Prevalidation of the CULTEX® method (BMBF project 0315710):

The air-liquid interface exposure of human lung cells in the CULTEX® Radial Flow System (RFS)

N. Möhle[1], D. Steinritz[2], C. Pohl[3], Sebastian Hoffmann[4], M. Aufderheide[1]

[1]Cultex Laboratories GmbH, Hannover Medical Park, Hannover, Germany, [2]Institute of Pharmacology and Toxicology, Bundeswehr München, Germany and Walther-Straub-Institute of Pharmacology and Toxicology, LMU Munich, Germany, [3]Johannes Gutenberg University, Institute of Pathology, Mainz, repair-lab, Germany, [4]seh consulting + services, Paderborn, Germany

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Introduction

The respiratory tract is the main portal of entry to the human body for inhaled particles and toxic substances. Over the last decade, nanotechnology has developed at a tremendous pace, although the safety and risk assessment of nanoparticles is a great challenge for current toxicological approaches. The EU regulation REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) thus demands the development and implementation of alternative methods to examine these substances for potential hazardous effects. In the field of inhalation toxicology, a variety of different in vitro models have been developed for screening and mechanistic studies. However, these approaches are mainly performed with submerged cultures. In order to simulate the in vivo situation in a realistic manner, an adequate exposure device which enables the direct exposure of cultivated lung cells at the air-liquid interface (ALI) is necessary. The CULTEX® Radial Flow System fulfills these requirements and is subject of a prevalidation study (BMBF project number 0315710), designed to demonstrate the stability, robustness and reproducibility of the exposure of cells to aerosolized suspensions, gases, volatile compounds and especially micro-/nano-sized particles by guiding the test atmosphere directly to the surface of the cell cultures (air-lifted cultures).

Using CFD (Computational Fluid Dynamics) analyses, the aerosol flow within the system was simulated and thus the technical design was optimized in order to realize a stable, reproducible and homogeneous deposition of airborne particles. The efficiency of the exposure procedure is demonstrated by exposing A549 cells to different micro- and nano-sized particles with varying dose.

Materials and Methods

For exposure experiments, the A549 cell line was used, which originate from human lung carcinoma tissue from a 58-year old Caucasian man. These cells exhibit features of alveolar type II cells, but on account of the production of mucins also show characteristics of bronchial cells. For the exposure in the CULTEX® system, A549 cells were seeded on cell culture inserts with a microporous membrane which allowed the separation of two compartments (Figure 1).

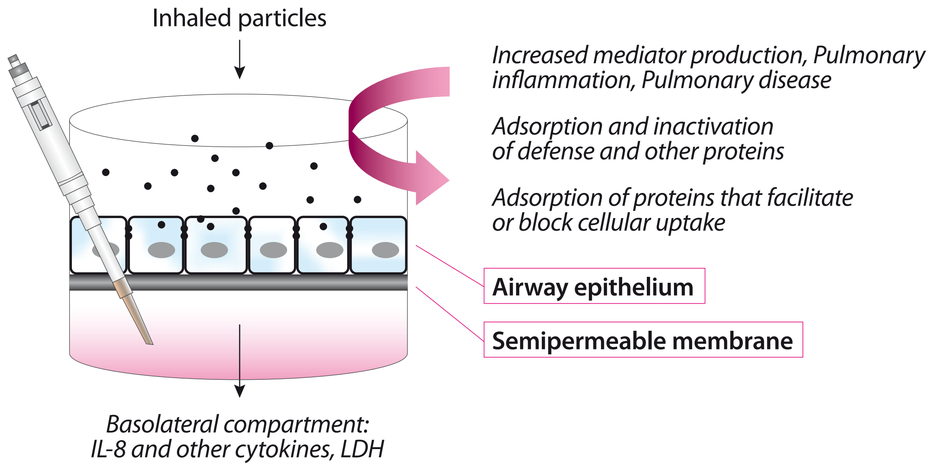

Figure 1: A549 cells on a cell culture insert, which allows the separation of the apical and the basolateral compartment

The CULTEX® RFS allows a dose-dependent exposure via a central inlet from which three radial tubes guide the atmosphere to the cell culture inserts. During the experiments, cells were fed with medium from the basolateral side and tempered at 37 °C by the integrated water heating circuit. A particle containing aerosol was generated with the CULTEX® DG – Dust Generator over a rotating scraper and 8 l/min compressed air (DIN 12021). The elutriator, integrated into the system, retains larger particles and serves as reservoir for a uniform aerosol (Figure 2). A549 cells were seeded on 6-well cell culture insert (BD Falcon™) and after 24 hours exposed at the air-liquid interface with direct contact to the test atmosphere.

