Evaluation of biological effects of airborne material using an improved cell-based *in vitro* approach

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Introduction

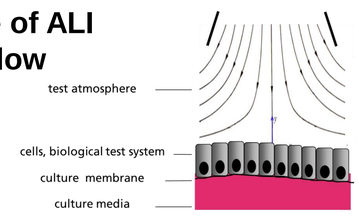
In vitro approaches for evaluation of biological effects of airborne materials are under development for several years. Meanwhile, biological test systems including cell-lines, primary cultures or *ex vivo* tissues such as PCLS and air-lifted interface (ALI) cultures are used for these purposes. However, until now, there have been still limitations with regard to the applicability and relevance during exposure of ALI cultures towards airborne material. These limitations for airborne testing lead to extended and unfavorable practical needs compared to usual work with liquid test compounds. Therefore, they represent a clear obstacle for harmonization, standardization, further dissemination and faster development of *in vitro* methods for testing inhalable compounds in their airborne state.

Objectives

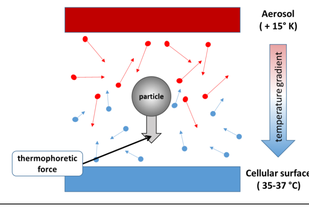
By simultaneous realization of (1) an efficient exposure alignment (stagnation flow), (2) an efficient particle deposition from aerosols without harming the exposed tissue (thermophoresis technique), (3) complete work in standard consumable multiwell-plates (4) the possibility to observe the biological 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®.

Results

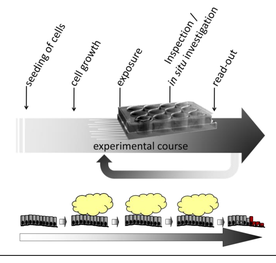
Efficient individual exposure of ALI cultures in a stagnation flow alignment



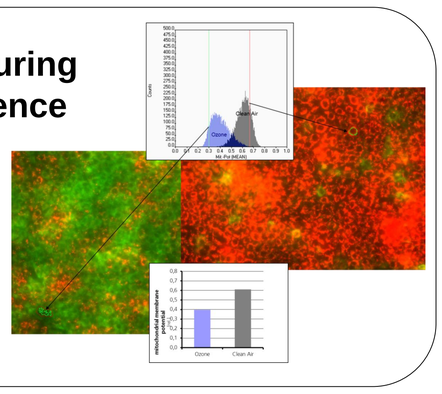
Thermophoresis for efficient particle exposure from aerosols



Exposure of ALI-cultures in consumable labware

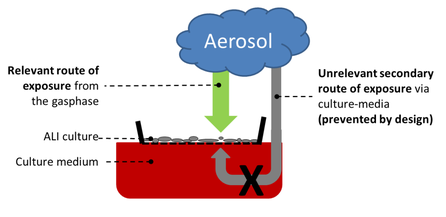


Observation of cells during exposure by fluorescence



P.R.I.T. ExpoCube®

Prevention of irrelevant secondary route of exposure



Efficient deposition of small particles from aerosols

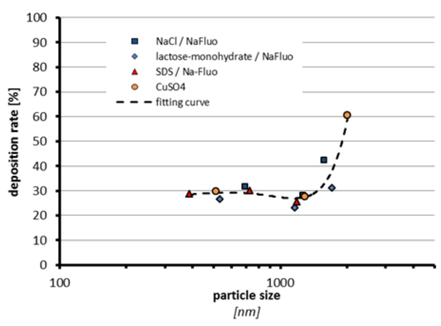


Figure 1: particle deposition rates from different aerosols in the size range below 2000 nm

Acute toxicity testing of volatile organic / gaseous compounds

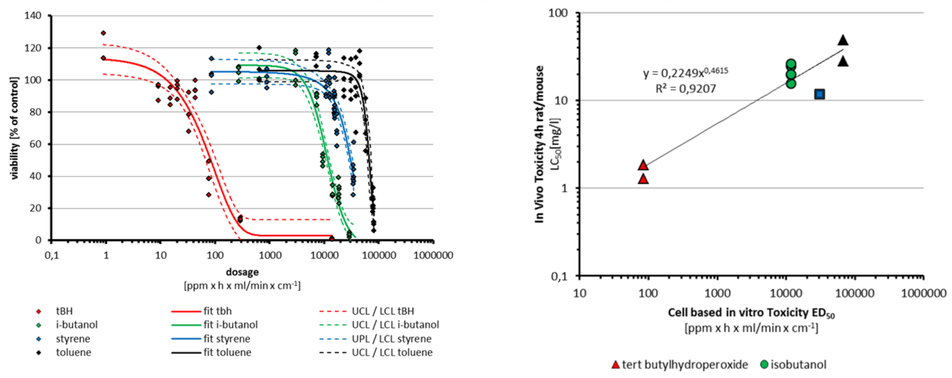


Figure 2: Acute toxic response from human lung cells (A549) to a set of volatile organic compounds and in vitro/in vivo correlation according to data from the ECHA data base.

