Improvements for testing of airborne materials using a cell-based *in vitro* approach for a more reliable data collection

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Introduction

Adverse toxic effects on humans by inhalation of airborne substances, e.g. chemicals or pharmacologically active substances have been determined in extensive animal experiments, so far. Among the aspects of animal protection, high costs and unexpected adverse effects in humans, a paradigm shift in the risk assessment to *in silico* approaches and *in vitro* and *ex vivo* models based on human cells and tissues is ongoing. Meanwhile, *in vitro* methods for the evaluation of the biological effects of airborne substances have been under development for several years. However, until now there have been still limitations with regard to the applicability and relevance during exposure of air-liquid interphase (ALI) cultures towards airborne substances. These limitations lead to extended and unfavorable practical needs compared to traditional *in vitro* testing of liquid test compounds. Therefore, they represent a fundamental obstacle for harmonization, standardization, further dissemination and faster development of *in vitro* methods for testing inhalable compounds in their airborne state.

Objectives

The continuing development of the P.R.I.T.® ExpoCube® applications is directed to reduce these limitations. The current improvement includes (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. The versatility and significance of results using this strategy has been studied in a range of applications.

Results

- **Compact design, versatility and exposure of ALI-cultures in consumable labware allow smooth exposure, accessibility of different test compounds and repeated exposure designs**

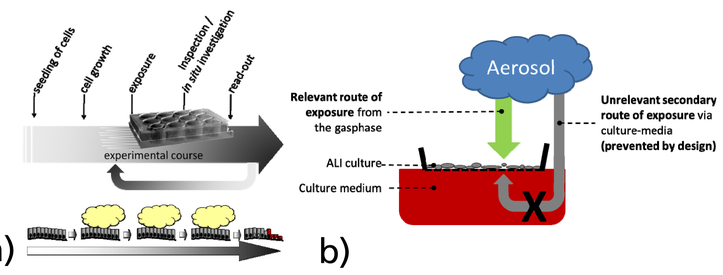

Figure 1: Experimental course of repeated aerosol exposures (a). Prevention of irrelevant secondary route of exposure supports the relevance and significance of toxicity testing *in vitro* (b)

- **Thermophoresis in a stagnation flow alignment allows efficient deposition of small particles from aerosols**

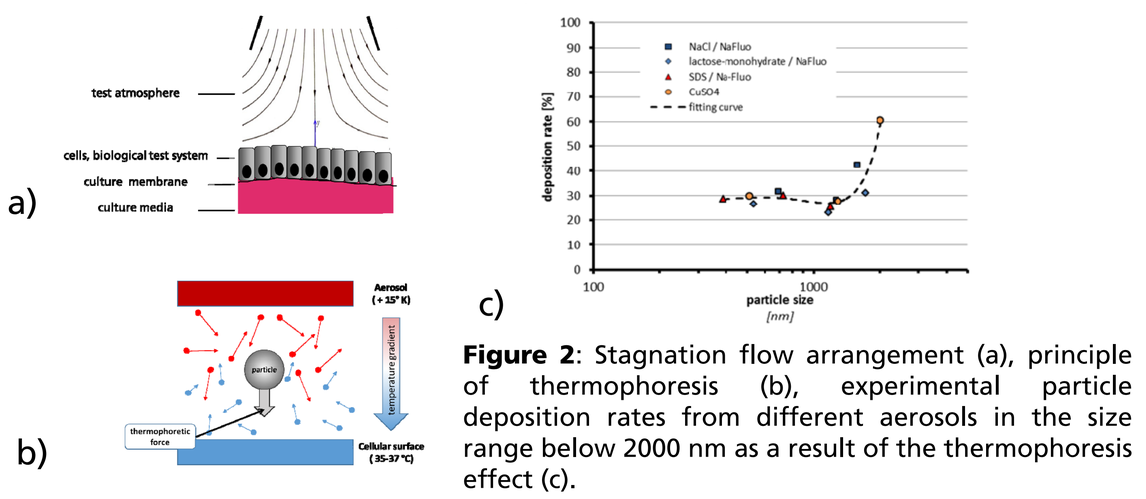

Figure 2: Stagnation flow arrangement (a), principle of thermophoresis (b), experimental particle deposition rates from different aerosols in the size range below 2000 nm as a result of the thermophoresis effect (c).

