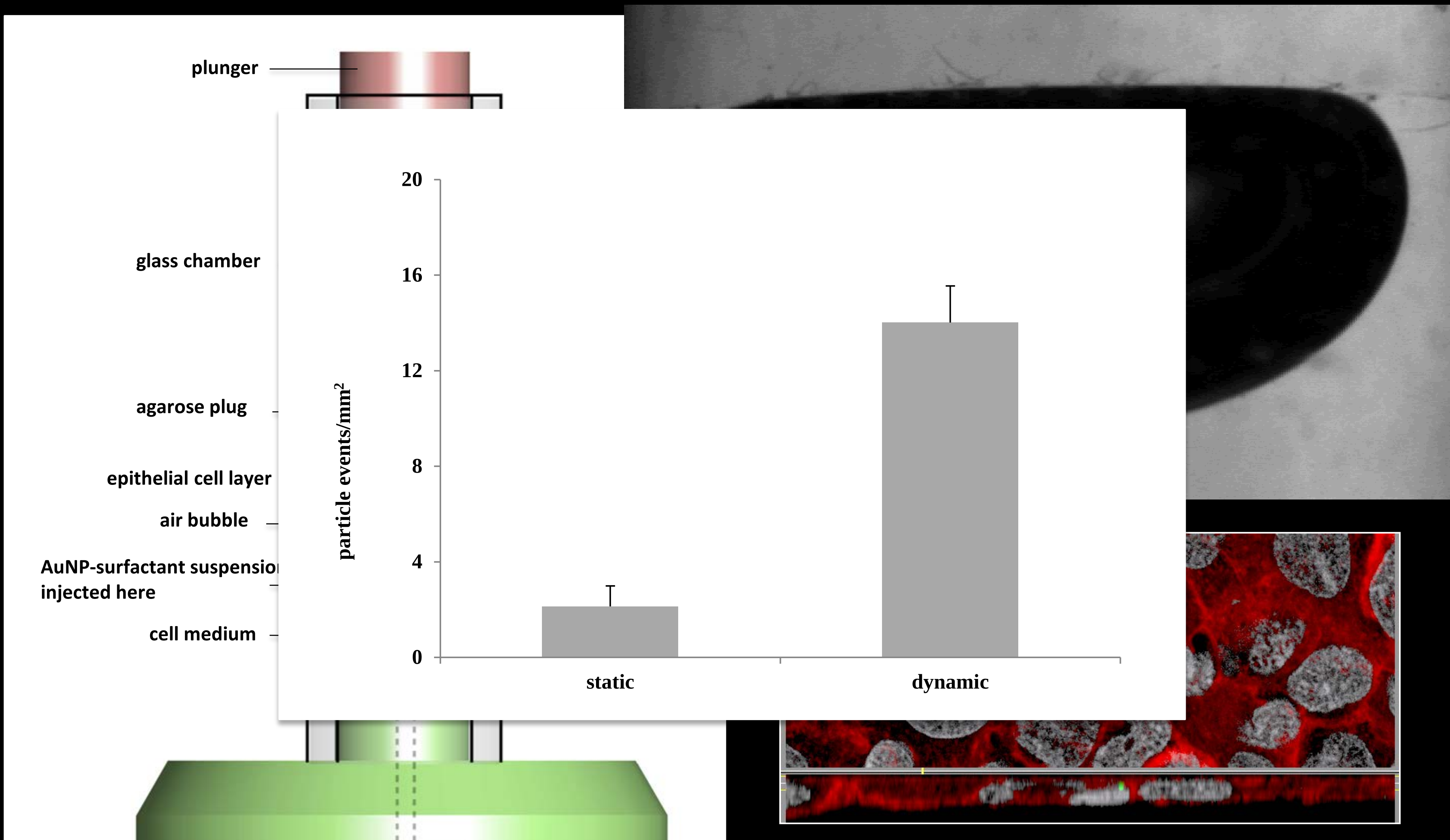






Schürch et al. Langmuir 2014

Captive bubble system – Breathing conditions



Schürch et al. Langmuir 2014

Printing of lung tissue – layer by layer a

SCIENTIFIC REPORTS

OPEN

Engineering an in vitro ai
by 3D bioprinting

SUBJECT AREAS:
TISSUE ENGINEERING
EXTRACELLULAR MATRIX

Lenke Horváth[1], Yuki Umehara[1], Corinne Jud[1]*, Fabian Blank[2], Alk
& Barbara Rothen-Rutishauser[1]

