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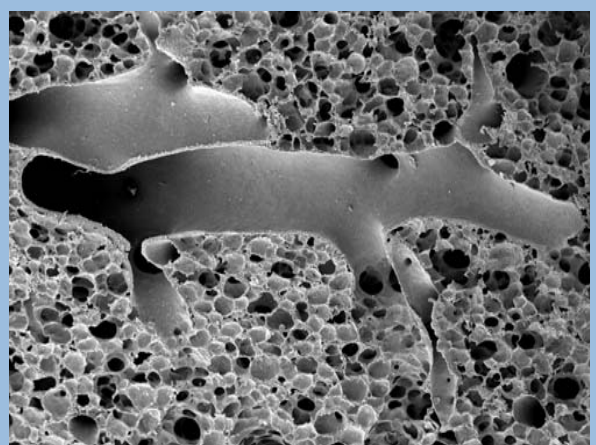
9th ETH Conference on CGP: 16. August 2005

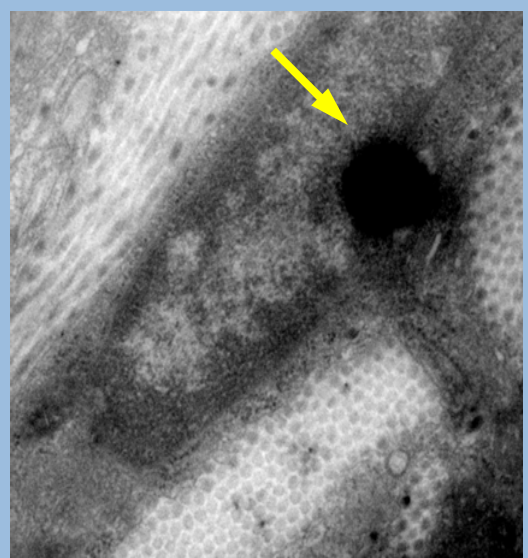
Distribution and Clearance of Inhaled Ultrafine TiO_2 Particles in Rat Lungs

Marianne Geiser

University of Bern



Airways & alveoli - rodent lung



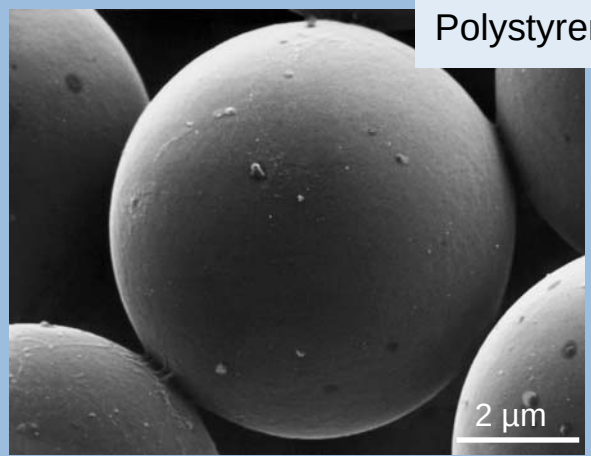
TiO_2 particle in fibroblast – rat lung

Aim of studies at the ultrastructural level

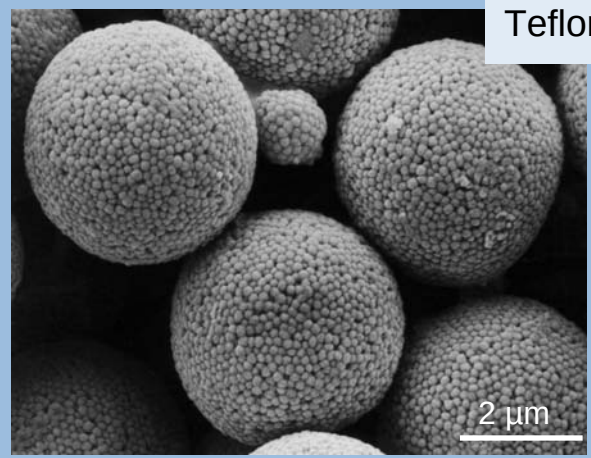
- Resolve distribution patterns, retention and clearance pathways at the individual particle level
- Increase knowledge of particle-lung (cell) interaction to better understand adverse and beneficial effects by inhaled particles

Particle types (insoluble PM 10)

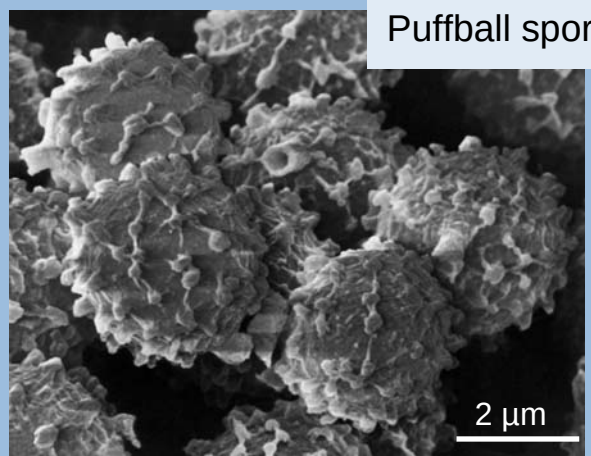
Polystyrene



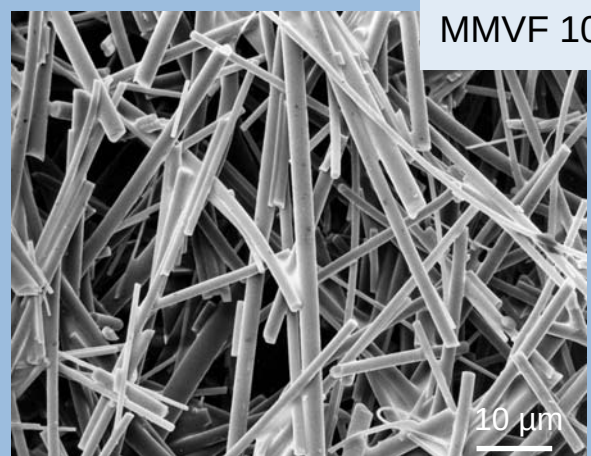
Teflon



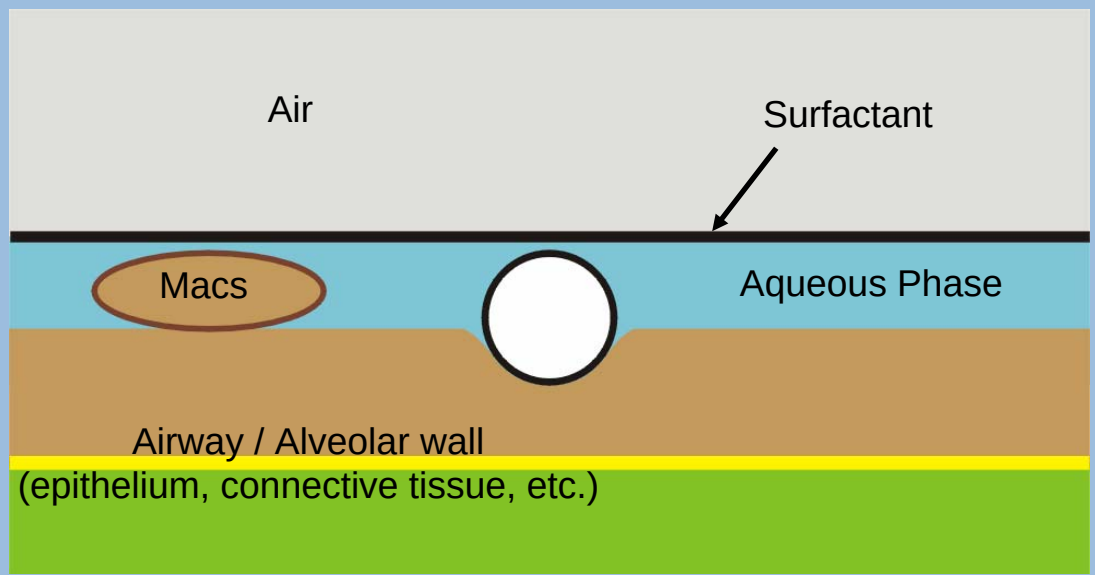
Puffball spores



MMVF 10a



Displacement of PM 10 by surfactant



Inhaled and deposited PM 10

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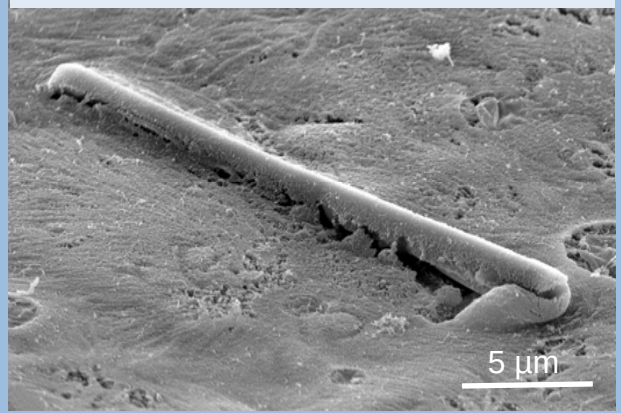
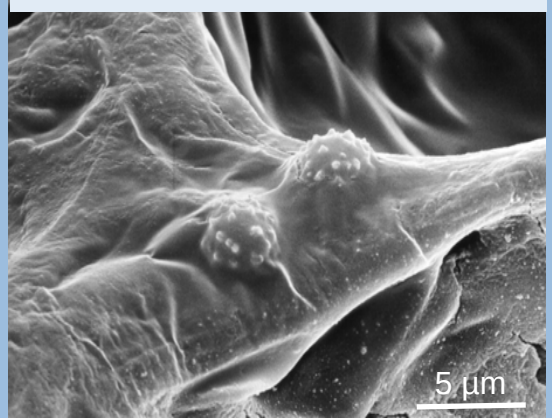
Glass fiber in conducting airway

