EFFECTS OF INHALED NANOPARTICLES IN HEALTH AND DISEASE BY IN VITRO TECHNOLOGY

N Jeannet[1], M Fierz[2], D Egli[2], P Steigmeier[2], ZS Juranyi[2], M Kalberer[3], H Burtscher[2], M Geiser[1]

[1]*Institute of Anatomy, University of Bern*, BERN, *Switzerland*
[2]*Institute of Aerosol and Sensor Technology, University of Applied Sciences Northwestern Switzerland*, WINDISCH, *Switzerland*
[3]*Department of Chemistry, University of Cambridge*, CAMBRIDGE, *United Kingdom*

Safe application of the promising and quickly growing nanotechnology requires a comprehensive clarification of the effects on human health and environment. Especially, nanoparticles in form of powders or sprays can easily be inhaled and for this reason the lungs are the main target organ of undesired exposure. Due to their small size, nanoparticles can deposit in different compartments of the respiratory tract from the head airways to the alveoli, mainly due to diffusion. Inhalation of engineered nanoparticles (ENPs) in industrial processes, in consumer products, but also in medicine poses a still unknown risk. Individuals with chronic lung diseases are expected to be more vulnerable than healthy adults. Safety testing thus needs to include studies in susceptible populations. Furthermore, for efficient, economical and ethically sound evaluation of health hazards by inhaled nanomaterials, animal-free and realistic in-vitro test systems are desirable.

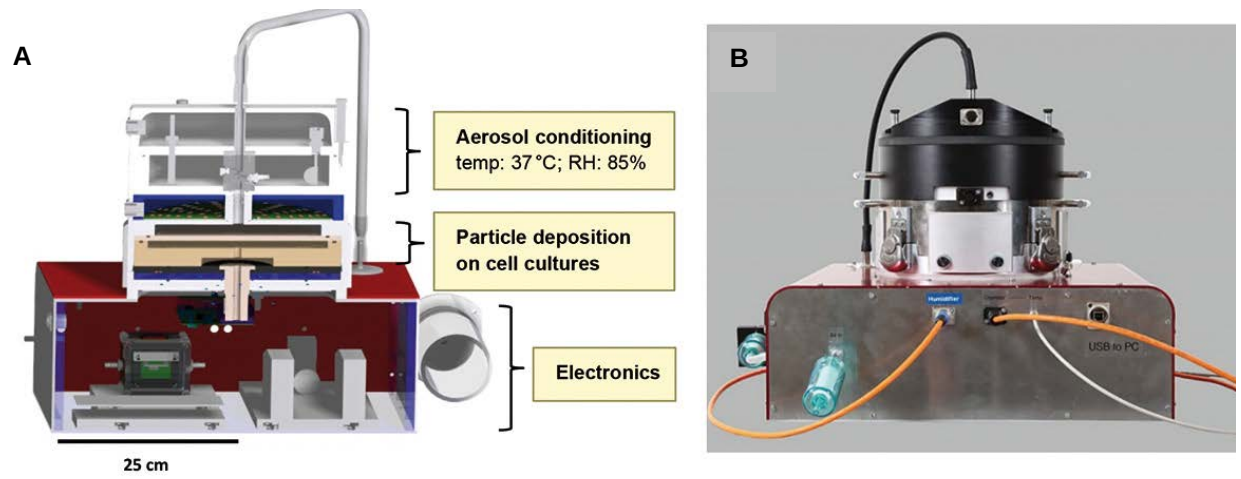

Figure 1: Design of NACIVT. A: Cross section of NACIVT, showing the base with the electronics and top part where aerosol conditioning (i.e. 37 °C and 85% RH) and particle deposition on cell cultures take place. B: Compact configuration of NACIVT.

Based on previous work [1] a chamber for efficient deposition of (nano)particles out of a continuous air-stream on cells cultured on Transwell® inserts has been developed (Figure 1). ENPs can be deposited under controlled conditions (85-95% RH and 37°C) simultaneously on 24 individual cell cultures, allowing high-throughput screening of hazards posed

by nanomaterials. Moreover, NACIVT utilizes electrostatic deposition as deposition mechanism, which is the most efficient way to precipitate particles.

In order to allow for reliable exposure experiments the new chamber has been thoroughly characterized. In different experiments NACIVT has been investigated in terms of deposition efficiency, spatial distribution of particles and compatibility for cell exposures. In first exposure experiments, re-differentiated healthy and diseased human bronchial epithelia (HBE) with established air-liquid interface and the human bronchial epithelial cell line BEAS-2B were exposed to silver (Ag)- and carbon (C)-NPs.

Particles were evenly distributed on all analysed Transwell® inserts. Towards the edge, there was a sharp decrease of deposited particles due to the gas flow pattern. Particle deposition efficiency on all 24 inserts was very similar and in the range of 15%. The exposure treatment itself was not cytotoxic, as lactate dehydrogenase release in BEAS-2B cells exposed to chemically inert polystyrene-latex particles was not different from unexposed controls. Preliminary results from Ag- and C-NP exposures indicate a greater sensitivity of HBE from a donor with cystic fibrosis compared to healthy HBE and BEAS-2B cells.

