Responses of lung cell cultures after realistic exposure to primary and secondary carbonaceous aerosols

M. Geiser[1], L. Künzi[1], S. Schneider[1], P. Mertes[2], J. Dommen[2], U. Baltensperger[2], A.S.H. Prévot[2], M. Kalberer[3]

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Epidemiology has provided consistent evidence for the link between adverse health effects and increased concentrations of ambient (ultra)fine particles. The interaction of particles with the lung, the main pathway of undesired particle uptake, however, is still poorly understood (e.g. Pope et al., 2006). There is an urgent need for biological experiments aimed directly at the cause-effect relationship. A major fraction of ambient aerosol particles is composed of organic material, whereby oxidizing organic compounds like peroxides in such particles, are possibly responsible for the observed health effects.

We examined the responses of lung cells to primary (POA) and secondary organic aerosols (SOA) originating from diesel exhaust and wood combustion processed and aged in a large-scale smog chamber. Thereby, aerosols were generated from a VW transporter and an Attika wood stove. Secondary aerosol formation was initiated in the smog chamber by irradiation. Prerequisites for studies in vitro are i) particle application as aerosols, ii) cell cultures closely reflecting the target organ, iii) exposure under realistic and physiological conditions (aerosols, cell cultures) and iv) meaningful endpoint measurements for homeostatic lung function.

We have developed a nanoparticle deposition chamber to expose lung cells mimicking closely the particle deposition conditions in the lung (Savi et al., 2008). In this deposition chamber, particles are deposited very efficiently, reproducibly, and uniformly onto the cell culture, a key aspect if cell responses are quantified in respect of the deposited particle number. Before entering the deposition chamber, particles pass a bipolar Kr-85 charger where they reach an equilibrium charge distribution. After the charger 41 - 70% of all particles in the size range of 50 – 600 nm carry one to about five positive or negative net elemental charges, whereas the rest is uncharged (Wiedensohler, 1988). Thus, the particles are not highly charged on their surface as would be the case for other charging processes, e.g., in corona chargers. The resulting charge distribution is very close to that of particles in ambient air (Hinds, 1982). Particles are deposited by an alternating, square-wave electrical field of 4 kV/cm applied between the end of each particle delivery tube and a circular electrode placed directly beneath each filter insert, assuring that the majority of the charged particles are deposited. Before the aerosol is delivered to the cells, the gas composition is adjusted to physiological conditions (i.e., 75% N_2, 20% O_2, 5% CO_2) and the aerosol is heated to 37°C and humidified to 85 - 95% relative humidity. The aerosol is then directed individually to one of 12 cell cultures grown on filter inserts (24 mm diameter) in multiwell plates. The main cell culture systems used are i) re-differentiated human airway epithelia with established air liquid interface (ALI), ii) porcine lung surface macrophages, and iii) the lung epithelial cell line BEAS-2B derived from human bronchi. Re-differentiated airway epithelia and macrophages represent on the one hand the immediate target of inhaled particles but they provide also barrier function and defense, i.e. the crucial mechanisms to maintain homeostatic lung function. The cells of the BEAS-2B cell line are proliferating and can be used to gain information on the capacity of cell replacement.

Cells were exposed at ALI conditions to the aerosols or to particle-free air for 2 hours; controls were left untreated in the incubator. Cellular responses were measured within 24 hours after exposure to the aerosol. A set of meaningful biological endpoints were chosen to assess impaired lung homeostasis (Gaschen et al., 2010). The measurements included assessing i) cytotoxicity by release of lactate dehydrogenase, LDH, ii) structural and functional cell and tissue integrity by microscopy and maintenance of the air-liquid interface, iii) clearance function, i.e., ciliary beating, mucus production and phagocytic activity of macrophages, and iv) release of inflammatory mediators such as interleukin-1β (IL-1b); IL-6, IL-8, IL-10, tumor necrosis factor alpha (TNF-α) and monocyte chemotactic protein-1 (MCP-1).

Our data indicate that the exposure of lung cells to diesel exhaust and wood burning aerosols under realistic conditions leads to mild but distinct cellular responses: In comparison to POA, the phagocytic activity of macrophages was decreased after exposure to SOA from wood burning, but increased after exposure to SOA from diesel. A trend to increased cytotoxicity was found after exposure to SOA of both aerosol types. The release of inflammatory mediators tended to be increased after exposure to SOA; this effect was more pronounced for diesel exhaust.

Overall, there is evidence for i) more pronounced cellular responses upon exposure to SOA than to POA, ii) different effects of aerosols originating from diesel and wood burning and for (iii) lung-cell donor specific responses. Although the short-term exposure of lung cells to these aerosols at ambient-air concentrations of about 10^4 particles/cm^3 leads to only moderate cellular responses, there is consistent evidence for potential induction or aggravation of adverse health effects by these aerosols.

