Cellular interplay and inflammatory response after exposition of an epithelial airway model to fine an nano-particles

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INTRODUCTION

A number of epidemiological studies give evidence that the inhalation of fine particles (0.1-2.5μm) and nanoparticles (< 0.1μm) may cause increased pulmonary morbidity and mortality (Pope *et al*., 1995; Schulz *et al.*, 2005). Of particular interest are nanoparticles, which according to recent epidemiologic studies are particularly toxic (Peters *et al*., 1997). So far, little is known about the interaction and entering of nanoparticles by lung cells and their transport through the blood stream to other organs. A series of structural and functional barriers exists in the airway and alveolar wall which protects the respiratory system against harmful and innocuous particulate material (Nicod, 2005). It is still not clear how highly immunocompetent dendritic cells (Nicod, 1997; Holt, 2005) located in or at the base of the airway epithelium take up inhaled antigens and how macrophages on top of the epithelium may collaborate.

METHODS

The entering of different nanoparticles consisting of different materials and of different charges was studied in a triple cell co-culture model of the human airway barrier to simulate the cellular part of the epithelial barrier represented by macrophages, epithelial cells, and dendritic cells (Rothen-Rutishauser *et al*., 2005, Blank *et al*., 2006). In addition we visualized the cellular interplay by laser scanning microscopy and determined the cellular response by measurement of tumor necrosis factor-α, a pro-inflammatory mediator, produced upon nanoparticle addition.
Since nanoparticles have the size of small cell components (for example ribosomes), their identification in cells is very difficult. We combined different microscopic techniques to visualise nanoparticles in cultured cells:

- fluorescent particles were analysed by laser scanning microscopy combined with digital image restoration
- gold particles were analysed by conventional transmission electron microscopy and energy filtering transmission electron microscopy
- titanium dioxide particles were analysed by energy filtering transmission electron microscopy

RESULTS

By using these differing microscopic techniques we were able to visualize and detect particles nanoparticles in epithelial cells, macrophages and dendritic cells. The surface charge and the material of the particles did not influence their interaction with the cells, however, we found an increase of TNF-α in the supernatants after applying gold particles, but not for ultrafine polystyrene and titanium dioxide particles.

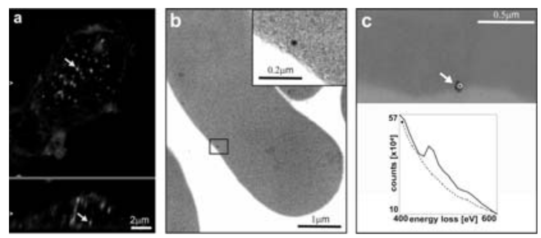

Figure 1. Visualization of nanoparticles. (a) Laser scanning micrograph. Single particles can only be detected after deconvolution (arrows). (b) Transmission electron micrograph of a 0.025μm gold particle within a red blood cell. (c) Electron energy loss spectroscopy image of titanium dioxide (arrow). The white open circles mark the positions where the energy loss analysis was performed. The corresponding energy loss spectra (black lines) is shown, the dotted lines represent the background.

By combination of laser scanning microscopy with advanced visualisation techniques we showed in the triple cell co-culture model that dendritic cells made processes between the epithelial cells through the tight junctions or transmigrate through the epithelium towards the "luminal side" to capture deposited particles on the epithelial surface.

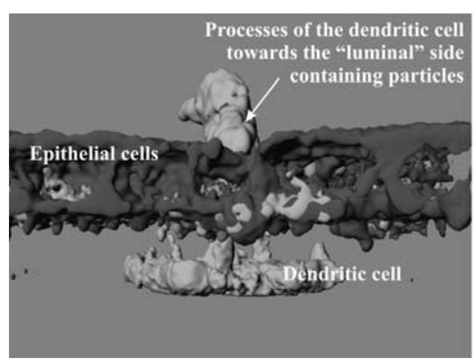

Figure 2. Laser scanning micrograph of a particle exposed triple cell co-culture. A dendritic cell residing underneath the insert membrane pushed processes between the epithelial cells upwards into the "luminal space" to take up 1 µm particles (arrow).

Dendritic cells also interacted with particle loaded airway macrophages or other dendritic cells in order to take up particles, and airway macrophages containing particles form interepithelial processes towards the base of the epithelium.

CONCLUSIONS

Using the triple cell co-culture model of the epithelial airway barrier and advanced microscopic techniques we have been able to visualize and detect nanoparticles within single cells. The surface charge and the material of the particles did not influence their entering and internalized particles are not membrane bound. However, inflammatory response is induced depending on the material in the co-culture model. The laser scanning micrographs illustrate the interplay of cells of the epithelial airway wall when exposed to particles. With this *in vitro* system we clearly show how dendritic cells gain access to the apical side of the epithelial cells (i.e. the lumen of the lung) where they may sample particulate antigens and interact with airway macrophages. Furthermore, the processes of airway macrophages extending into the epithelium, suggest an interaction of airway macrophages and dendritic cells within the epithelial wall.

Keywords: *Nanoparticles, epithelial airway model, cellular interplay, inflammation*

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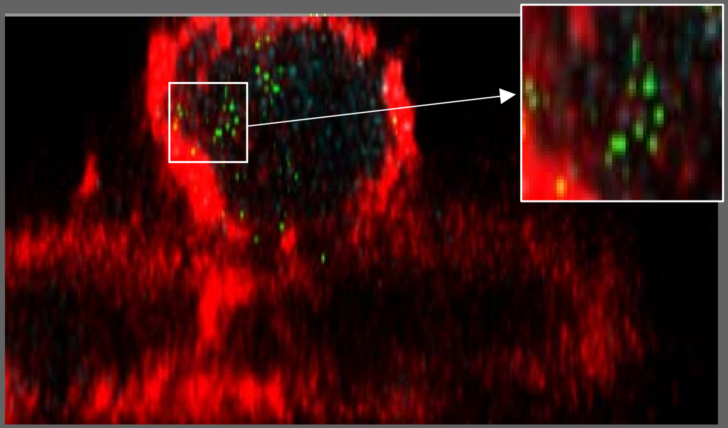
An epithelial airway model to visualize cellular interplay and to determine cellular responses after nanoparticle exposure