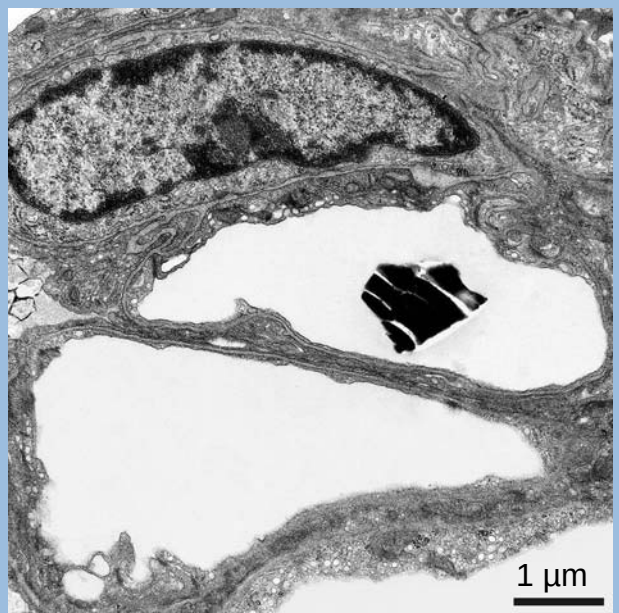


Puffball spores in alveoli



Puffball spore in alveolus

MMVF 10a in blood capillaries



Most profiles with

> diameter ≤ 1µm
> angular surface

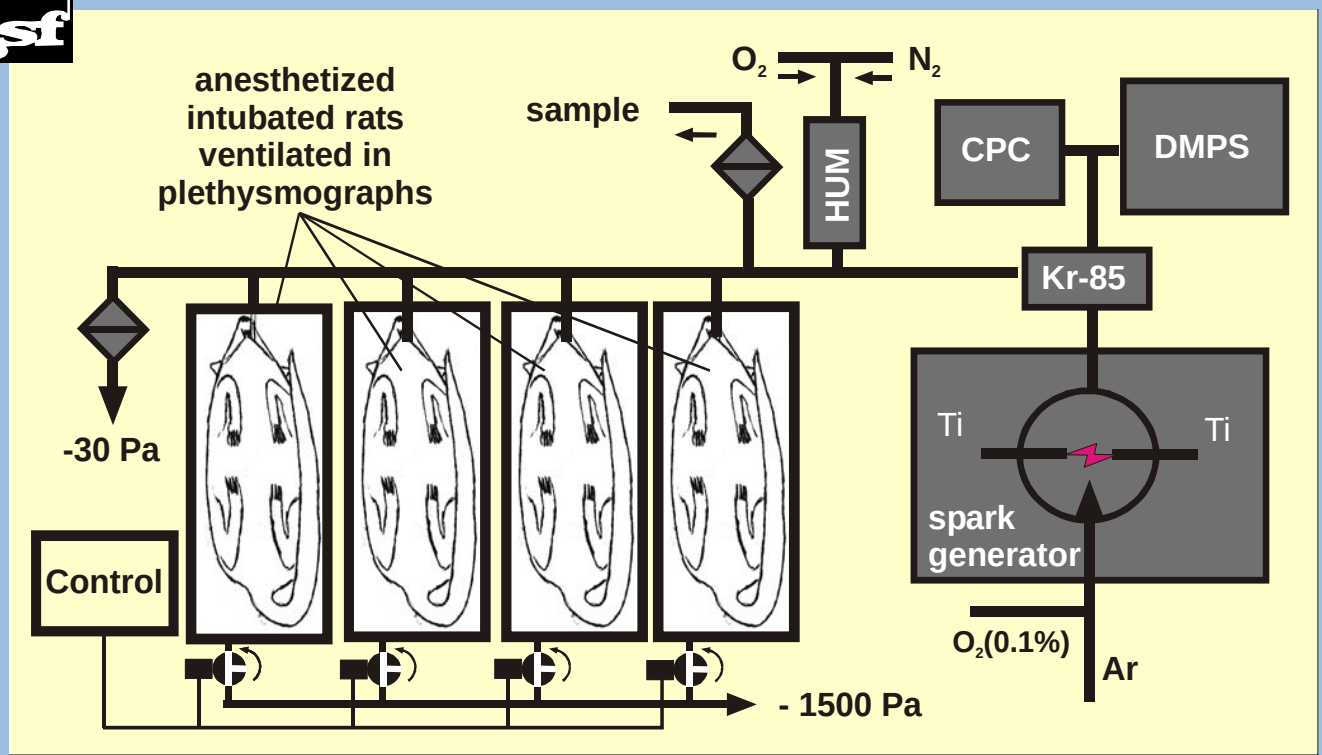
Translocation of the particulate fraction of glass fibers beyond the epithelial barrier

Inhalation experiment with ultrafine particles

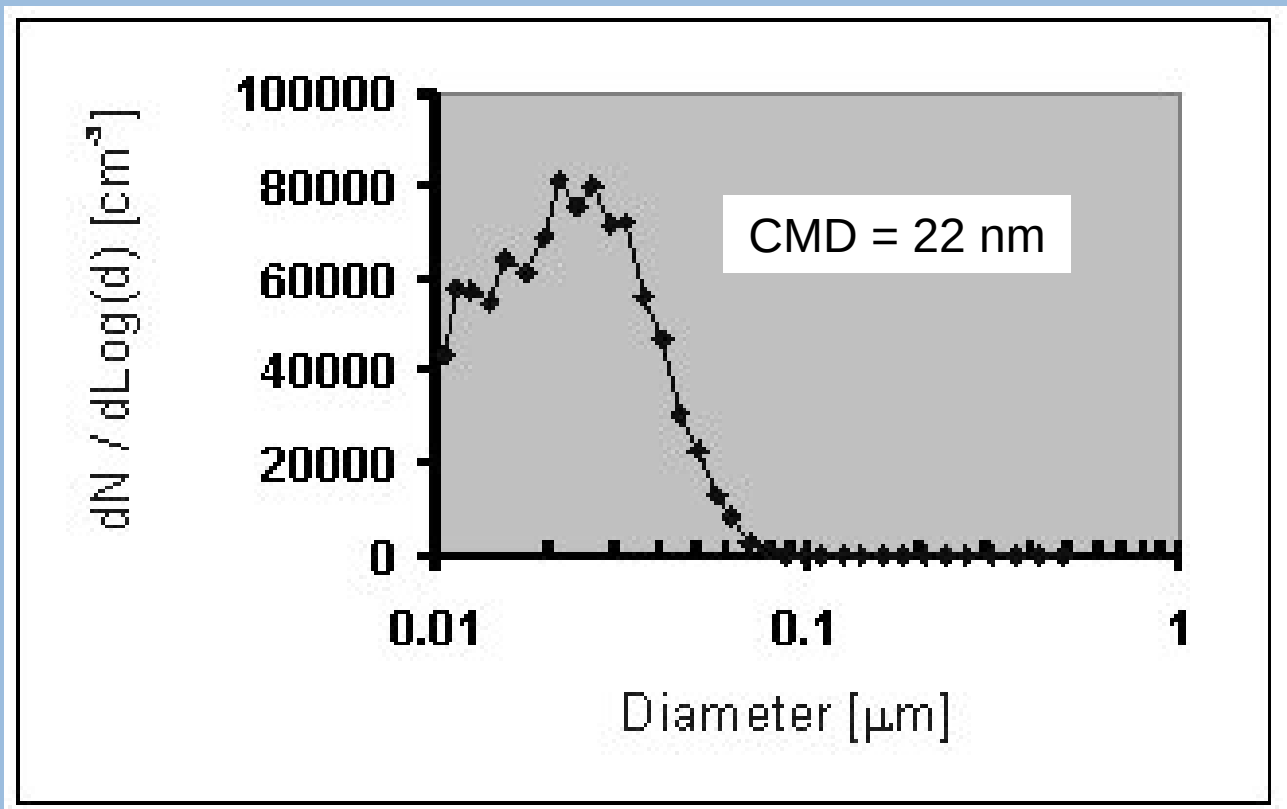
- Titanium dioxide (TiO_2)
- Aerosol: 22 nm CMD
- Inhalation: 1 h
- Deposition: 4-5 μg, ~2×10^{11}particles
- Lung fixation: 1 h / 24 h after inhalation
- Systematic tissue sampling
- Energy filtering TEM (EFTEM): Electron-energy loss spectroscopy (EELS)

Aerosol generation and inhalation

Dr. Wolfgang Kreyling



Size distribution of TiO_2 particles in the aerosol



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Localization and elemental micro-analysis of ultrafine TiO_2 particles

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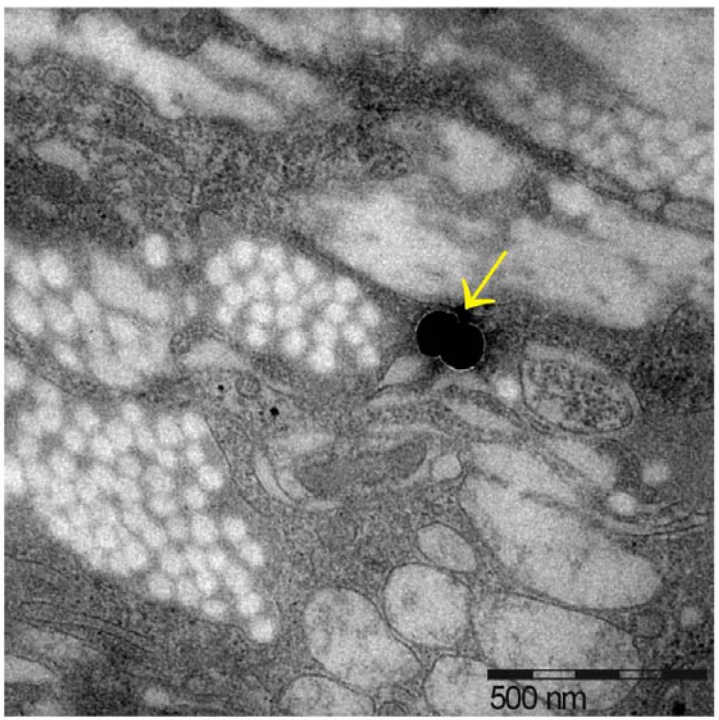
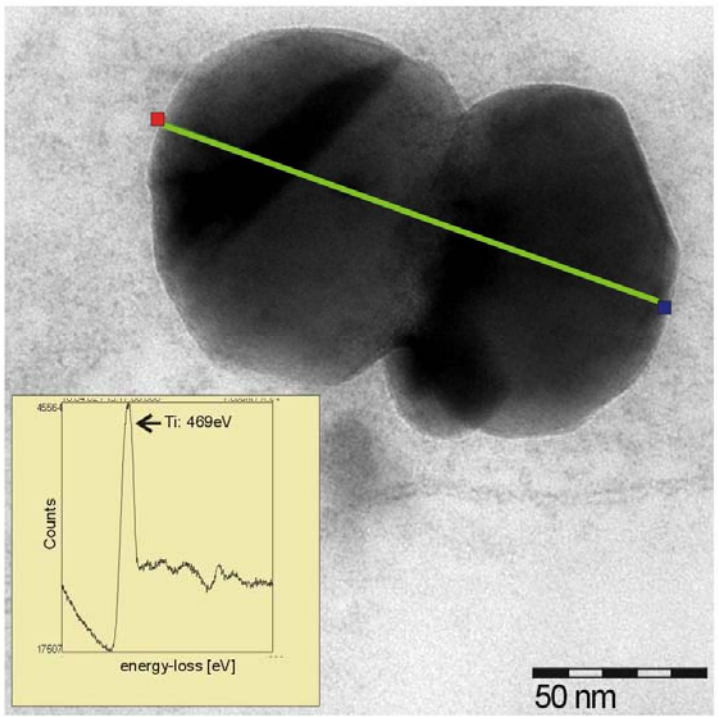
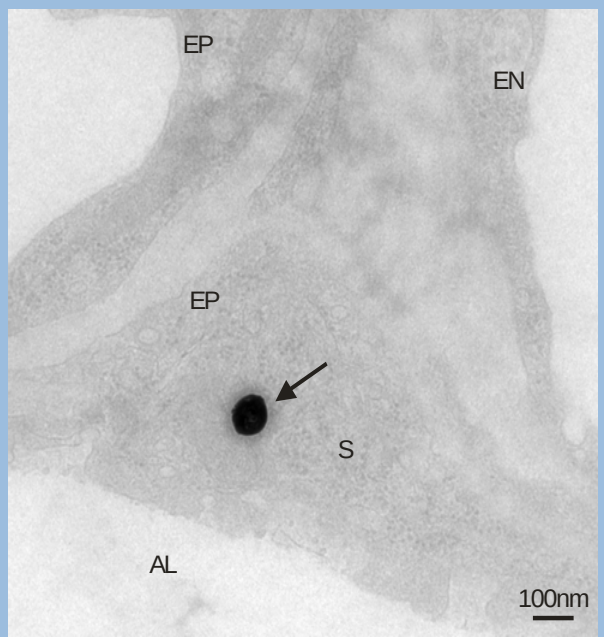
Localization in lung tissue