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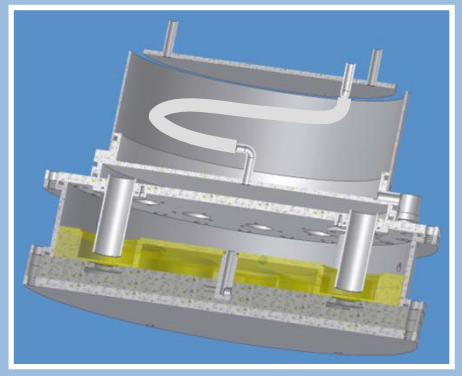
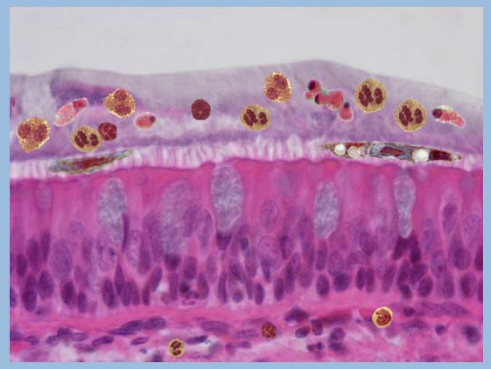


Schematic of aerosol deposition chamber



Schematic of inner lung surface in disease

Background and Aims

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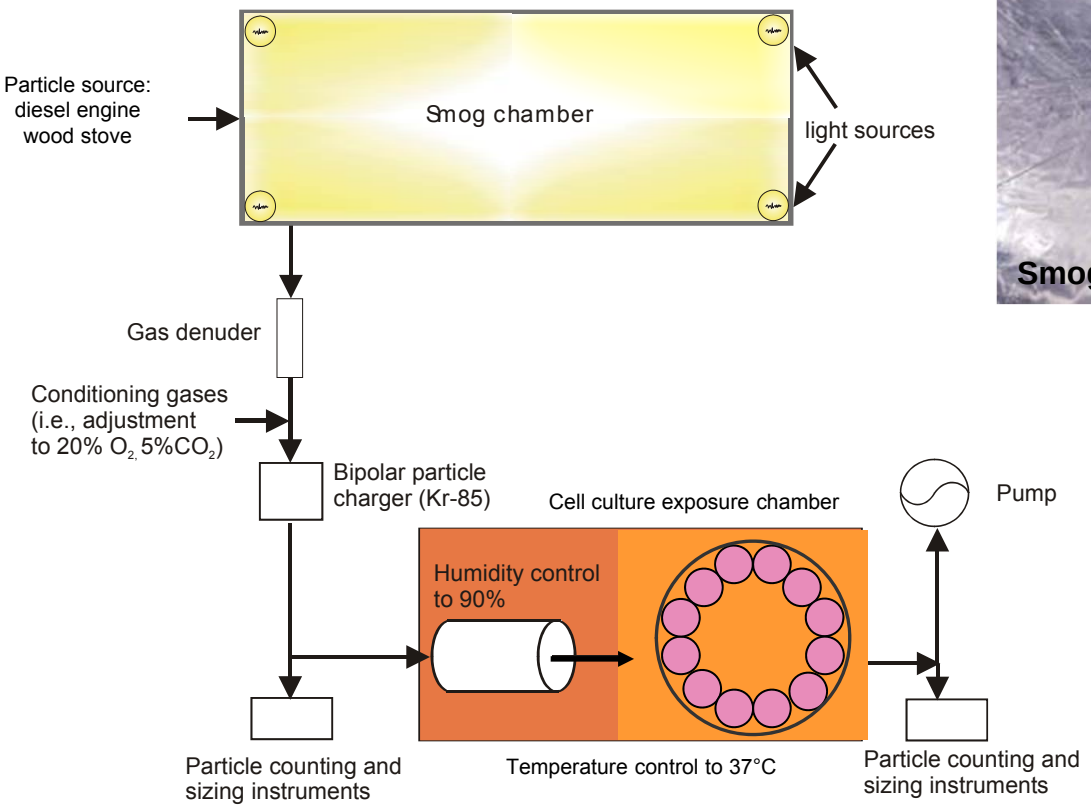
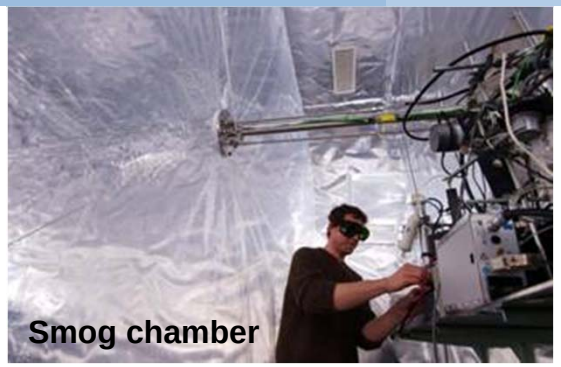
- Ambient particles (< 1µm) → adverse health effects
- Health effects still poorly characterized
- Organic aerosols → substantial fraction of ambient aerosols
- Peroxides in such particles → responsible for adverse effects