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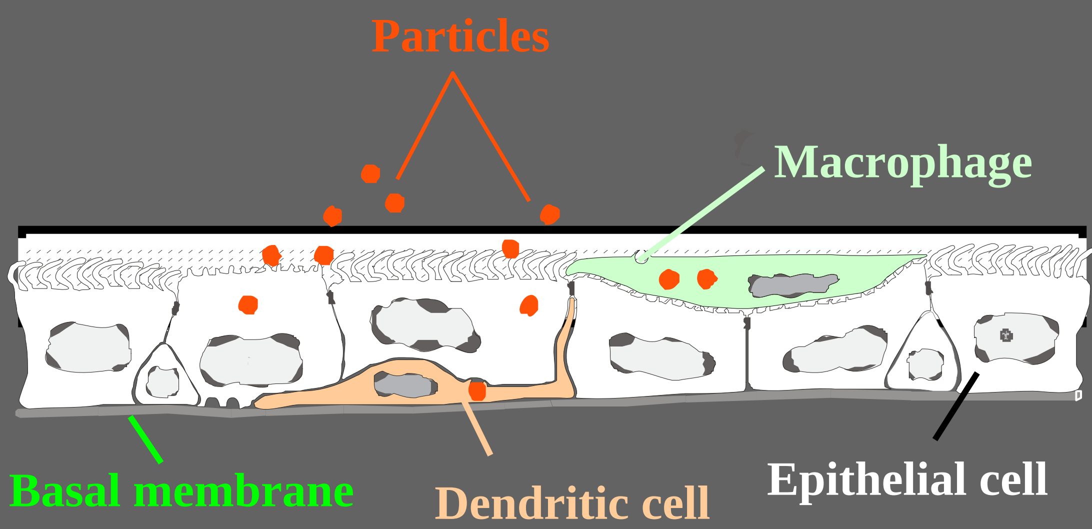
10th ETH Conference on Combustion Generated Nanoparticles
22th August 2006, Zürich

Bollwerk in Bern, Der Bund, 02.02.06

Ambient PM:

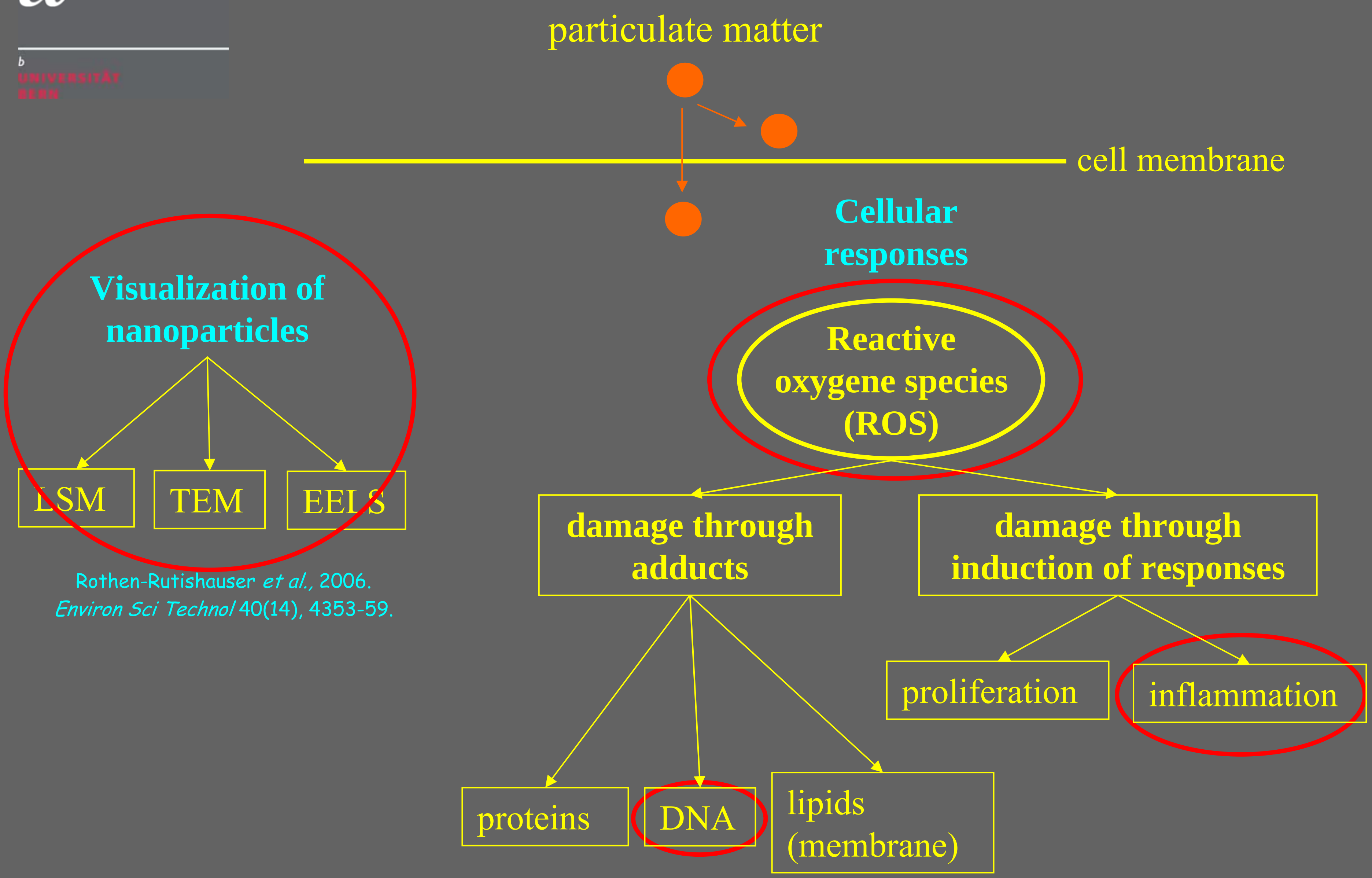
- Natural sources
- Combustion processes

Nanoparticles from combustion processes and nanotechnology



P. Gehr

PM_{10} (< 10 µm ∅) particles are associated with increased morbidity and mortality. Recent studies indicate a specific toxicological **role of nanoparticles!**



Rothen-Rutishauser *et al.*, 2006. *Environ Sci Technol* 40(14), 4353-59.

Donaldson *et al*, 2003. *Free Radic Biol Med.* 34(11)1369-82.; Donaldsonn *et al.*, 2005. *Part Fibre Toxicol.* 210.

