

Toxic effects of nanoparticles from biomass combustion

18th ETH Conference on Combustion Generated Nanoparticles

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Outline

- 1) Combustion source: Private-owned heat generators**
 - subsidised employment of renewable fuels

- 2) Particulate matter: Nanoparticles (< 1 μm) in focus**
 - Karlsruhe Exposure System

- 3) Biological model: A549 and SK-MES-1 lung tumour cell lines**
 - liquid-air interface vs. submerge culture

- 4) Results & discussion**

Model sources of combustion particles: Two commercially available heat generators

Shortlisted by *Bundesamt f. Wirtschaft u. Ausfuhrkontrolle (BAFA)* to be eligible for state subsidies.

Pellet stove (7.6 kW)



Buderus Blueline Pellet 1

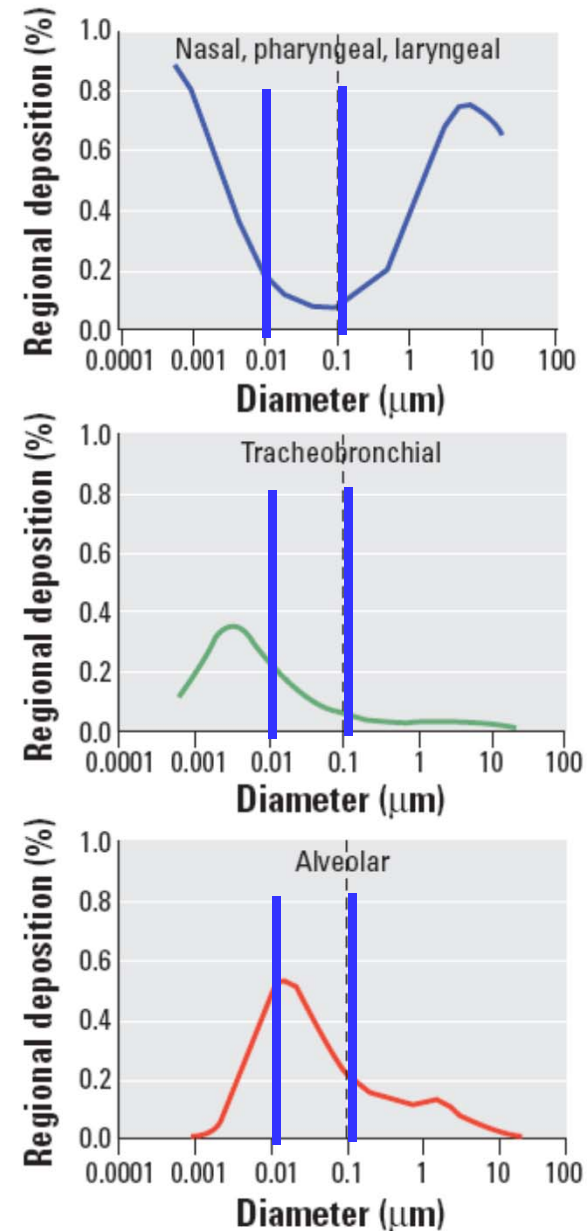
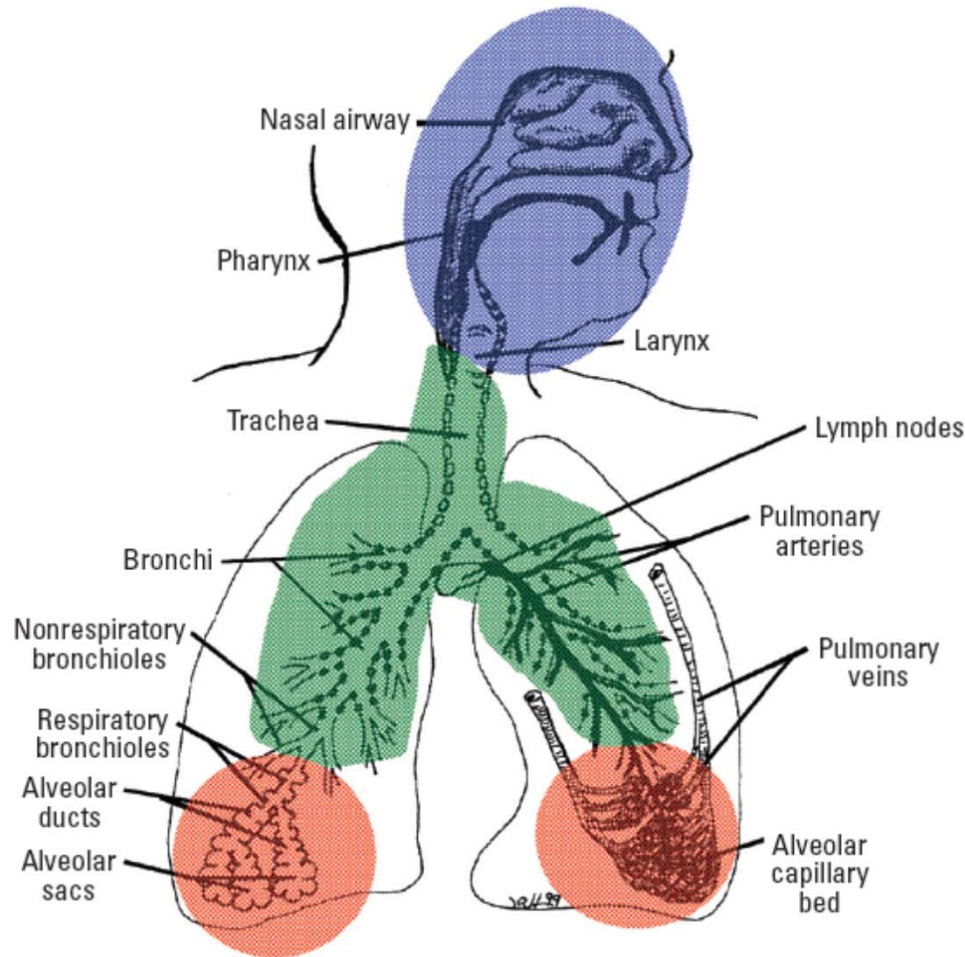
Wood-burning stove (8.0 kW)