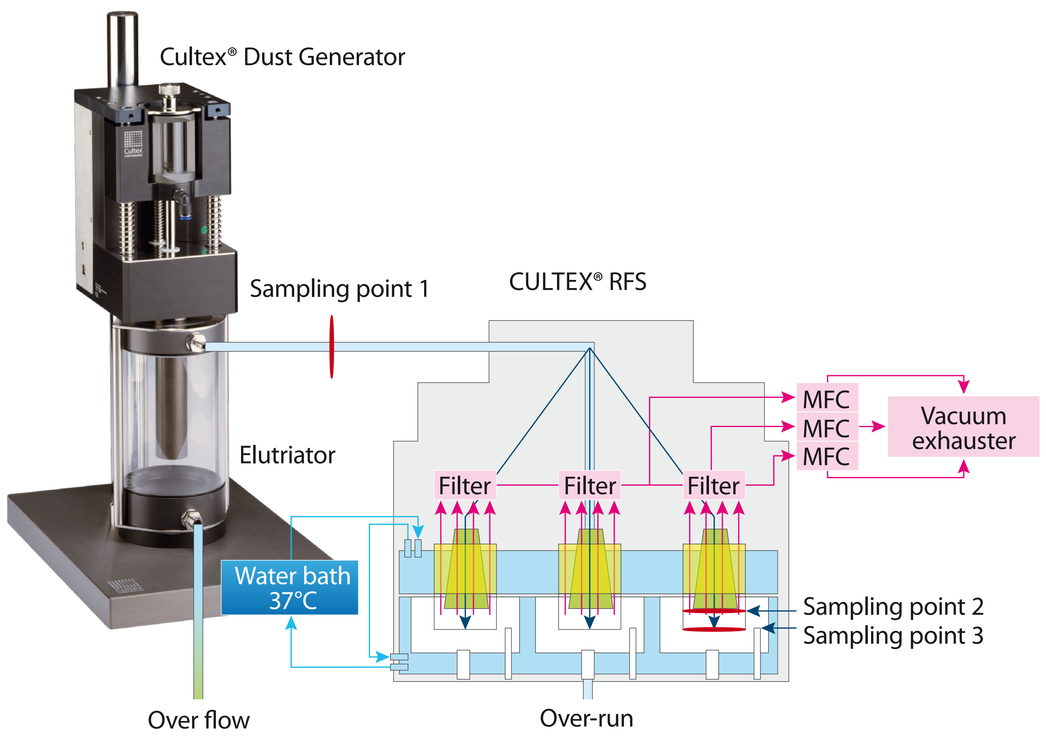

Figure 2: Schematic assembly of the experimental setup for the exposure at the air-liquid interface.

For prevalidation of the system, the exposure experiments were performed in three independent laboratories with different particles. Eleven different test substances were tested and the within- and between-laboratory reproducibility was investigated. Among others, lactose monohydrate, Aeroxide® TiO2-P25, Printex 90®, copper(II) sulphate and copper(II) oxide were tested. The cytotoxic potential of the test substances was determined by the WST-1 assay in all three labs.

In principle, ALI cultures offer the possibility to analyze a variety of biological endpoints adressing morphological alterations, cytotoxicity, biochemical and functional changes, modifications in the cytoskeleton as well as the analysis of molecular biological effects.

Results

Computational Fluid Dynamic (CFD) simulations were calculated to characterize the distribution of the chambers and the deposition rate by analyzing the behavior of the particles within the CULTEX® Radial Flow System. The pathway of 50 particles was tracked from each of the three chambers to the beginning of the inlet tube behind the aerosol generator. The data demonstrated that the particles in each chamber came from a defined region within the inlet tube, that an homogenous distribution depends on an evenly distributed aerosol (Figure 3a). A flexible nozzle was installed in the inlet adapter in order to ensure an improved distribution over the three chambers, even for uncharacterized aerosols, aggregates or agglomerates (Figure 3b).

Measurements of the deposited mass of various powder aerosols showed that the nozzle reduced the variations between the chambers constantly to less than 3 – 4 %. Especially for the exposure with powder aerosol, this is a substantial improvement to the older linear glass modules (25 – 30 %) or the CULTEX® RFS without nozzle (8 – 9 %).