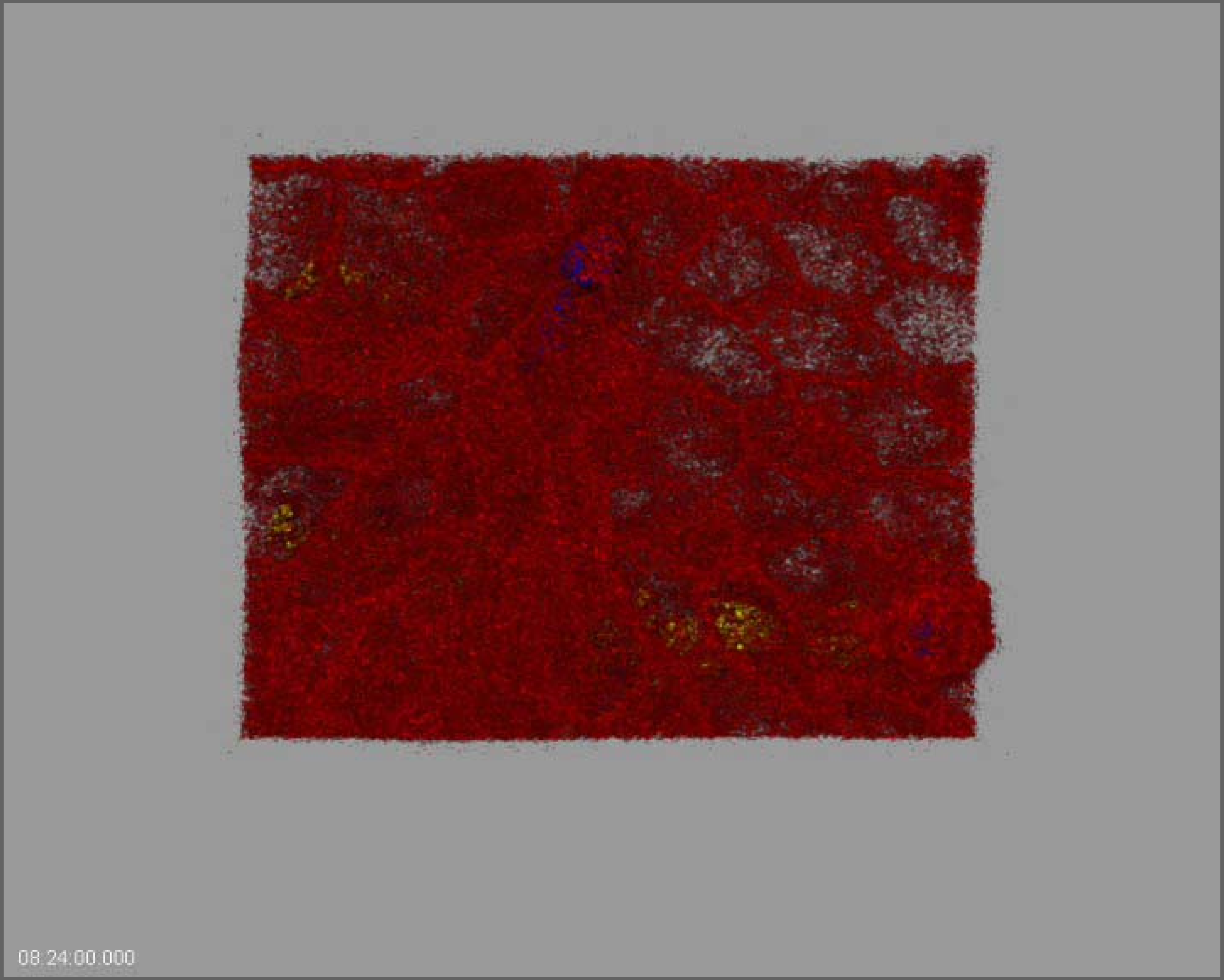
Universität Bern

Epithelial airway barrier *in vitro*:

Triple cell co-cultures:

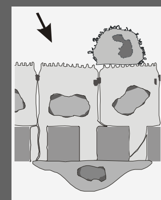
- Epithelial cells
- Macrophages
- Dendritic cells

Rothen-Rutishauser *et al.*, 2005. *Am J Respir Cell Mol Biol.* 32(4):281-9.