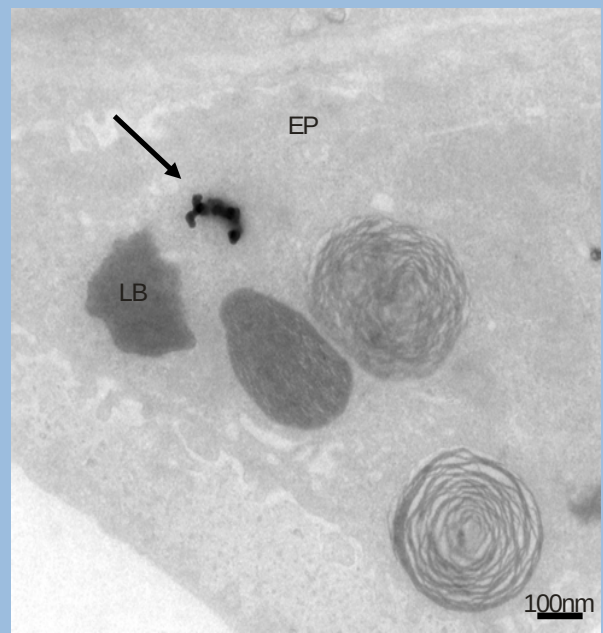
Elemental microanalysis



TiO_2 particles in the lung parenchyma

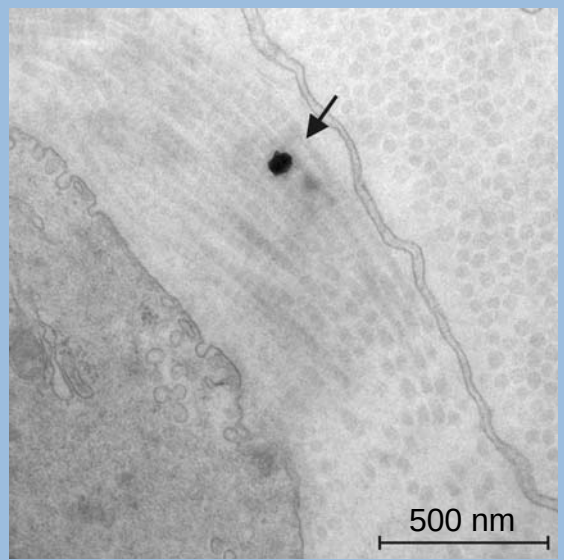


On the alveolar surface

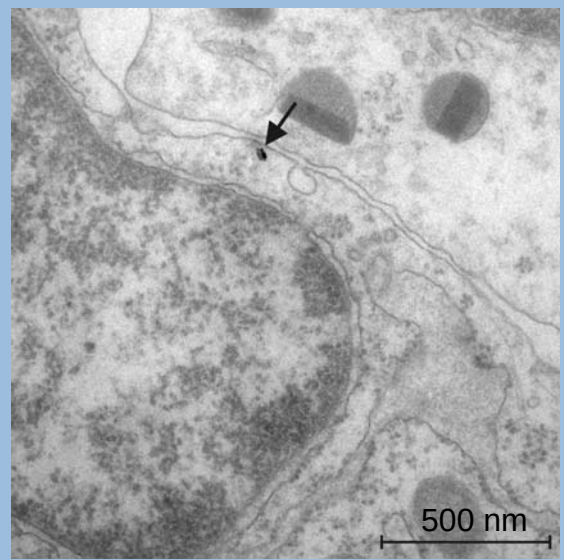


Within pneumocyte Type 2

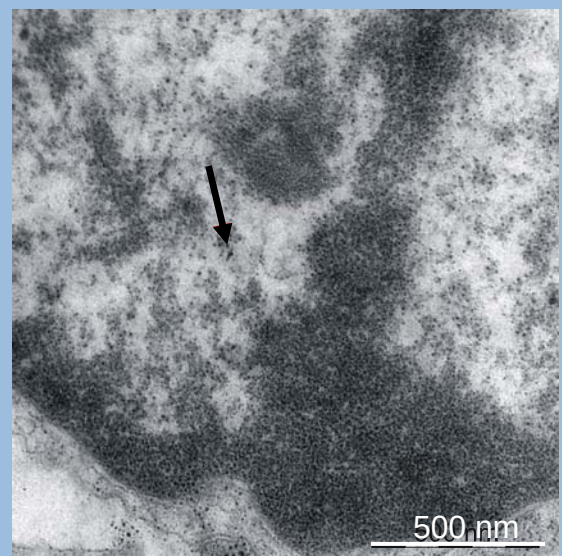
TiO_2 particles in the connective tissue



Between collagen fibrils

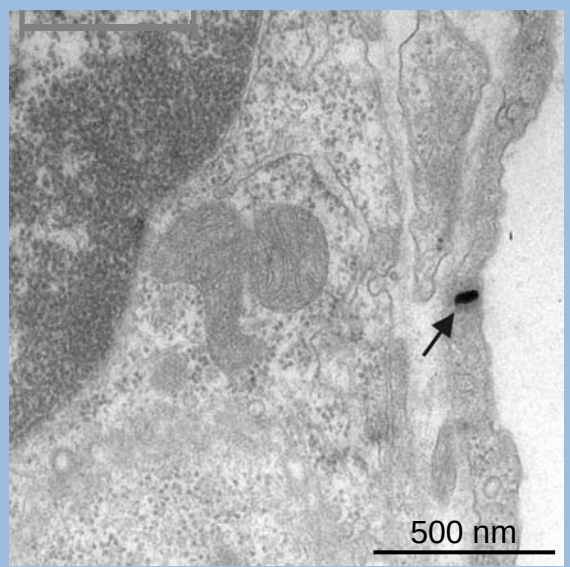


Within fibroblast (cytoplasm)

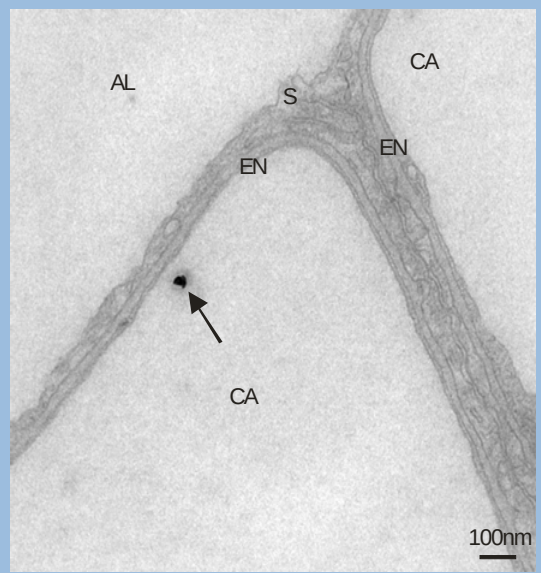


Within fibroblast (nucleus)

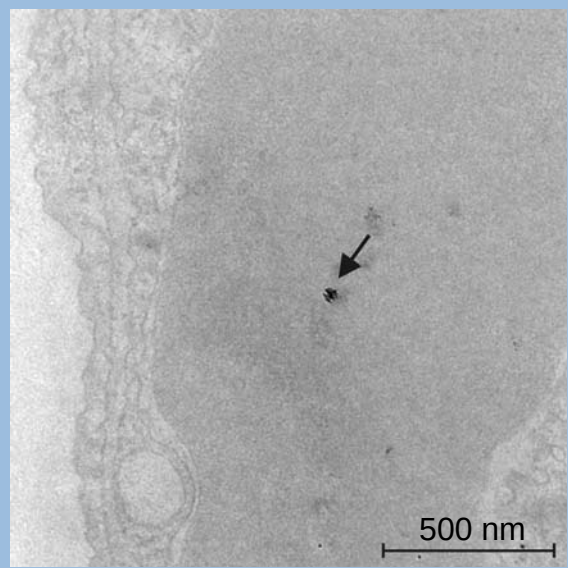
TiO_2 particles in blood capillaries



Within endothelial cell

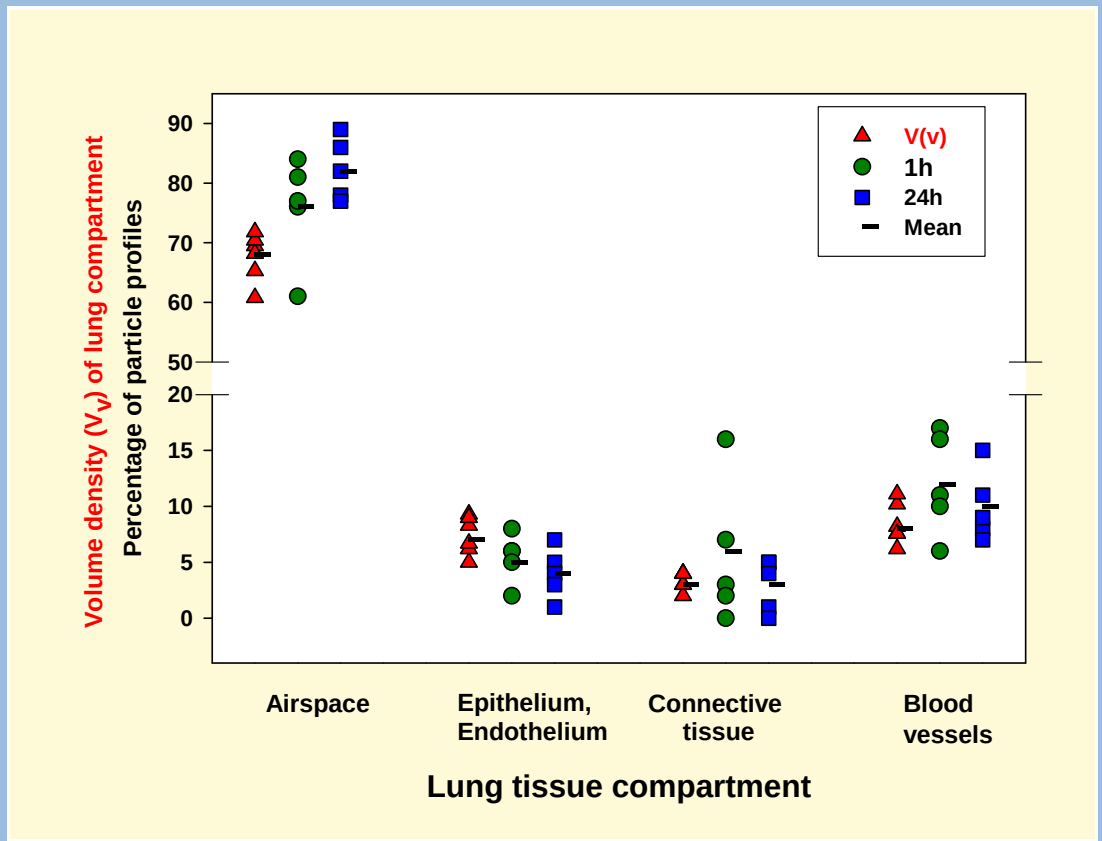


In blood capillary



Within erythrocyte

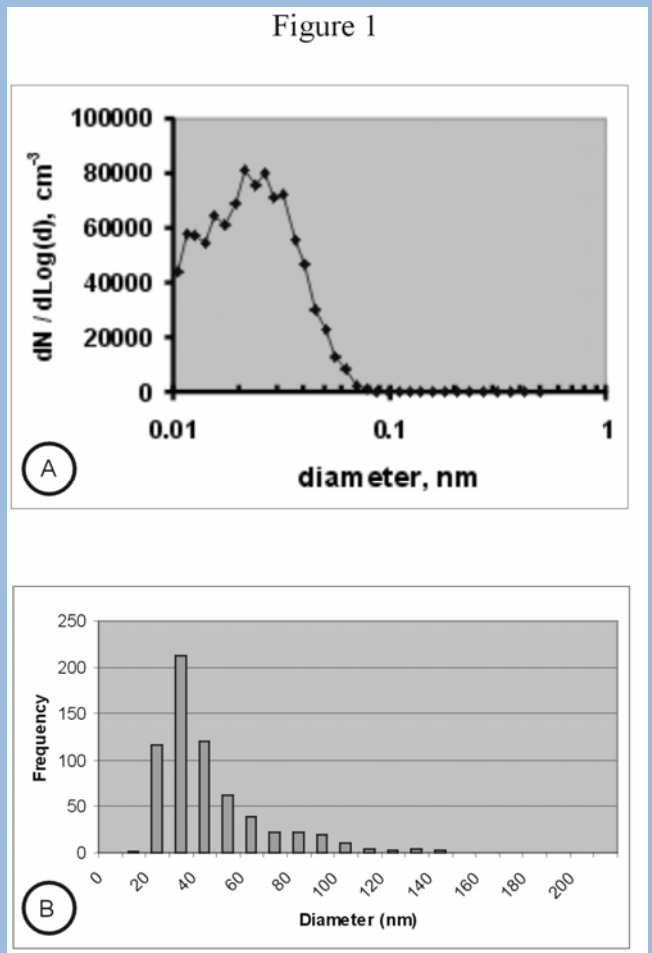
Distribution of TiO_2 particles in the lungs



