Establishing the Vitrocell® Powder Chamber as a particle size-selective platform for *in vitro* dry powder testing

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Introduction and Objectives

- The human respiratory tract, from the head airways (nasal, oral, and nasopharyngeal cavities) over the conducting airways (trachea, bronchi, bronchioles), down to the respiratory regions (respiratory bronchioles and alveolar sacs), is structurally and functionally heterogeneous *(1)*.
- The deposition efficiency of inhaled particles varies strongly between the different regions of the respiratory tract, whereby particle size is a key determinant *(2)*.
- The biological response to particle deposition in the respiratory tract is a function of the combined action of deposited particle mass, surface, and number *(2)*.
- *In vitro* aerosol exposures should therefore aim at simulating aerosol doses, not only with respect to the applied aerosol mass but also with respect to the applied particle size distribution.
- The Vitrocell® Powder Chamber was developed for the size-selective deposition of dry powders on *in vitro* test systems in order to simulate the regionally different deposition efficiency of various particle sizes.
- In the presented work, a dry powder consisting of trehalose and containing nicotine was used in cell-free exposures for testing the system's ability to uniformly and reliably deliver dry powders in a particle size-specific manner.

System Description and System Testing

The Vitrocell® Powder Chamber: System overview

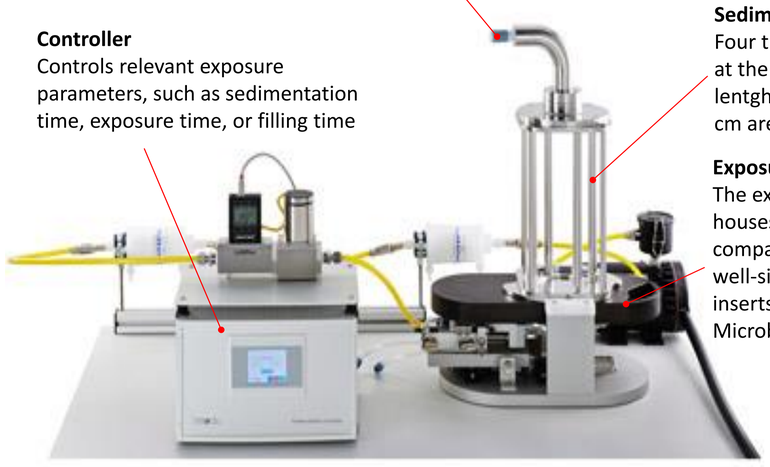
Powder inlet
For optimal powder aerosolization, different inlet modules are available

Distribution unit
Distributes the powder aerosol generated at the inlet to the four sedimentation tubes and removes large particle agglomerates

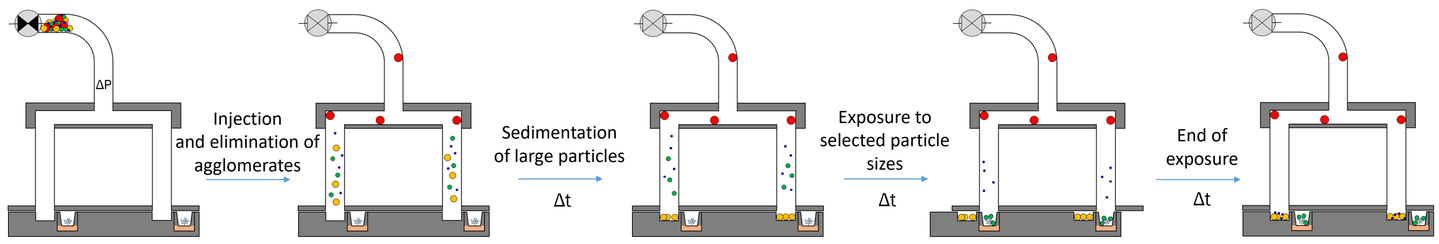
Controller
Controls relevant exposure parameters, such as sedimentation time, exposure time, or filling time

Sedimentation tubes
Four tubes are present at the system. Tube lentghs between 10–30 cm are available

Exposure tray
The exposure tray houses four compartments for 12 well-sized cell culture inserts or Quartz Crystal Microbalances (QCM)



Working principle



Step 1
The test powder is loaded at the inlet of the system, and a negative pressure is built up in the system.

Step 2
A controlled flow, driven by the negative pressure in the system, aerosolizes the powder and transports it into the sedimentation tubes.
Large particle agglomerates are removed from the aerosol by impaction in the distribution unit.
Powder sedimentation within the sedimentation tubes is driven by gravity in absence of gas or aerosol flow.