Thus, the Nano Aerosol Chamber for In-Vitro Toxicology (NACIVT, www.nacivt.ch) combined with advanced cell models, i.e. HBE from healthy and diseased donors provides a highly realistic in vitro system for safety testing of ENPs. In addition to toxicology applications, the combined system allows also studies with novel therapeutic aerosols with particle diameters up to micrometer size.

[1] Mertes et al., JAMPDD 3013, DOI: 10.1089/jamp.2012.0985.

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EFFECTS OF INHALED NANOPARTICLES ON THE SUSCEPTIBLE POPULATION – IN VITRO EVOLUTION

N Jeannet[1], M Fierz[2], D Egli[2], P Steigmeier[2], Zs Juranyi[2], M Kalberer[3],H Burtscher[2] and M Geiser[1]

[1]**Institute of Anatomy, University of Bern, Switzerland**

[2]**Institute of Aerosol and Sensor Technology, University of Applied Sciences Northwestern Switzerland, Switzerland**

[3]**Department of Chemistry, University of Cambridge, United Kingdom**

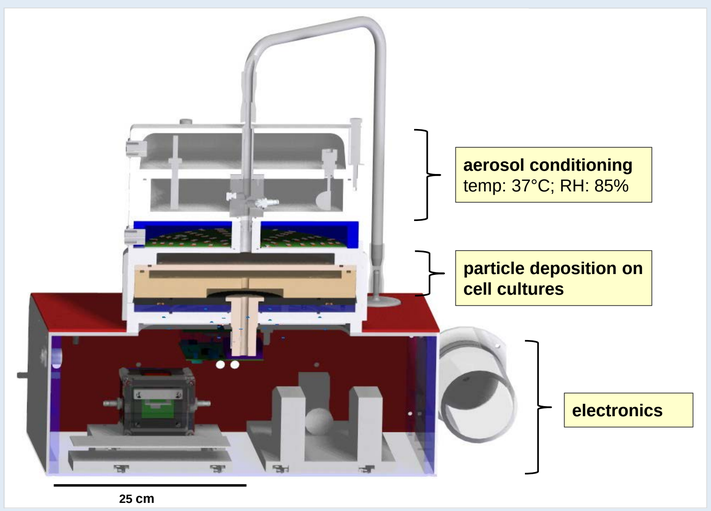
Introduction

Inhalation of engineered nanoparticles (ENP) in industrial processes and consumer products poses a still unknown risk. Individuals with chronic lung diseases are expected to be more vulnerable than healthy adults. Safety testing thus needs to include studies in susceptible populations. Furthermore, for efficient, economical and ethically sound evaluation of health hazards by inhaled nanomaterials, animal-free and realistic in-vitro test systems are desirable.
Based on previous work [1] an aerosol deposition chamber for efficient and quantitative ENP deposition out of a continuous air-stream on cell cultures has been developed and characterized regarding its performance. Cellular responses of healthy and diseased respiratory epithelia to silver (Ag) and carbon (C) NP were examined using the new aerosol deposition chamber.

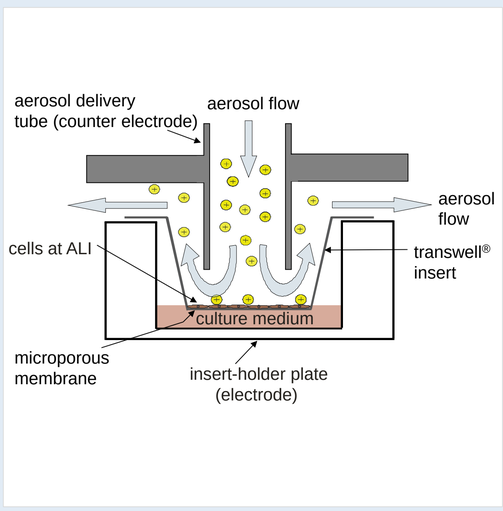
Nano Aerosol Chamber In-Vitro Toxicity (NACIVT)

Characteristics

- exposure conditions closely reflecting the physiological situation in the respiratory tract
  → 37°C, 85-95% RH
- electrostatic particle deposition (2.5 kV)
  → particle charging by unipolar diffusion charger
- high through-put analysis
  → simultaneous particle deposition on 24 individual cell cultures on Transwell® inserts (6.5 mm)
- exposure of cells at the air-liquid interface (ALI)



Setup of NACIVT (www.nacivt.ch). After entering the deposition chamber particles are charged by a unipolar diffusion charger. By passing a heated water bath the aerosol is humidified and temperature adjusted. The conditioned air stream is equally divided onto 24 aerosol delivery tubes and particles are precipitated on cells by electrostatic deposition.



Electrostatic particle deposition. Cells are cultured on the Transwell® insert and exposed to the NP at the ALI.