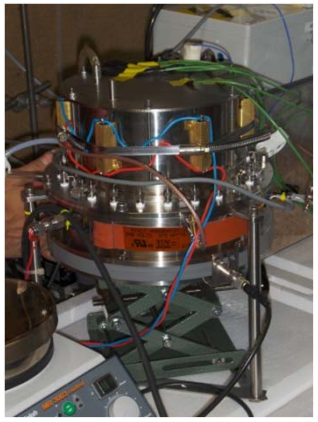
- Cellular responses of lung cells *in vitro* to organic aerosols from diesel exhaust and wood burning

- Prerequisites
  - Particle application as aerosols
  - Cell cultures reflecting the target organ
  - Exposure under realistic and physiological conditions (aerosols, cell cultures)
  - Endpoint measurements for homeostatic lung function

Particle generation and aerosol exposure chamber



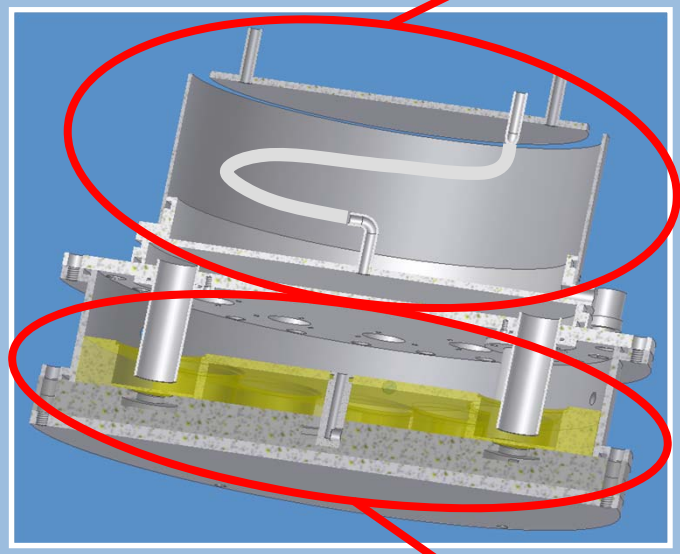




Savi et al., *Environ. Sci. Technol.*, 2008

Deposition chamber

Conditioning chamber
(90% RH, heated to 37°C)



12 cell cultures
(heated to 37°C)

Deposition chamber

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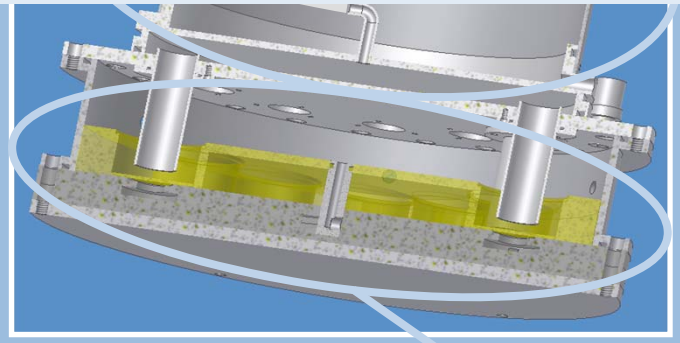
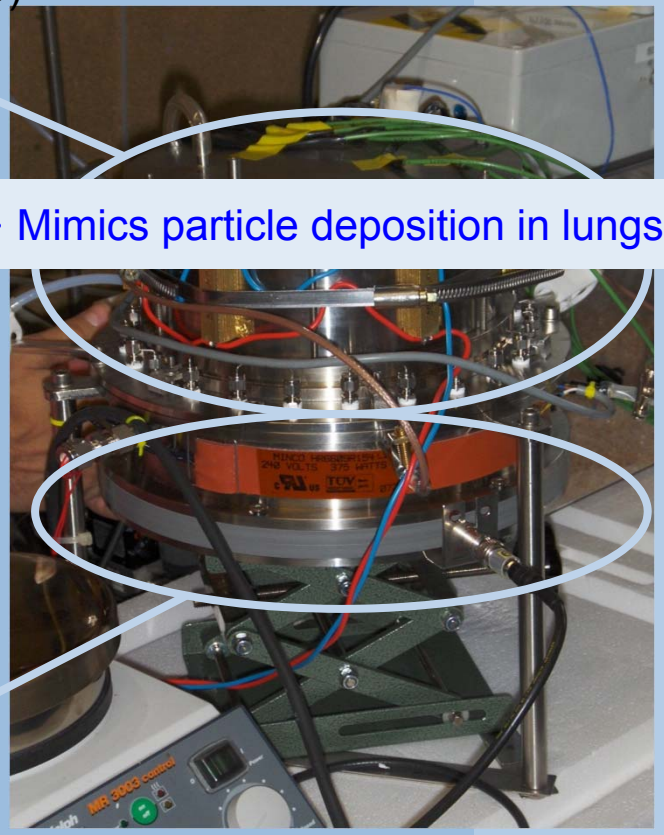
Conditioning chamber
(90% RH, heated to 37°C)