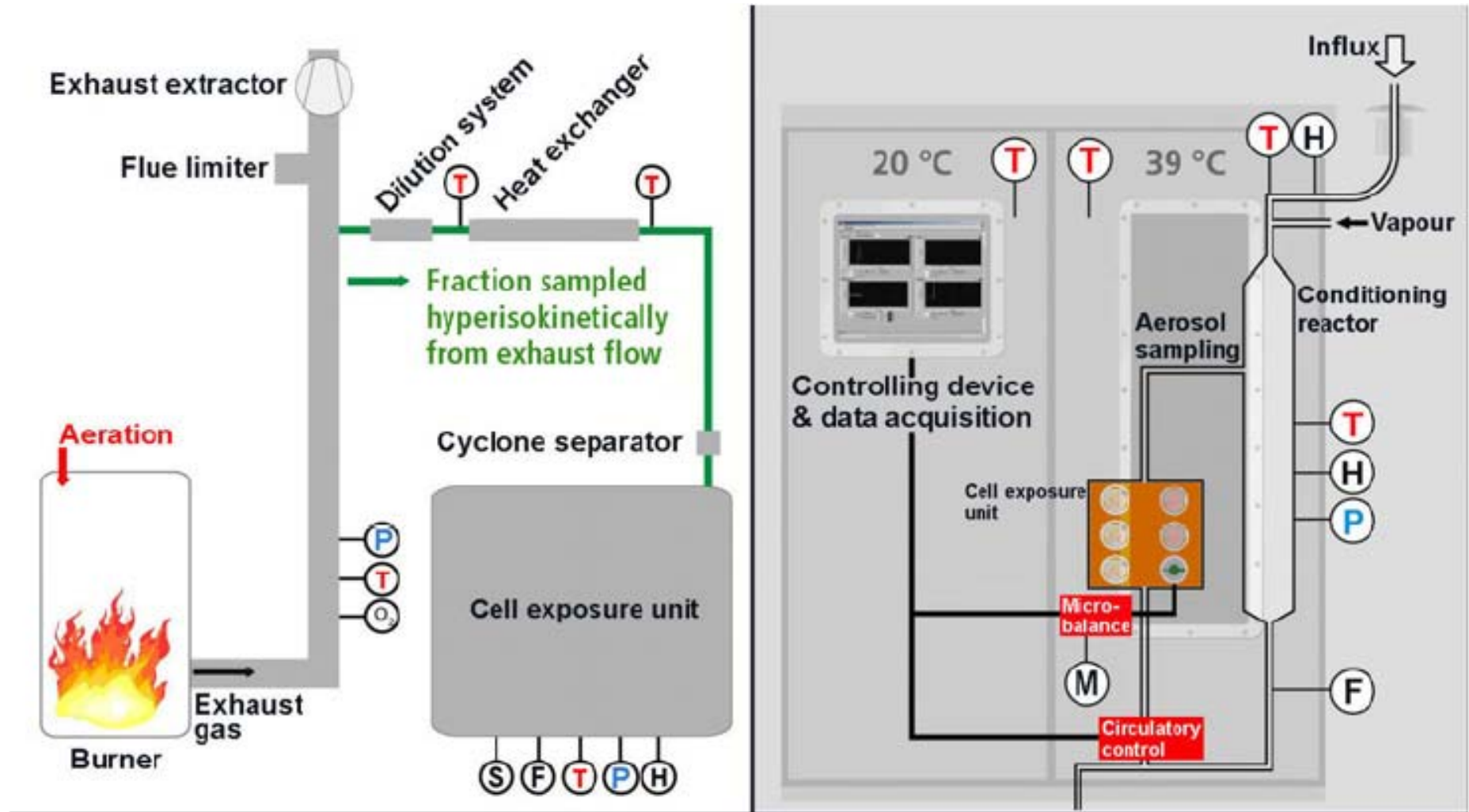
Buderus Blueline 4W

Why focus on nanoparticles?

They reach the very termini of the respiratory system – the alveoles



Nanoparticle selection for exposure: Sketch of the experimental set-up



- Individual mass flow w/ 250 hPa underpressure
- Gas flow: 37 °C; 85 % relative humidity
- Two sampling outlets (e.g. for scanning mobility particle sizer (SMPS), impactor)
- Exposure chamber: 39°C
- Five exposures in parallel

