Prevalidation of the CULTEX® method:
in vitro analysis of the acute toxicity of inhalable fine dusts and nanoparticles after direct exposure of cultivated human cells from the respiratory tract

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Introduction

The respiratory tract is the main portal to the human body for inhaled particles and toxic substances. Over the last decade, nanomedicine and nanochemistry have developed at a tremendous pace, not without realizing that the safety and risk assessment of nanoparticles will challenge current toxicological approaches. The CULTEX® system provides a method for screening such particles for their cytotoxic and therapeutic potency at an air-liquid interface on epithelial cells. **The aim of the project is to prevalidate the CULTEX® system and method regarding the usefulness as a toxicological screening method in a multi-laboratory study.**

Materials and Methods

In our experiments, we used the cell line A549 (ATCC: CCL-185). A549 cells are from human lung carcinoma tissue from a 58-year old Caucasian man, in which the tumor cells exhibit features of alveolar type II cells, but on account of the production of mucins also show characteristics of bronchial cells. For the CULTEX® system, A549 cells were seeded on transwells with a semipermeable membrane which allows the separation of two compartments (Figure 1).

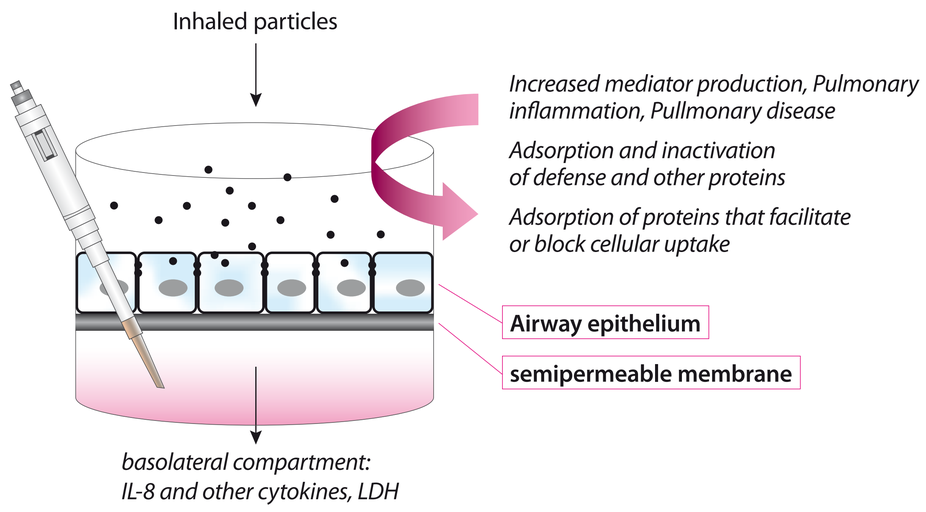

Figure 1: A549 cells on a transwell, which allows the separation of the apical and the basolateral compartment

To generate a test atmosphere, we used the CULTEX® Radial Flow System (RFS) module which allows a dose-dependent exposure via a central inlet, from which three radial tubes guide the atmosphere to the inserts. During the experiments, cells were fed with medium basolaterally and maintained at 37 °C by the integrated water heating circuit. Particles were generated with the dust generator over a rotating scraper. The elutriator, integrated into the system, retains bigger particles and serves as a reservoir for a uniform and ideal particle-containing atmosphere (Figure 2). A549 cells were seeded into 6-well transwell inserts (BD Falcon) for 24 h. Immediately before the experiment, cells in the apical well were subject to an air-liquid interface and could thus directly contact the test atmosphere.

