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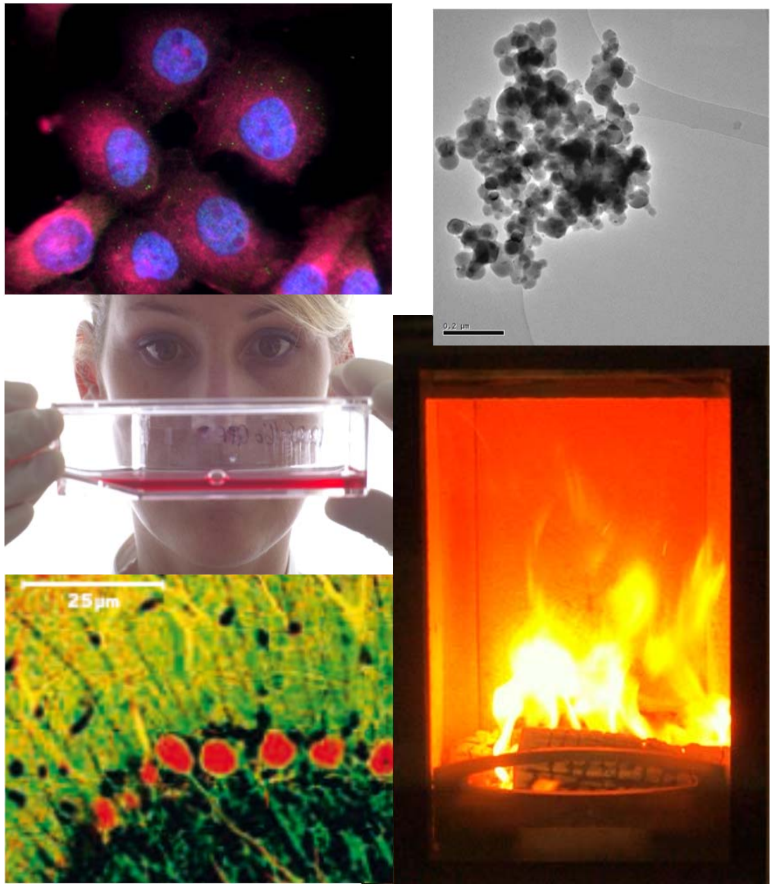

Toxic effects of nanoparticles from biomass combustion

18th ETH Conference on Combustion Generated Nanoparticles

June 24th, 2014

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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. submerse culture

4) **Results & discussion**

Model sources of combustion particles:
Two commercially available heat generators

NMI achieving results

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

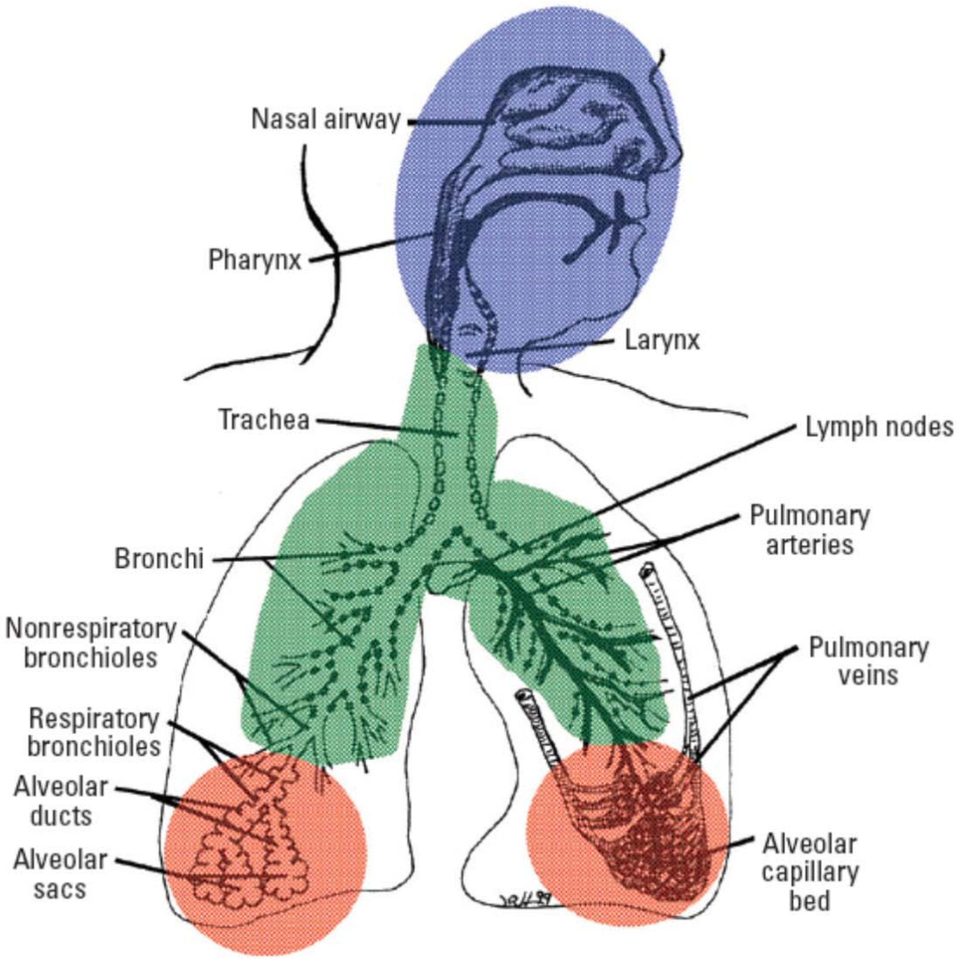
Pellet stove (7.6 kW)