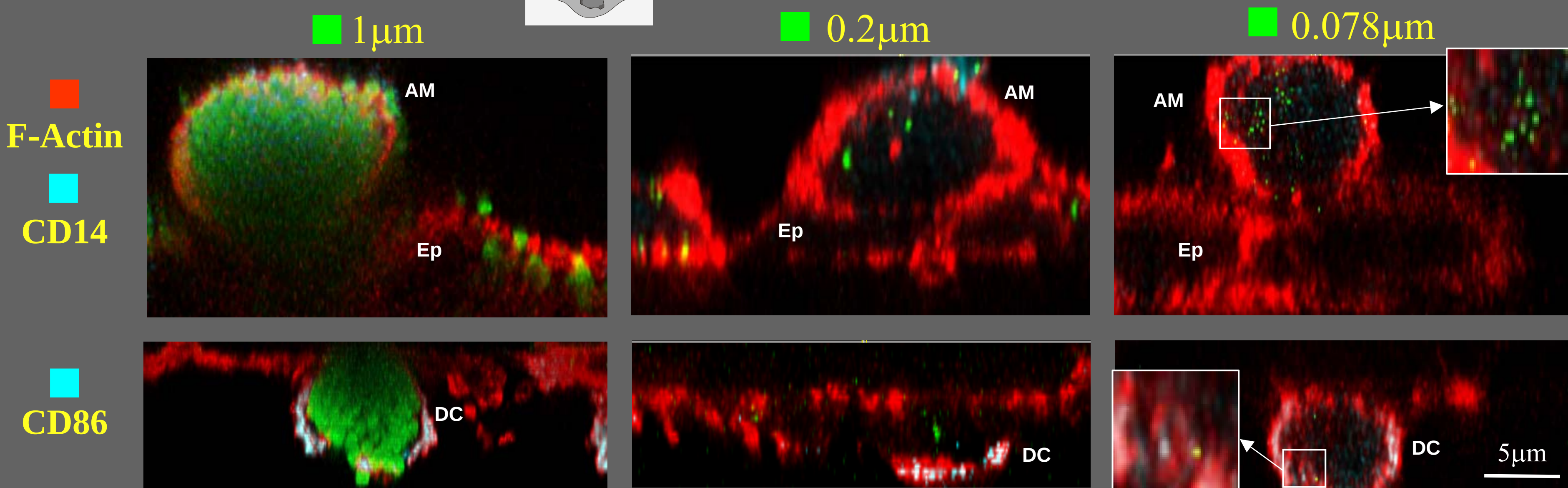


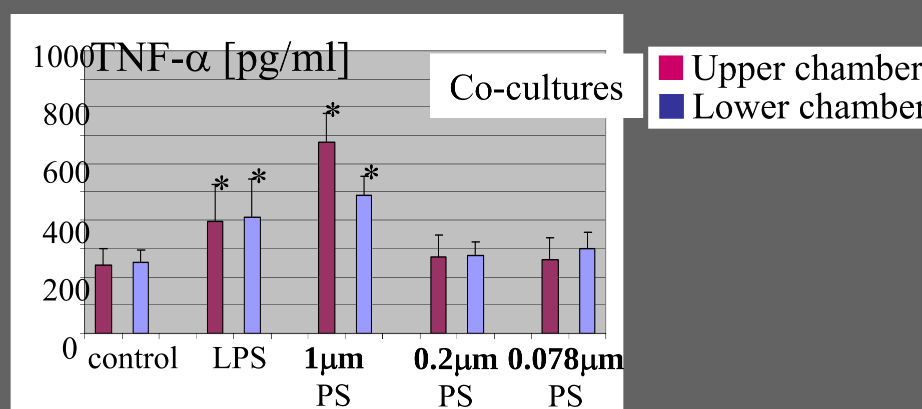
F. Blank

Epithelial airway wall – *in vitro* system: localization of particles and inflammation



Polystyrol particles



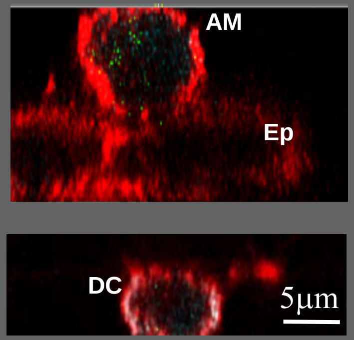


Epithelial airway wall – *in vitro* system: localization of particles and inflammation

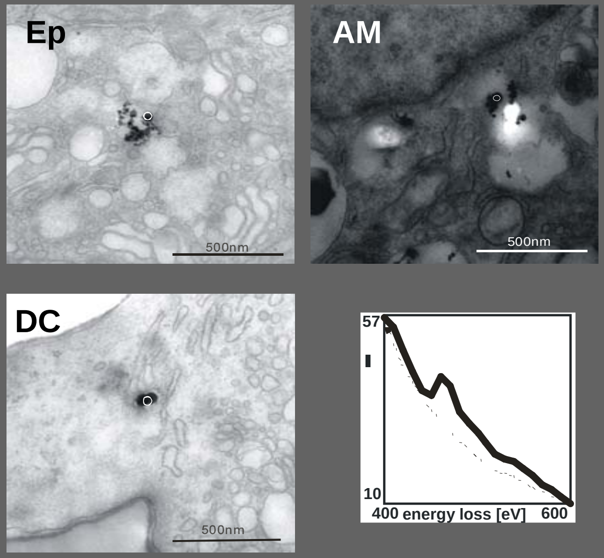
UNIVERSITÄT BERN

Polystyrol particles

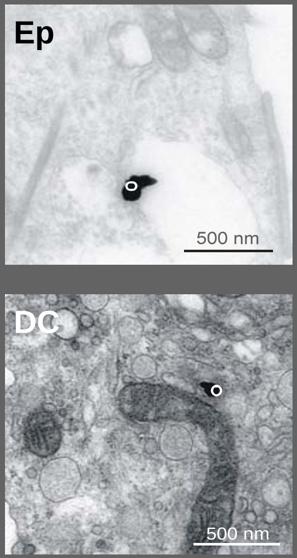
0.078µm

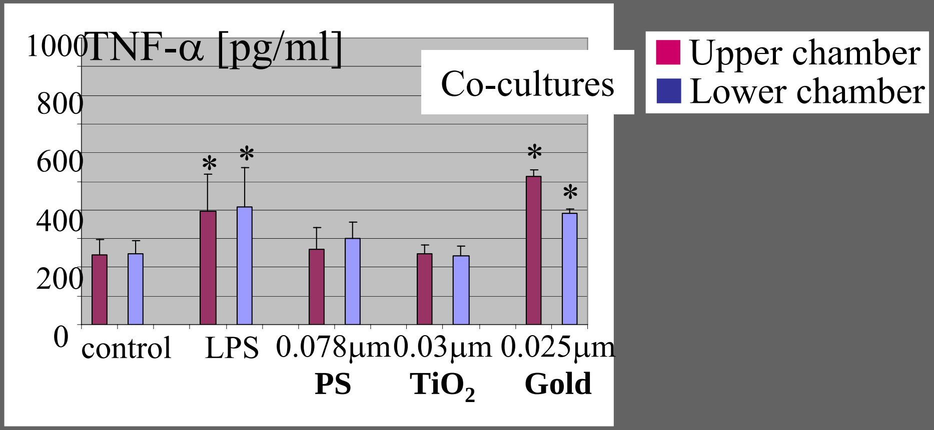


0.03µm TiO_2 particles



0.025µm Gold particles





Nanoparticle-related oxidative stress and pro-inflammatory responses in the triple cell co-culture system

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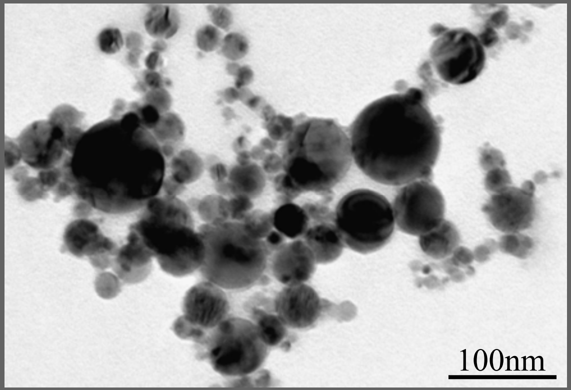
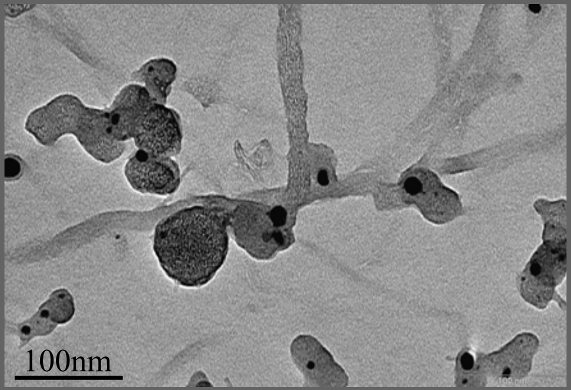
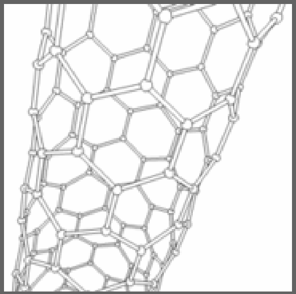
Epithelial airway wall – *in vitro* system:
Nanoparticle-related oxidative stress and pro-inflammatory response

