Use of Computational Fluid Dynamics for optimization of cell-based in vitro methods in inhalation research

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Introduction

In order to understand effects of inhalable substances in the lung, it is reasonable to investigate these substances using biological models. Until recently tests for this purpose had to be performed mainly in-vivo with experimental animals. Due to ethical and political, but also scientific reasons, alternative **in-vitro test methods** are of increasing importance. At the Fraunhofer Institute ITEM, a testing procedure based on an **air-liquid interface** culture technology was developed for this purpose.

By simultaneous realization of (1) an efficient exposure alignment (stagnation flow), (2) an efficient particle deposition from aerosols, (3) complete work in standard consumable multiwell-plates (4) the possibility to observe the biological effects during exposure non-invasively (live fluorescence staining) and (5) technically safeguarding the relevant route of airborne exposure exclusively, these limitations are specifically addressed and improved for exposure of ALI cultures to airborne material in the **P.R.I.T.® ExpoCube®.**

Objectives

In cooperation with Fraunhofer Institute SCAI this in-vitro testing procedure was revised by applying **Computational Fluid Dynamics (CFD)** for the layout of the **aerosol conduction** and an **aerosol sampling box**.

The aim of the study was to characterize and improve:

(1) the deposited amount of test aerosols on the cultured human cells without harming the exposed tissue (thermophoresis technique),

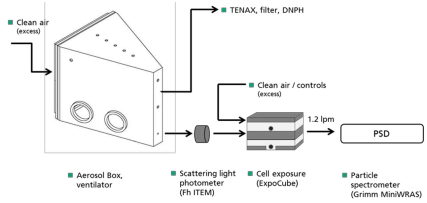
(2) and furthermore to increase the amount of aerosols extracted from a sampling box.

Simulation Set-up

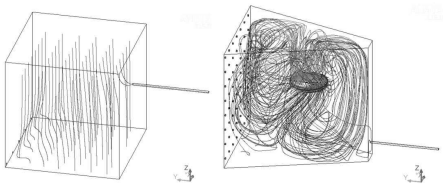
- Carrier fluid air: incompressible, laminar, not influenced by particles, heat distribution, stationary flow
- Aerosols: spherical particles Ø10nm – 3000nm, Lagrangian approach, thermophoresis, Brownian motion, gravity, Stokes-Cunningham drag
- Sampling Box Propeller: Frame Motion (Frozen Rotor), turbulent (k-ε enhanced wall)

Simulation Results

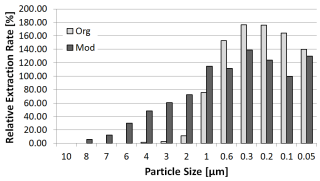
Aerosol Sampling Box - Improvements



Aerosol Box Set-up

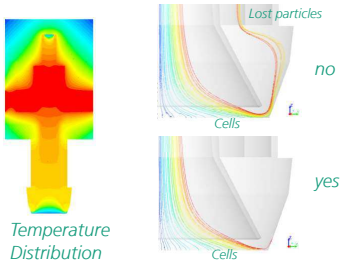


Exemplary Particle Tracks Original vs. Modified Design



Improved Sampling of Larger Particles

ExpoCube – Thermophoresis

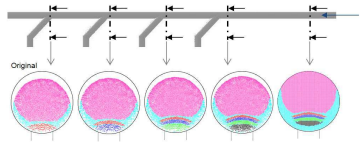


Temperature Distribution

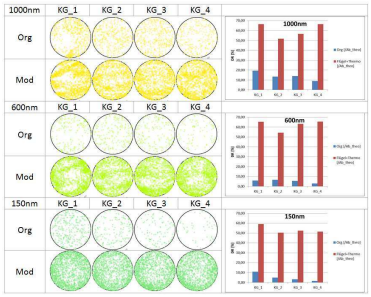
Thermophoresis

P.R.I.T. ExpoCube®

ExpoCube - Branching and Deposition of Aerosols

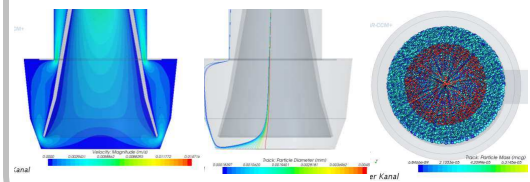


Particle Distribution in Supply Pipe



Improvement of Deposition on Cell Membranes

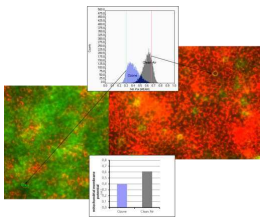
ExpoCube – Particle Deposition Device



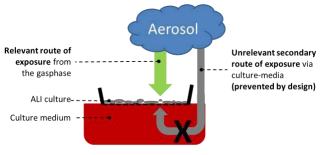
Velocity *Particle Tracks* *Particle Imprint*

ExpoCube® Features

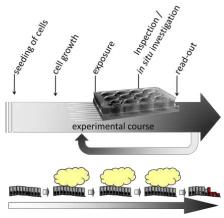
- **Effective particle deposition** also for small particles > 1 µm
- **Handling and testing** quality is clearly **improved** by use of commercial consumables.
- The **compactness of the design** and a large range of applicability improve the *in vitro* potential to test inhalable materials in a relevant way.
- Cell-based *in vitro* testing using ALI cultures can be applied to a **range of different testing materials** and testing scenarios including gases, aerosols an complex mixtures (gaseous compounds, volatile organic chemicals, aerosols from nebulized liquids, aerosolized particles and aerosols generated by consumer products)
- *Ex vitro* testing of inhalable compounds using **precision cut lung slices (PCLS).**
- **Online observation** of exposure effects during exposure by live fluorescence stains.



Observation of cells during exposure by fluorescence



Prevention of irrelevant secondary route of exposure



Exposure of ALI-cultures in consumable labware

Conclusions

- The simulation results gave a meaningful **insight into the aerosol particle behavior** and provided ways to **enhance the amount of deposited particles** on the cell cultures through geometrical and physical modifications. The enhanced constellation was confirmed in experiments.
- For the purpose of generating custom-designed particle samples an aerosol extraction box was reworked resulting in a **broader spectrum of sampled particle sizes**.

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