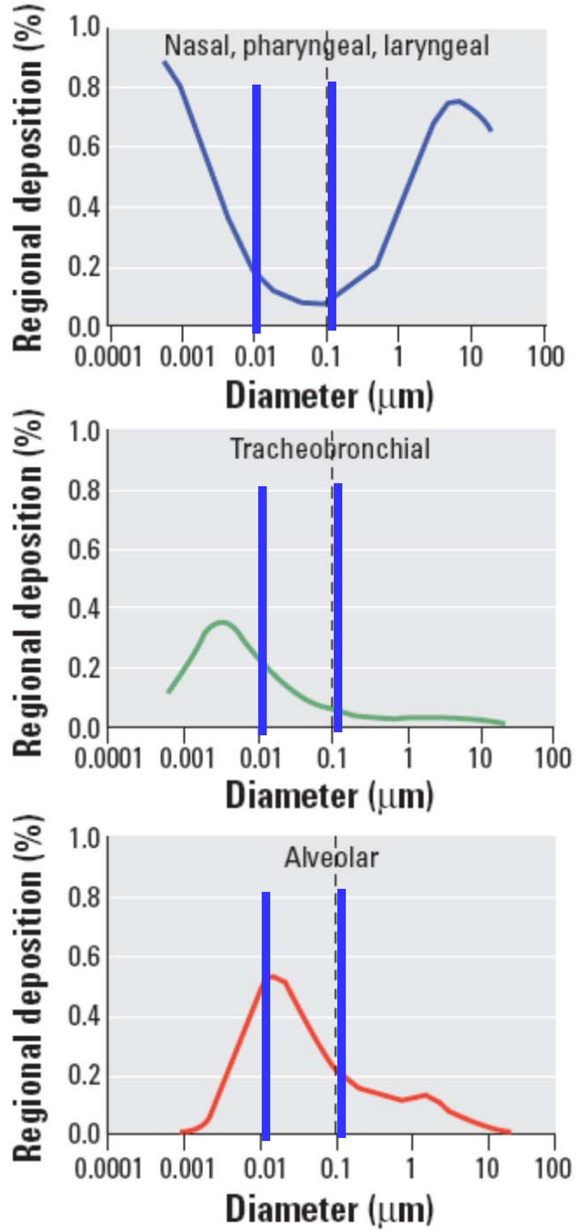
Buderus Blueline Pellet 1

Wood-burning stove (8.0 kW)

Buderus Blueline 4W

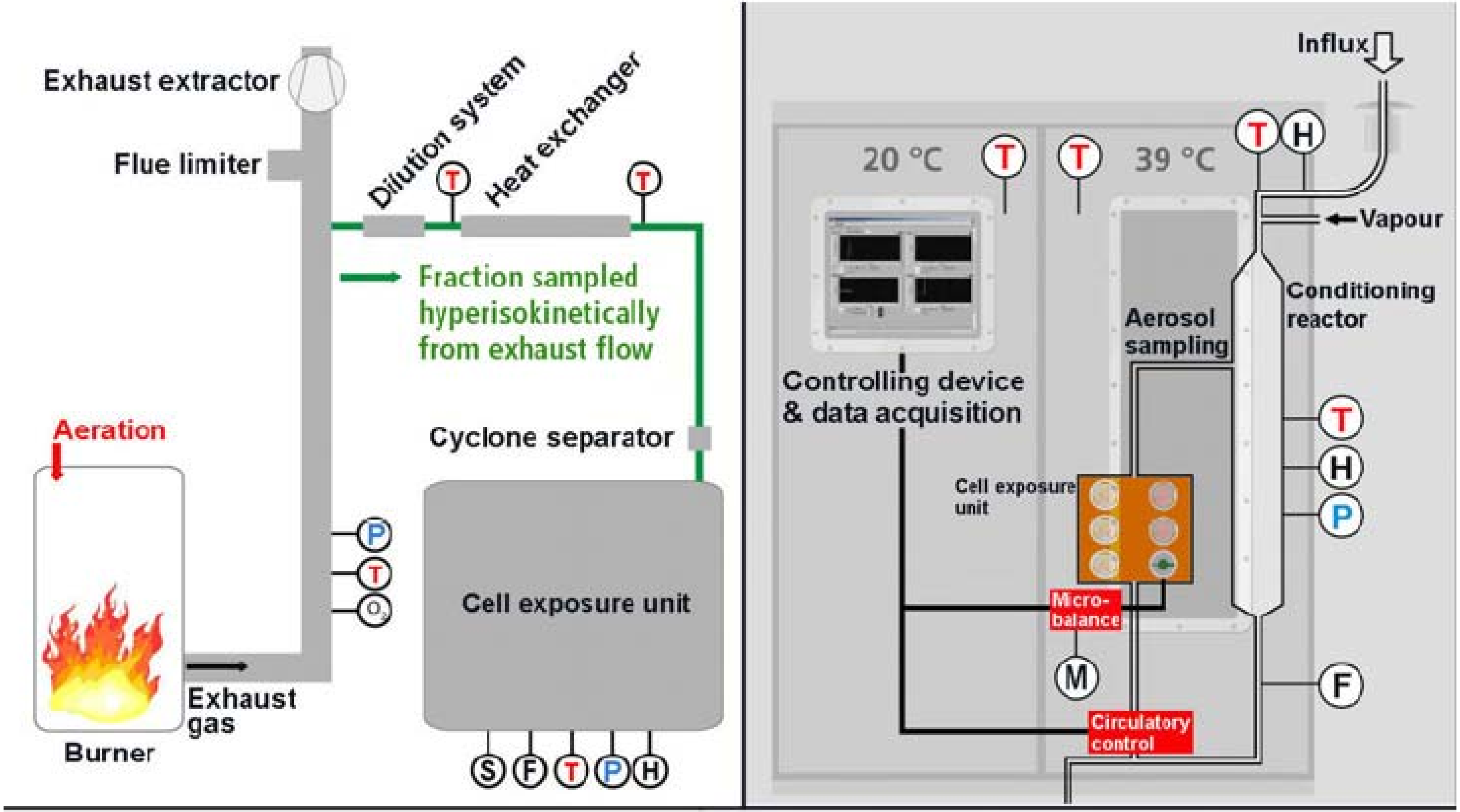
Why focus on nanoparticles?
They reach the very termini of the respiratory system – the alveoles





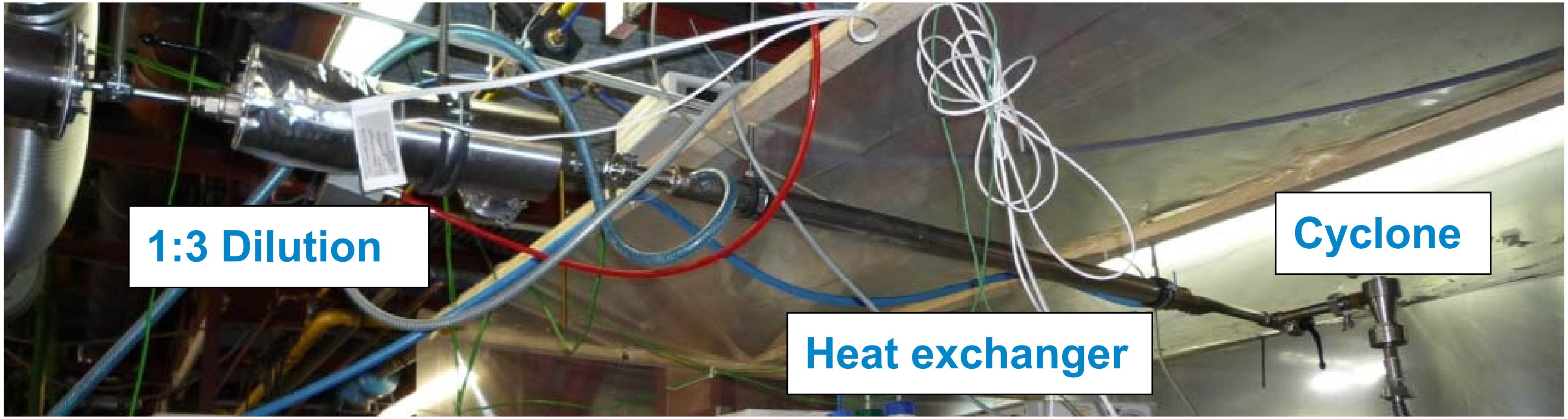
Oberdörster (2005) *Environ Health Perspect* 113: 823-839

