Biological effects of inhalable compounds – improvements of the *in vitro* testing method

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Background

The air-liquid interphase (ALI) cell culture technology is the state-of-the-art method for *in vitro* testing of airborne substances. Round-robin prevalidation studies with model gases demonstrated the sensitivity and relevance of the basic technique in principle. Until now major limiting factors still exist including robustness, practicability, applicability and efficacy of the method regarding aerosol applications.

Objectives

Improvement of the ALI technique concerning

(1) integration of the complex exposure process in a smooth workflow,
(2) incorporation of a cell culture model with an artificial lung surfactant,
(3) deposition of airborne particulate matter for aerosol testing.

Approach

Integration of the complex exposure process in a smooth workflow

Design of a user friendly process. For work with inhalable compounds comparable to work with water soluble substances, typically used culture membranes in standard multiwell plates are integrated.

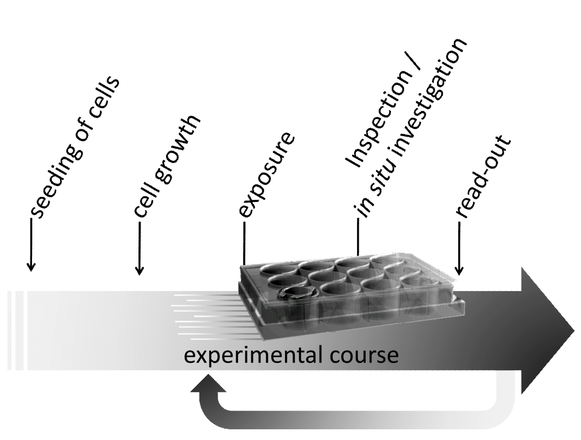

Figure 1
"All in one plate" optimized workflow

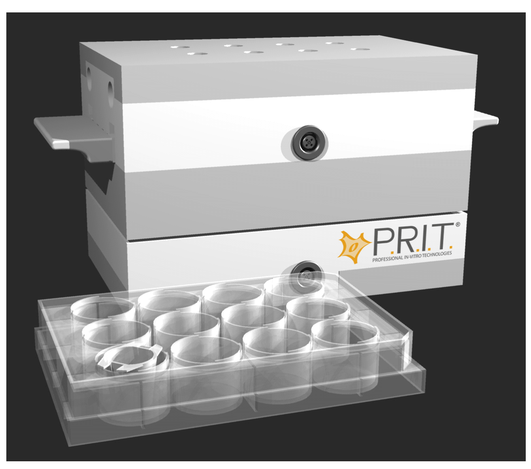

Figure 2
Exposure device "ExpoCube"

Cell culture model with an artificial lung surfactant

A549 cells were pre-cultured and exposed at the ALI without moving the permeable filter supports throughout the whole process from the corresponding commercially available multiwell plates. One group of ALI cell layers was covered with an artificial lung surfactant ([1]Jalava et al. 2013) before use. Exposures were carried out to synthetic air or ozone (5.2 ppm) for 1hr. Corresponding plate controls exposed to ambient experimental conditions only were processed in the same manner. Cell viability was quantified by measuring the amount of living cells using an electronic cell counter directly after exposure.

Deposition of airborne particulate matter for aerosol testing

As a new strategy for optimization of particle deposition in stagnation flow cell exposure, thermophoresis was applied additionally to gravitational settling and diffusion by generating a thermal gradient between the aerosol and the cellular surface.
Numerical simulations (CFD) were applied for the optimization of the particle transportation and deposition using [2]STAR-CCM+ and [3]FLUENT software packages.

[1]Jalava, Pasi, Jan Knebel, Detlef Ritter, Building up a co-culture and surfactant model for routine use in ALI-exposures, Poster SSCT-Workshop 2013
[2]STAR-CCM+ is registered trademark of CD-adapco
[3]FLUENT is registered trademark of ANSYS, Inc.

Results

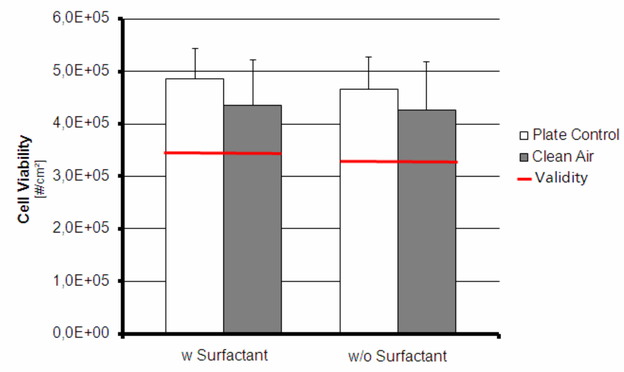

Figure 3

Cells were successfully exposed under these conditions without significant loss of viability.
Artificial lung surfactant did not decrease the viability of the cells.