Geiser et al., Environ Health Perspect: doi:10.1289/ehp.8006, 2005

Size distribution of TiO_2 particles in the aerosol and in lung sections

Figure 1



Summary

Ultrafine TiO2 particles

- Penetrate through the surface lining layer and the epithelial barrier
- Distribute rapidly and evenly in all lung tissues and cells
- Are not membrane bound within cells
- Overwhelm the biological membranes by a yet unknown mechanism

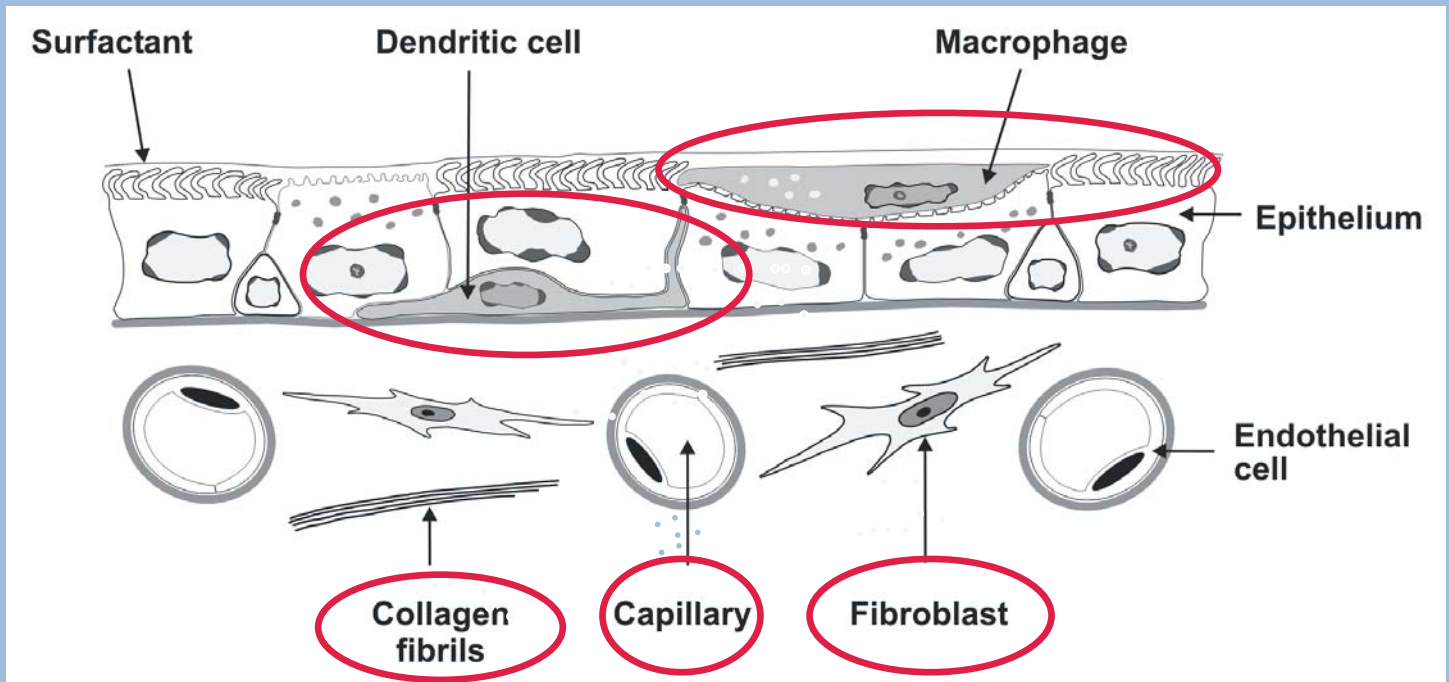
Collaborators, Support and most recent References

- P. Gehr, V. Im Hof, N. Kapp
  S. Frank, B. Kupferschmid *(University of Bern, Bern, CH)*
- W. Kreyling, H. Schulz, M. Semmler
  (GSF-Neuherberg, Munich, FRG)
- L.M. Cruz-Orive *(University of Cantabria, Santander, E)*

- Swiss National Science Foundation *(SNF)*
- Silva Casa Foundation
- Swiss Agency for the Environment, Forest and Landscape *(BUWAL)*

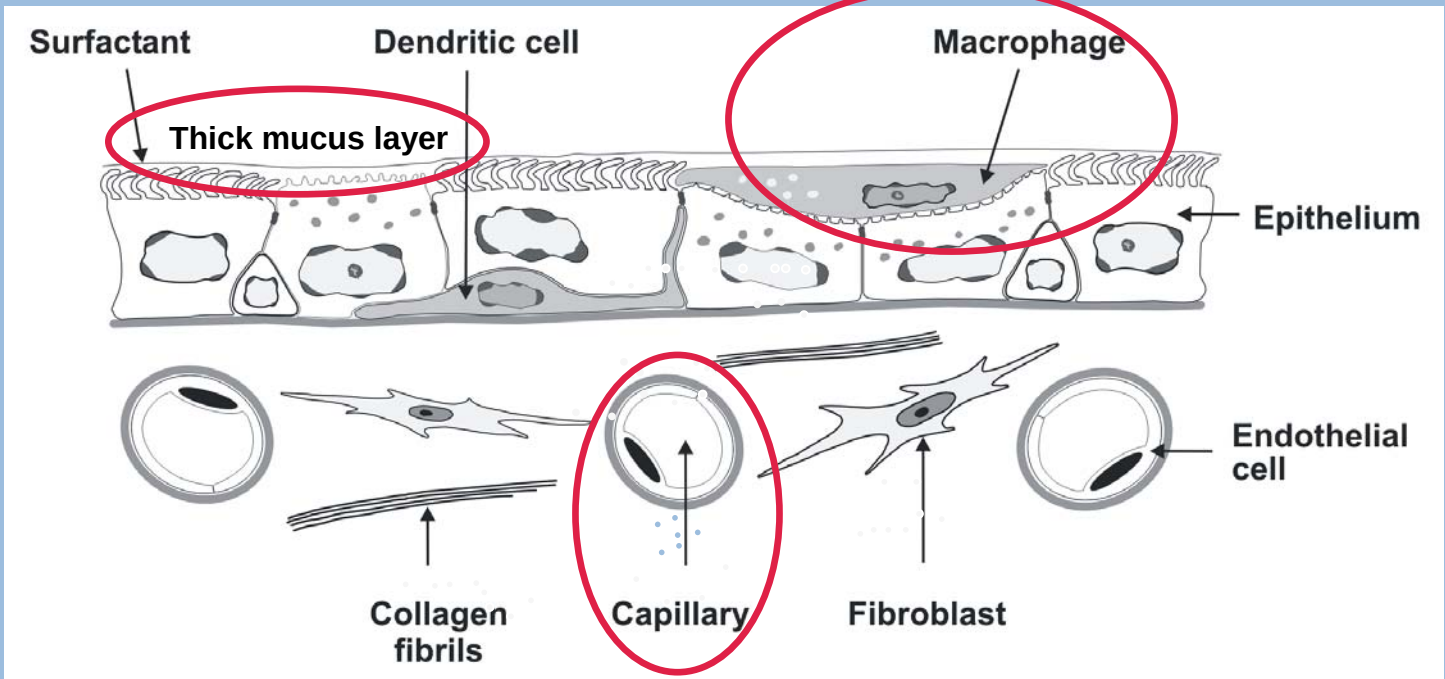
- Geiser M. et al., *J Appl Physiol* 94, 2004
- Kapp N. et al., *Micr Res Techn* 63, 2004
- Geiser M. et al., *Environ Health Perspect,* doi:10.1289/ehp.8006, 2005

Possible consequences from particle translocation into the lung tissue



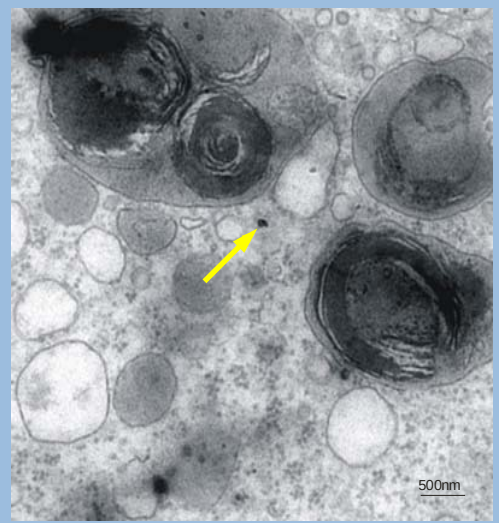
Adapted from Gehr et al., Phil. Trans. R. Soc. Lond. A, 2000

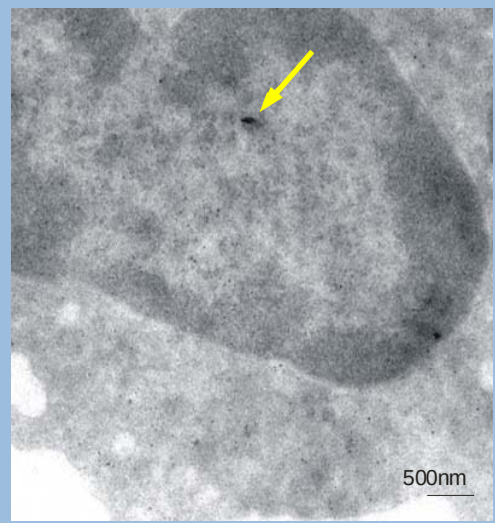
Focus of current and future in vivo studies



Adapted from Gehr et al., Phil. Trans. R. Soc. Lond. A, 2000

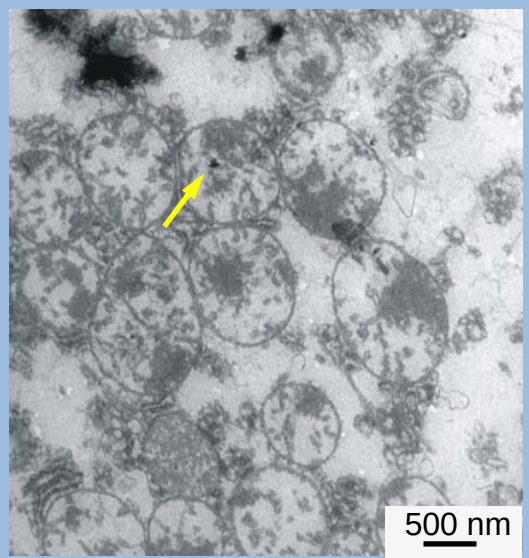
Clearance of ultrafine TiO_2 particles by macrophages



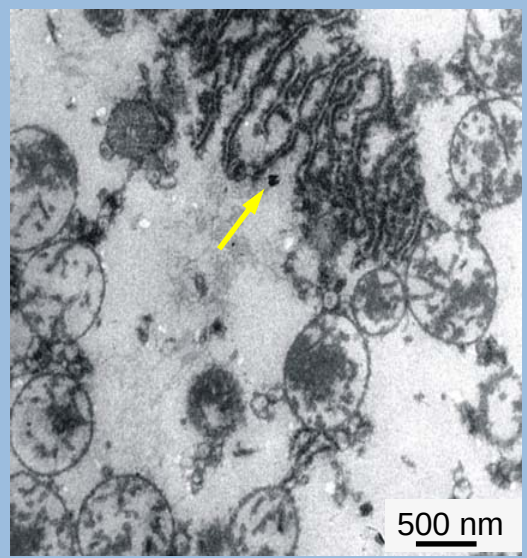


	N_{macs}	N_{part}	$P_{cytoplasm}$	$/P_{vesicles}$	$P_{nucleus}$
1 h	264	37	35	1	1
24 h	246	64	53	3	8
Total	**510**	**101**	**87**	**4**	**9**