Nanoparticle selection for exposure: Experimental set-up

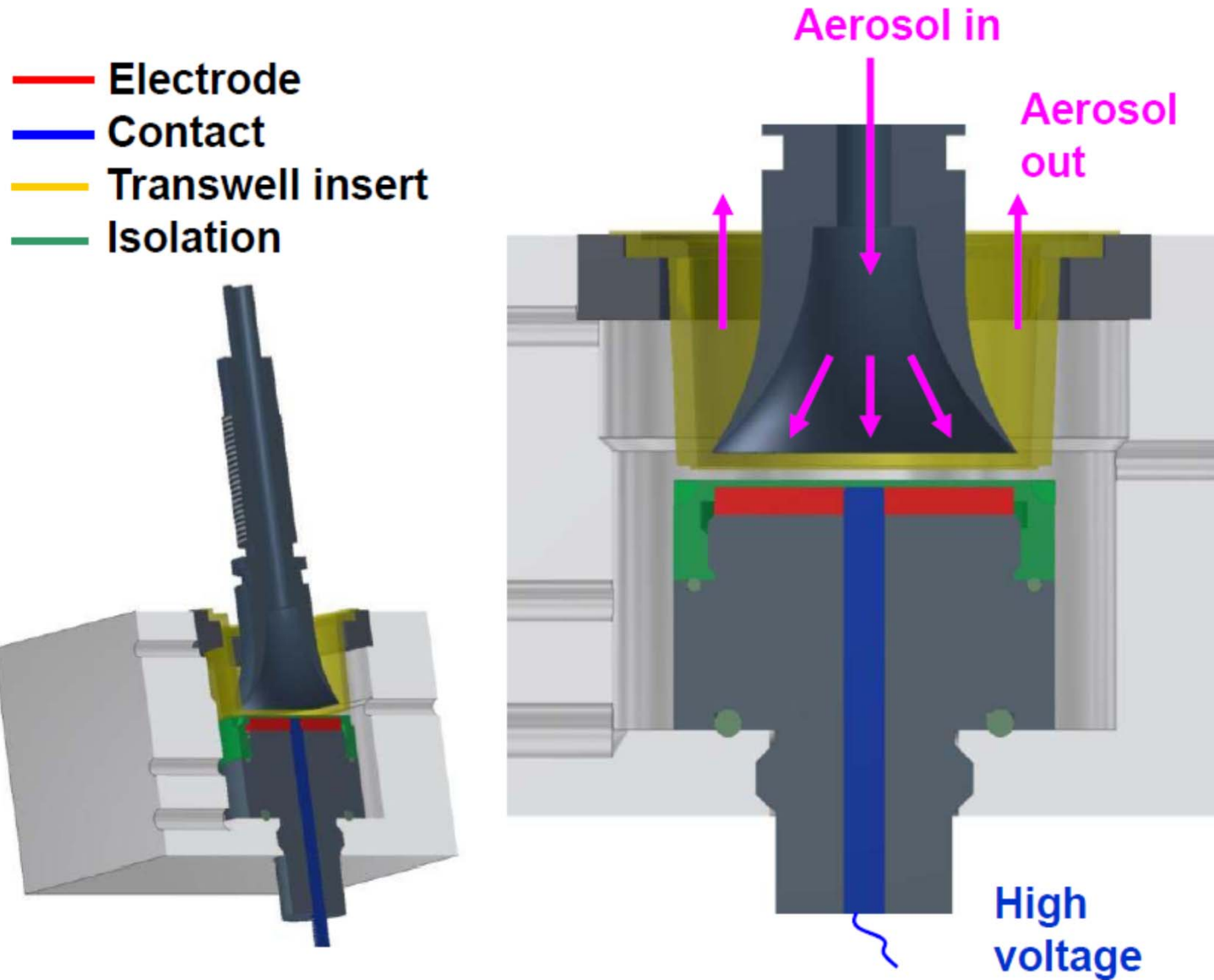


Monitoring unit:

- temperature
- CO₂
- CO
- O₂



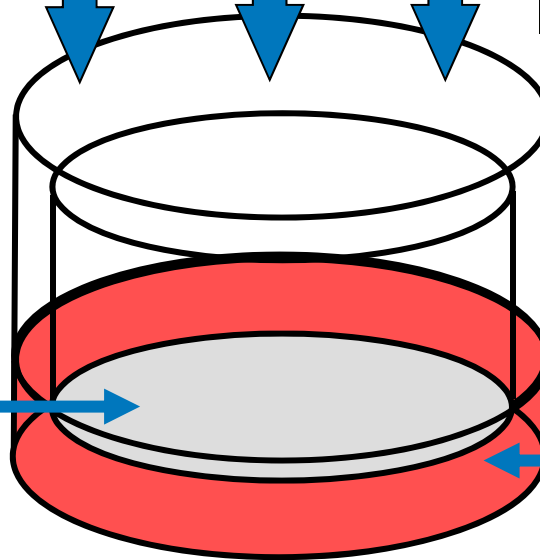
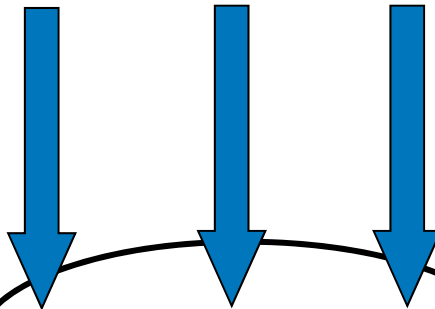
Cell exposure unit – schematic overview



