Dosimetry in inhalation: an in vitro inhalation model to study the relevance of dose parameters related to particle mass and particle number

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An ongoing discussion exists on the relevant metric relating dose and biological effects regarding the toxicity of inhaled particles. Deposited particle surface versus number or mass are under discussion correlated to different types of aerosols (e.g. poorly soluble, combustion, cigarette smoke). An experimental model was established to study the otherwise unchanged toxicological properties of an aerosol in defined states regarding particle size, mass- and number concentration, giving the unique opportunity to test the most relevant dose-metric based on biological response in vitro.

Methods

Aerosol generation and determination of dose

Variation of the number dose at constant mass dose from highly concentrated aerosol of condensed lubrication oil of a two-stroke engine was achieved by Brownian particle coagulation ageing of the pre-diluted exhaust gas within a laminar flow conditioning tube. The design of the conditioning unit allowed for loss-free coagulation resulting in the increase of the particle size and reduction of the number concentration without any change, neither in the mass concentration of the particle phase nor the concentration and composition of the gas phase (figure 1). Variation of mass dose during exposure was achieved by changing exposure time. Deposited material from the aerosol was evaluated analytically after spiking the lubrication oil with nano-MoO_3.

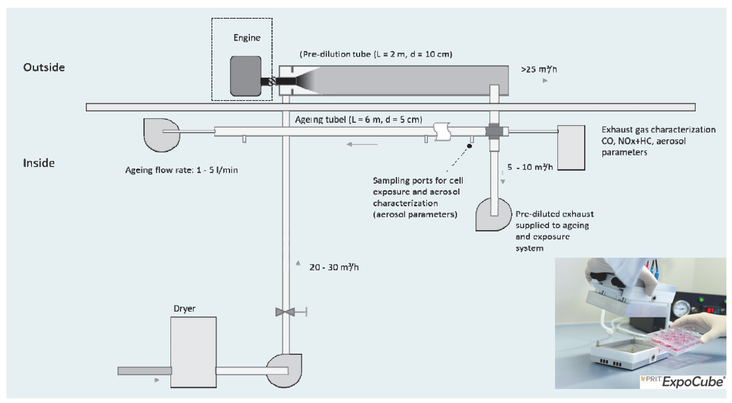

Figure 1: Experimental set-up for generation of complex test atmospheres for nano-particle dose-metric studies. Taking advantage of Brownian coagulation, number dose can be varied independently from mass dose.

Cell exposure and read-outs

Acute local biological effects were studied by application of an optimized exposure device for air-lifted interface cultures (ALI, P.R.I.T.® ExpoCube®), a human lung cell line (A549) and read-outs for viability (WST-1), mitochondrial membrane potential (JC-1) and Interleukin-8 release.

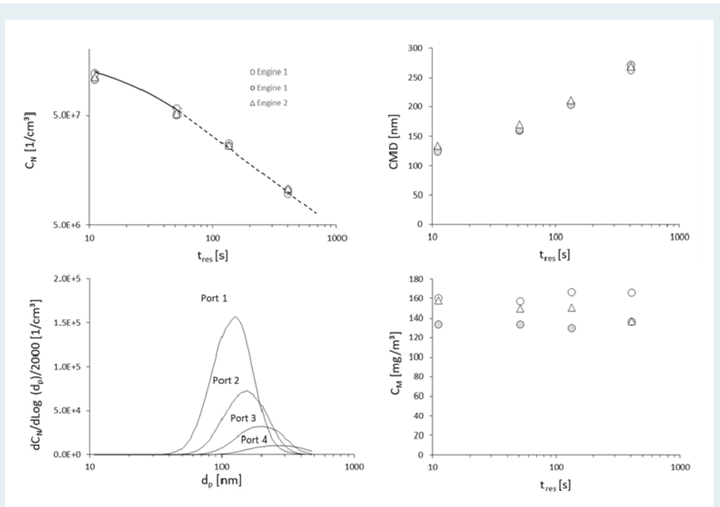

Figure 2: Aerosol properties at the sampling ports representing different stages of ageing: number concentration, count mean diameter, number distribution density (SMPS-measurements), and mass concentration (gravimetric analysis).