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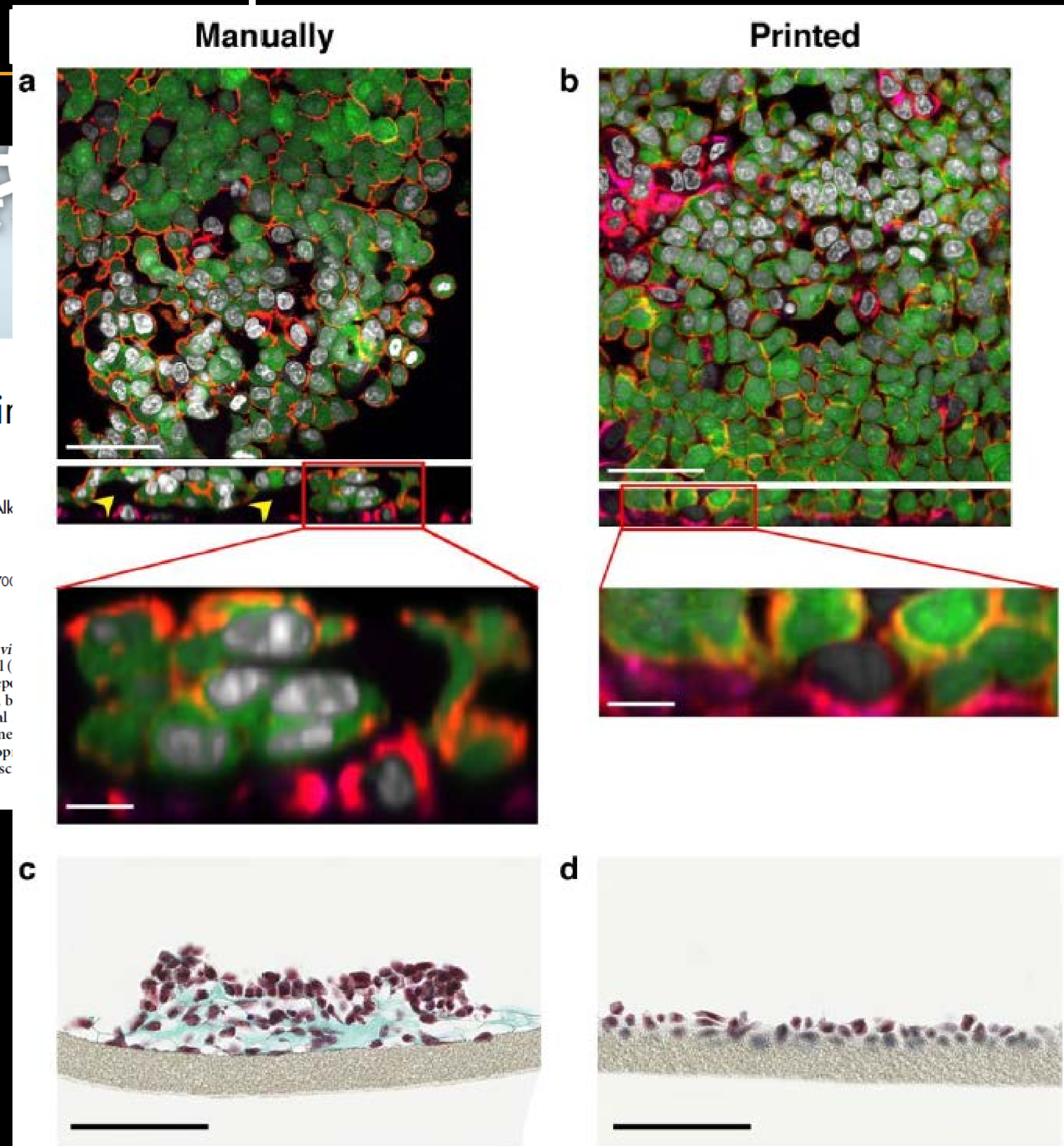
Respiratory Medicine, Bern University Hospital, CH-3010 Bern, Switzerland.