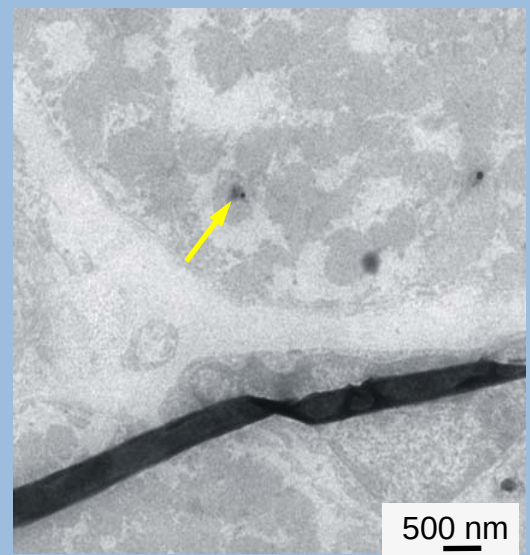
Translocation of ultrafine TiO_2 particles into secondary organs



Liver
Hepatocyte (mitochondrium)



Liver
Hepatocyte (cytoplasm)



Heart
Between myofilaments

Collaborators and Support

- M. Casaulta, B. Kupferschmid *(University of Bern, Bern, CH)*
- W. Kreyling, H. Schulz, M. Semmler *(GSF-Neuherberg, Munich, FRG)*
- L.M. Cruz-Orive *(University of Cantabria, Santander, E)*

- Swiss National Science Foundation *(SNF)*
- *?*

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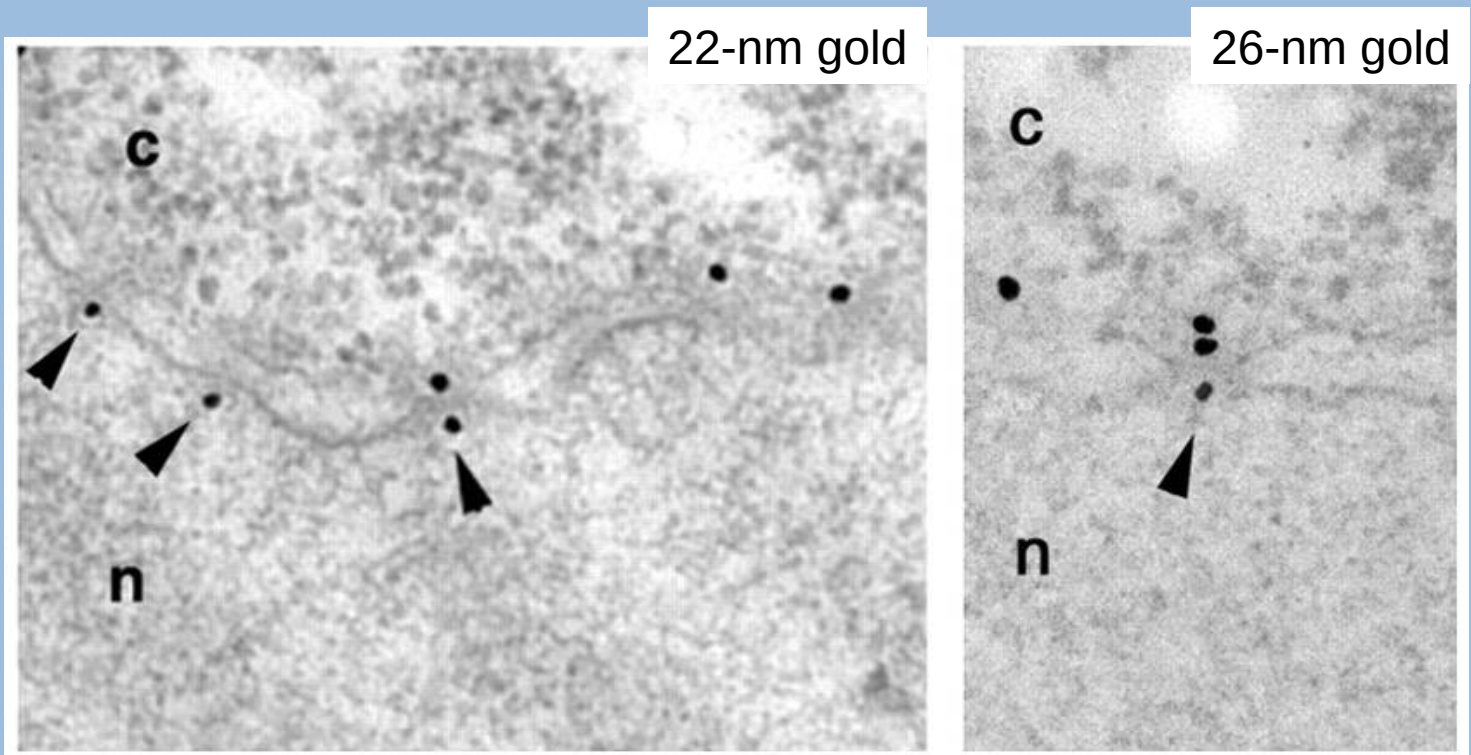
Transport of ultrafine particles through the nuclear pore complex



Panté & Kann, Mol Biol Cell, 2002

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Distribution and Clearance of Inhaled Ultrafine TiO_2 Particles in Rat Lungs

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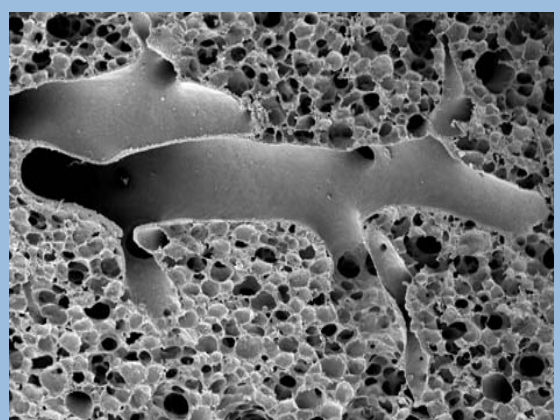
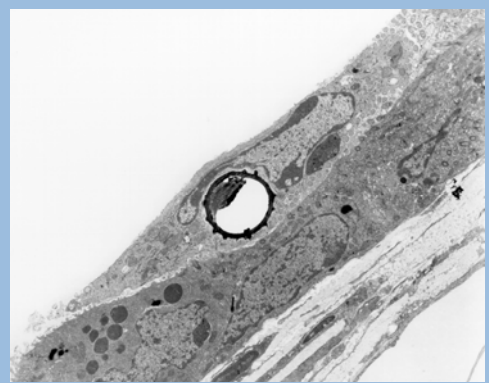
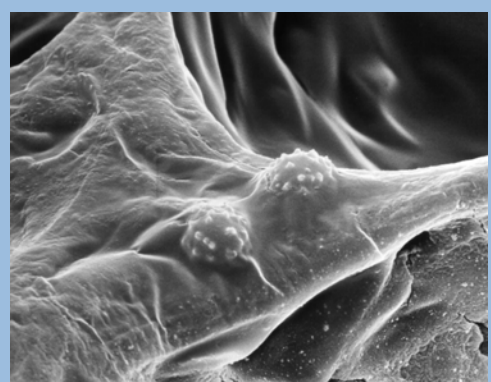