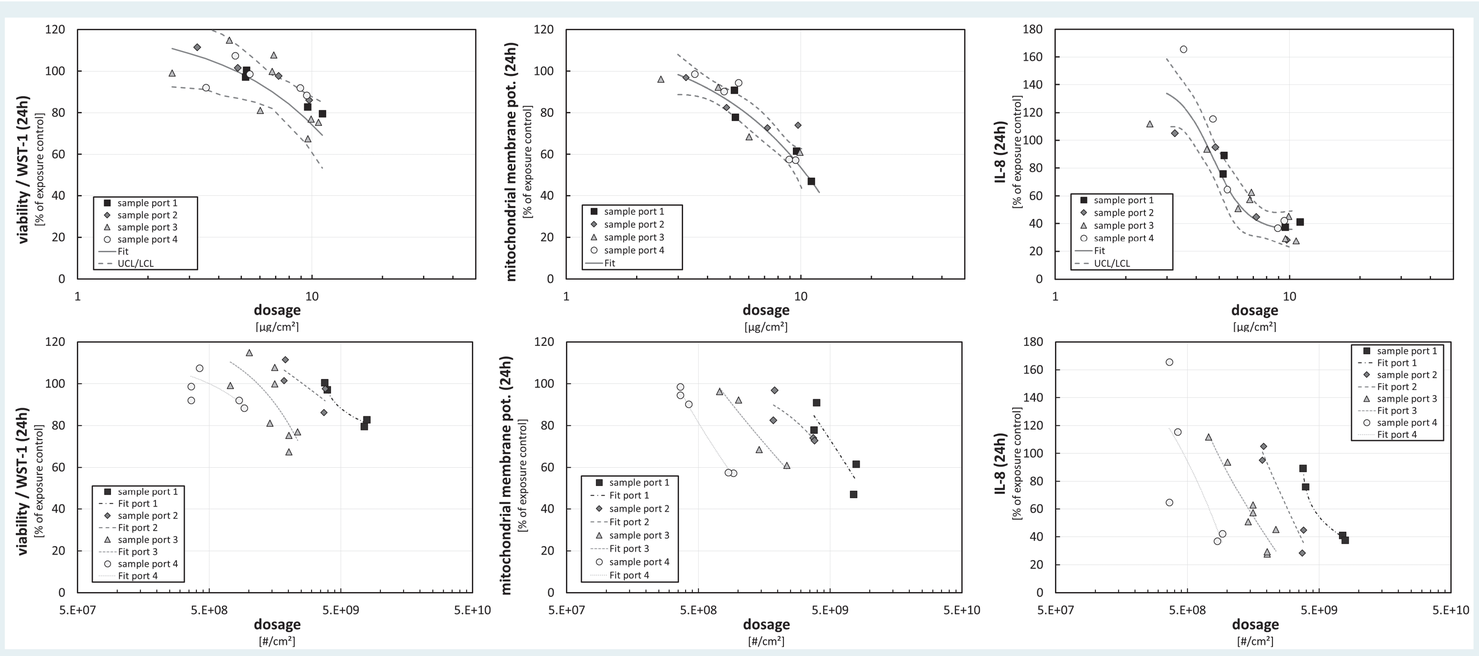

Figure 4: Cell exposures to whole aerosols from sample ports 1, 2, 3 or 4; analysis of viability (left), mitochondrial membrane potential (middle) or Interleukin 8 release (right panel) 24 hours after exposure. Percentage of control (clean air) using particle mass-based dosage [µg/cm²] (upper or particle number-based dose-metrics [#/cm²] (lower panel). Each dot represents one independent exposure experiment (mean of 4 replicate cultures).