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



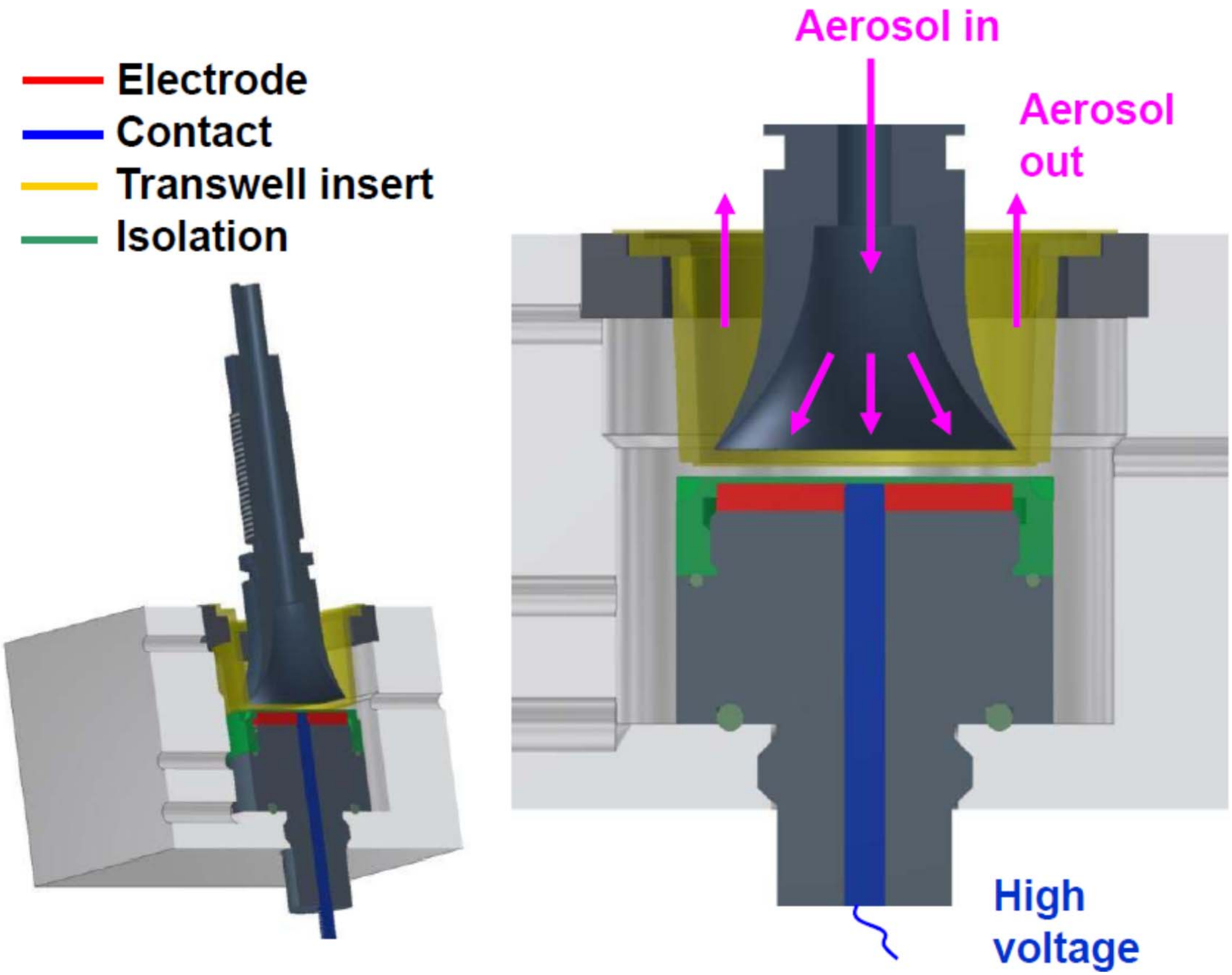
- Individual mass flow w/ 250 hPa underpressure
- Gas flow: 37 °C; 85 % relative humidity
- Two sampling outlets (e.g. for scanning mobility particle sizer (SMPS), impactor)
- Exposure chamber: 39°C
- Five exposures in parallel

Nanoparticle selection for exposure: Experimental set-up



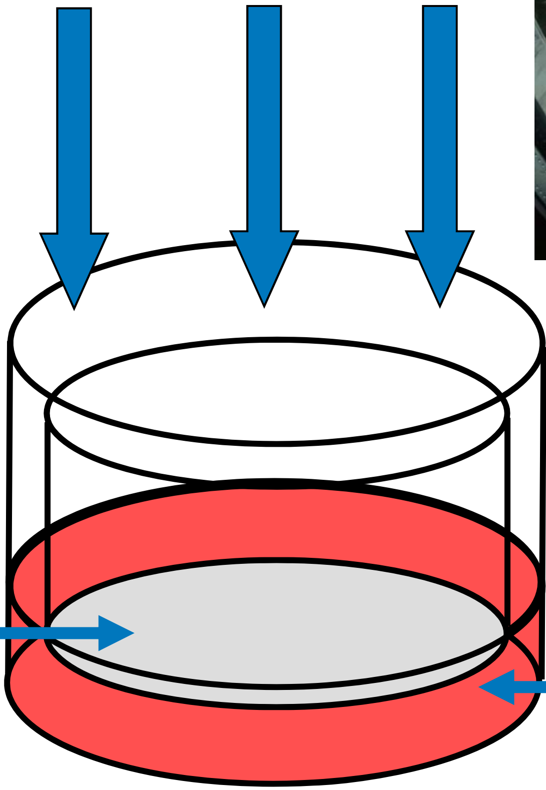
Monitoring unit:
- temperature
- CO_2
- CO
- O_2