Step 3
The settling velocity of particles is a function of particle size:

$$v_s = \rho_p \times d_p^2 \times g \times C_c / 18\eta$$

v_s: terminal settling velocity
ρ_p: Particle density
d_p: Particle diameter
g: gravitational constant
C_c: Cunningham slip correction
η: dynamic viscosity of the carrier gas

Variation in the sedimentation time therefore allows depositing largest particles selectively before starting the exposure.

Step 4
Once the desired upper cutoff of particle sizes has been reached, the exposure tray is moved sidewards, thereby positioning the cell cultures under the sedimentation tubes.
Gravitational sedimentation of the aerosolized powder remaining in the tubes after removal of the largest particles continues.

Step 5
Once the desired lower cutoff of particle sizes is reached, the exposure is terminated by removing the cell cultures from the system or by moving the exposure tray back.

***Figure 1**: Overview of the Vitrocell® Powder Chamber and the system's working principle.*

Experimental procedures

Test Powder

- The test substance was a dry powder consisting of trehalose and nicotine (5% by mass).
- The mass median aerodynamic diameter of the primary particles in the powder was 2.6 µm.
- Irregularly shaped agglomerates with sizes above 100 µm in their largest dimension and large aspect ratios were formed during storage of the powder.

Test exposures

Three exposure modes were defined for test exposures:

- Mode A: deposition of the whole range of particle sizes reaching the sedimentation tube
- Mode B: removal of particles larger than about 35 µm (in humans reaching the head airways with low efficiency *(2)*
- Mode C: removal of particles larger than about 15 µm (in humans exclusively deposited in the extrathoracic airways *(2)*

The applied system parameters are summarized in **Table 1**.

Table 1: system parameters used during dry powder exposures

Exposure mode	Loaded powder mass (mg)	sedimentation time (seconds)	Exposure time (minutes)	sedimentation tube length (cm)
Mode A	20	0	30	30
Mode B	20	10	10	20
Mode C	20	30	30	20

Powder deposition in the exposure chambers was determined in terms of total powder mass, nicotine mass, and particle number and size distribution

- Monitoring of total powder mass deposition:

  QCMs were placed into the exposure chambers. Powder mass deposition was monitored in real time during the exposures.

- Quantification of nicotine deposition in the exposure chambers:

  500 µL of phosphate-buffered saline (PBS), pipetted into empty cell culture inserts, was exposed in the exposure chambers. Nicotine concentrations in the exposed samples were determined by high-performance liquid chromatography (HPLC) with diode array detection.

- Number and size distribution of the deposited particles:

  Scanning electron (SE) microscopy (SEM) carbon discs were placed in the exposure chambers. Once roughly 500 ng of powder were deposited per cm^2 (estimated based on QCM measurements), particles were counted, and their Feret diameters were determined. Image processing was performed manually, using the image processing software ImageJ.

Results and Discussion

Online monitoring of powder mass deposition by QCMs
The QCMs clearly showed the expected differences in the kinetics of mass deposition and in the absolute total deposited masses, but also showed significant differences in the masses deposited in individual exposure chambers **(Figure 2)**.

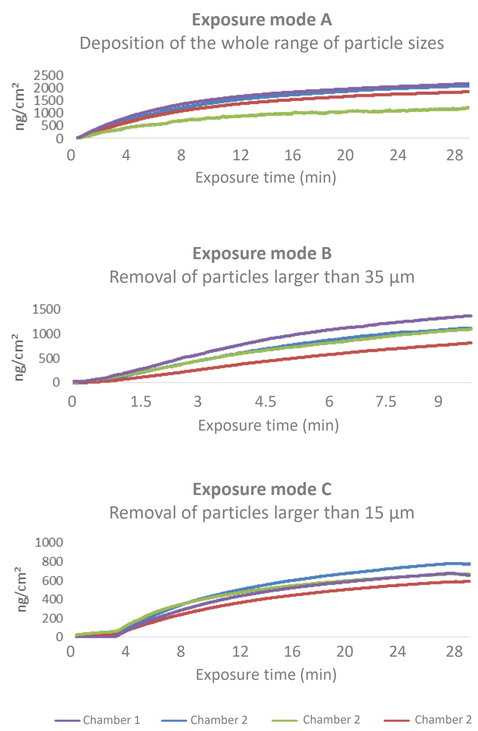


***Figure 2**: Real-time powder mass deposition during the exposures. The figure shows one representative repetition for each tested exposure mode.*

Nicotine quantification in exposed PBS samples
In line with the QCM mass deposition data, nicotine quantification showed a clear effect of the exposure mode toward the powder mass delivery. Differences in the mass delivery to individual exposure chambers as well as inter-repetition variations were observed **(Figure 3)**. A quantitative match between HPLC and QCM data is not given.