- **Observation of cells during exposure based on fluorescence live staining allows detection of immediate cellular effects by aerosols**

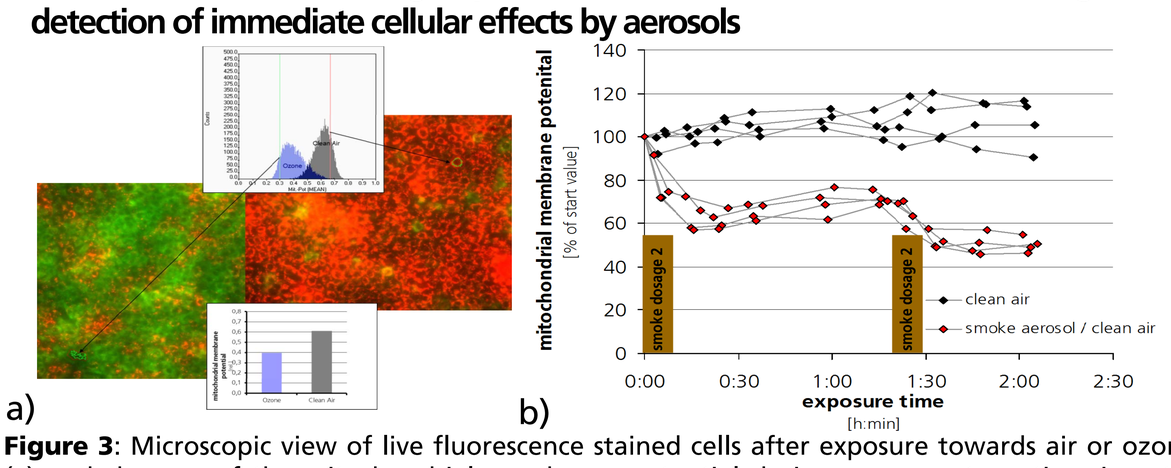

Figure 3: Microscopic view of live fluorescence stained cells after exposure towards air or ozone (a) and changes of the mitochondrial membrane potential during exposure to native cigarette smoke aerosol (b)

- **Acute toxicity testing of volatile organics (VOCs) / gaseous compounds**

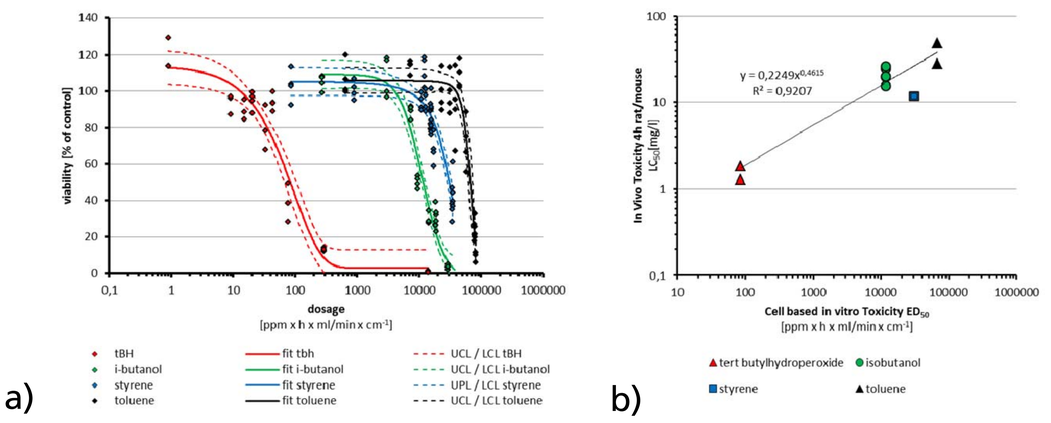

Figure 4: Acute toxic response from human lung cells (A549) to a set of volatile organic compounds (a) and in *vitro*/*in vivo* correlation based on *in vivo* data from the ECHA data base (b).

