Risk assessment of nanoparticle exposure at working places

Barbara Rothen-Rutishauser[1)], Robert N. Grass[2)], Fabian Blank[1)], Ludwig K. Limbach[2)], Christian Mühlfeld[1)], Peter Gehr[1)] and Wendelin J. Stark[2)]
[1)] Institute for Anatomy, Division of Histology, University of Bern, Bern, Switzerland
[2)] Institute for Chemical and Bioengineering, D-CHAB, ETH Zurich, Zurich, Switzerland

The rapid proliferation of many different manufactured nanomaterials requires defined screening strategies for the characterization of the potential human health effects from exposure to nanomaterial. The application of in vitro methods to analyse the effects of nanoparticles on the cellular level is still limited due to the difficulties of exposing cultured cells of the respiratory tract directly to the test atmospheres.
In a completely new approach we exposed A549 lung epithelial cells grown at the air-liquid interface to manufactured cerium oxide (CeO_2) nanoparticles generated by flame spray directly at the place where the particles have been produced, i.e. at the working place. The hydrodynamic particle size distribution was measured by X-ray disc centrifugation and particle distribution was investigated by transmission electron microscopy. The epithelial tightness of the cells was measured before the cells were fixed for analysis with laser scanning microscopy. Cellular response was assessed by investigating DNA damage induced by oxidative stress.
Transepithelial electrical resistance of control cultures was 290 ± 116 (SD) Ωcm^2, whereas in cultures exposed for 30 min to CeO_2 values decreased to 156 ± 84 (SD) Ωcm^2. The cellular morphology was not affected. However, we found, in coincidence with the TEER measurements, that the tight junction protein occludin was found to be absent at the cell-cell contacts in cells exposed for 30 min. We also observed that exposure for 30 min with CeO_2 induced DNA damage, which is an indicator for oxidative stress.
Exposure of lung epithelial cells to nanoparticles generated by flame spray synthesis in a glove box allowed to study particle toxicity in a simple and reproducible way under environmental conditions.

Risk assessment of nanoparticle exposure at working places

B Rothen-Rutishauser[1], R N Grass[2], F Blank[1], L K Limbach[2], CH Mühlfeld[1], P Gehr[1], and W J Stark[2]
[1]Institute of Anatomy, University of Bern, Bern, Switzerland
[2]Functional Materials Laboratory, ETH Zurich, Zurich, Switzerland

Introduction

There are progressively more **manufactured nanoparticles (NP)**, defined as manufactured structures with 1-100 nm size dimensions, released into the air, into water and soil every year (1, 2). With nanotechnological production processes many novel applications progress rapidly by the use of NP (reviewed in 3).

Understanding the possible functional and pathological disorders induced in the respiratory tract by NP requires the investigation of their intracellular localization and the direct effects of these particles on the state and activity of **lung cells**. The toxicity of industrially important representative cerium oxide (CeO_2; frequently used as a catalysator) nanoparticles has already been tested on human cells (4) and on bacteria (5). **However, the occupational risk of human exposure to CeO_2 has not been considered yet.**

→ We have started to work with a completely new approach, simulating an occupational exposure scenario: **Cultures of human lung epithelial cells are exposed to CeO_2 nanoparticles generated by flame spray synthesis in a glove box**

Particle exposure

Cell cultures

- A549 cells were grown on inserts submersed in medium for 7 d to grow to confluence. The cells were then exposed to air for 1d as described [5].

Cell exposure

- The industrially important representative cerium dioxide (CeO_2) NP was chosen [4].
- Exposition of cell cultures within the glove box. The 6-er well plates were placed on a heating plate for the CeO_2 exposition the plates were opened for 10, 20, or 30 min.


Experimental set-up of the production of CeO_2.


Flame spray synthesis of CeO_2.


Exposition of cells.

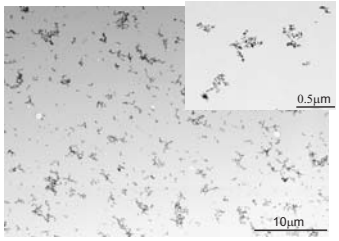
- After exposition the cells were placed for 24 h in the incubator.

Cell analysis

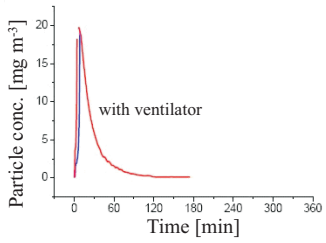
- Before fixation of the cells the transepithelial electrical resistance (TEER) was measured [6].
- After fixation cells were stained for F-Actin, the tight junction protein occludin and 8-oxoguanine.
- A Zeiss 510 META (LSM) was used for imaging, and for image processing and visualization the IMARIS software (Bitplane AG, Zurich, Switzerland).

Results

Particle monitoring

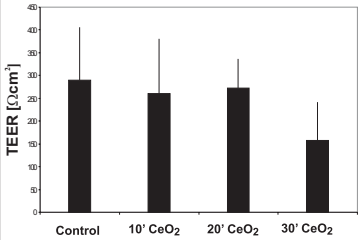


Transmission electron micrography shows a homogenous distribution of agglomerates and nanoparticles after 10 min.