- Direct deposition of particles from gas flow
- Efficient & even deposition of particles
- Experiment under well controlled conditions

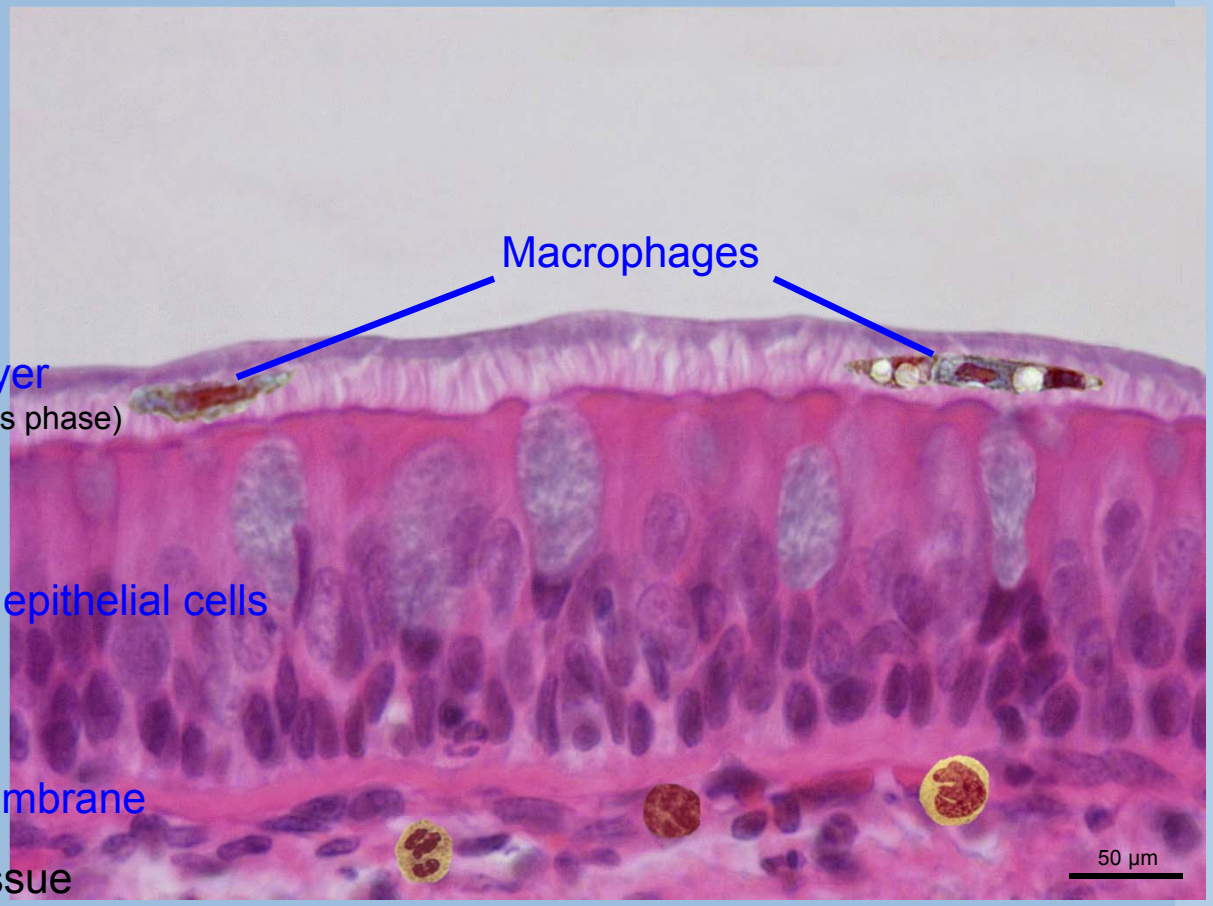


Mimics particle deposition in lungs



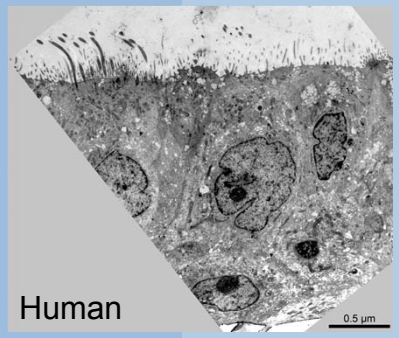
12 cell cultures
(heated to 37°C)