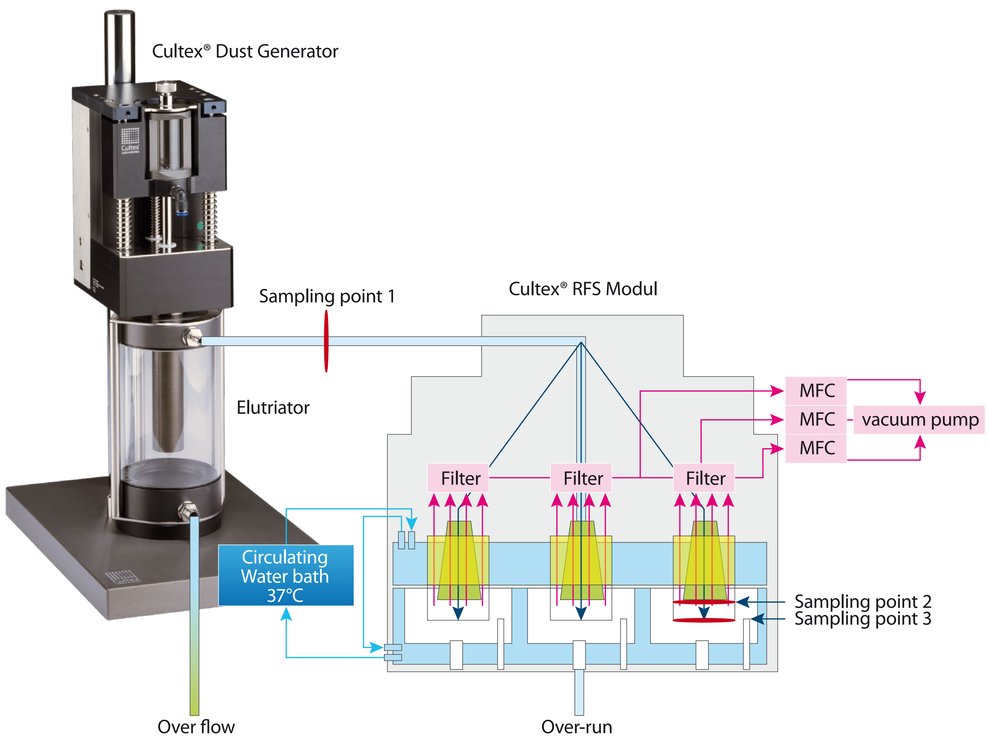

Figure 2: Schematic assembly of the experimental setup for the air-liquid interface exposure

Via the individual medium supply for all three inserts and the separation through the semipermeable membrane into the two compartments, measurement of different endpoints like the morphological behavior of the cells or the analysis of biological markers are possible.

In our study, we expose the cells in the system in three independent laboratories to different particles to prevalidate this system. In the different labs, up to twelve different nanoparticles will be tested and the within- and inter-laboratory reproducibility investigated.

Particles to be tested are, among others, DQ12, TiO2-P25, CB14, ZnO, BaSO4, ALOOH I, CeO2, ZrO2, and CuO. Endpoints to be determined in all three labs are the cytotoxicity via the proliferating assay WST-1 and the LDH leakage. Proinflammatory markers such as IL-8 could be measured in the medium below the membrane.

Results

The first results which could be reproduced independently in all three labs are presented in the following figures. A549 cells were seeded on filter membranes and exposed in the CULTEX® system with a CuO atmosphere. Control filters with clean air treated in the same way as for the CuO atmosphere and incubator controls which were placed in an air-liquid interface were determined in each experiment.

After exposure to the test atmosphere, cells were cultivated for a further 24 h period at 37 °C and 5 % CO_2 before the WST-1 assay was performed and the supernatants were taken for the other tests.

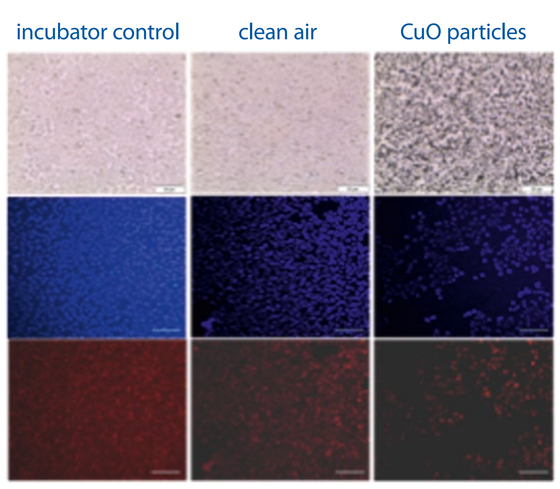

Figure 3: Microscopic images of the cells after 1 h treatment with the control cells and the CuO particles. Immunofluorescent staining shows the A549 cells on the membranes with the proliferation marker, Ki-67 (red fluorescence), and the parallel images of the nuclear counter stain (in blue) (scale bar = 50 µm).