Cell exposure unit – schematic overview



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

Exhaustive gas
w/ nanoparticles



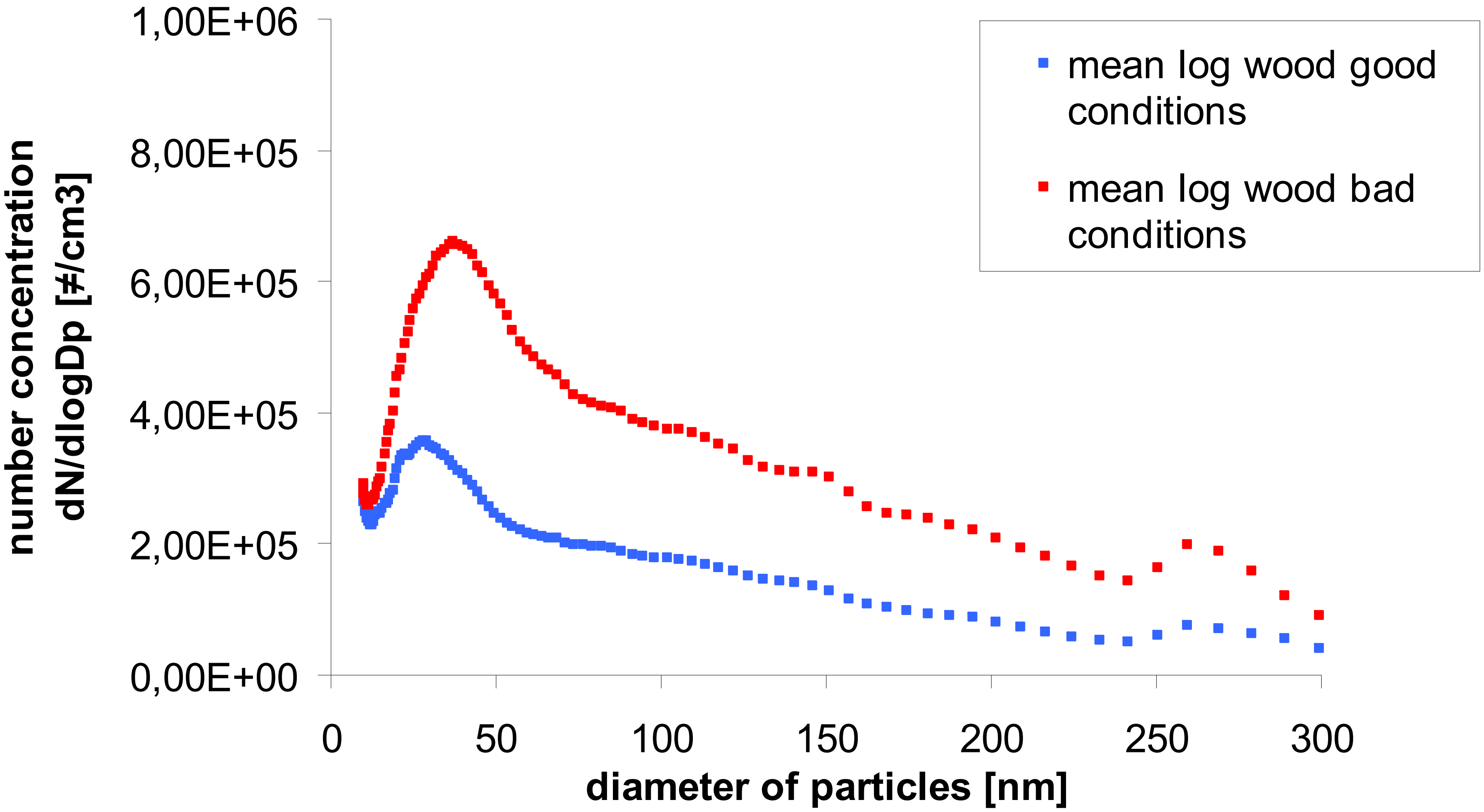
Lung cells
on a semi-permeable
membrane

Cell culture
medium

VITROCELL SYSTEMS

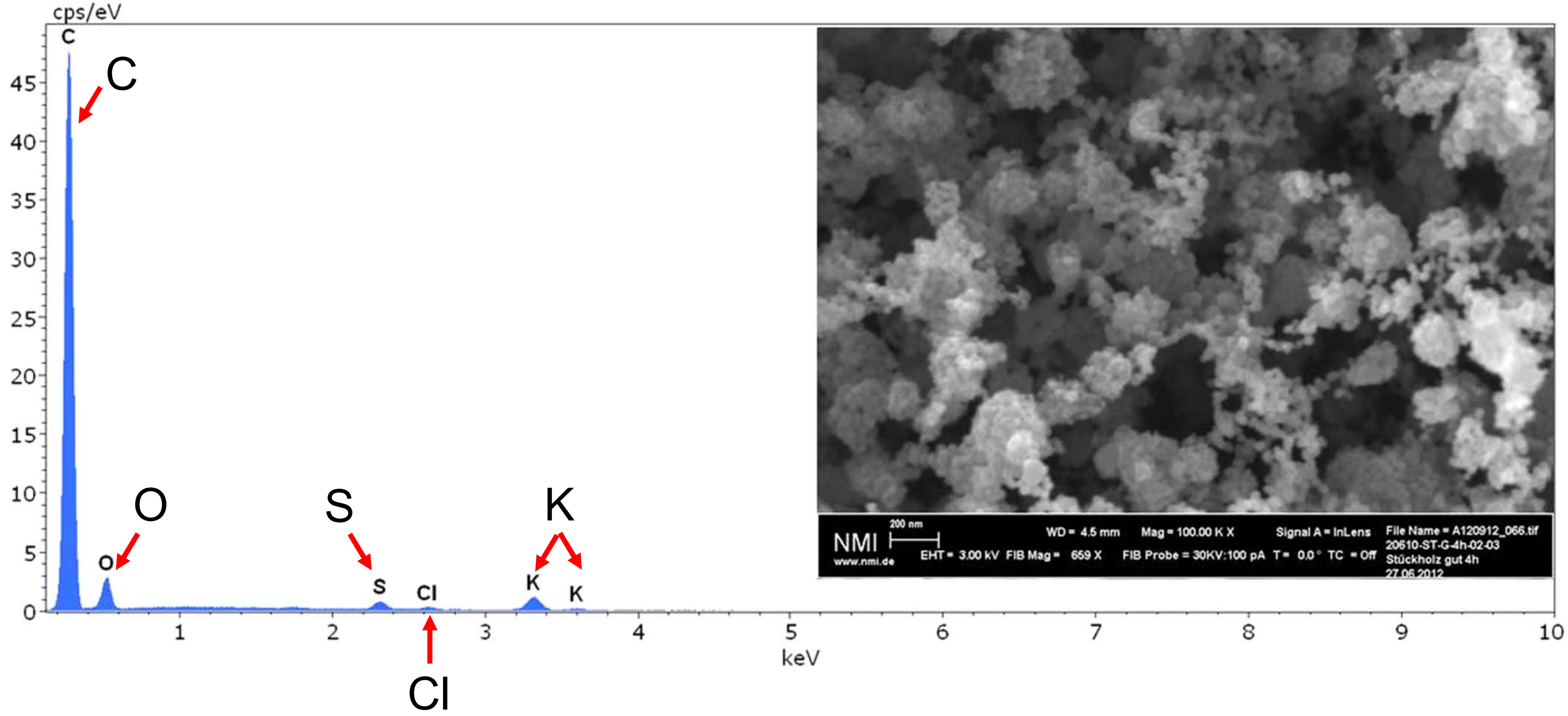
Size distribution of nanoparticles applied