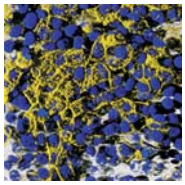
Monitoring of particle concentration inside the glovebox

Epithelial integrity and tight junctions

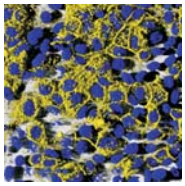


TEER values of control cultures (30 min without particle generation) were between 290 ± 116 (SD) Ωcm^2, whereas in cultures exposed for 30 min. to CeO_2 the TEER values decreased to 156 ± 84 (SD) Ωcm^2.

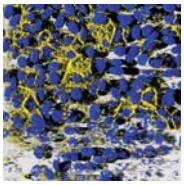
Control
(30' exposure in glove box, But no particles)



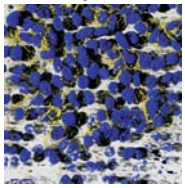
10' CeO_2
≅ $4x10^{12}$ particles/membrane
≅ 0.05mg/membrane



20' CeO_2
≅ $6x10^{12}$ particles/membrane
≅ 0.08mg/membrane



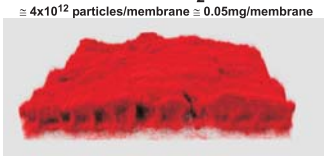
30' CeO_2
≅ $8x10^{12}$ particles/membrane
≅ 0.1mg/membrane


Labelling of the tight junction protein occludin (yellow). Considerable loss in structure of tight junctions was monitored after 30 min of exposure (cell nuclei: blue).

Cellular morphology

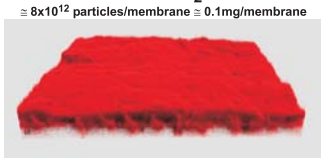
Control
(30' exposure in glove box, but no particles)

10' CeO_2
≅ $4x10^{12}$ particles/membrane ≅ 0.05mg/membrane



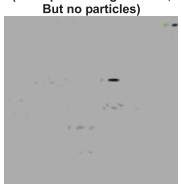
20' CeO_2
≅ $6x10^{12}$ particles/membrane ≅ 0.08mg/membrane

30' CeO_2
≅ $8x10^{12}$ particles/membrane ≅ 0.1mg/membrane

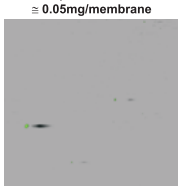

Morphology of the cytoskeleton (F-actin) which was studied by LSM was not affected.

Oxidative DNA damage

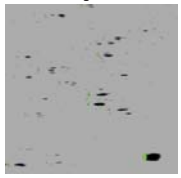
Control
(30' exposure in glove box, But no particles)



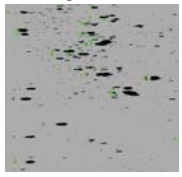
10' CeO_2
≅ $4x10^{12}$ particles/membrane
≅ 0.05mg/membrane



20' CeO_2
≅ $6x10^{12}$ particles/membrane
≅ 0.08mg/membrane



30' CeO_2
≅ $8x10^{12}$ particles/membrane
≅ 0.1mg/membrane


Exposure for 20 min and 30 min with CeO_2 induces DNA damage (8-oxoguanine detection in green by LSM), an indicator for oxidative stress, in the cells.

Conclusions

CeO_2 shows cytotoxic effects:
While cytoskeletal morphology was not affected, epithelial integrity was impaired by nanoparticles in a dose or time dependent manner.

DNA damage:
Induced by CeO_2 nanoparticles after 30min of exposure

Exact simulation of an occupational exposure:
The nanomaterial was made exactly as in the industrial process with the same degree of agglomeration/size/surface coating

➨ **The toxicity of almost any nanoparticle type can be studied with this system in an easy and reproducible way**

Acknowledgements

We thank **B. Tschirren**, **S. Frank,** and **B. Haenni** for their excellent technical assistance.

- **Work was supported by**
- The Federal Office for the Environment
- The Swiss National Science Foundation
- The Silva Casa Foundation
- The Novartis Foundation

Literature

[1] Mazzola L. Commercializing nanotechnology. Nat Biotechnol: 21:1137-43, 2003.
[2] Paull R, Wolfe J, Hebert P, Sinkula M. Investing in nanotechnology. Nat Biotechnol 21:1144-1147, 2003.
[3] Gwinn MR, Vallyathan V. Nanoparticles: Health Effects-Pros and Cons. EHP, doi:10.1289/ehp.8871 (available at http://dx.doi.org/), 2006.
[4] Lin W, Huang YW, Zhou XD, Ma Y.Toxicity of cerium oxide nanoparticles in human lung cancer cells. Int J Toxicol. 2006 Nov-Dec;25(6):451-7.
[5] Thill A, Zeyons O, Spalla O, Chauvat F, Rose J, Auffan M, Flank AM. Cytotoxicity of CeO2 nanoparticles for Escherichia coli. Physico-chemical insight of the cytotoxicity mechanism. Environ Sci Technol. 2006 Oct 1;40(19):6151-6.
[6] Limbach LK, Li Y, Grass RN, Brunner TJ, Hintermann MA, Muller M, Gunther D, Stark WJ. Oxide nanoparticle uptake in human lung fibroblasts: effects of particle size, agglomeration, and diffusion at low concentrations. Environ Sci Technol 39:9370-9376, 2005.
[7] Blank F, Rothen-Rutishauser B, Schurch S, Gehr P. An optimized in vitro model of the respiratory tract wall to study particle cell interactions. J Aerosol Med, 19:392-405, 2006.