Set-up of the exposure system - relevant cells at the air-liquid interphase



Exhaustive gas
w/ nanoparticles



Lung cells
on a semi-permeable
membrane

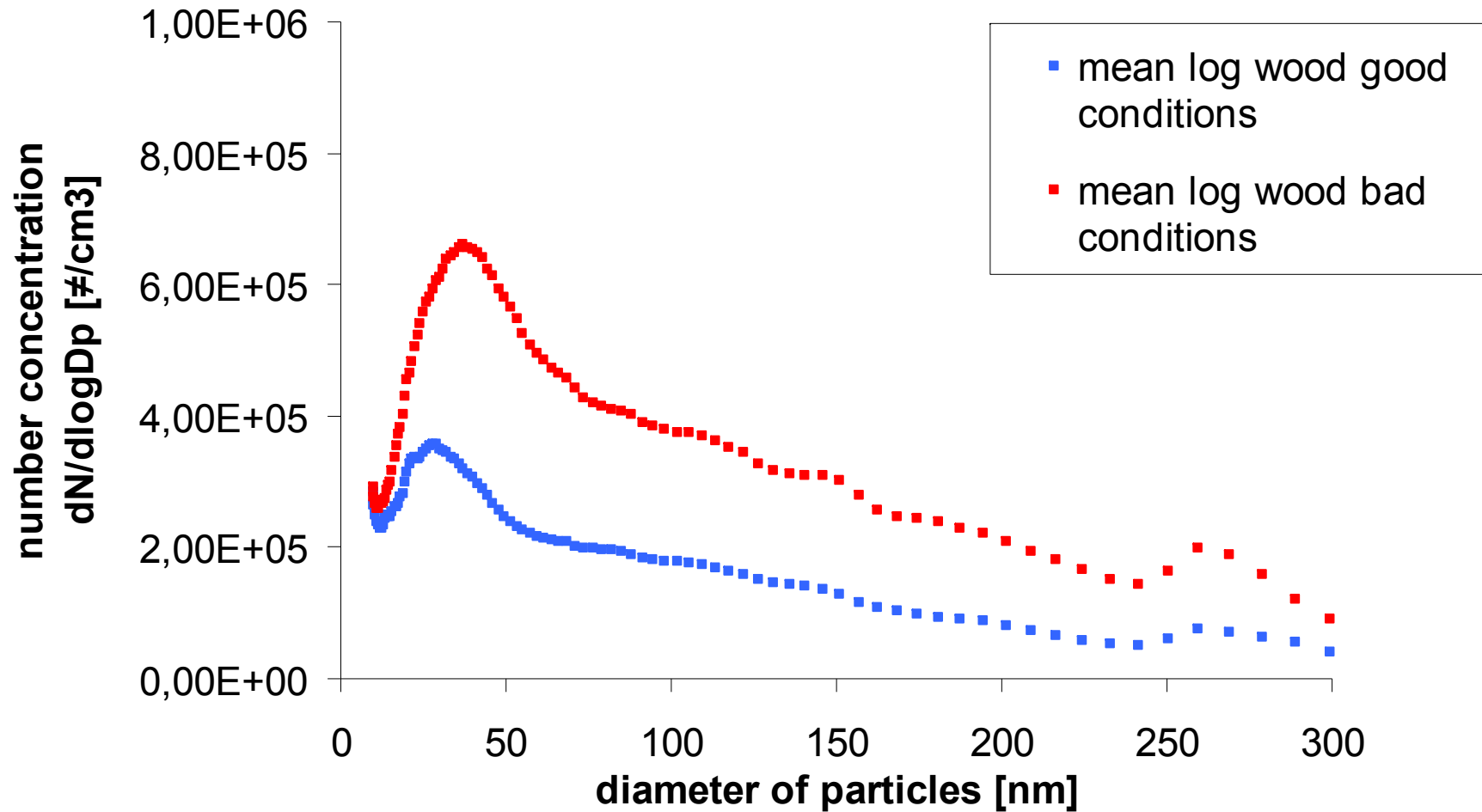


Cell culture
medium



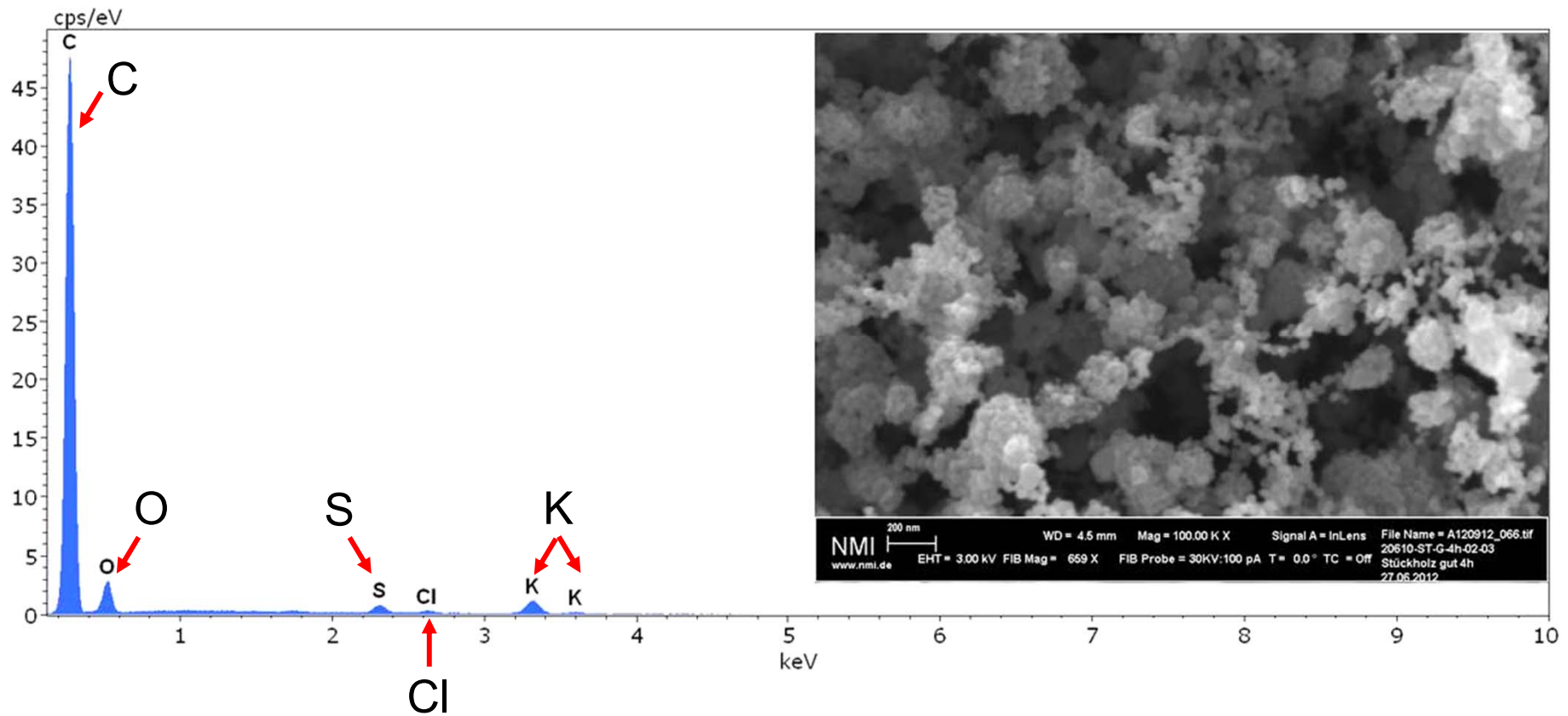
Size distribution of nanoparticles applied

Mean values from SMPS-Measurements of log wood



Elementary analysis of particles using energy dispersive X-ray spectroscopy (EDX)

Nanoparticles arising from combustion of log wood are rich in carbon but also contain salts (KCl, K₂SO₄)



Cell types employed represent cells present in human alveoles

- **Type I pneumocytes:**

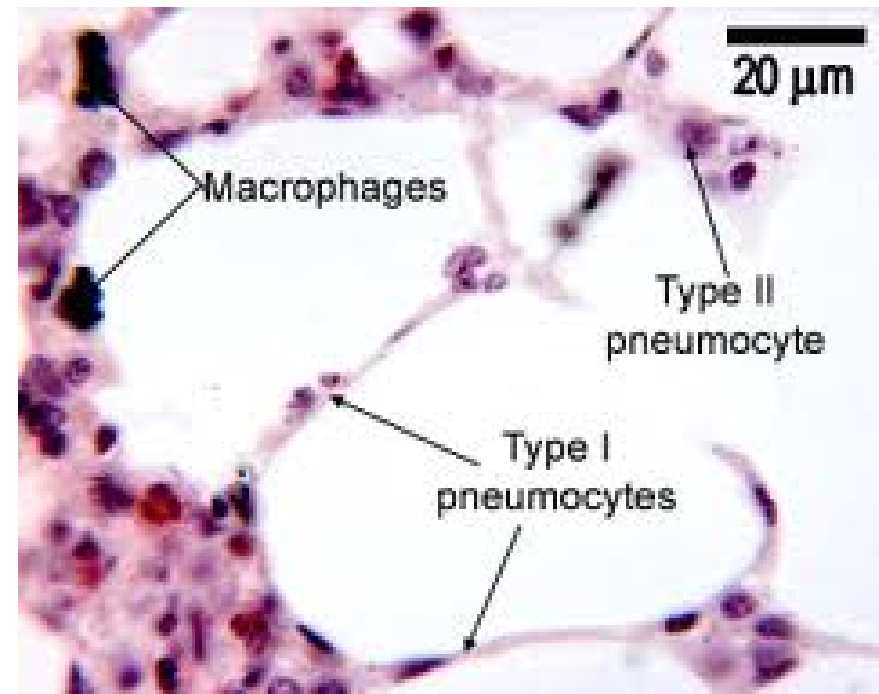
Flat epithelial cells lining the alveoles,
constituting the blood-air interface: **SK-MES-1**