Titanium dioxide (TiO_2)



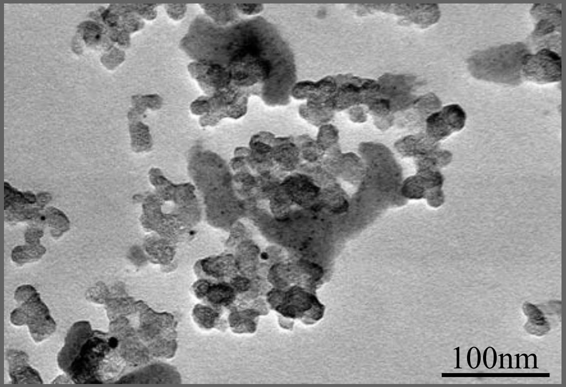
Carbon nanotubes (CNTs)





Wikipedia

Diesel particles (DEP)



Epithelial airway wall – *in vitro* system:
Nanoparticle-related oxidative stress and pro-inflammatory response

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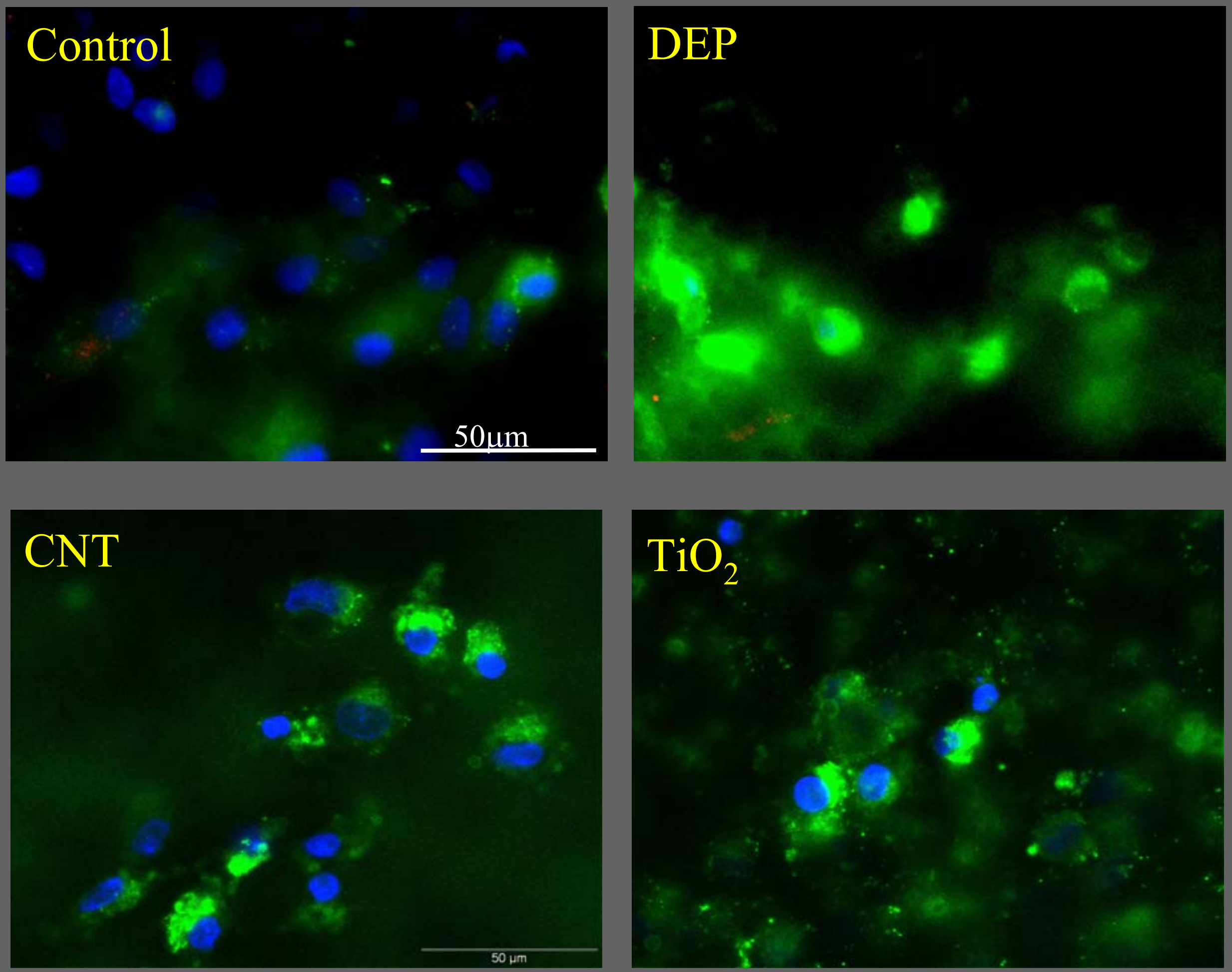
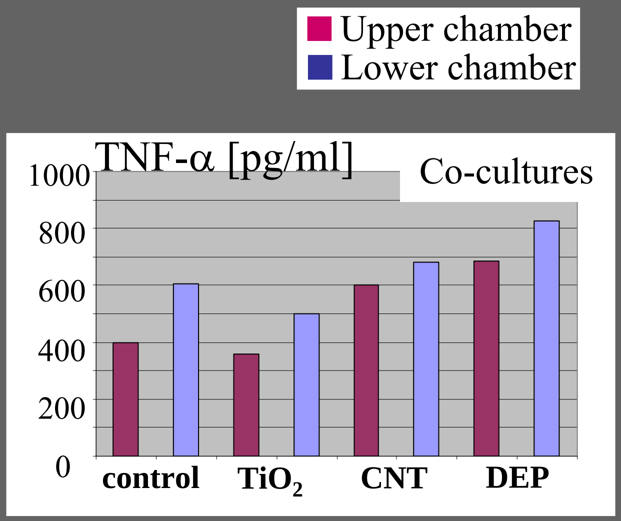
ROS Zellkerne