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, K_2SO_4)



Cell types employed represent cells present in human alveoles

- **Type I pneumocytes:**

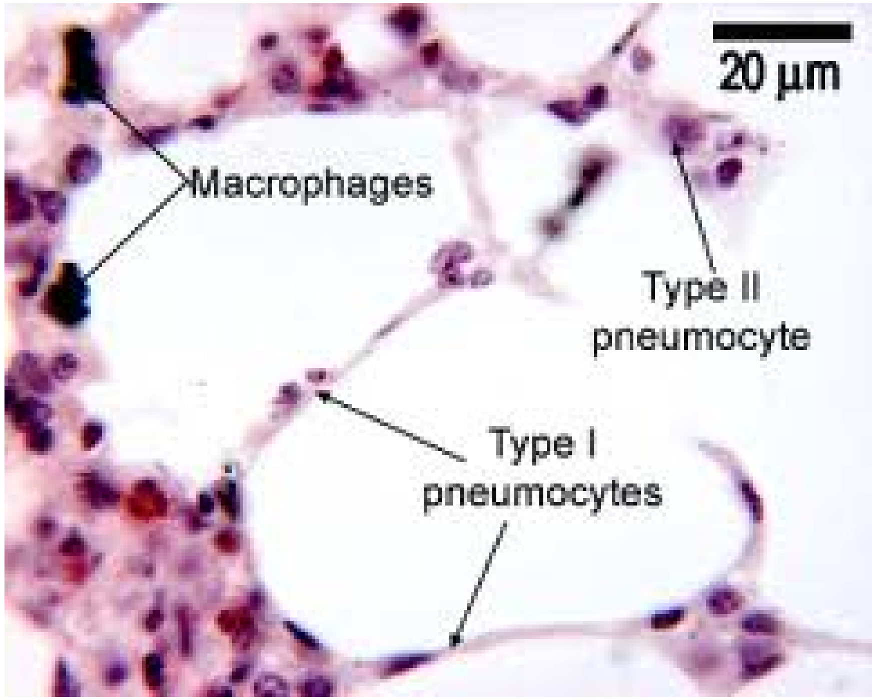
  Flat epithelial cells lining the alveoles, constituting the blood-air interface: SK-MES-1

- **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; precursers (monocytes): THP-1



Consecutive exposure of the same cell sample – circumventing desiccation stress

NMI achieving results

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 % CO_2
- cells were harvested for analysis one day after the last exposure
- expression of stress markes 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 oxigenase 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)

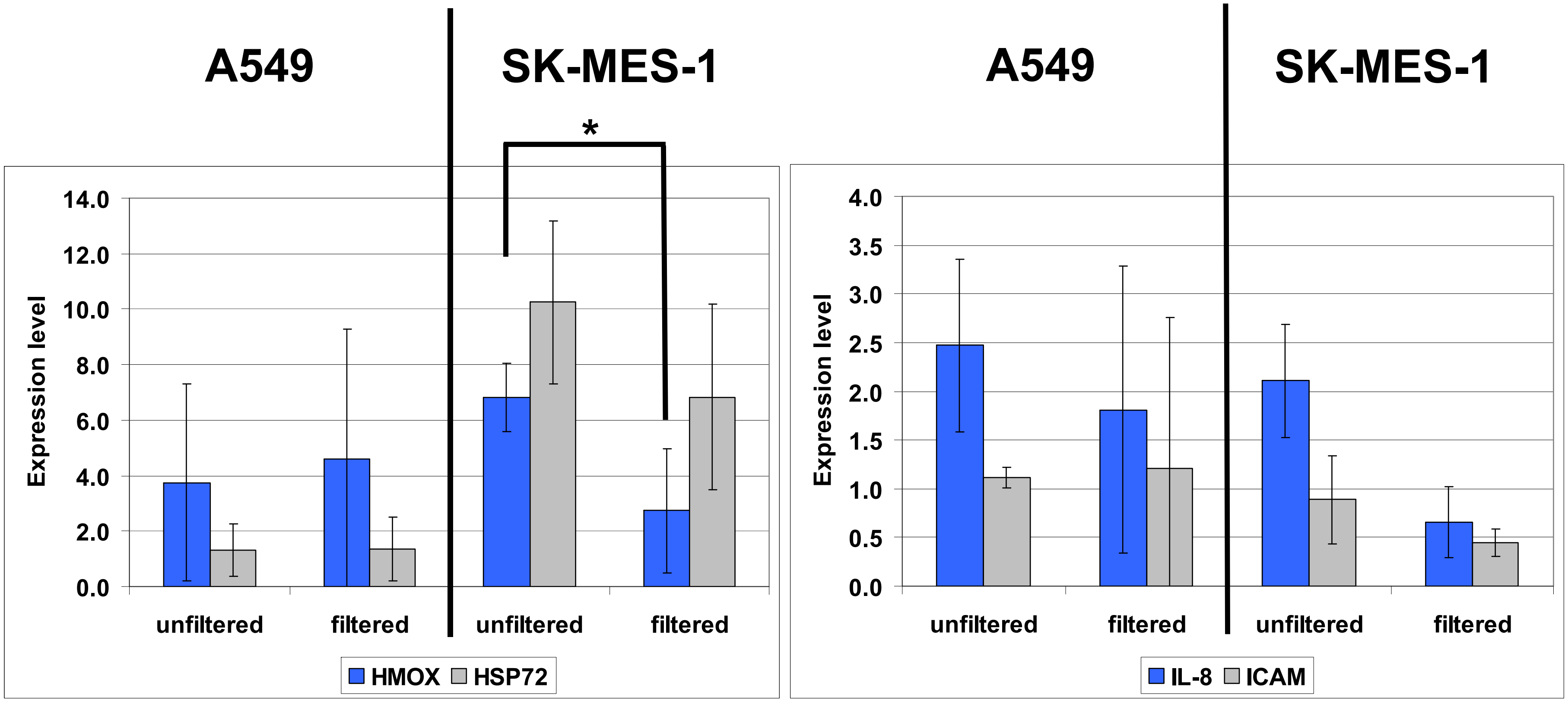
- proinflammatory glycoprotein of the cell surface
- binds macrophages and leukocytes
- induced by TNF-α; IL-27

HSP72 (*heat shock protein* 72; HSPA1A)

- inhibits apoptosis (programmed cell death)