- **Type II pneumocytes:**

Cubical epithelial cells, secrete surfactant, can proliferate and differentiate to repair injury: **A549**

- **Macrophages:**

Cells of the immune system,
specialised in the removal of
microbes and debris;
precursors (monocytes): **THP-1**



Consecutive exposure of the same cell sample – circumventing desiccation stress

In order to minimise desiccation stress, samples were exposed 3 x 2 h on three consecutive days:

Exposure series	Day 1			Day 2			Day 3		
1	Sample 1			Sample 1			Sample 1		
2		Sample 2			Sample 2			Sample 2	
3			Sample 3			Sample 3			Sample 3

- between exposures, cells were cultivated w/ medium at 37 °C, 5 % CO₂
- cells were harvested for analysis one day after the last exposure
- expression of stress markers was analysed using qRT-PCR

Stress markers analysed

Oxidative stress

Interleukin-8 (IL-8; CXCL8)

- proinflammatory cytokine,
- indicative of oxidative stress
- secreted by epithelial cells
- induced by p38 MAP kinase pathway

HMOX1 (heme oxygenase 1; HO-1)

- alleviates oxidative stress,
due to its antioxidative function,
degrading heme into biliverdin
and carbon monoxide
- induced by p38 MAP kinase pathway

General stress

ICAM-1 (*intercellular adhesion molecule 1*; CD54)

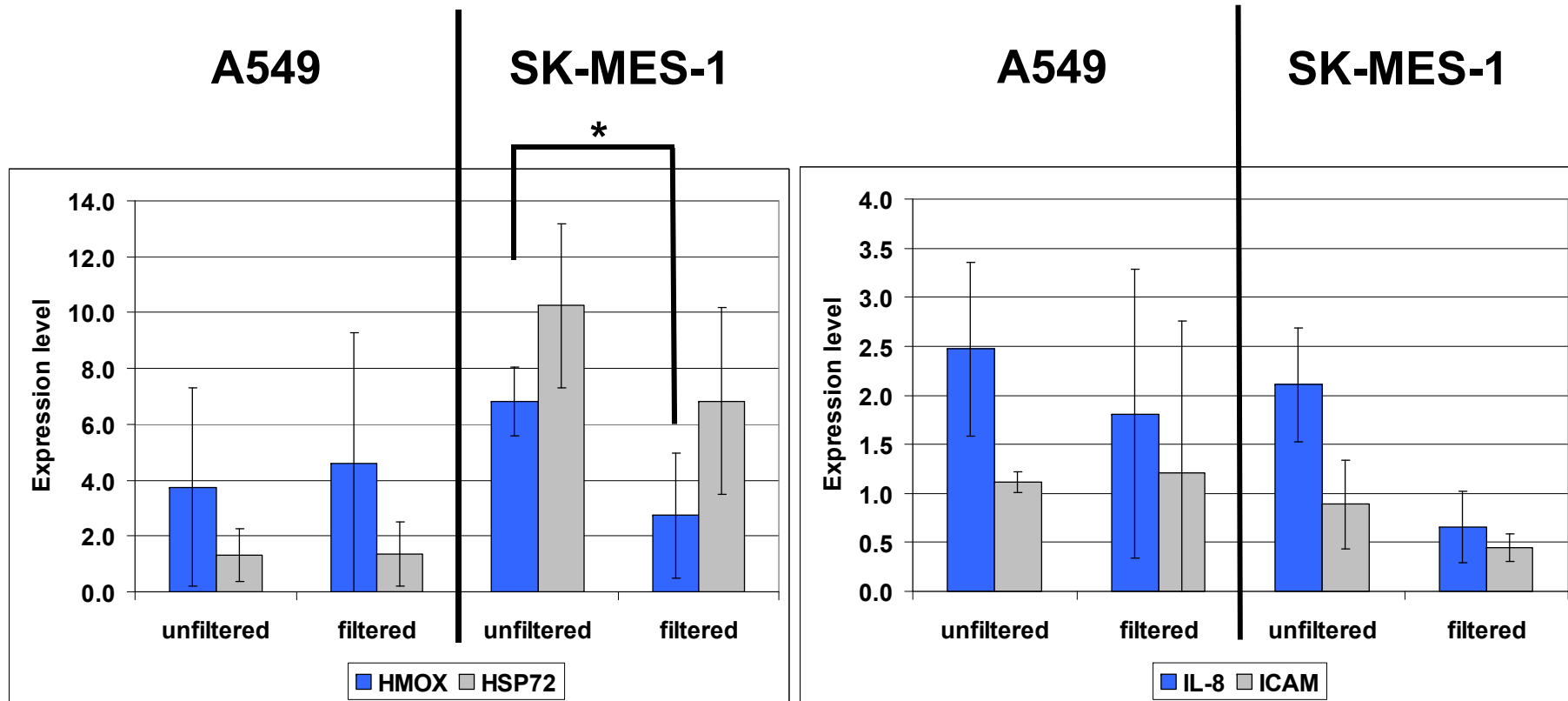
- proinflammatory glycoprotein of the cell surface
- binds macrophages and leukocytes
- induced by TNF- α ; IL-27

HSP72 (*heat shock protein 72*; HSPA1A)

- inhibits apoptosis (programmed cell death)