L. Müller

Conclusions (I):

Visualization of nanoparticles with advanced microscopic techniques is possible

Particles ≤ 1µm are found within all three cell types

=> number of particles within the cell types is different

=> number of particles within one cell type depends on particle size

TNF-α release is dependent on size and material

Nanoparticles may induce inflammation and/or oxidative stress

Deposition of nanoparticles on lung cell cultures at the air-liquid interface : A new exposition setup

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Institute for Chemical and Bioengineering
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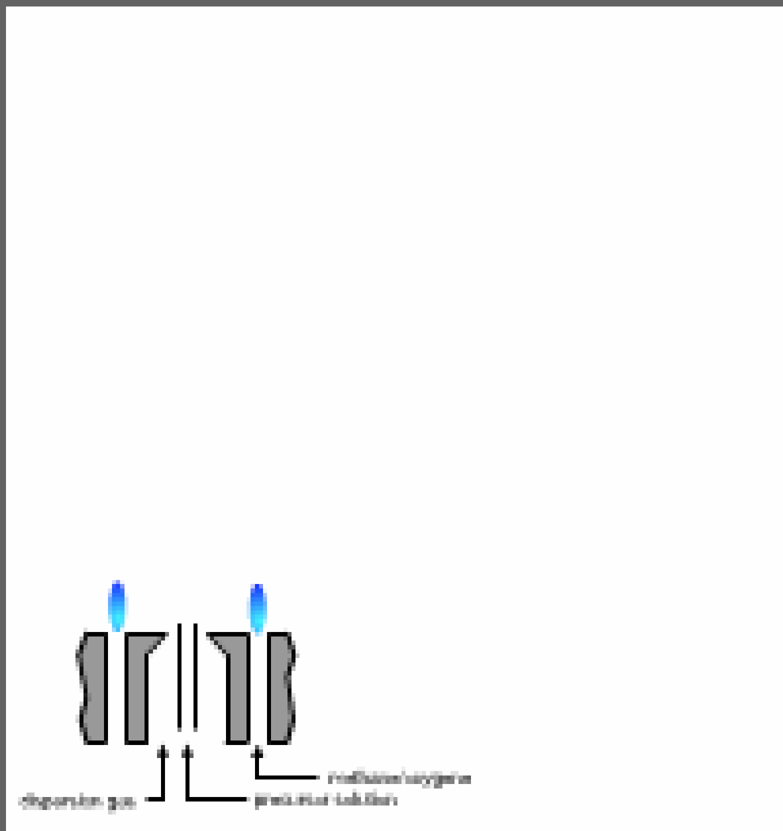
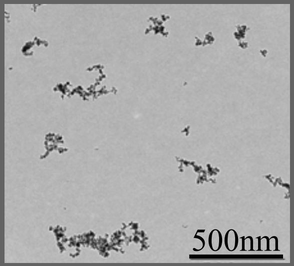
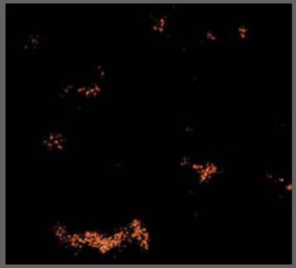
Wendelin Stark
Robert Grass
Ludwig Limbach