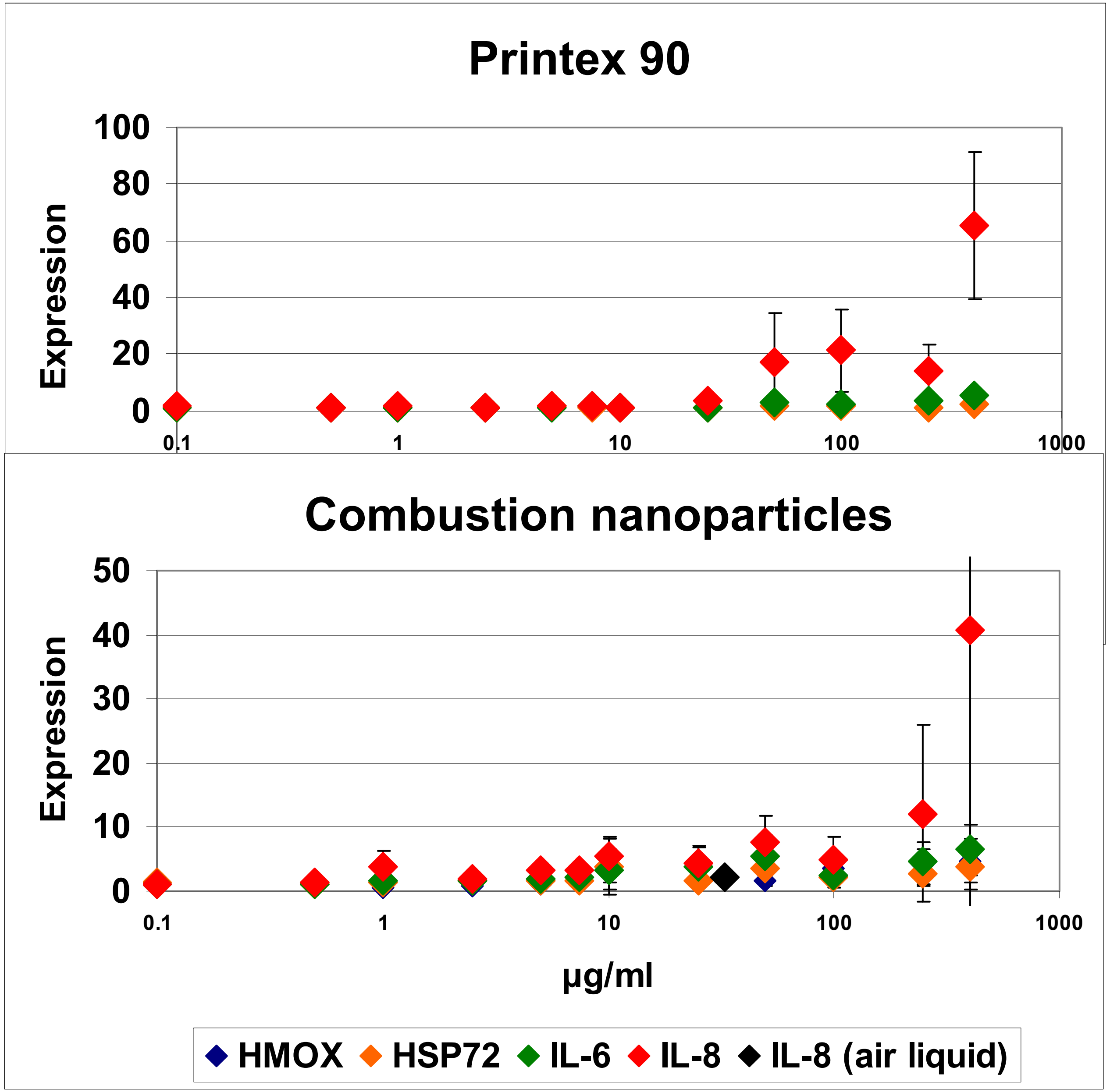
Cellular response to nanoparticle exposure

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



NB: Expression was normalised to untreated controls

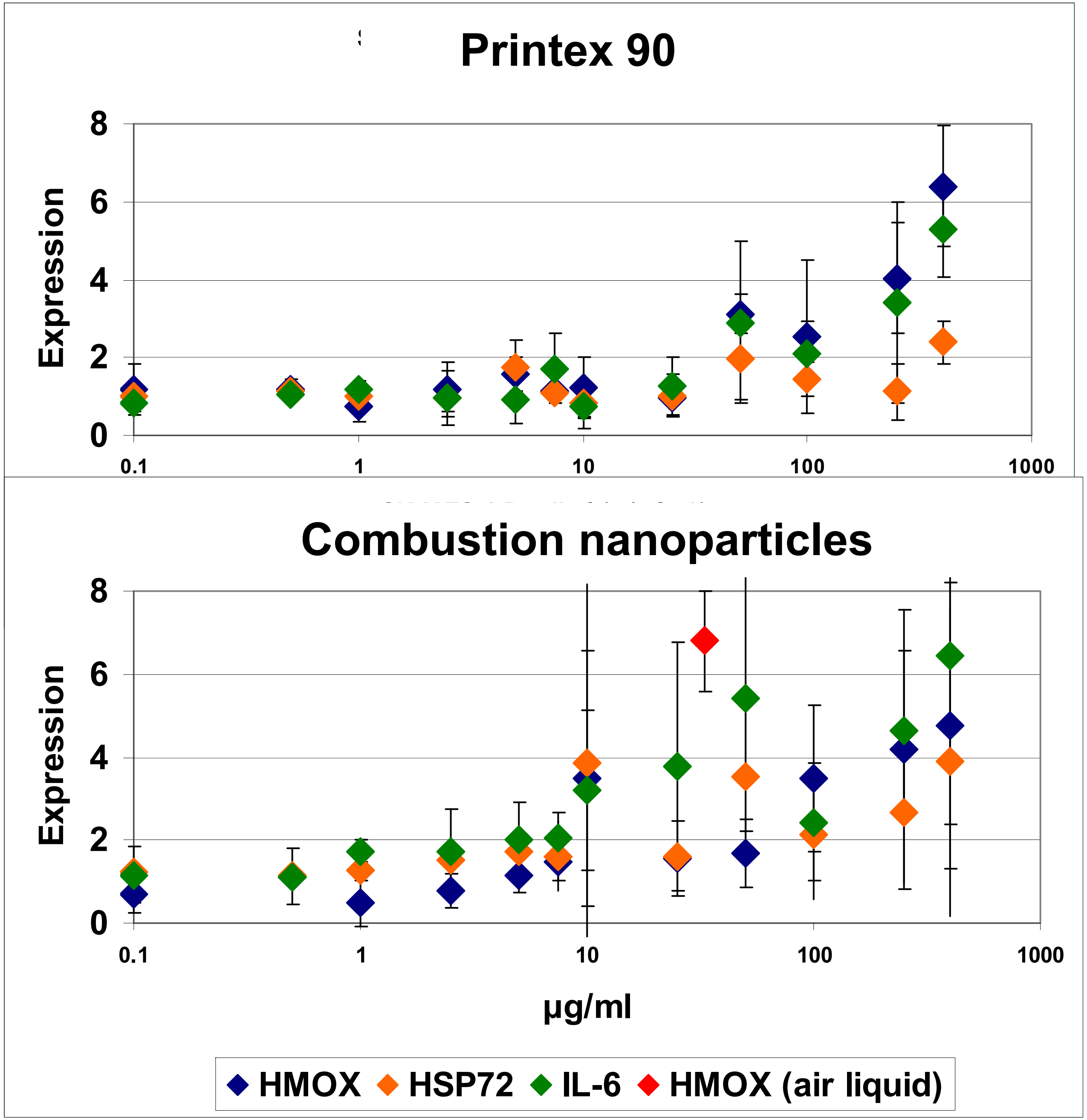
Submerse exposure of SK-MES-1 cells (1): Printex 90 vs. combustion nanoparticles