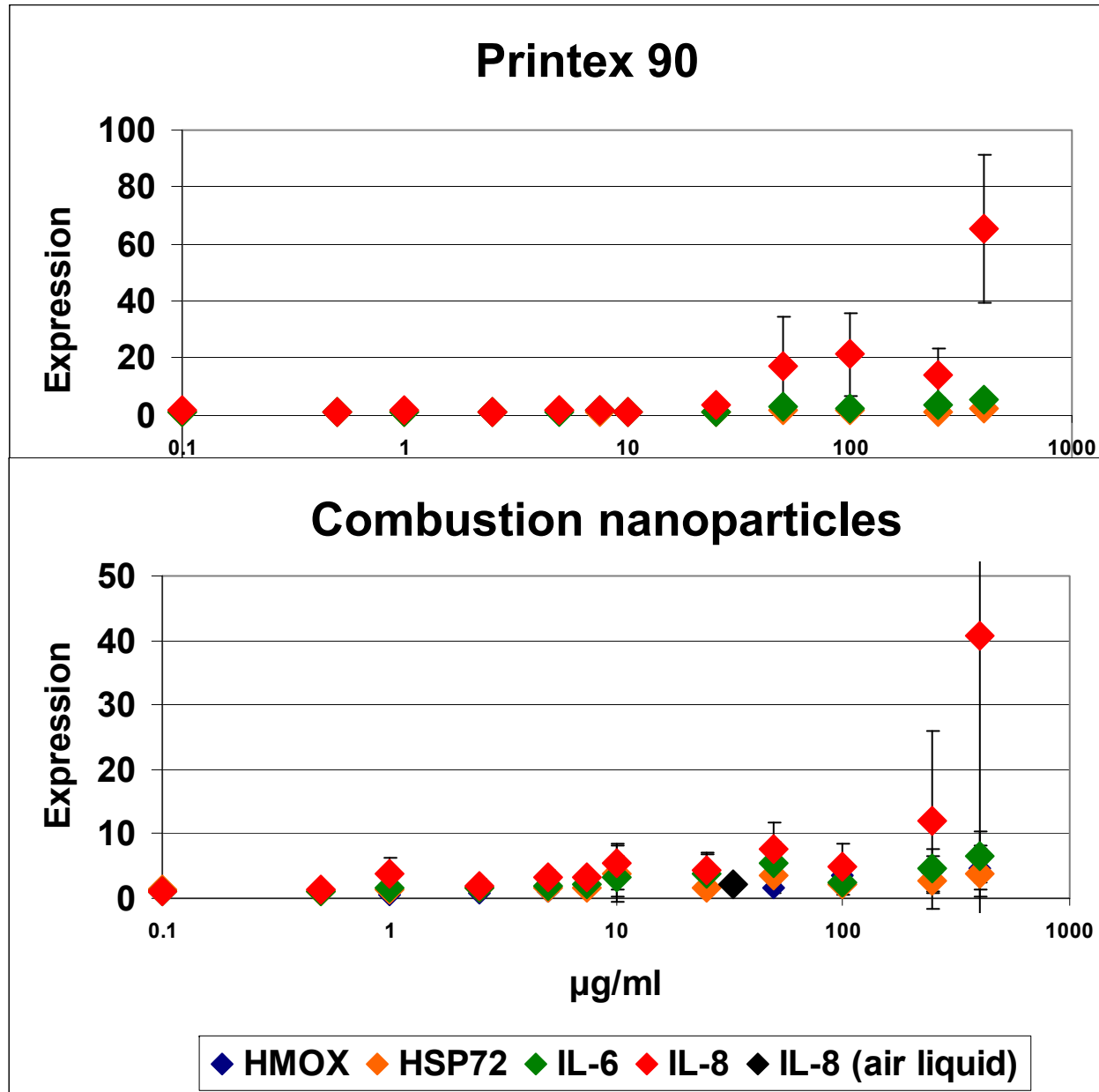
Cellular response to nanoparticle exposure

SK-MES-1 cells show a 2.5-fold increase in HMOX expression (mRNA level)



NB: Expression was normalised to untreated controls

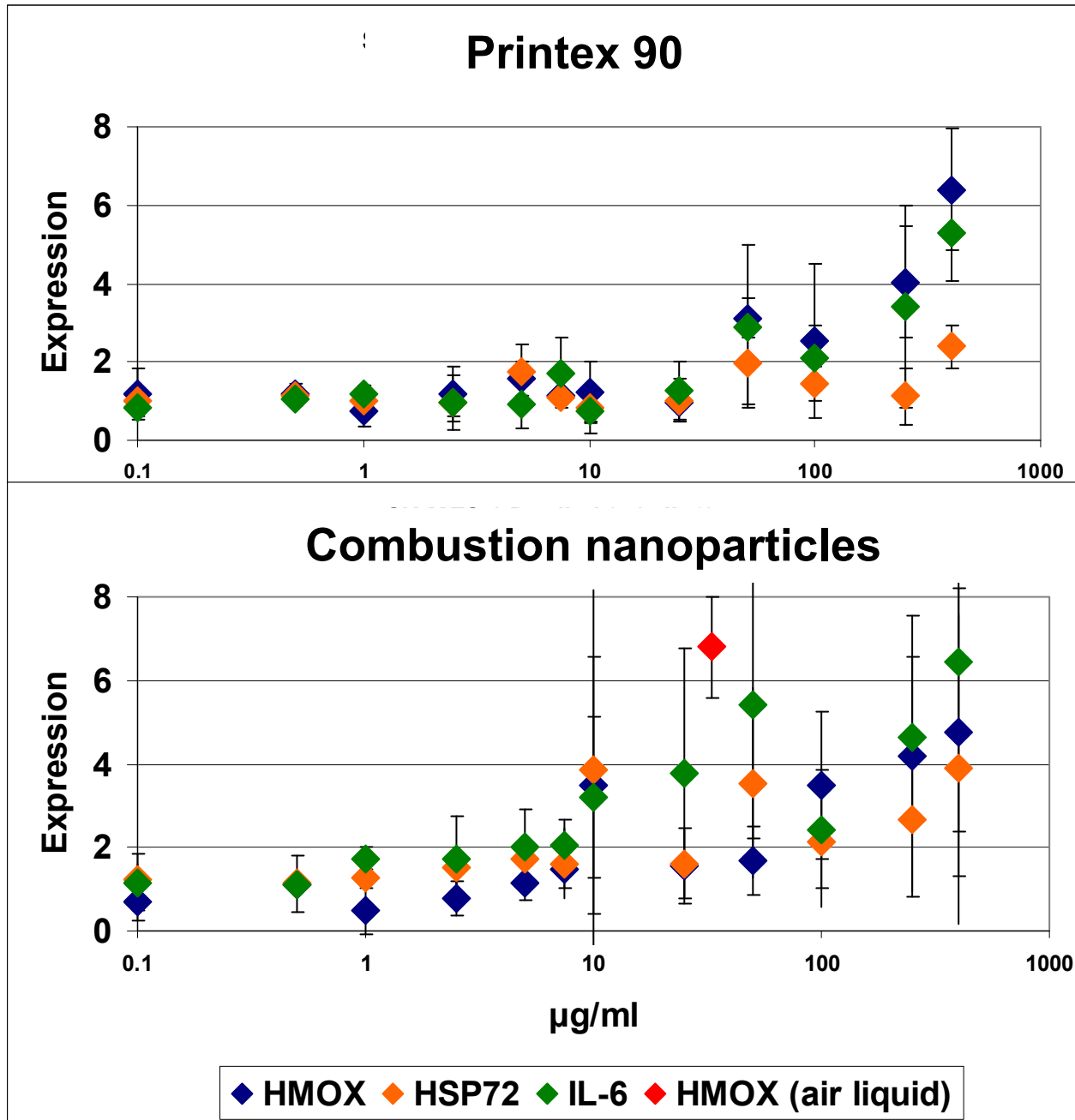
Submerge exposure of SK-MES-1 cells (1): Printex 90 vs. combustion nanoparticles