Inner lung surface in health: target, barrier, clearance

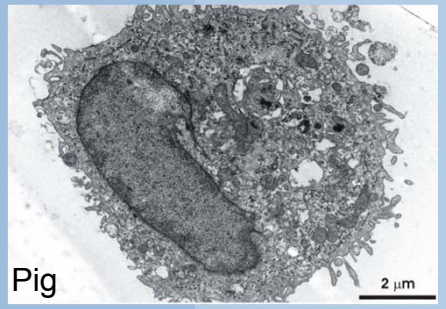


Airway model:
cell cultures on filter inserts

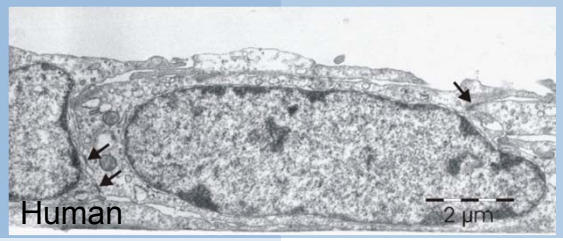
- Redifferentiated airway epithelia with established air liquid interface (ALI)
  - Barrier
    - Junctional complexes, basement membrane
  - Defence
    - Beating cilia, mucus production



- Bronchoalveolar lavage macrophages
  - Defence / particle clearance
    - Phagocytosis



- Bronchial epithelial cell line BEAS-2B
  - Proliferating cells



Analysis: impaired lung homeostasis

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- Endpoints
  - Cytotoxicity
    - LDH release
  - Function of lung surface
    - Barrier (epithelium)
      - Structural integrity (electron microscopy)
      - Maintenance of air-liquid interface
    - Defense
      - Ciliary beating and mucus production
      - Phagocytic activity of macrophages
  - Inflammatory reactions
    - Cytokine release (TNF-α, IL-1β, IL-6, IL-8, MCP-1, IL-10)

Gaschen et al., *Environ. Sci. Technol.*, 2010

Experimental Design

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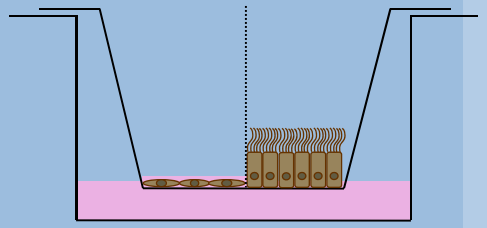
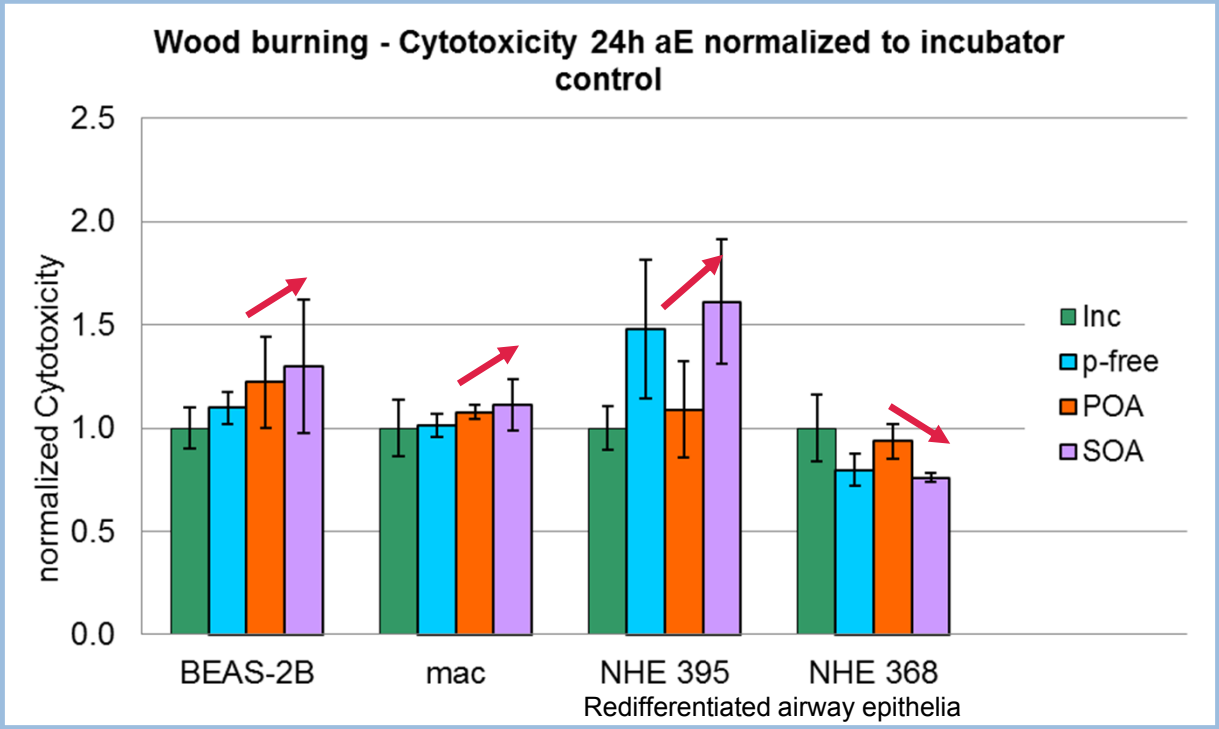
- Cell culture
  - Microporous filter inserts
  - Air-liquid interface (ALI)