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

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

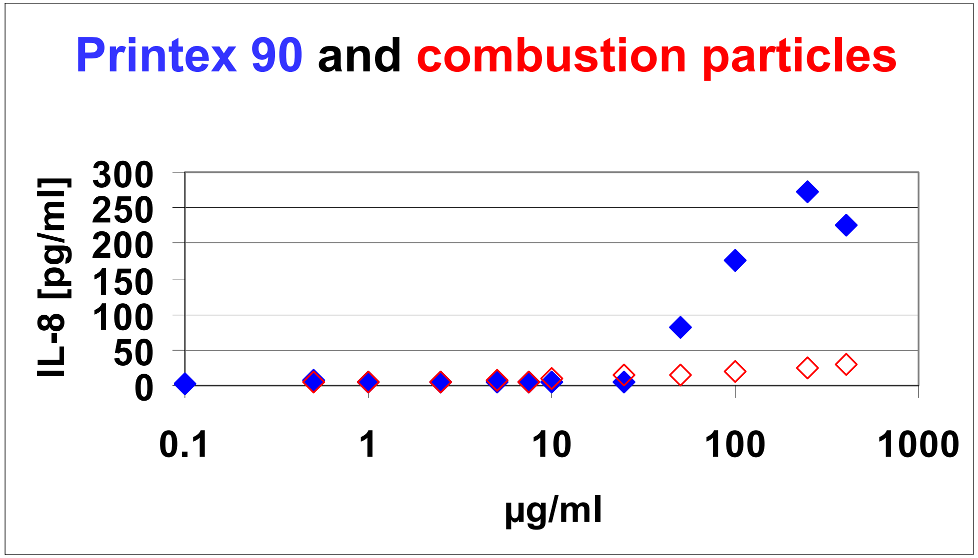
Submerse exposure of SK-MES-1 cells (2): Printex 90 vs. combustion nanoparticles

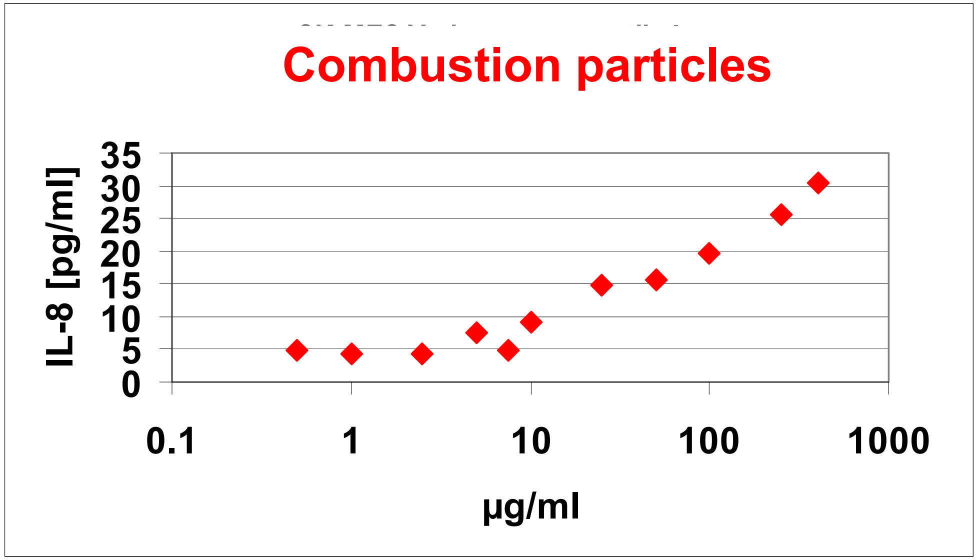


- Both types of particles elicit a comparable response for HMOX, HSP27 and IL-6 at the mRNA level.

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

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





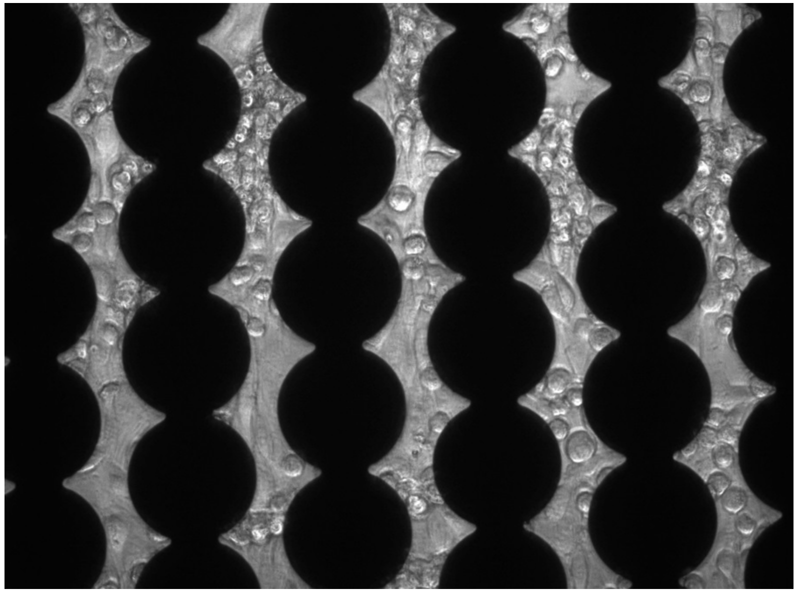
- At the protein level, the secretion of IL-8 caused by Printex® 90 is approx. one order of magnitude higher than that caused by combustion particles.