The viability of the cells was measured with the WST-1 assay. After exposure to the test atmosphere, cells were cultivated for a further 24 h period at the air-liquid interface before the proliferation assay was performed. After 1 h incubation time, the amount of reduced WST-1 was quantified at 450 nm (Figure 4). Although the incubator control and the clean air provided comparable results at all time points, the vitality of the CuO-treated cells decreased after 1 h incubation time, thus confirming the findings from the immunofluorescence-labeled cells in Figure 3.

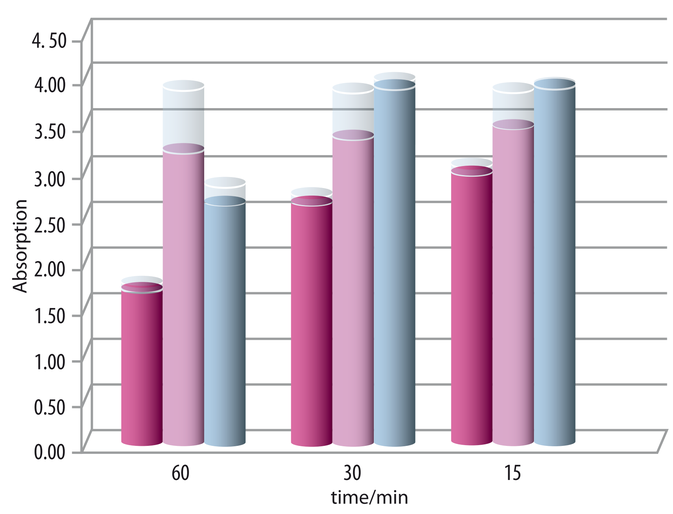

Figure 4: Cytotoxic effects of CuO particles in the test atmosphere on a cell layer of A549 in the WST-1 assay. (CuO: magenta bar, clean air: light magenta bar; incubator control: blue bar). Data are depicted as means ± S.D. from n = 4 independent experiments.

Inflammatory markers such as IL-8 were measured in the medium below the membrane and the release of IL-8 increased in comparison to the controls (Figure 5).

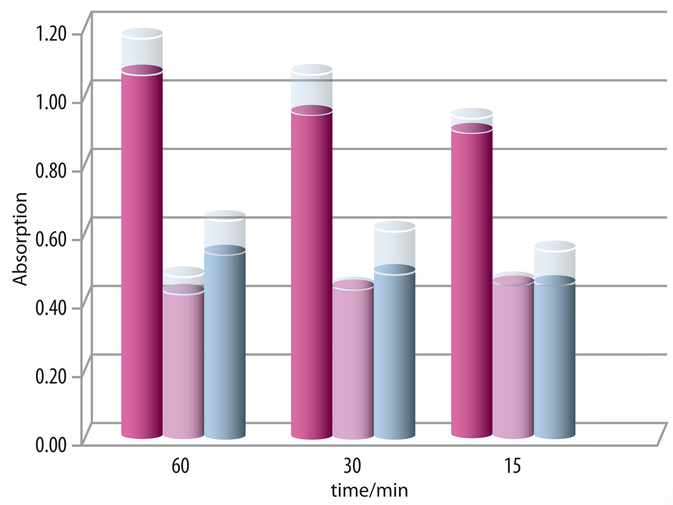

Figure 5: Proinflammatory release of IL-8 after exposure with the different atmospheres. (CuO: magenta bar, clean air: light magenta bar; incubator control: blue bar). Data are depicted as means ± S.D. from n = 4 independent experiments.

Conclusions and Outlook

- Drug delivery via the respiratory tract is a possibility for systemic therapy.
- The main focus was to create a test atmosphere which approximates the in vivo situation and which could be useful for safety and risk assessment of nanoparticles and current toxicological approaches.
- It is hoped to validate a system which could be a possibility for toxicological drug screening and to provide an opportunity to reduce the number of animals in drug research and development.

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