- Aerosol
  - Wood burning (Attika stove) & diesel (VW transporter) exhaust
  - Primary & secondary organic aerosols (POA & SOA)
  - Ambient air concentration (~10^4/cm^3)
  - Flow: 50 mL/insert/min
- Exposure
  - Single, 2h (short term)
  - Cell groups: aerosol, particle-free air, incubator
- Cell analysis
  - Within 24h after exposure (acute)

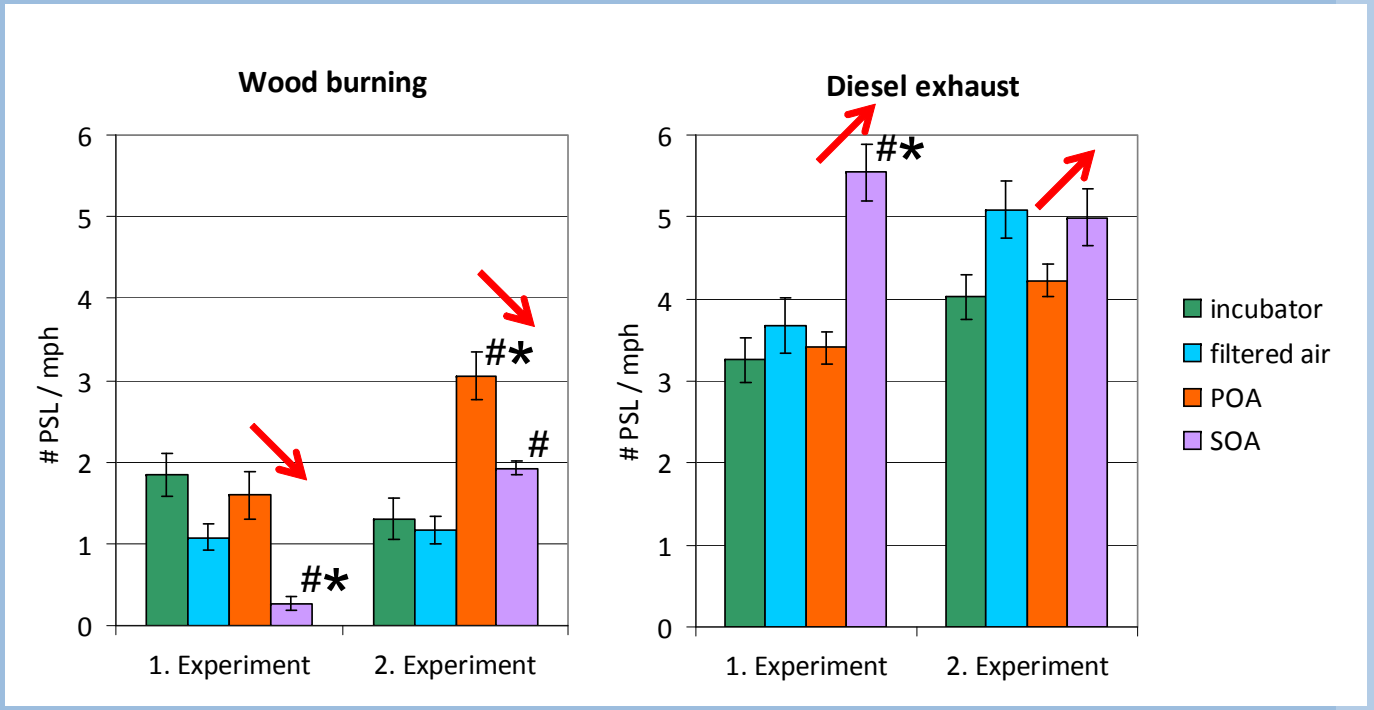
Cytotoxicity

- LDH release by epithelia and macrophages



Phagocytic activity

- Particle uptake by macrophages



Summary of results

- Cytotoxicity (LDH)
  - Generally low
  - Cytotoxicity ↑ after exposure to SOA (wood & diesel)
- Cell and tissue integrity
  - Not affected
- Inflammatory mediators
  - IL-6 and MCP-1: ↑ after exposure to SOA (wood & diesel)
  - IL-6 and TNFα: donor specific response
- Phagocytic activity of macrophages
  - Aerosol specific response

	POA	SOA
wood	↑	↓
diesel	→	↑

Conclusions

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- Single acute exposure to the aerosols at ambient-air concentrations leads to only moderate cellular responses