- Both types of particles elicit a roughly comparable response at the mRNA level.

- Printex 90 is approx. twice as potent as the combustion particles.

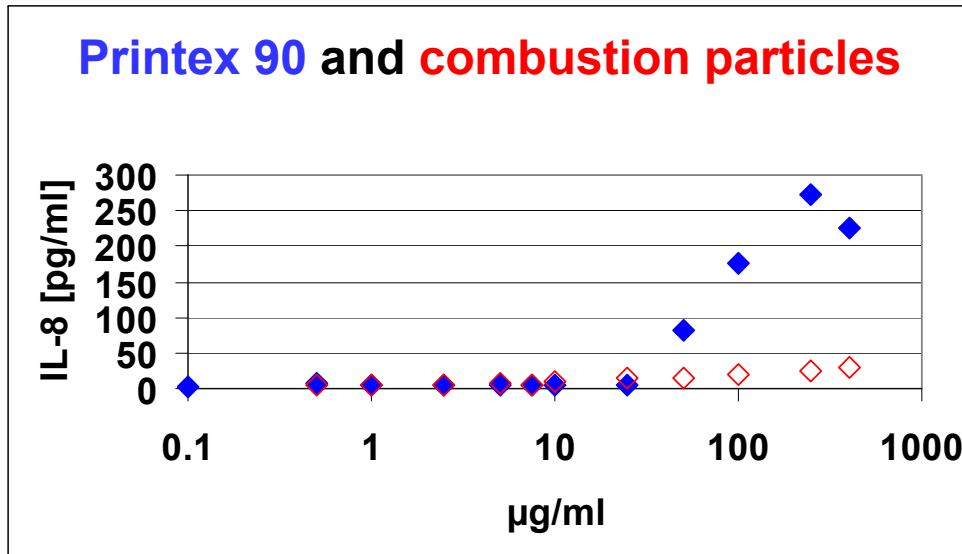
Submerge exposure of SK-MES-1 cells (2): Printex 90 vs. combustion nanoparticles



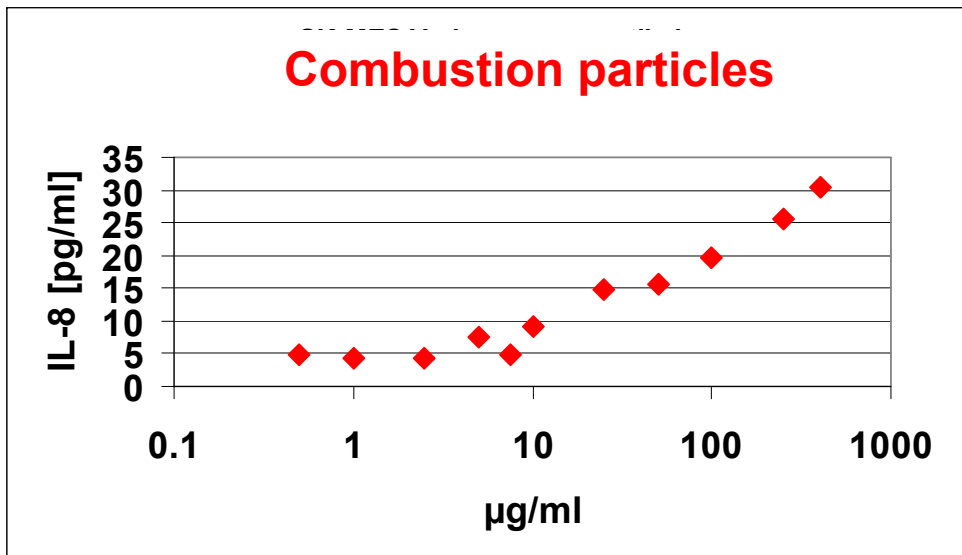
- Both types of particles elicit a comparable response for HMOX, HSP27 and IL-6 at the mRNA level.

- The HMOX response caused by exposure at the air-liquid interface is approx. twice as high as under submerge exposure (33 µg/ml).

Submerge exposure of SK-MES-1 cells (3): Printex 90 vs. combustion nanoparticles



- At the protein level, the secretion of IL-8 caused by Printex® 90 is approx. one order of magnitude higher than that caused by combustion particles.