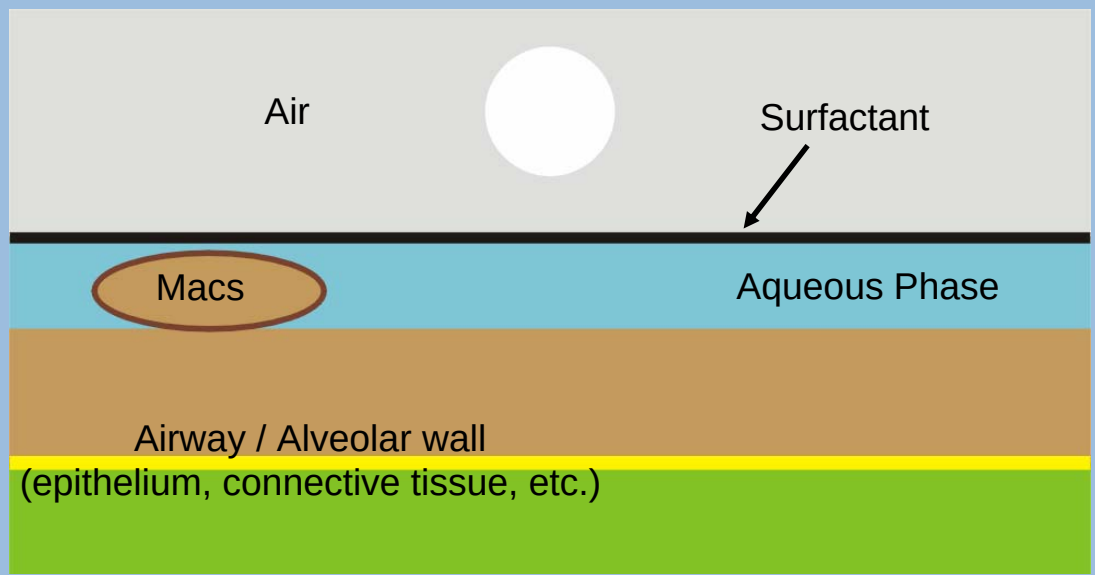
Airways & alveoli - rodent lung



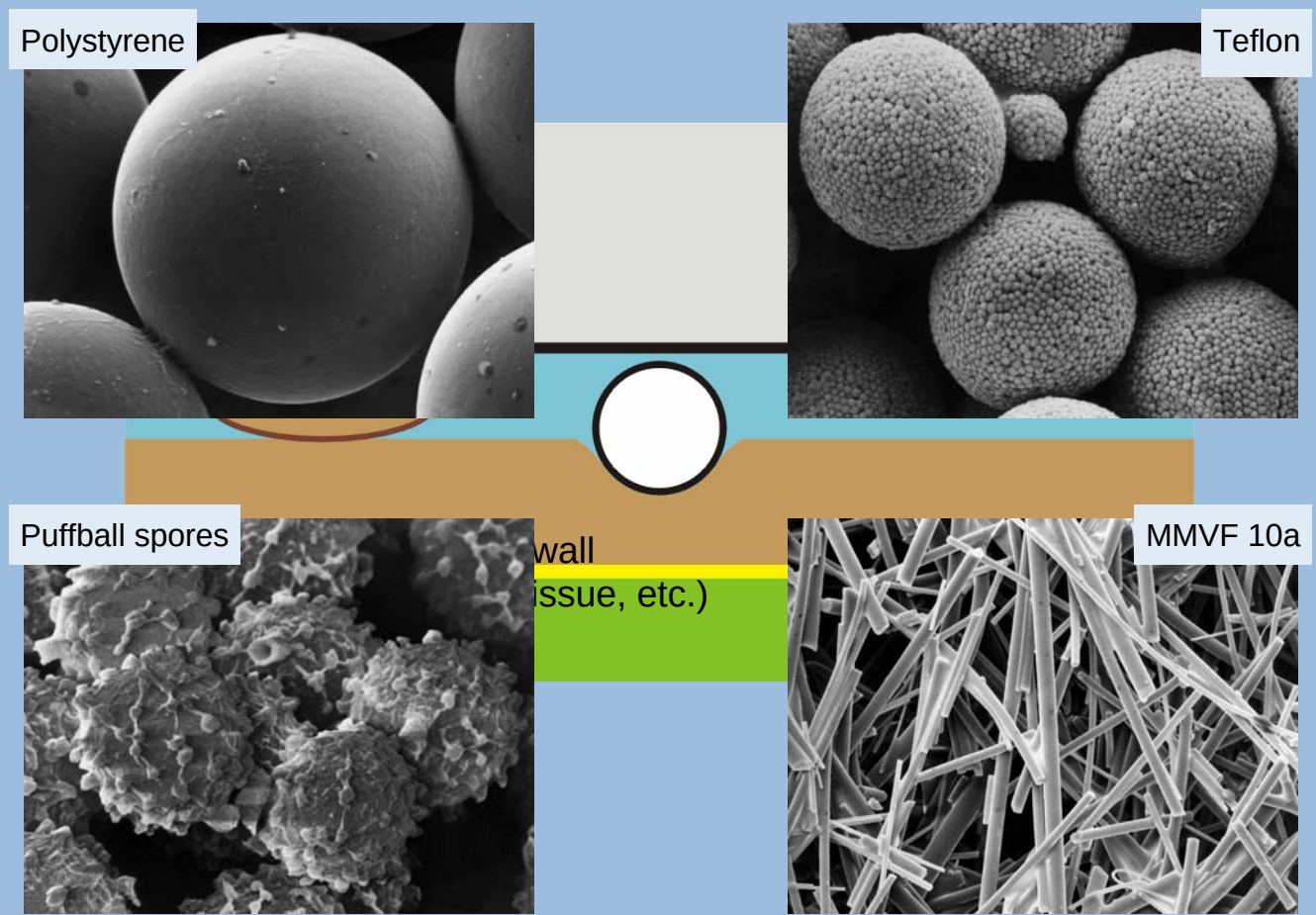


Puffball spores in airways and alveoli

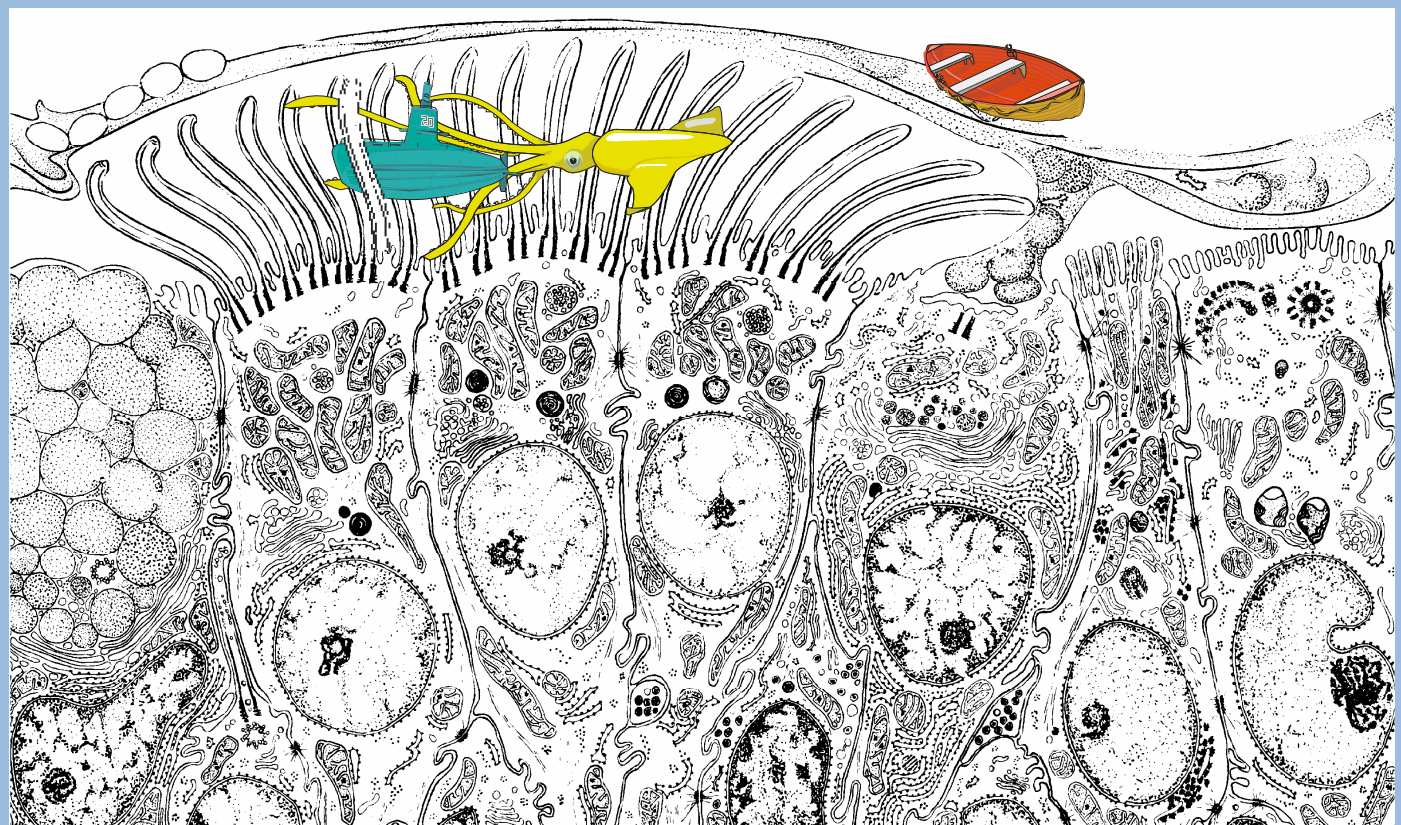
Components of the inner surface of the lungs



Displacement of PM 10 by surfactant



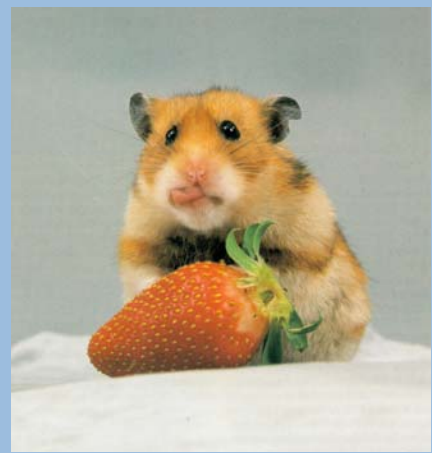
The concept of PM 10 retention and clearance



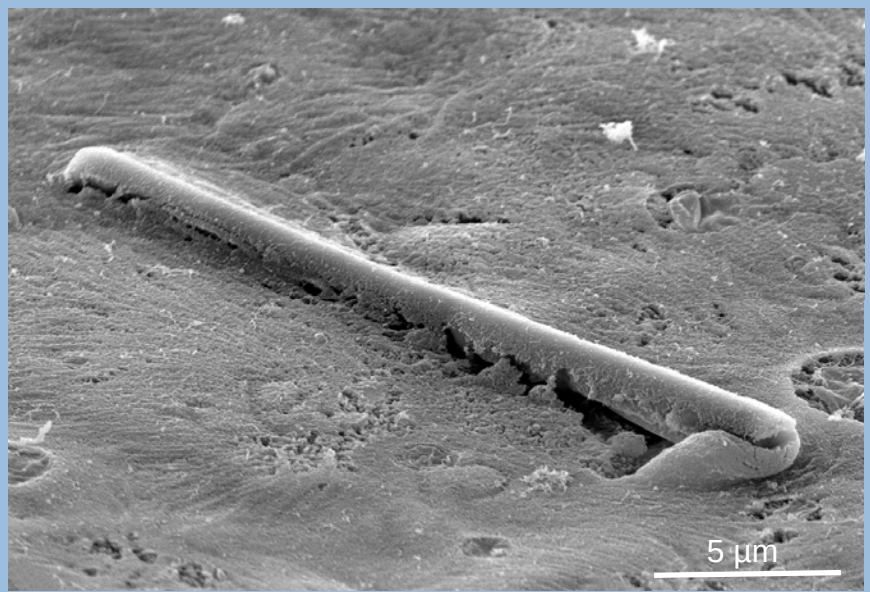
Adapted from Cell and Tissue Biology by L. Weiss, Urban & Schwarzenberg Inc. 1988

In vivo: Rodent model

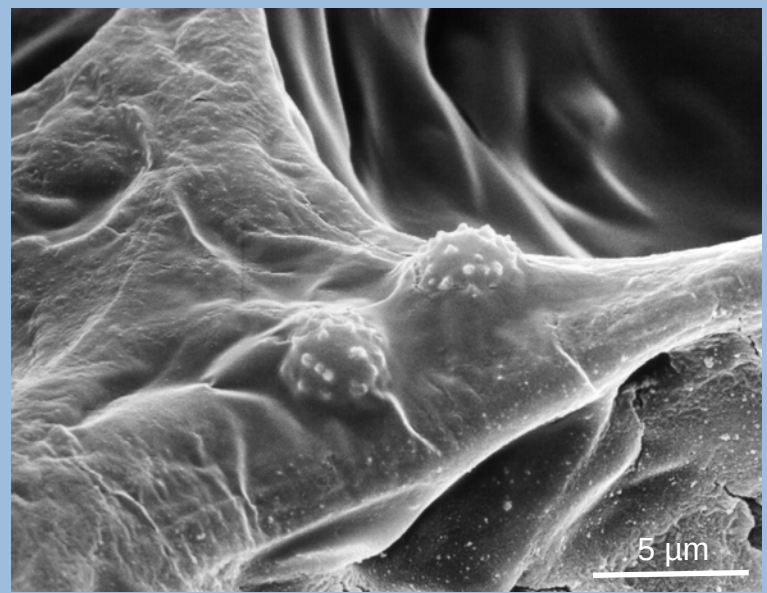
- Aerosol generation by nebulization
- Aerosol inhalation via endotracheal tube
- Continuous negative-pressure ventilation
- Lung fixation by vascular triple perfusion
- Systematic tissue sampling
- Light and electron microscopy