- Evidence for
  - Different effects of POA and SOA
  - Different effects of aerosols originating from diesel and wood burning
  - Subtle changes in cellular functions that are essential for lung homoeostasis
  - Donor specific effects

Collaborations and Support

- Core group
  - L. Künzi, S. Schneider, N. Jeannet
    University of Bern, CH
  - P. Mertes, J. Dommen, U. Baltensperger, A.S.H. Prévot
    Paul Scherrer Institut, Villigen, CH
  - M. Kalberer
    University of Cambridge, UK

- Chamber development
  - H. Burtscher, M. Fierz
    Univ. Applied Sciences Northwestern Switzerland, CH

- Human cells
  - M. Salathé
    University of Miami, USA

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Schweizerischer Nationalfonds
Fondo nazionale svizzero
Swiss National Science Foundation

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Thank you for your attention

Experimental conditions of wood and diesel experiments

– Particle types, duration of cell exposure to the aerosols, time point of exposure (time after lights on = photochemical age of particles)

Particles	Exposure of cells	Time of aerosol sampling
Primary organic aerosols (POA)	90 min	Before irradiation, i.e. primary emission
Secondary organic aerosols (SOA)	120 min	2 h after irradiation, i.e. formation

Aerosol parameters

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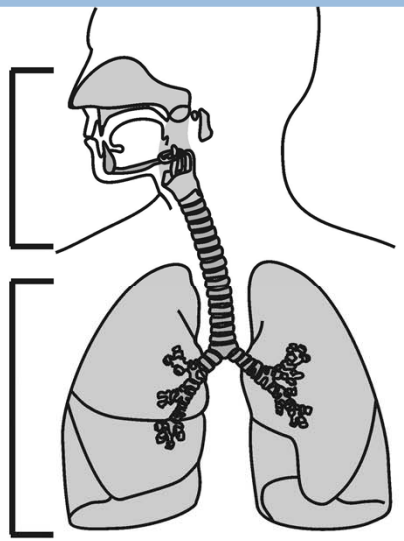
- Particle concentration
  - 1. POA, 2. SOA
  - Number concentration (SMPS) measured at the beginning and end of the exposure

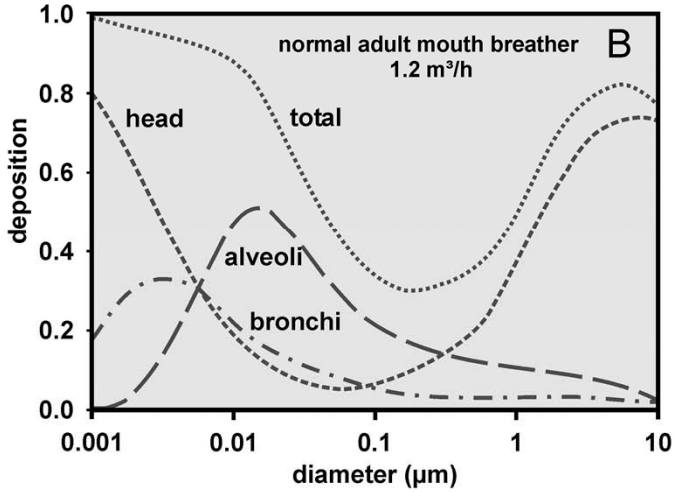
	Source	Exhaust dilution with pure air	Particle conc. [#/cm^3]
1st wood	Wood stove "AVANT" (Attika Feuer AG) heat output: 4.5 kW room heating capacity: 215 m^3	approx. factor 7	4.5×10^4 - 2.9×10^4 2.0×10^4 - 1.6×10^4
2nd wood			2.1×10^4 - 1.3×10^4 8.3×10^3 - 5.9×10^3
3rd wood			1.7×10^4 - 1.2×10^4 8.6×10^3 - 6.6×10^3
4th wood			2.0×10^4 - 1.4×10^4 1.0×10^4 - 7.5×10^3
1st diesel	VW Transporter TDI Syncro (2 461 cm^3), Dec. 2000, no exhaust after treatment	approx. factor 7	3.7×10^4 - 1.7×10^4 1.1×10^4 - 7.9×10^3
2nd diesel			1.8×10^4 - 9.8×10^3 6.0×10^3 - 4.4×10^3
3rd diesel			2.3×10^4 - 1.5×10^4 7.9×10^3 - 5.8×10^3

Deposition of inhaled (nano)particles

Extrathoracic
nose, pharynx, mouth, larynx

Intrathoracic
extrapulmonary:
trachea & stem bronchi
intrapulmonary:
bronchi, bronchioles, alveoli