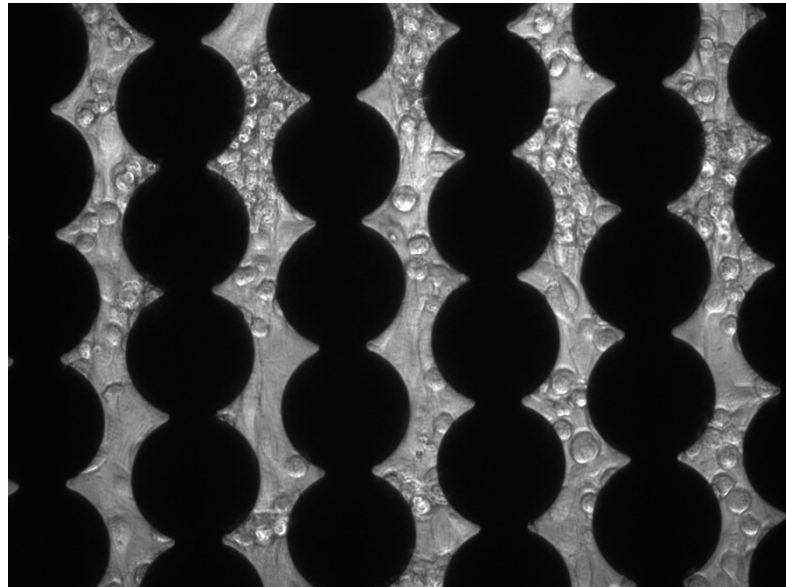


Impedance measurements as a proxy for long-term cell proliferation

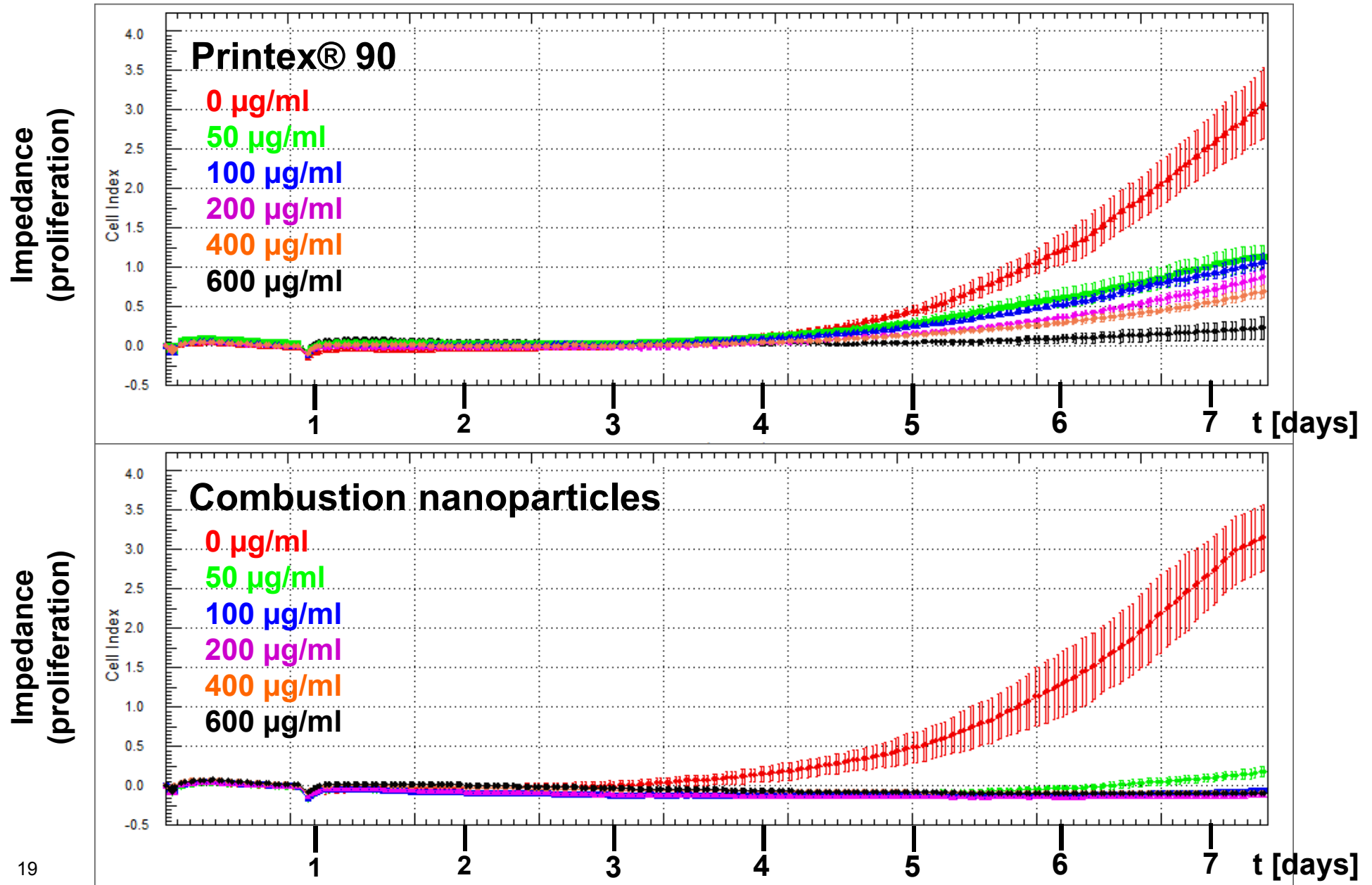
Attachment of cells on electrodes => **increase in impedance**
(resistance of alternate current)

Impedance is affected by **cell proliferation** (growth rate) and **morphology**, both influencing the coverage of electrode surface.

Thus, impedance measurement allows for a **real-time**, label-free and **non-invasive** analysis of key cellular events .



Printex 90 is less anti-proliferative than combustion nanoparticles



Summary of stress marker analysis

- 1) **A549 cells** (Type II pneumocytes) show a **weaker** response than **SK-MES-1 cells** (Type I pneumocytes).
- 2) Exposure at the air liquid interphase: induction of **HMOX expression** is significantly higher in the presence of combustion-derived particles (SK-MES-1 cells; $p = 0.05$).
- 3) At submerge exposure, Printex® 90 elicits a response of **similar** strength at the **mRNA level** as combustion-derived particles.
- 4) At submerge exposure, the secretion of **IL-8** caused by Printex® 90 is approx. **one order of magnitude higher** than that caused by combustion particles.
- 5) At submerge exposure, the anti-proliferative effect of Printex® 90 is **lower** than that of combustion particles.

Acknowledgments

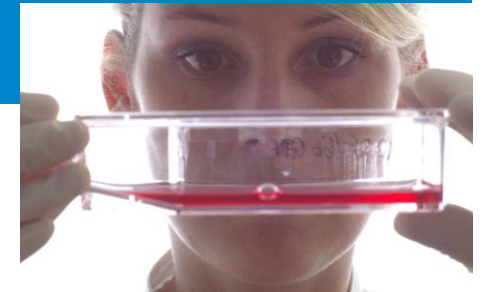
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