Inhaled and deposited PM 10, by SEM



Glass fiber in conducting airway



Puffball spores in alveoli

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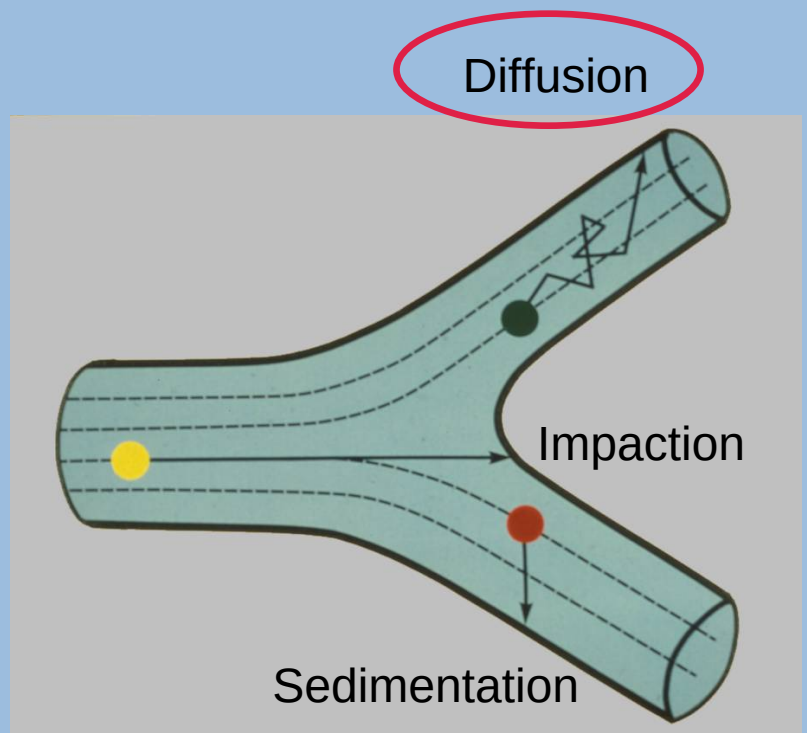
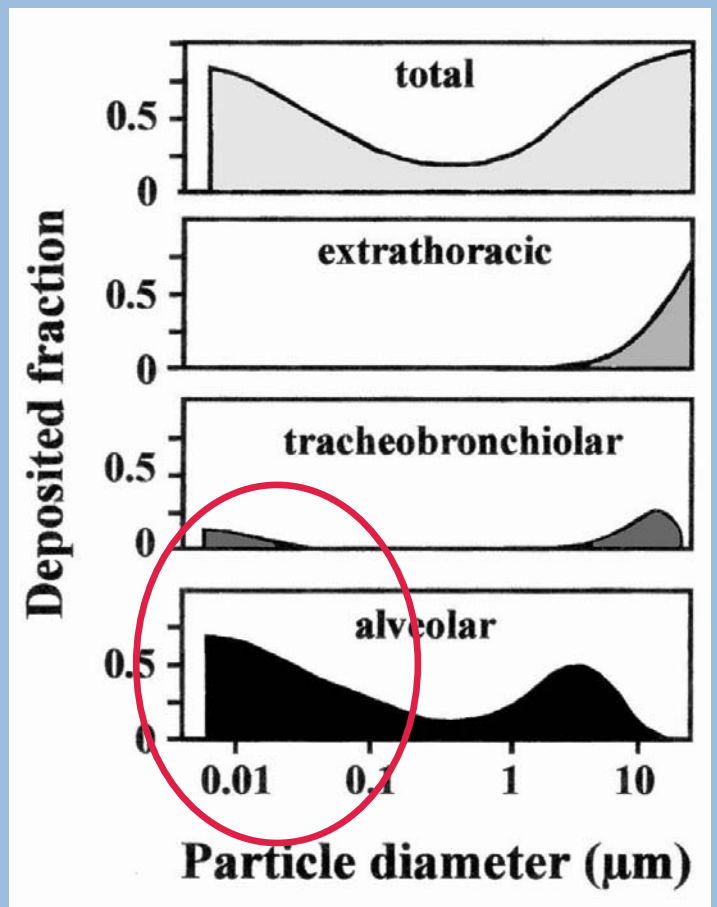
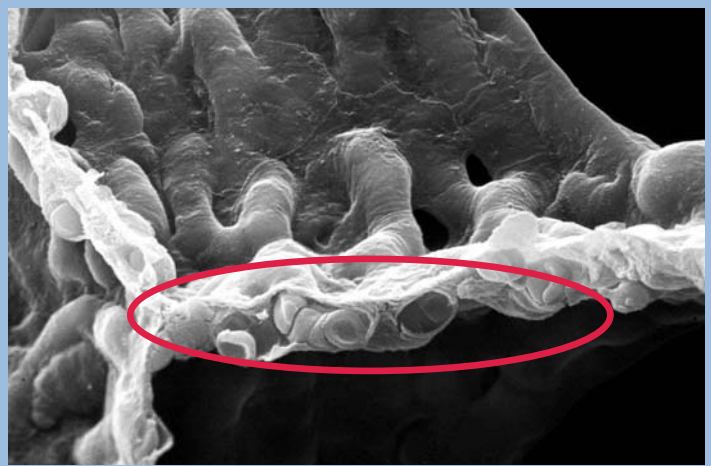
Deposition of ultrafine particles

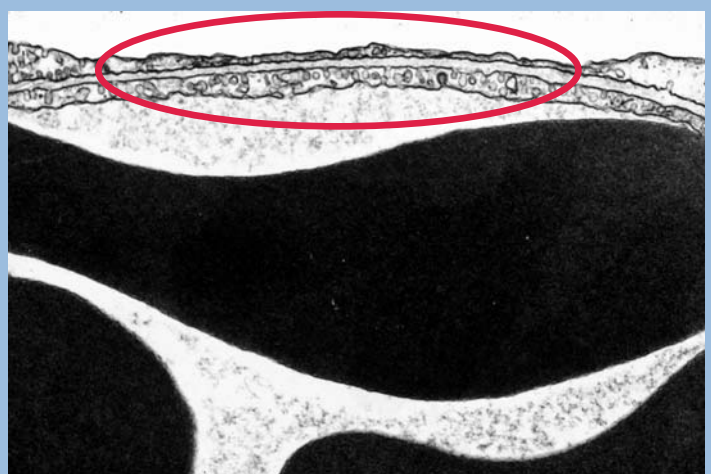


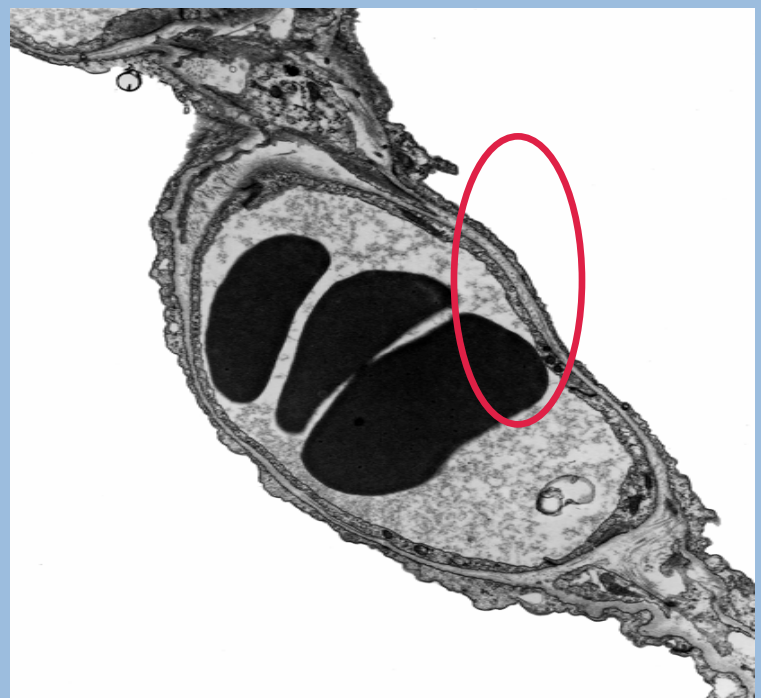


From Schulz et al., Lenfant Series, 2000

Ultrastructure of the lung parenchyma



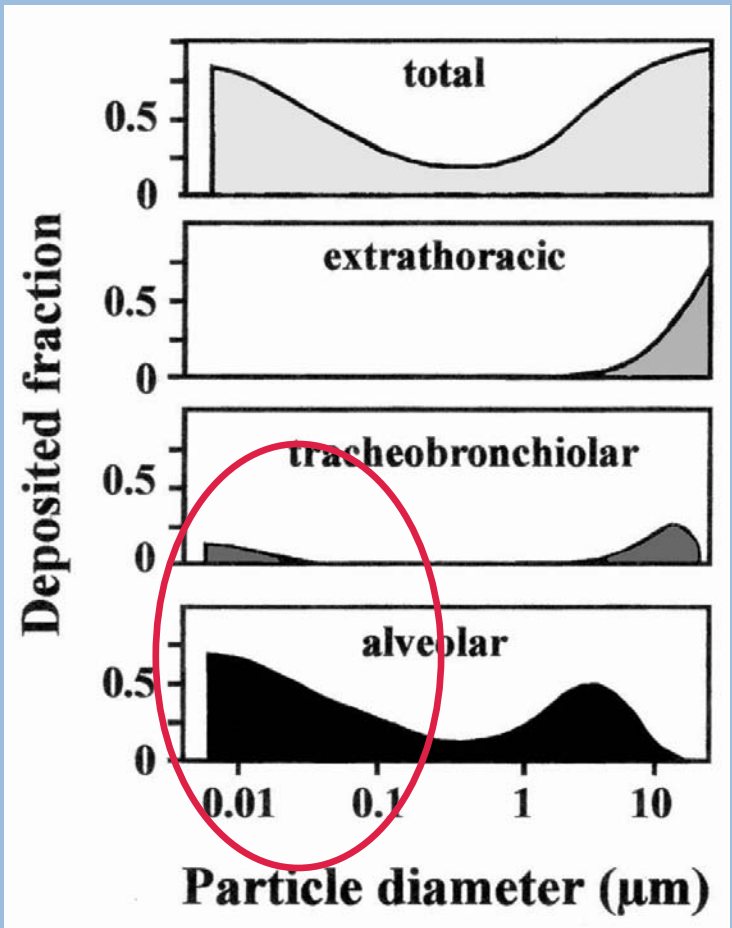




Air-blood barrier = 2 μm

1/50 Air mail paper

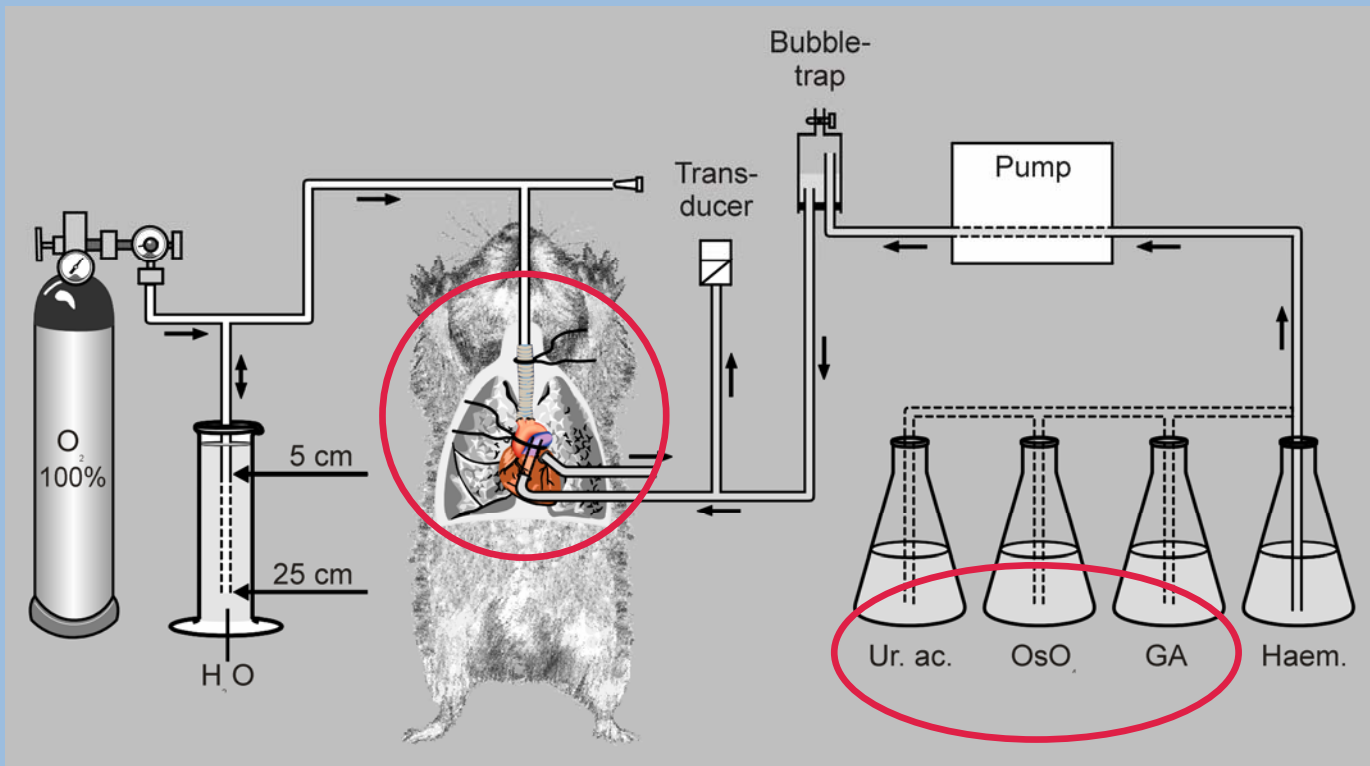
Deposition of ultrafine particles in lungs