- **Acute toxicity testing of dry particle aerosols and complex aerosols**

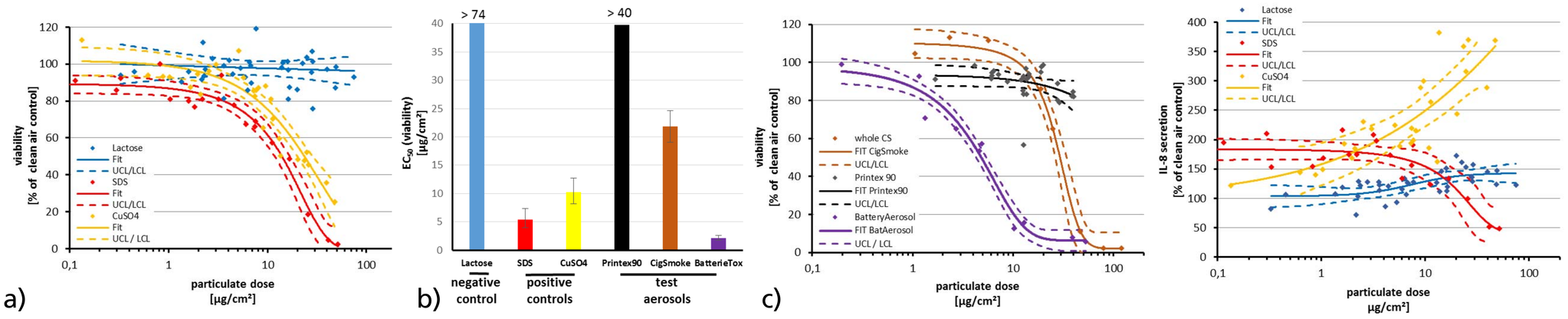

Figure 5: Acute toxic response from human lung cells (A549) to a set of dry particle aerosol negative (lactose) and positive (SDS, $CuSO_4$) control substances (a). Dry particle aerosol (Printex 90) and complex aerosol (Li-Ion failure/nail worst-case test and native cigarette smoke) test compounds (c). EC_{50} values based on cell viability in compare of 6 aerosols (b)

Figure 6: IL-8 secretion dose response as an indicator of irritative potential of control dry particle aerosols after aerosol exposure of A549-cells

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

- Cell-based *in vitro* testing of gaseous compounds / volatile organic chemicals.
- Cell-based *in vitro* testing of aerosols from nebulized liquids.
- Cell-based *in vitro* testing of aerosolized dry particles.
- Cell-based *in vitro* testing of aerosols generated from consumer product use (hair care product).
- Cell-based *in vitro* testing of technical worst-case scenarios (aerosols from Li-ion battery failure situation)
- *Ex vivo* testing of inhalable compounds using precision cut lung slices (PCLS).
- Online observation of aerosol exposure effects by live fluorescence stains.

Conclusions

- Improvement of handling and versatility by multiwell-plate based concept.
- Effective particle deposition also for small particles < 1 µm.
- Observation of exposed cells during exposure documents fast cellular response to aerosol compounds.
- Testing of volatile chemicals resulted in a promising in *vitro* / *in vivo* correlation.
- Assessment of toxicological potential based on *in-vivo*/*in vitro* correlation or use of relevant positive and negative controls.
- Cell-based *in vitro* testing using ALI cultures was applied to a range of different testing materials and testing scenarios including gases, aerosols and complex mixtures.

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