Pre-validation of the CULTEX® system

assessing the acute inhalation toxicity of nanoparticles by direct exposure of cells via the air-liquid interface

Sebastian Hoffmann[1], Niklas Möhle[2], Mirko Papritz[3], Christine Pohl[4], Michaela Aufderheide[2]

[1] seh consulting + services, Köln, Germany, [2] Cultex® Laboratories GmbH, Hannover, Germany, [3] Institute of Pharmacology and Toxicology, German Armed Forces Munich, Germany, [4] Institute of Pathology, Repair-Lab, University Medical Center, Johannes Gutenberg University Mainz, Germany

Cultex® Laboratories
The Airborne Exposure Experts

REPAIRlab. MAINZ

Sebastian Hoffmann consulting + services

Introduction

Over the last decade, nanochemistry has developed tremendously, not without realising that the safety and risk assessment of nanoparticles will challenge current toxicological approaches. As for many nanoparticles, the respiratory tract is the main portal to the human body, and the inhalation exposure route requires special attention. Therefore, the CULTEX® Radial Flow System (RFS) has specifically been designed to model inhalation *in-vitro*. Furthermore, aerosol generation, particle distribution and deposition have also been taken into consideration, in order to optimize and standardize the *in-vitro* exposure of cells at the air-liquid interface.

Objectives

The aim of the research project is the reduction of animal studies in the field of acute inhalation toxicology by a standardized *in-vitro* direct exposure method for studying particulate atmospheres.

In this pre-validation study, the 4 modules of the modular approach, which primarily focus on assay reliability, are as follows:

1. Test definition
SOPs have been developed for cell culturing and handling, exposure-related methods and procedures as well as for analytical methods like the WST-1 (cytotoxicity) and LDH assay (membrane integrity).

2. Transferability
Identical laboratory setups have been installed (e.g. CULTEX® RFS module, particle press, aerosol generator) in the 3 laboratories. The two unskilled laboratories have been trained in handling the instruments and performing the experiments.

3. Within-laboratory reproducibility
Currently, the first substances are being tested. The overall analysis of all test substances will be performed threefold in all laboratories. The within-laboratory reproducibility will be assessed independently.

4. Inter-laboratory reproducibility
Based on the data on all substances of the 3 laboratories the inter-laboratory reproducibility will be assessed independently.

Nanoparticle selection

In total, 12 nanoparticles have been selected, e.g. DQ12, TiO2-P25, CB14, ZnO, BaSO4, ALOOH I, CeO2, ZrO2 and CuO. The selection has been based on availability and quality of toxicological animal data from inhalation studies as well as availability of samples. As the nanoparticles show very different behaviour when being compressed for use in the CULTEX® Dust Generator, substance-specific compressing procedures had to be developed and transferred to the other laboratories.

The CULTEX® Radial Flow System

The CULTEX® RFS module distributes the generated test aerosol via a central inlet into 3 radial tubes directly to 3 transwell inserts (Fig. 1) for dose-dependent exposure of cultivated cells at the air-liquid interface (ALI). During the experiment, the cells are supplied with medium from below the membrane. An integrated water heating circuit maintains the temperature constantly at 37 °C. Particles are generated in the specially developed CULTEX® Dust Generator according to Wright (1955), connected with the elutriator. Here, the larger particles are retained whereas the air-transported fraction provides the particle test atmosphere.

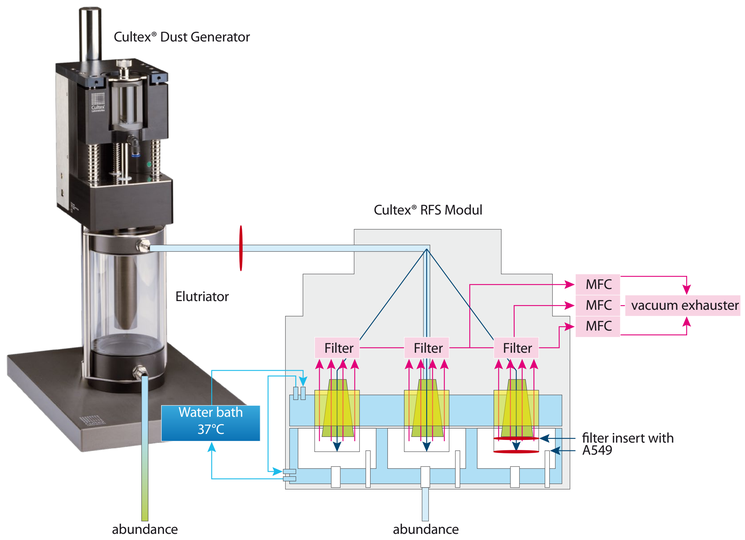

Figure 1: Scheme of the experimental setup

In the study, A549 cells (ATCC CCL-185) are used, showing features of alveolar type II and bronchial epithelial cells. They are seeded on the surface of semipermeable transwell membranes, which can be exposed directly on the apical side (ALI) (Fig. 2).

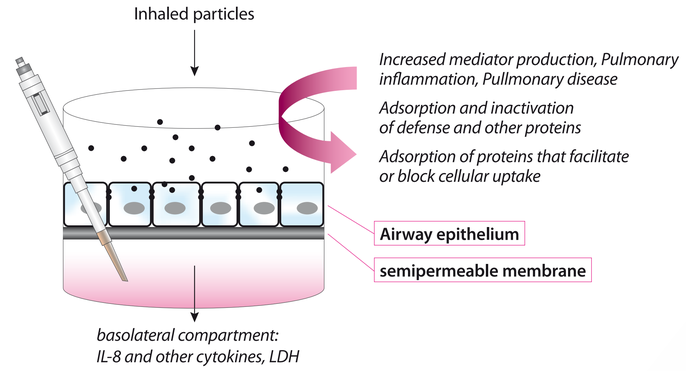

Figure 2: A549 cells on a transwell

The pre-validation team

Three laboratories and an independent statistician form the team of this pre-validation study, which is funded by the German Ministry of Education and Research (BMBF grant 0315710):

- **Cultex® Laboratories GmbH**
  Co-ordinating and lead laboratory as well as provider of the Cultex® technology.
- **Institute of Pharmacology and Toxicology, German Armed Forces, Munich** and
- **Institute of Pathology, Repair-Lab, University Mainz**
  These institutes are well experienced in the field of inhalation toxicology *in-vitro* and represent the unskilled laboratories in this study.
- **seh consulting + services**
  This competent partner in the validation process is responsible for data handling, assessment and supports the study management.

Study progress

Activity	(tentative) date
Development of the SOPs	Sep 2010 – Mar 2011
Transfer	Sep 2010 – Jul 2011
Training & transferability	Jan 2011 – Sep 2011
Testing of nanoparticles	Oct 2011 – Apr 2012
Data analysis	May 2012 – Aug 2012

www.cultex-laboratories.com