Electrostatic particle deposition

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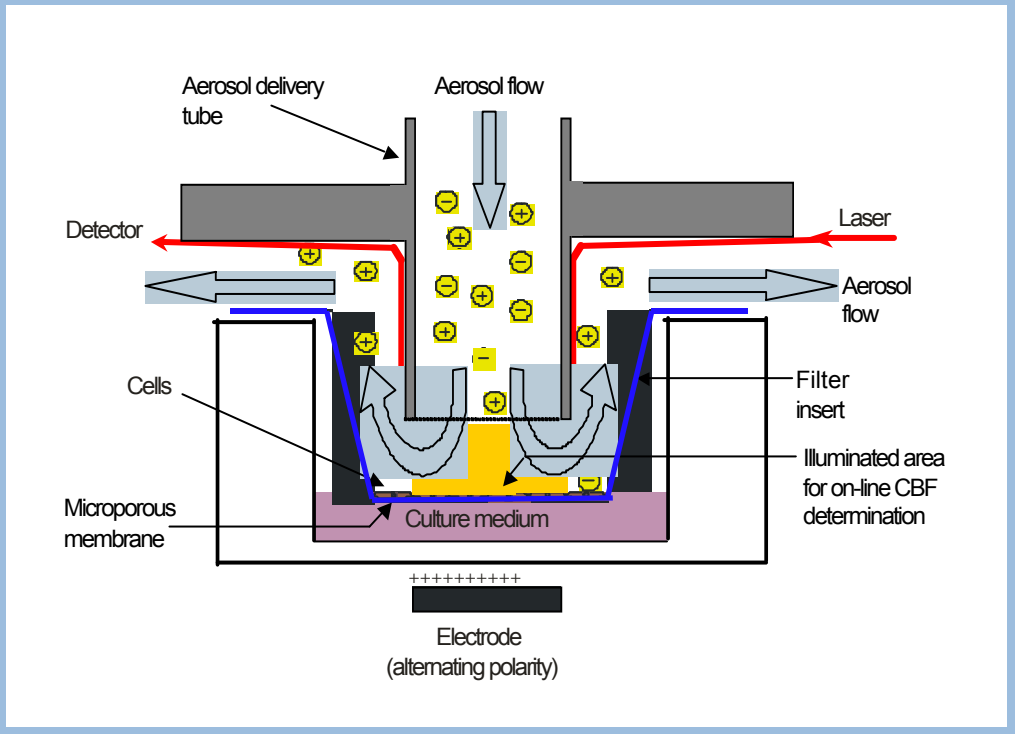
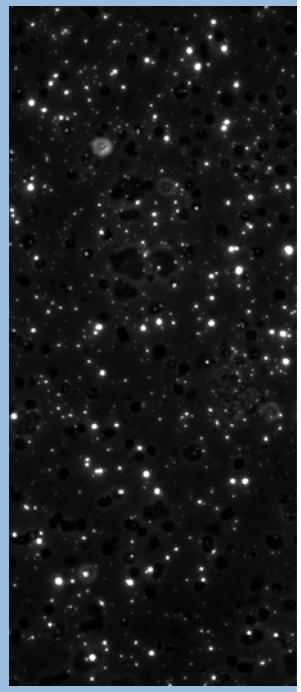
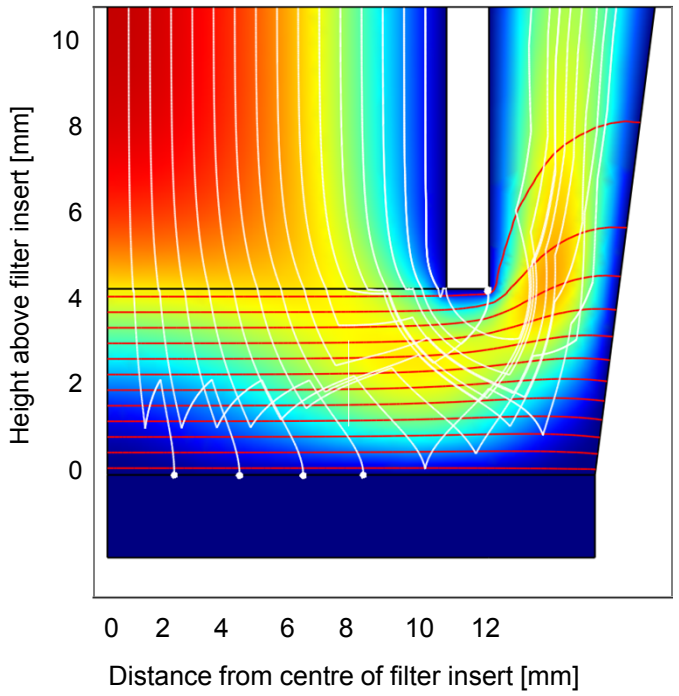
- Defined aerosol (Boltzmann equilibrium) with bipolar Kr^{85} particle charger → *Controlled particle deposition*
- Efficient particle deposition



The new particle deposition chamber: even particle deposition





➡ Even deposition of 200 nm and 50 nm PSL particles

The new particle deposition chamber: efficient particle deposition