The CULTEX® RFS Compact:

The Next Generation of CULTEX® Systems for the Direct Exposure of Cells at the Air-Liquid Interface

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Introduction

Over the last decades the atmospheric pollution showed a tremendous pace. The increasing concentration of airborne particles leads to a higher burden for the respiratory tract resulting in acute or chronic effects. The influence of inhalable substances is generally determined in animal experiments. Only a few alternative strategies to investigate the effects of such chemicals have been developed so far.

A method for the direct exposure of cells at the air-liquid interface is for example the CULTEX® Radial Flow System (CULTEX® RFS). The cells are exposed to a constant flow of atmosphere from the apical and the nutrient medium from the basal side.

The newest generation, the CULTEX® RFS Compact is designed for the parallel exposure of three cell culture inserts to test atmosphere and three to carrier gas (or six to test atmosphere/clean air). The special construction of the inlet in combination with the radial distribution system guarantees a homogenous distribution into the chambers. An electric heating system ensures optimal conditions for the cells even for longer exposure times. The suitability of this system is demonstrated by dose-response investigations using different atmospheres. The dose-dependent cytotoxicity of the test material was verified after different exposure times. The high reproducibility of the results indicate the reliability of the new system.

Materials and Methods

The modular design of the CULTEX® RFS Compact allows a dose-dependent direct exposure via a central inlet from which six radial tubes guide the atmosphere to the chambers with the cell culture inserts (6.5 mm or 12 mm membrane diameter). High-end mass flow controllers (Bronkhorst Mättig) are used to control the flow within the system and through each of the chambers. The unique design of the CULTEX® RFS Compact and the precise regulation of the atmosphere flow guarantee excellent and highly reproducible results.

The integrated heating device provides a constant temperature of the cell culture medium even under unsteady conditions. An overflow tube ensures a controlled medium level under each of the cell culture inserts and therefore guarantees reproducible conditions for the cells during the exposure.

For a higher stability and comfortable handling, the system is integrated into a rack with a specially designed locking device. All parts that get in contact with the test atmosphere or the cell culture medium are tested for cell compatibility and are fully autoclavable.

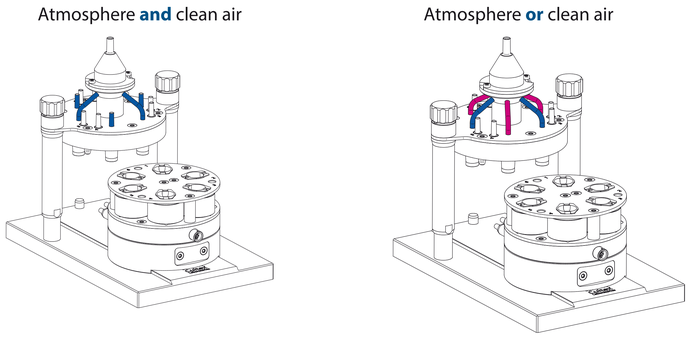

Figure 1: CULTEX® RFS Compact with two different distributors.
Left picture: System for the parallel exposure of three cell culture inserts to test atmosphere and three to clean air.
Right picture: System for the exposure of six cell culture inserts either to test atmosphere or clean air.

In our experiments we used the cell line A549 (ATCC: CCL-185) and isolated primary normal human bronchial epithelial (NHBE) cells.

For the exposure in the CULTEX® RFS Compact, the cells were seeded into 12-well cell culture insert (COSTAR®/Corning) for 24 h and exposed at the air-liquid interface with direct contact to the atmosphere. During the experiments, cells were fed with medium basolateral and tempered at 37 °C by an integrated electric heating system.

Cigarette smoke (whole smoke/gas vapour phase – K3R4F) and a 1.6 % nicotine liquid with tobacco flavour (electronic cigarette) were used as test atmospheres to characterize the system.