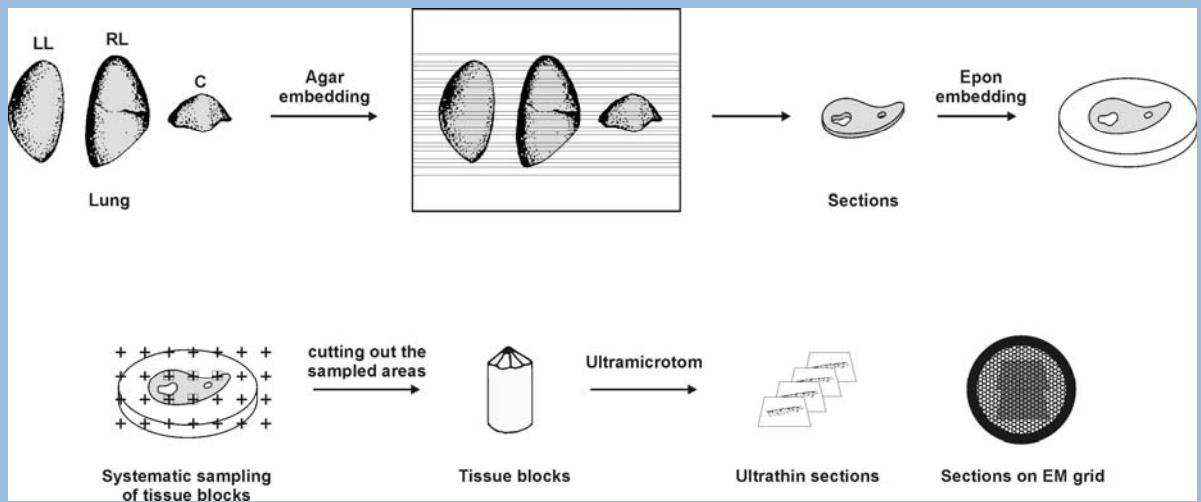
From Schulz et al., Lenfant Series, 2000

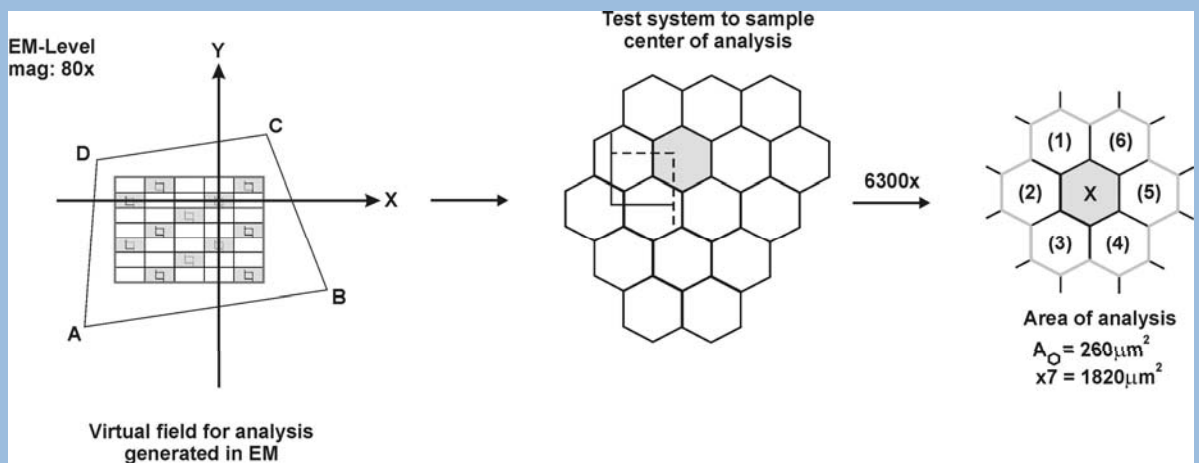
> High deposition rate
> Alveolar surface = 140 m^2
> Air-blood barrier = 2 μm

Animal model: Lung fixation

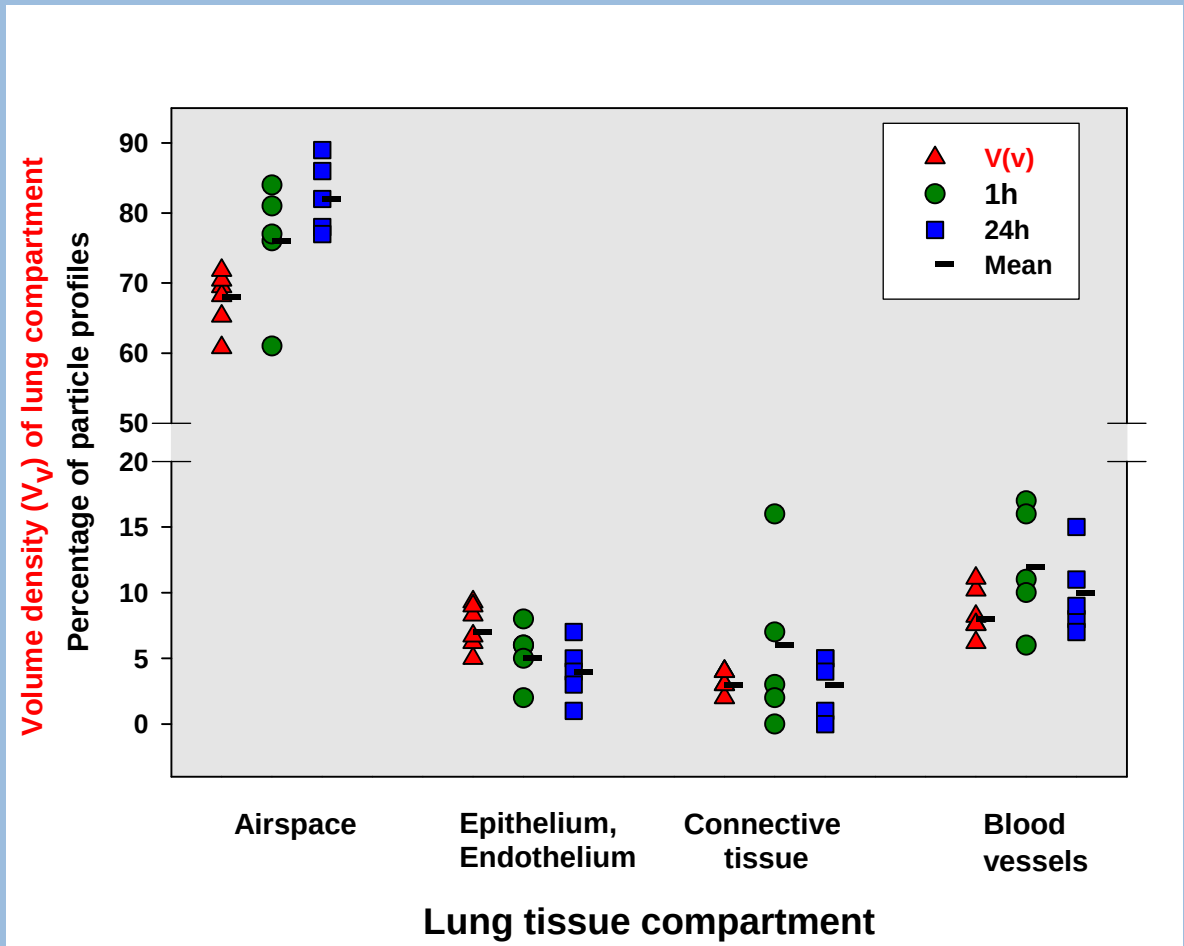


Systematic tissue sampling for stereologic analysis

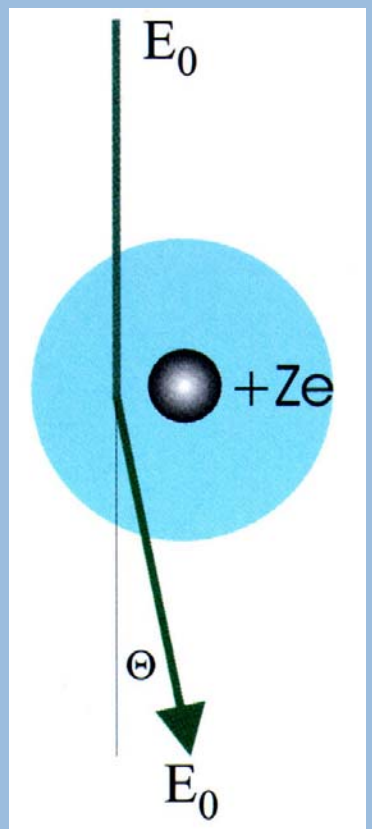


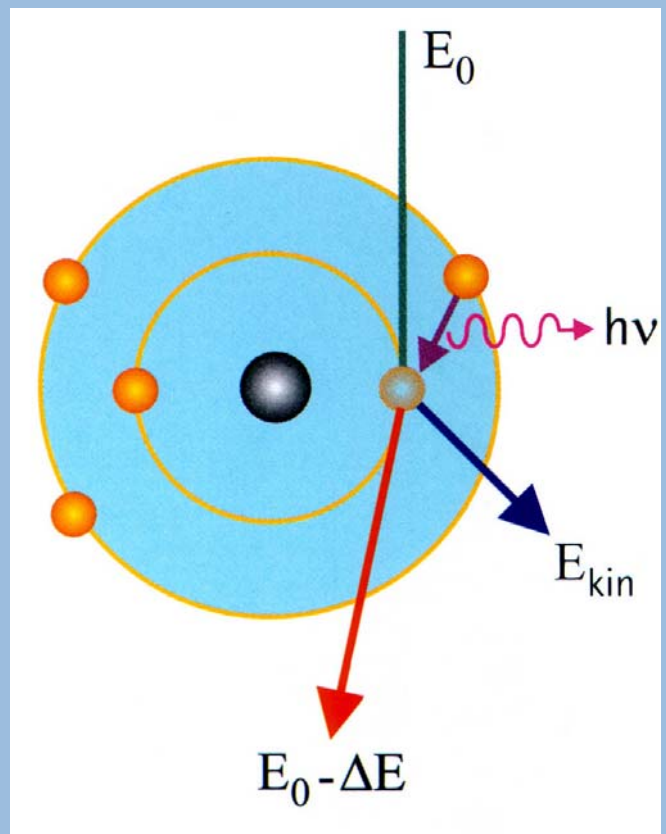


Distribution of TiO_2 particles in the lungs

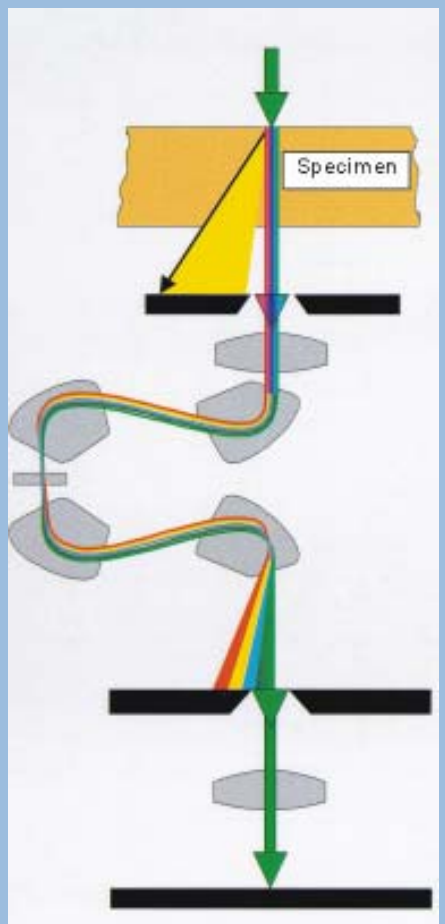


Electron beam - specimen interactions





Electron filtering TEM



Poly-energetic electrons

Objective aperture → Angle selection

+

Slit aperture → Energy selection

Mono-energetic electrons