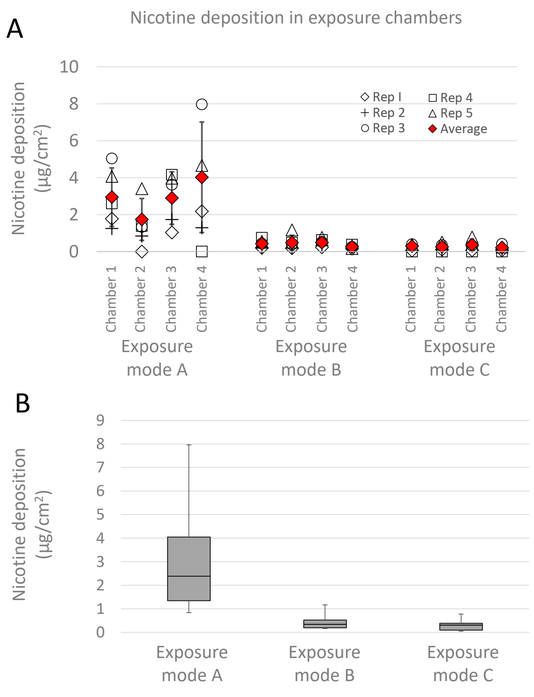


***Figure 3**: Nicotine deposition in the exposure chambers. **A)** resolved by experimental repetition and exposure chamber, **B)** global across the four exposure chambers and five experimental repetitions. Error bars in **A)** indicate standard deviations, error bars in **B)** indicate the lowest and the highest measured values.*

SEM analyses of particle number deposition and particle size distribution
SEM quantitatively revealed a clear effect of the exposure modes on the deposited particle size distributions.

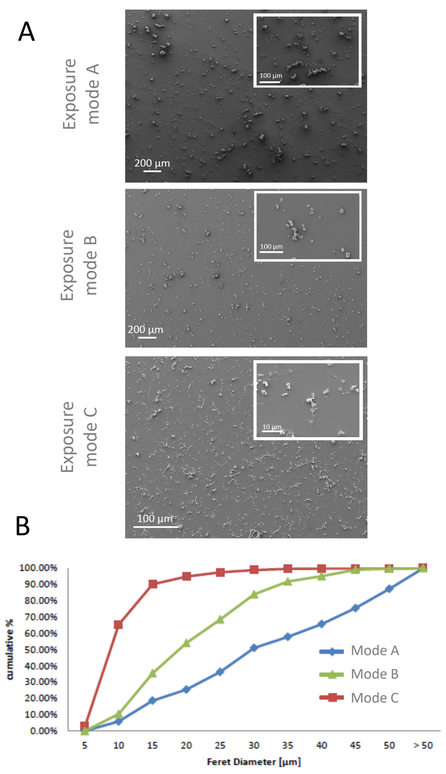


***Figure 4: A)** SE micrographs of particles deposited under the three exposure modes. **B)** cumulative particle size distribution measured in the SE micrographs.*

- **The Vitrocell® Powder Chamber allows for particle size-selective *in vitro* exposures to dry powders.**
- **Based on the quantification of nicotine delivery to the exposure chambers, application of 20 mg dry powder at the system inlet resulted in deposition of up to 80 µg/cm² in the exposure chambers (depending on the exposure mode).**
- **For dry powders with particle sizes ranging from 2 – >50 µm, the resolution with which different particle sizes could be efficiently separated was in the range of 15 – 20 µm.**
- **For powders of significantly smaller particle sizes (e.g., nanoparticles), additional testing is required.**
- **Large inter-repetition variations in powder mass deposition were observed (up to >100%). These are suspected to be mainly caused by variations in the aerosolization efficiency and therefore powder-related.**
- **Relatively large variations in the mass deposition across different exposure chambers (intra-repetition variations) were observed. These are considered mainly the result of system properties, which are currently addressed by adaptations in system design.**

*References: **1)** Fishman AP: **Pulmonary diseases and disorders**. In.: New York: McGraw-Hill; 2008. **2)** Carvalho TC, Peters JI, and Williams Iii RO. International Journal of Pharmaceutics. 2011;406:1-10. **3)** Oberdorster G, Oberdorster E, and Oberdorster J. Environmental health perspectives. 2005;113:823-839.*

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Competing Financial Interest
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