Results

The viability of the cells was measured with the WST-1 assay. For the e-cigarette exposed cells, the oxidative stress level in the cells was additionally analyzed. Both assays were performed 24 hours post-exposure. The results were normalized to the values determined for the clean air control (figure 2 and figure 3). Significant differences between two groups were evaluated by Student's unpaired t-test, whereas the symbols (asterisks) are defined as followed:

**** p < 0.0001 extremely significant, *** p = 0.0001 – 0.001 extremely significant,
** p = 0.001 – 0.01 very significant, * p = 0.01 – 0.05 significant

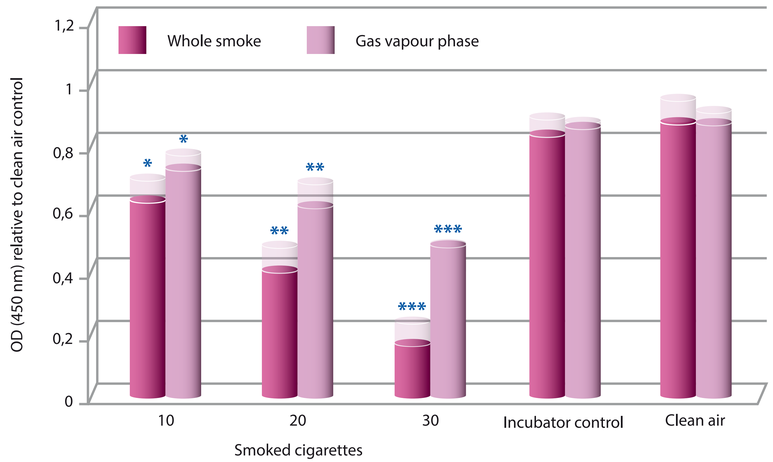

Figure 2: Cytotoxic effects of cigarette smoke (whole smoke/gas vapour phase) on a cell layer of A549 in the WST-1 Assay.
The results were normalized to the values determined for the clean air control.
Data are given as means + S.D. from 3 independent experiments with 3 samples each.

Mainstream smoke of 10 and more cigarettes reduced the viability of A549 cells significantly compared to clean air exposed cells. The viability decreases with increasing numbers of smoked cigarettes, whereas the effects of whole mainstream smoke are even greater than those of the gas vapor phase. Clean air exposed cells show no viability reduction compared to cells remained air-lifted in the incubator.

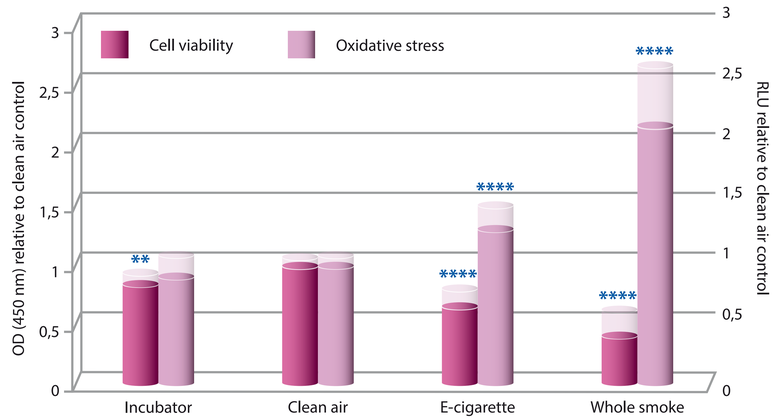

Figure 3: Cytotoxic effects and oxidative stress response of NHBE cells after the exposure to cigarette smoke and a 1.6 % nicotine liquid with tobacco flavour (electronic cigarette).
The results were normalized to the values determined for the clean air control.
Data are given as means + S.D. from 3 independent experiments with 3 samples each.

In NHBE cells, the effects regarding cell viability and oxidative stress level are opposed. The exposure to e-cigarette liquid leads to reduced cell viability and an increase in the oxidative stress level. Mainstream smoke exposure results in even lower cell viability and higher oxidative stress. The clean air control cultures show higher oxidative stress than the incubator control cultures, explainable by the mechanical stimulation caused by the airflow above the cells. However, the cell viability of incubator and clean air control are not significantly different.

Conclusions and Outlook

- The CULTEX® RFS Compact is a reliable system for the direct exposure of six 6.5 or 12 mm cell culture inserts to complex mixtures and gases.
- The CULTEX® RFS Compact allows the parallel exposure of three cell culture inserts to the test atmosphere and three to clean air (carrier gas).
- The present data show an excellent dose-effect-dependency and a high reproducibility.
- The next step will be the further characterization of the system with larger variety of substances and the determination of the effective dose.

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