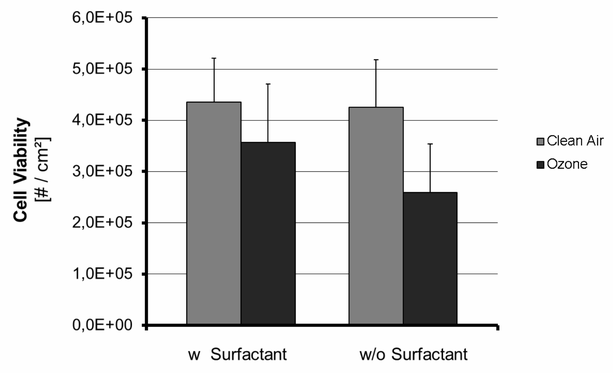

Figure 4

Artificial lung surfactant had protective effects on ozone exposed cells in the *in vitro* system as it is known from lung surfactant *in vivo.*

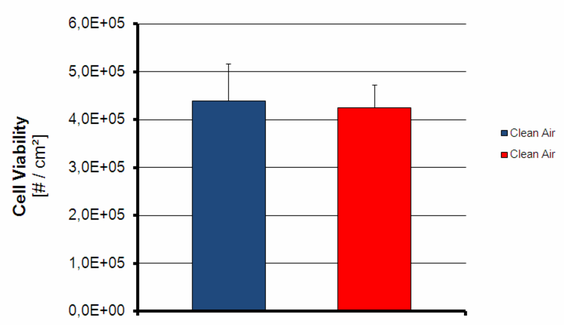

Figure 5

Appliance of thermophoretic exposure conditions did not affect the cellular viability in clean air exposures

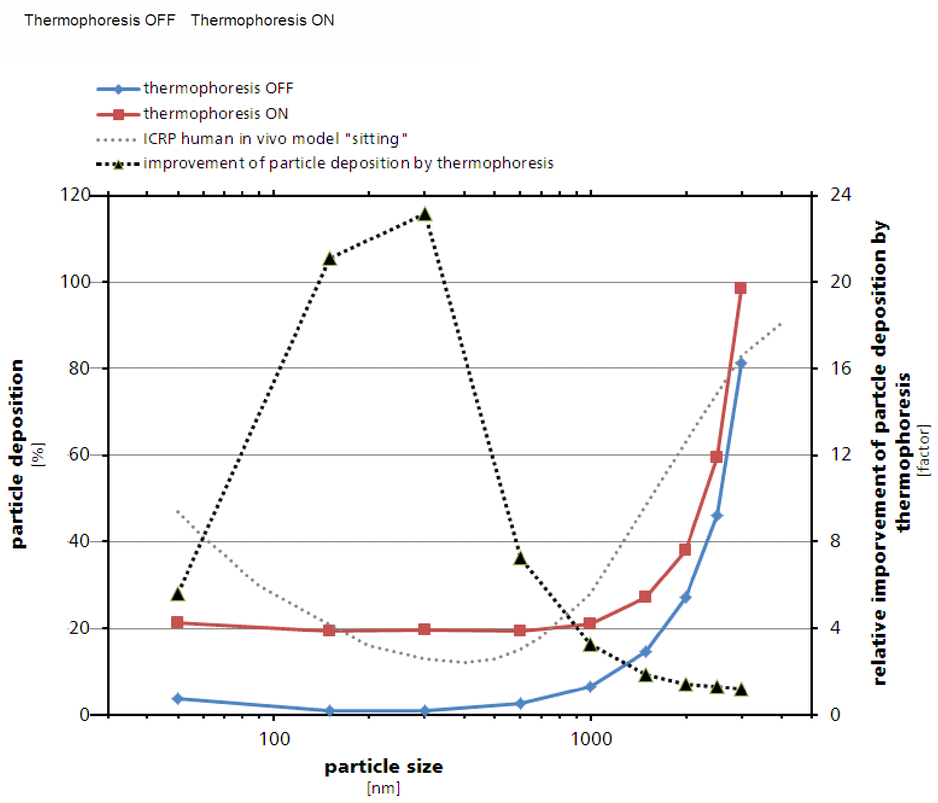

Figure 5

Results of CFD simulations indicate an increased particle deposition by thermophoresis in the exposure system which clearly **improves the comparability of the deposition characteristics from *in vitro* to *in vivo* conditions** for relevant particle sizes between 50 nm and 3000 nm

Conclusion

- "All in one plate" optimized workflow in an effectiv ALI exposure design
- Functional cell model including artificial lung surfactant
- Improved particle deposition characteristics by thermophoresis as indicated by CFD simulations

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