Flame spray synthesis of cerium dioxide





Element analysis



0.7g **CeO_2** in 1.5 Min
Mean particle diameter: 15nm

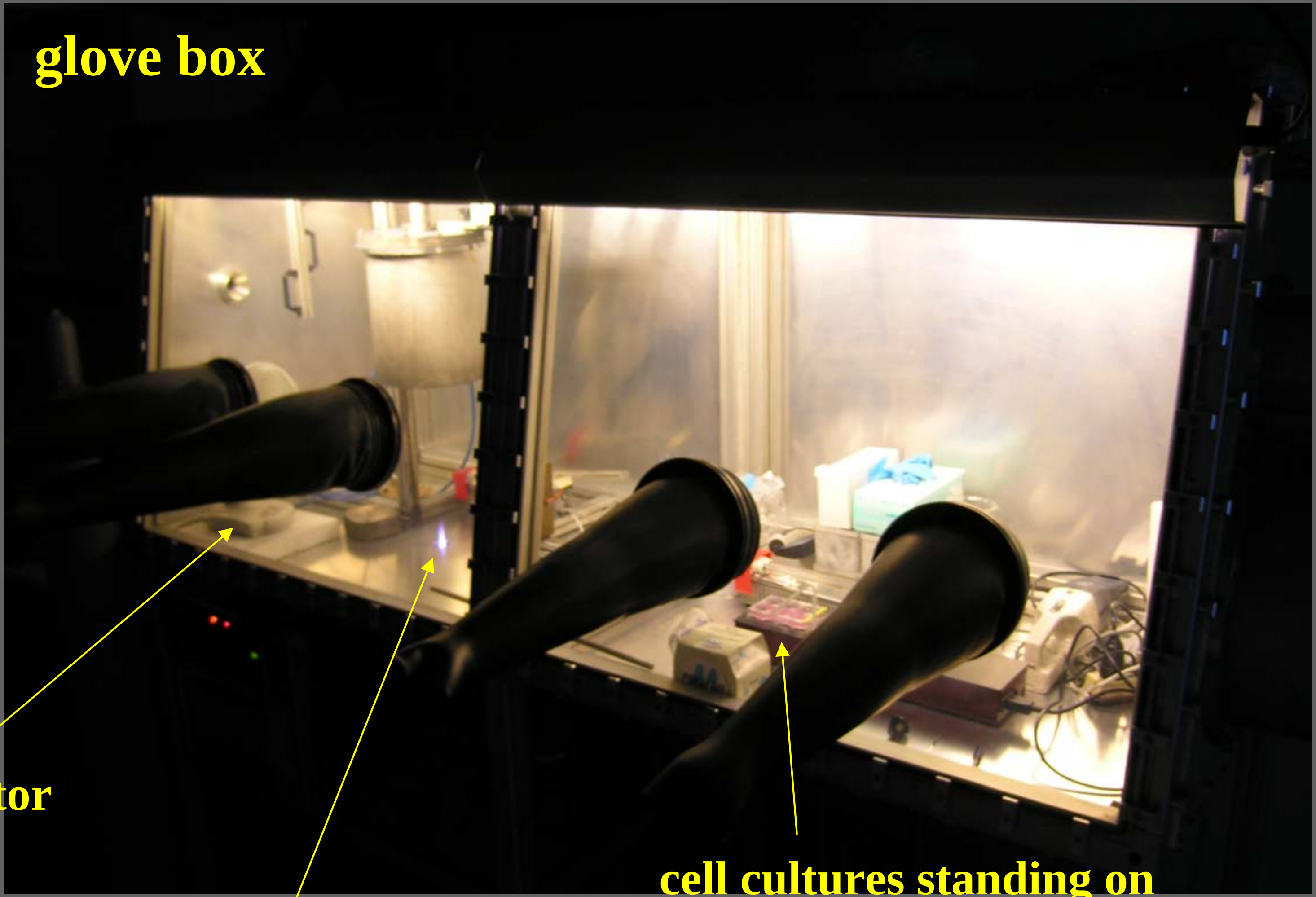
CeO_2

↑

Cerium-2-ethylhexanoate in Xylol

Animation by Reto Strobel, PTL, ETH Zürich b UNIVERSITÄT BERN

CeO_2 deposition: the experimental setup

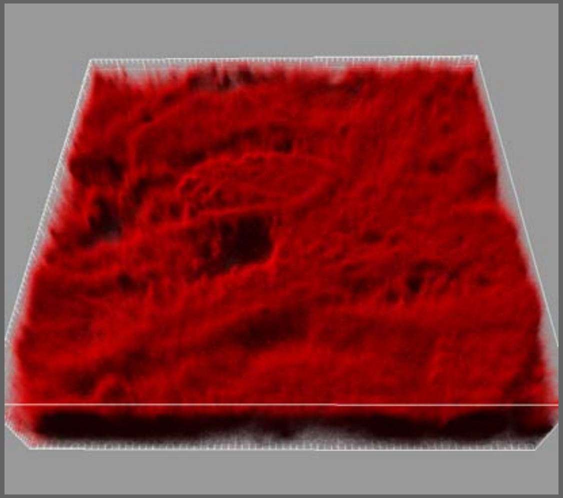


R. Grass

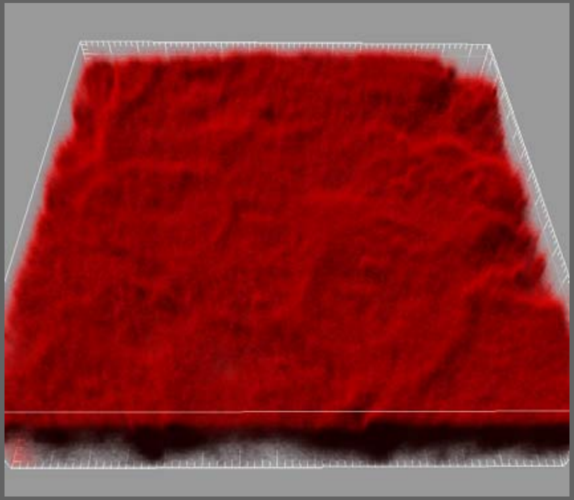
CeO_2 deposition on A549 cells: the cytoskeleton

F-Actin

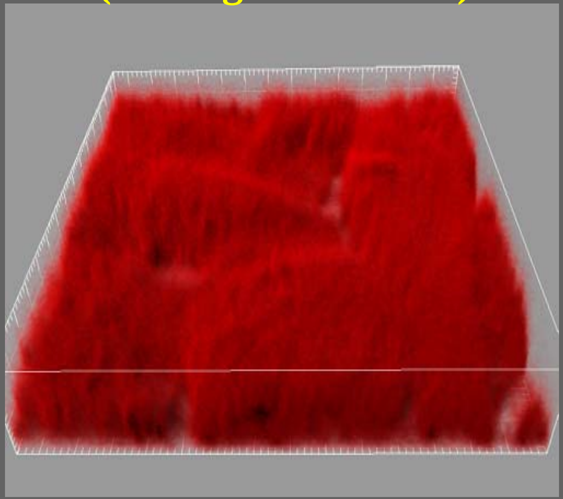
Control



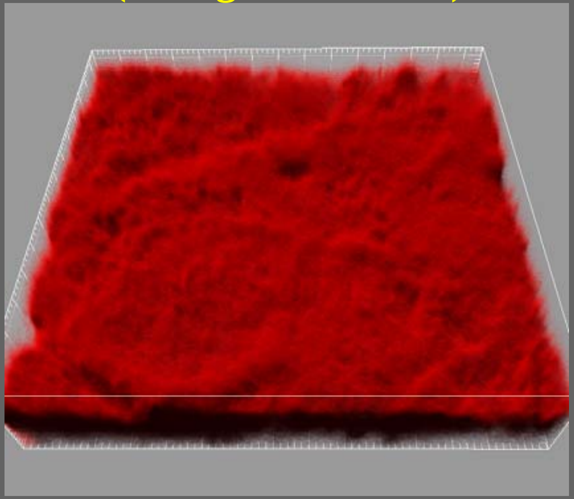
10 Min. CeO_2 (0.05mg/Membrane)



