Biological monitoring of inhalable substances *in vitro* – development of an improved test method based on the air-liquid-interface (ALI) cell culture technique

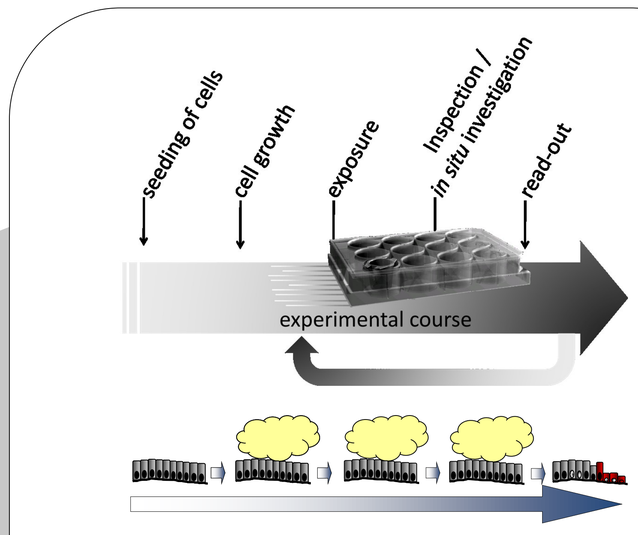
D. Ritter[1], C. Brodbeck[2], J. Knebel[1]
[1]Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover, Germany
[2]Fraunhofer Institute for Algorithms and Scientific Computing, Sankt Augustin, Germany

Situation

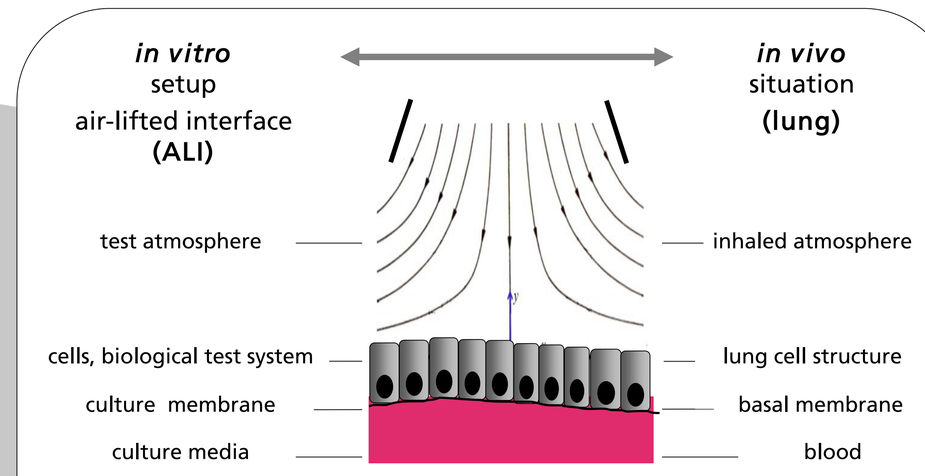
- The air-lifted interface (ALI) cell culture technique is the state-of-the-art method for lung related research *in vitro*.
- Biological barrier function represent „first site-of-contact“ models for airborne mixtures (lung, skin).
- Prevalidation studies of the basic ALI cell culture technique have proven good reproducibility and relavance for detection of biological effects of chemical gases.
- Limiting factors until now include practicability, applicability and efficiency in aerosol testing and insight into cellular changes during exposure.

Objectives

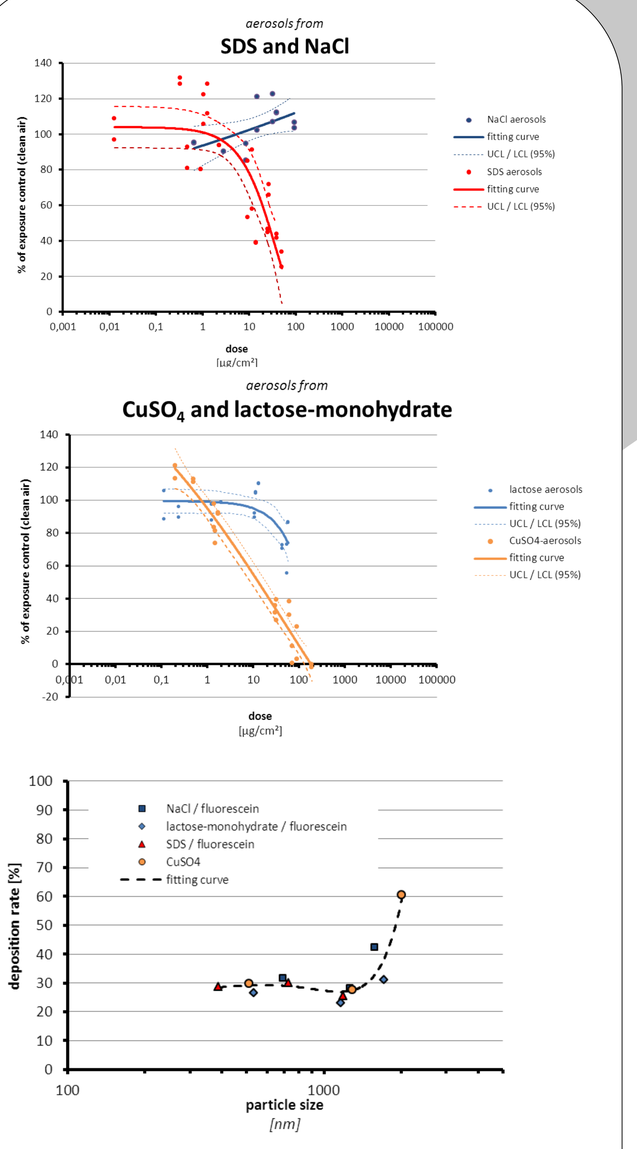
- The development of an improved exposure process with focus on
  - optimized *in vitro* exposure efficiency both to gases and aerosols (respirable fraction < 10 µm and < 1 µm),
  - a small and mobile device for efficient biological *in vitro* testing of various aerosol sources at environmental conditions,
  - non-invasive online observation of biological changes in the *in vitro* model,
  - an optimized workflow and *in vitro* procedure.