Impedance measurements as a proxy for long-term cell proliferation

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

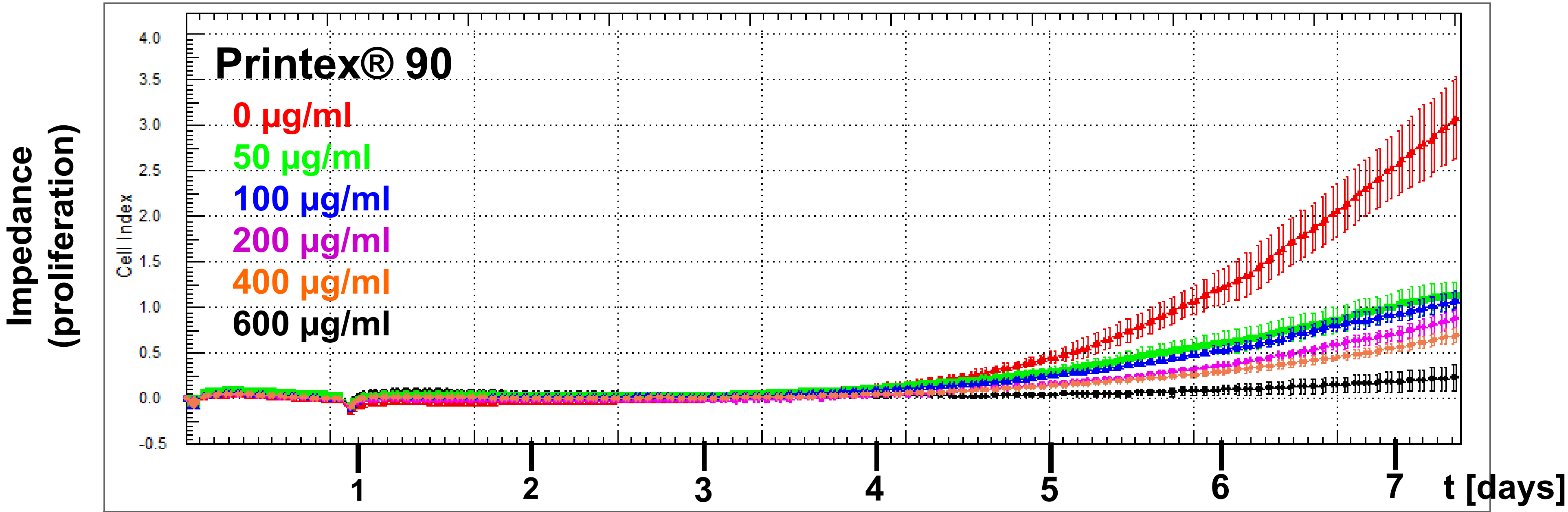
Impedance is affected by **cell proliferation** (growth rate) and **morphology**, both influencing the coverage of electrode surface.

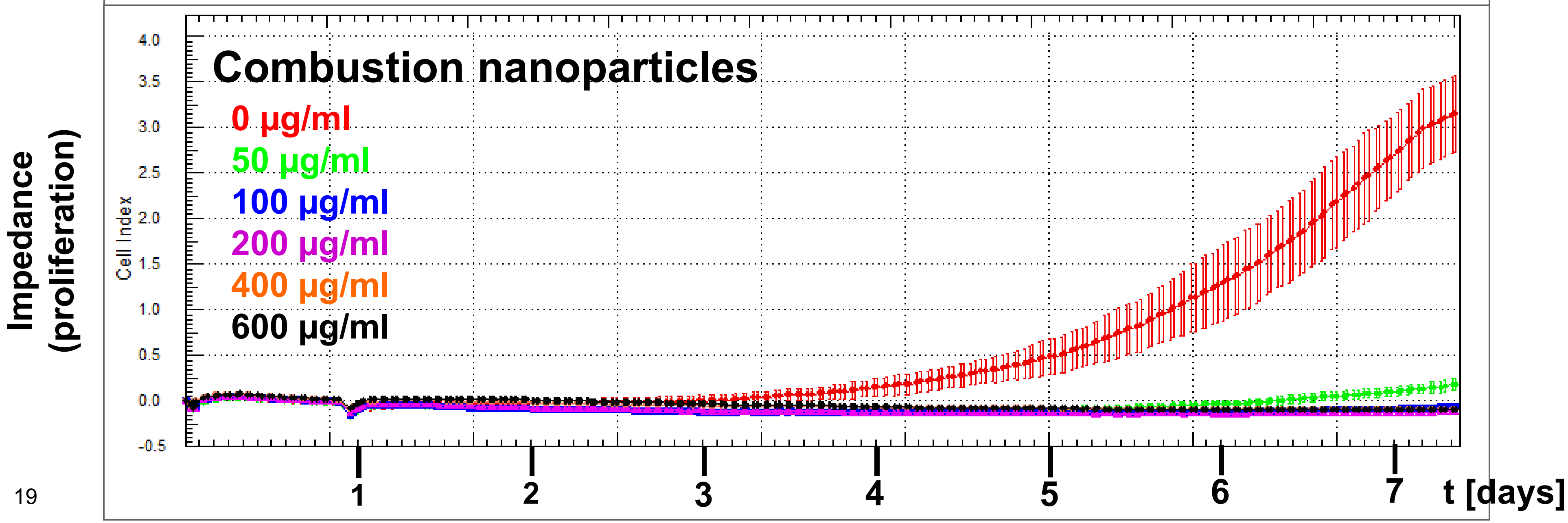
Thus, impedance measurement allows for a **real-time**, label-free and **non-invasive** analysis of key cellular events .



Printex 90 is less anti-proliferative than combustion nanoparticles

NMI achieving results





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 submerse exposure, Printex® 90 elicits a response of **similar** strength at the **mRNA level** as combustion-derived particles.

4) At submerse 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 submerse exposure, the anti-proliferative effect of Printex® 90 is **lower** than that of combustion particles.

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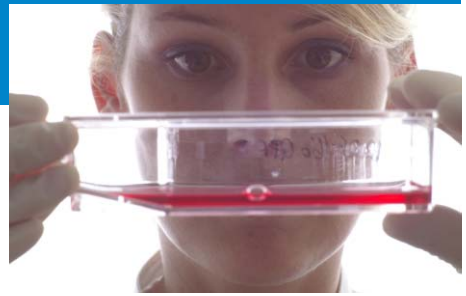

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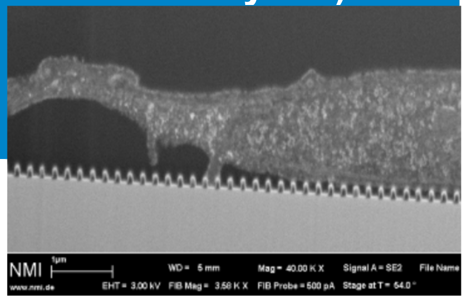

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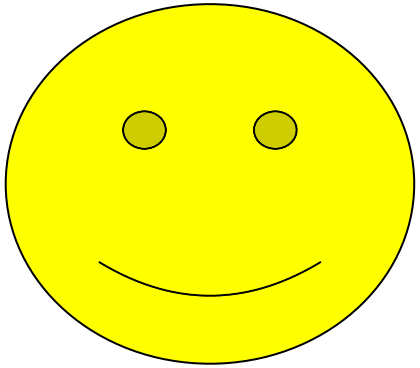

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