Intensive efforts in recent years to develop and commercialize *in vi*
assessment have yielded new promising two- and three dimensional (
realistic 3D *in vitro* alveolar model is not available yet. Here we rep
air-blood tissue barrier analogue composed of an endothelial cell, b
layer by using a bioprinting technology. In contrary to the manual
technique enables automatized and reproducible creation of thinne
which is required for an optimal air-blood tissue barrier. This biop
tool to engineer an advanced 3D lung model for high-throughput sc
efficacy testing.

Correspondence and
requests for materials
should be addressed to

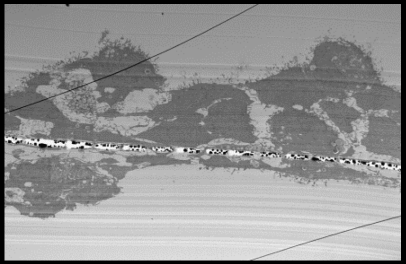


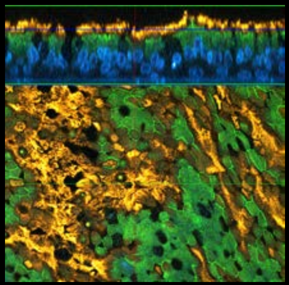
Horvath et al. Sci Report 2015

....where to go from here

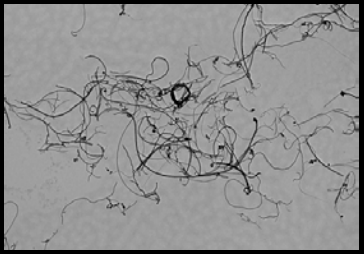
Lung cell models

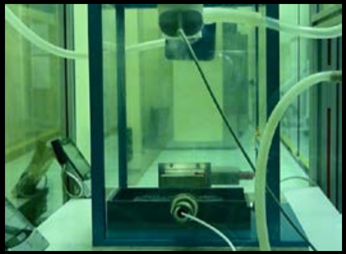
- Co-culture models
- Diseased cells



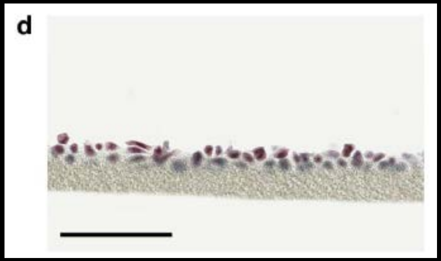


Air-liquid exposures mimicking realistic inhalations of nanomaterials (relevant dose)



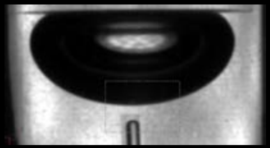


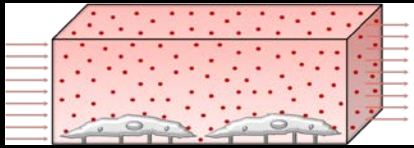
Standardization of protocols and **validation** of *in vitro* findings with *in vivo* data



More complex models including

- **breathing** patterns
- (blood) **flow**





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- PD Dr. von Garnier and Dr. Blank, Bern University Hospital

LUNGENLIGA Leben heisst atmen

Opportunities and Risks of Nanomaterials
National Research Programme NRP 64

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Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung

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Bundesamt für Gesundheit BAG

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