Material and Methods

	Chamber characterization	AgNP / CNP experiments		
test particles	yellow green (YG) fluorescent polystyrene latex (PSL, 200 nm)	spherical Ag and C (~20 nm)		
particle generation	nebulizer	spark generator		
aerosol flow	25 mL / insert / min			
# of deposited particles / insert (0.33 cm²)	5×10^6		AgNP	CNP
		low	7×10^6	7×10^7
		medium	7×10^7	7×10^8
		high	7×10^8	3.5×10^9
cell culture model	BEAS-2B cell line: bronchial epithelial cells, human origin	re-differentiated human bronchial epithelial (HBE) cells: healthy and diseased donors		
exposure time	1800 - 3600 s	36 - 3600 s		
endpoint analysis	• # of particles deposited on insert membrane: counting in optical fields using fluorescence microscopy • cytotoxicity: release of lactate dehydrogenase (LDH)	• cytotoxicity: release of LDH • inflammatory response: release of interleukin (IL)-6 and IL-8		

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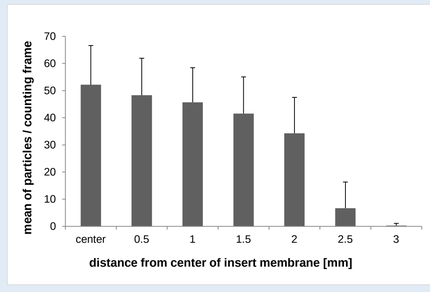
Results – Chamber Characterization

Distribution of deposited particles



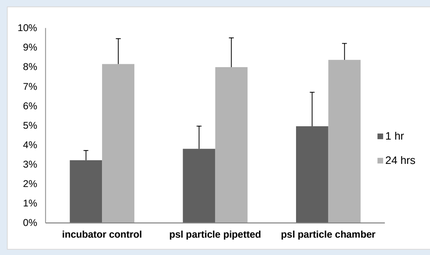
Distribution of PSL particles (YG, 200 nm in diameter) on Transwell® inserts (n=24); mean and SD.

The number of deposited PSL particles was assessed as function of the distance from the insert center. PSL particles were evenly distributed on all analyzed Transwell® inserts. Towards the edge, there was a sharp decrease of deposited particles due to the gas flow pattern.

Deposition efficiency

Independent experiments demonstrated a particle deposition efficiency in the range of 15% in all 24 inserts.

Cytotoxicity



Cytotoxicity in BEAS-2B cells after exposure treatment in the deposition chamber („psl particle chamber“ [n=26; 3 independent experiments]) in comparison to control cell cultures („incubator control“ [n=5] and „psl particle pipetted“ [n=3]); mean and SD.

Compatibility of NACIVT for aerosol exposure of cells. Cytotoxicity in BEAS-2B cells was generally low, as expected. LDH release from cells exposed in the chamber to aerosolized inert 200-nm PSL particles was comparable to control cells. The small increase in cytotoxicity at 1 hr after exposure treatment is biologically not relevant.

Results – AgNP and CNP Experiments

LDH release in HBE cells exposed to AgNP and CNP was generally low at 4 hrs and 24 hrs after treatment. Cells from a cystic fibrosis (CF) donor exposed to AgNP showed a distinct inverse correlation between cytotoxicity and NP concentration. This may be due to a hormetic effect [2,3]. Moreover, cells from a donor with CF showed increased cytotoxicity compared to the healthy donor.

% cytotoxicity (normalized to control)

	AgNP	CNP
healthy	< 1 %	< 4 %
CF	6-13 %	4-7 %

Cytotoxicity of different AgNP and CNP concentrations in HBE cell cultures from diseased (“CF” [n=4]) and healthy (“healthy” [n=4]) donors,24 hrs after exposureAbsorbance values were normalized to the corresponding untreated control (n=3).

In general the release of IL-6 and IL-8 was increased in cells exposed either to AgNP or CNP compared to controls. Cells from a CF donor tended to release higher amounts of both cytokines than cells from a healthy donor. This effect was more pronounced in IL-6 release. Moreover, in both donors a dose dependent release of IL-6 was observed upon exposure to AgNP and CNP.

IL-6 release [pg/ml]

	AgNP	CNP
healthy	< 50	30-100
CF	130-230	100-160

IL-8 release [pg/ml]

	AgNP	CNP
healthy	4000-7000	4000-9000
CF	6000-10000	4000-7000

Release of IL-6 and IL-8 from healthy (“healthy” [n=4]) and diseased (“CF” [n=4]) HBE cells 24 hrs after exposure to different concentrations [conc.] of AgNP and CNP.

Conclusions

Deposition chamber

- realistic and controlled particle delivery
- no adverse effects on cell cultures by the chamber
- efficient particle deposition

AgNP / CNP experiments

- low cytotoxicity; inverse correlation between cytotoxicity and AgNP concentration
- cytokine release increased after exposure to NP; dose dependent IL-6 release
- HBE cells of CF donor more vulnerable than cells of healthy donor

References: [1] Mertes et al., JAMPDD 3013, DOI: 10.1089/jamp.2012.0985.
[2] Kawata et al. *Environ Sci Techn* 43:6048-6051 (2009)
[3] Xiu et al. Nano Lett. 12:4271-4275 (2012)