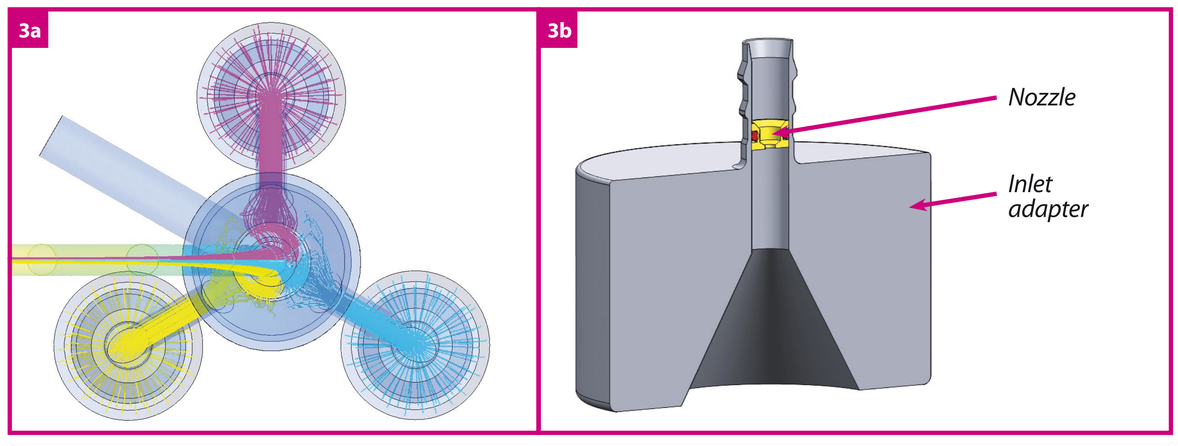

Figure 3a: Computational Fluid Dynamics (CFD) simulation with 50 particles starting in each chamber of the CULTEX® RFS.
Figure 3b: Inlet adapter for the CULTEX® Radial Flow System with integrated nozzle.

The viability of the cells was measured with the WST-1 assay. After exposure to the test atmospheres, the cultures were cultivated for another 24 h at the air-liquid interface before the proliferation assay was performed. After 30 min incubation time, the amount of reduced WST-1 was quantified at 450 nm. The results were normalized as percentage of the cell viability determined for the clean air control (Figure 4).

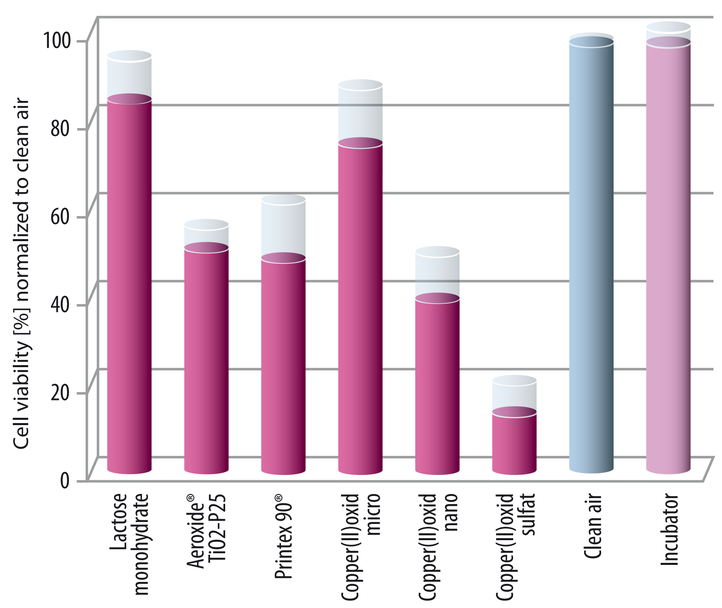

Figure 4: Cytotoxic effects of particles in the test atmosphere on A549 cells, determined by the WST-1 assay. The cell viability is shown as percentage of the clean air control. Data are depicted as means ± S.D. from n = 4 independent experiments.

Conclusions and Outlook

- The exposure setup, cell culture work, particle generation and handling of the substances within the three labs was optimized and harmonized during the project. Standard Operating Procedures for all these processes were defined.
- The CULTEX® Radial Flow System, the particle generation and the test substances were characterized prior to the actual exposures. The particle size and mass distribution within the aerosol as well as the deposited mass (defined dose 180 ng per cell) on the membranes were determined in advance.
- The results clearly demonstrated intra- and inter-laboratory variations between the different test substances.
- The advantages of the CULTEX® method, including the CULTEX® RFS, for the air-liquid interface exposure and the intensive characterization of the parameters were shown within the project.
- Additional test substances and end points are planned for the second phase of the prevalidation.

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