20 Min. CeO_2 (0.08mg/Membrane)



30 Min. CeO_2 (0.1mg/Membrane)

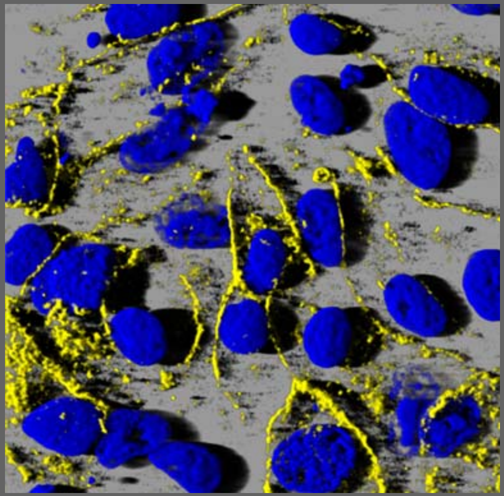


CeO_2 deposition on A549 cells: the tight junctions

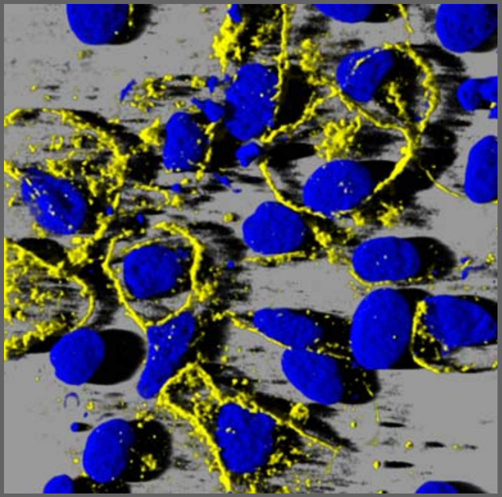
Occludin

Cell nuclei

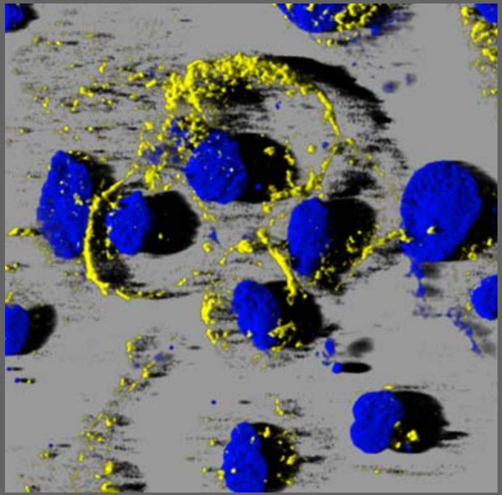
Control



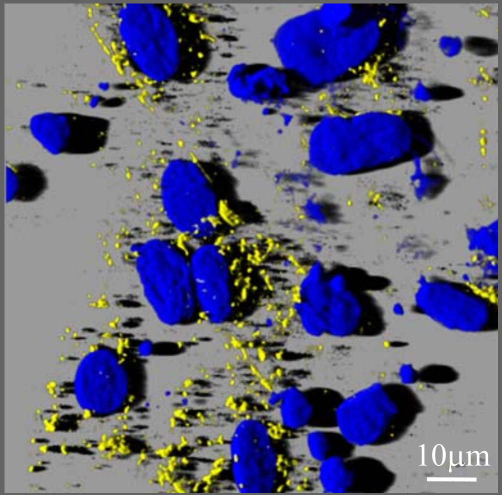
10 Min. CeO_2



20 Min. CeO_2



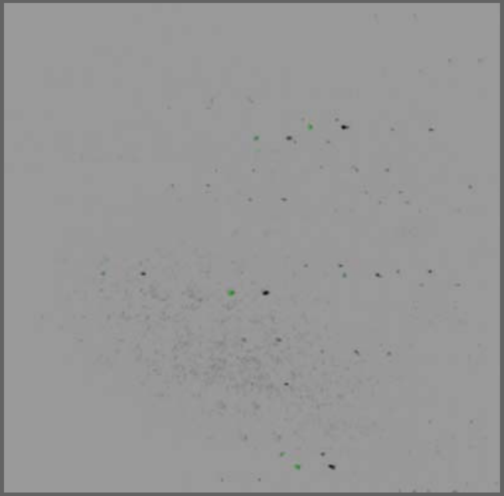
30 Min. CeO_2



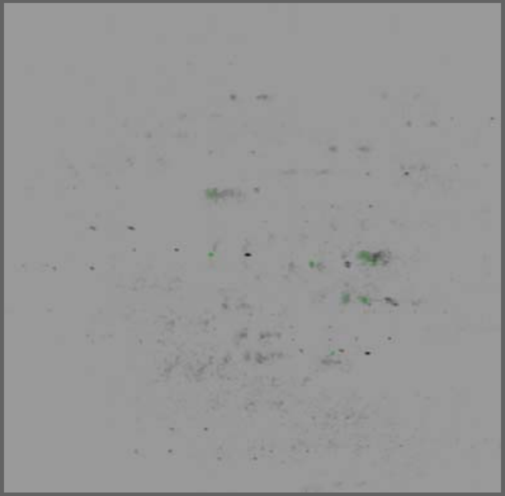
CeO_2 deposition on A549 cells: oxidative stress

8-oxoguanine

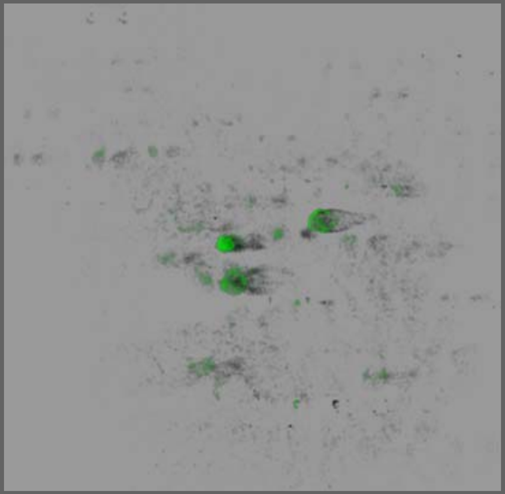
Control



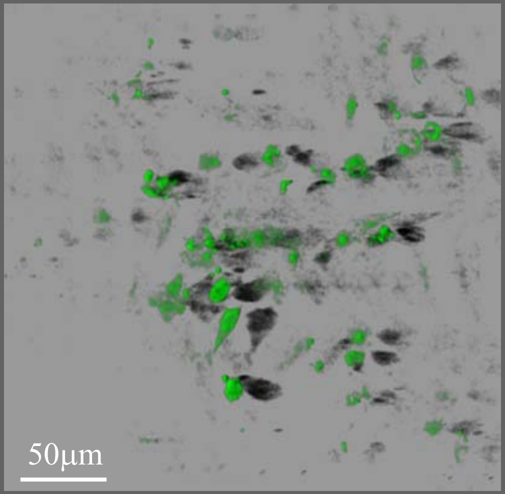
10 Min. CeO_2



20 Min. CeO_2



30 Min. CeO_2



Conclusions (II):

Production of nanoparticles under controlled conditions:

the nanomaterial is made exactly as in the industrial process with the same degree of agglomeration/size/surface coating

Most versatile setup for exposure to virtually any oxide or salt nanoparticle

Best simulation of an *in vivo* exposure to combustion generated nanoparticles

Exposition of A549 cells with CeO_2 nanoparticles:

a dose dependent impairmend of tight junctions and induction of oxidative stress

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Michael Riediker

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