Dose-responsive effects from dry particle aerosols

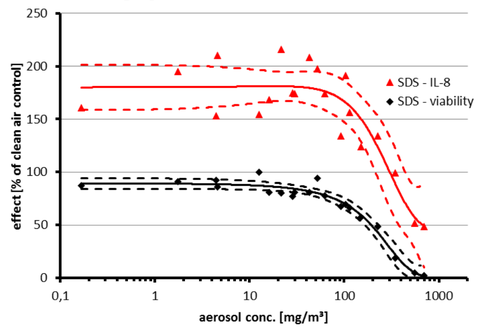


Figure 3: Toxic (cell viability, black) and irritative (IL-8 secretion, red) response from human lung cells (A549) to a dry particle aerosol (sodium dodecyl sulfate)

Application to testing of manually generated test atmospheres such as aerosols from use of consumer products

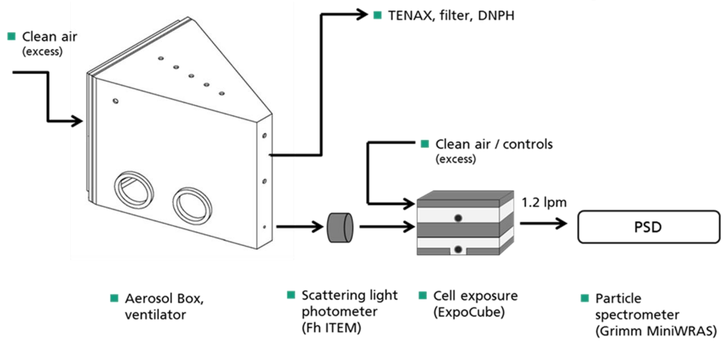


Figure 4: Experimental setup for generation, particle monitoring and exposure of ALI cultures to a variety of different test aerosols including aerosols emerging from consumer product use, such as heating applications of cosmetic products (e.g. hair care products)

Immediate cellular effects during exposure to aerosols monitored by live fluorescence staining

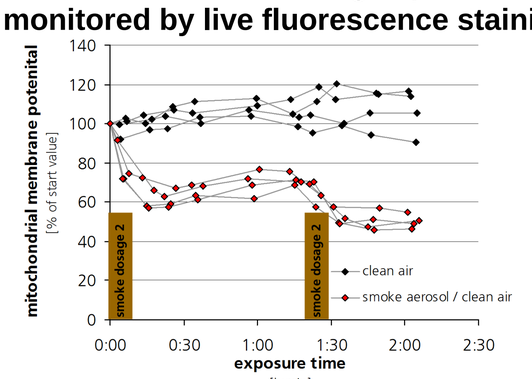


Figure 5: Kinetic effects of smoke aerosol on exposed human lung cells as followed by live fluorescence staining of the mitochondria.

Until now, the ExpoCube® based ALI exposure technology has been applied to:

- Cell-based *in vitro* testing of gaseous compounds and volatile organic chemicals.
- *Ex vitro* testing of inhalable compounds using precision cut lung slices (PCLS).
- Cell-based *in vitro* testing of aerosols from nebulized liquids.
- Cell-based *in vitro* testing of aerosolized particles.
- Online observation of exposure effects during exposure by live fluorescence stains.
- Cell-based *in vitro* testing of aerosols generated by consumer product use (hair care product).

Conclusions

- Handling and testing quality is clearly improved by use of commercial consumables.
- Testing of volatile chemicals resulted in a promising in vitro / in vivo correlation.
- Observation of exposed cells during exposure documents fast cellular response to aerosol compounds.
- Effective particle deposition also for small particles < 1 µm.
- 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.
- The compactness of the design and a large range of applicability improve the *in vitro* potential to test inhalable materials in a relevant way.

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