“All-in-one-plate”- *in vitro* workflow using ALI cultures in the optimized test method based on the ExpoCube. Repeated exposure experiments can reproducibly be conducted.

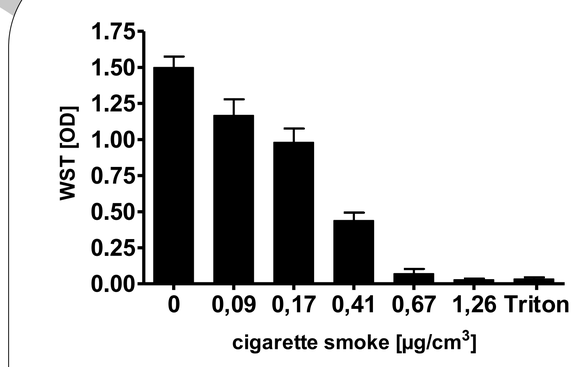


Stagnation flow setup for ALI cultures on membranes as used in the ExpoCube.

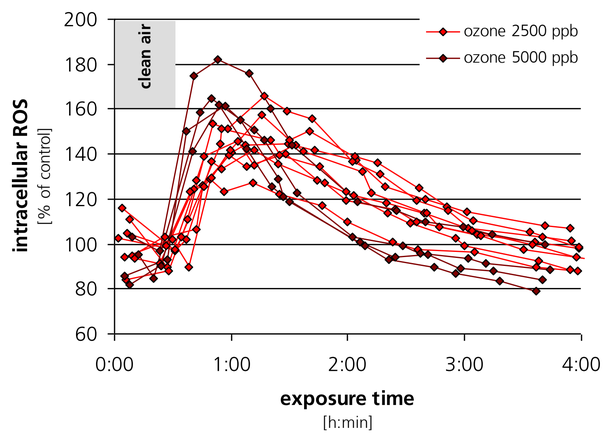


Studies using positive- and negative control aerosols show that **particle-dependent effects can be detected specifically** and in a dose-dependent manner due to **extraordinary high particle deposition rates** by use of thermophoresis. (Common deposition rates using standard ALI conditions are in the range of 1 to 2 % for particle sizes < 1000 nm.)

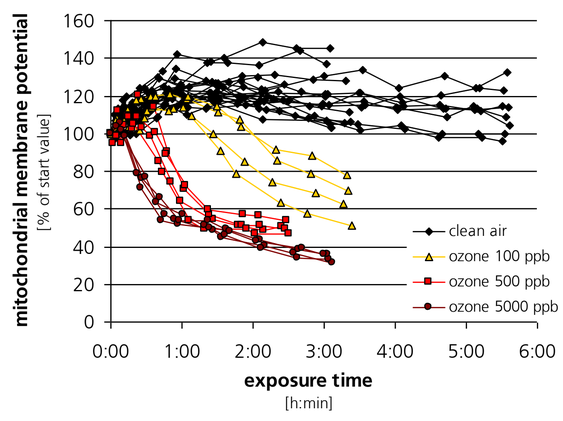
P.R.I.T. ExpoCube for *in vitro* investigations of inhalable substances with culture membranes and standard multiwell plate



Measurement of viability of **live *ex vivo* tissue (precision cut lung slices, PCLS)** after exposure to smoke aerosol from cigarettes.



Online observation of cellular changes in a **human lung cell line** (A549 cell, left) and **human primary lung cells** (above). Cells were stained using live fluorescence stains and observed during exposure using the ExpoCube.



in vivo data	NOEL	[mmol/kg bw/d]	1.1 x 10^{-3} to 1.1 x 10^{-2}		2.2 x 10^{-2} to 2.5 x 10^{-2}		0.18 to 1.8		0.45 to 1.2
			formaldehyde	**<**	**dimethylamine**	**<**	**acetaldehyde**	**<**	**isobutylene**
in vitro data	IC_{10}	[ppm]	5.97		150		210		>50000

***In vitro / in vivo* correlation** experiments using four chemical gases in comparison of *in vivo* no-observed-effect-levels (NOELs) and *in vitro* IC10 values.

Results

Conclusion

- Various types of atmosphere are applicable (including gases and aerosols from lab, environmental or other sources).
- Easy applicability to any test compound at atmospheric pressure.
- High sensitivity to biological effects of gases and aerosols including environmental relevant concentrations.
- High relevance for *in vivo* situation as documented by prevalidated *in vitro* technology and compare to *in vivo* data.
- High routine suitability by use of “all-in-one-plate” *in vitro* workflow.
- Applicable to varying biological models including cell lines, primary cells or *ex vivo* models such as PCLS.
- Applicable to a large range of biological endpoints including cellular toxicity, genotoxicity or specific pathways and mechanisms.

A highly relevant, robust and sensitive *in vitro* model for characterization of biological effects of inhalable compounds

Contact:
detlef.ritter@item.fraunhofer.de
jan.knebel@item.fraunhofer.de

P.R.I.T.® PROFESSIONAL IN-VITRO TECHNOLOGIES
www.prit-systems.de

Fraunhofer ITEM
www.item.fraunhofer.de