Results

Test aerosols and deposition

Four different test aerosols were generated (sample port 1-4) with decreasing number concentrations and increasing particle size distributions at constant mass concentration (figure 2).

Deposition analysis of Mo-spiked aerosols indicated a relative deposition rate under thermophoretic conditions, that fit very well into historical data of the exposure device using varying aerosol types (figure 3 (left)).

Gas-phase effects

Cells were exposed to whole or filtered aerosols (gas-phase only) from the sample ports. Comparison of effects indicated a relatively lower toxicity for the exposures to the gas-phase. Figure 3 (right) shows the respective results for the mitochondrial membrane potential which were resembled by results from viability measurements ad IL-8 release in a similar way (not shown).

Cell exposures to 4 test aerosols

Dose-response relationships were established after 30 or 60-minutes of exposure. Dose-responsive effects were found for viability, mitochondrial membrane potential and IL-8 release following to a 24 hour post-exposure incubation phase (figure 4).

Results were plotted against a mass-based dose-metric [µg/cm²] (figure 4 (upper)) or a number-based dose metric [#/cm²] (figure 4 (lower)). Whereas plotting against the mass-based dose-matric resulted into common dose-responses for the four test aerosols, plotting against the number-based dose-metric split data into individual dose responses for the test aerosols.

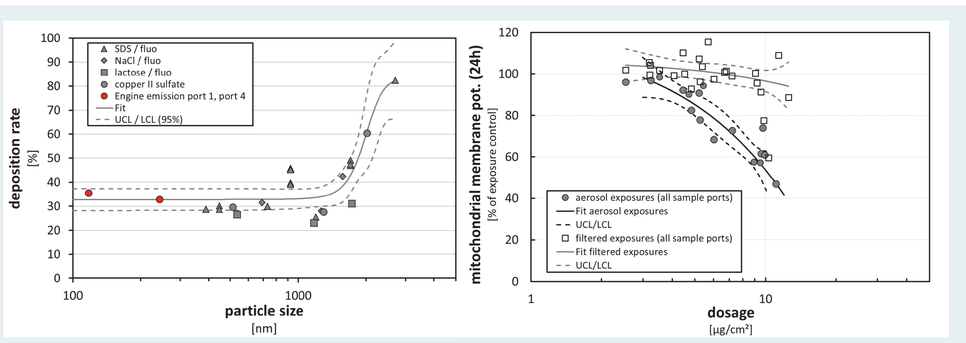

Figure 3: Deposition analysis using Mo-spiked engine exhaust in comparison to historical results (P.R.I.T.® ExpoCube®) (left). Cell exposures to aerosols from all sample ports using filtered (gas-phase) or non-filtered aerosol (right).

Conclusion

The specific method of aerosol generation enabled reproducible cell exposures to 4 aerosol types from a single source with different number but comparable mass concentration. Deposition analysis and comparison of results after exposures to whole or filtered aerosols validated aerosol deposition of the oily aerosol droplets on the cellular surface and the relevance of the aerosol phase for the biological effect. Plotting of the dose-responses using a mass-based versus a number-based dose-metric indicated the aerosol-droplet-mass dosage and not the droplet number dosage as the relevant dose-metric in this case.

Oily aerosol-droplets may lose their droplet character immediately after deposition on the biological surface making the dose-metrics number, size, or surface not relevant. In accordance to the current scientific discussion this is a different dosimetric situation in comparison to solid aerosol particles, e.g. poorly soluble particles, where particle number resp. the particle surface is under discussion as the relevant parameter for the dose.

Perspective

In summary, the novel approach allows for systematic nano-particle dose-metric studies of biological/toxicological effects of exhaust fumes in vitro. Extension of the model to different aerosol types such as non-oily might further support the understanding of acute particle dosimetry and toxicology on biological surfaces such as the lung.

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