Characterization of the Vitrocell® Cloud SEQ 24 System and its Application in Exposure to Aerosolized Bortezomib and Ibuprofen

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Introduction and Objectives

The Vitrocell® Cloud SEQ 24 System is an advanced version of the Vitrocell® Cloud 6 and the Vitrocell® 12, a series of aerosol exposure systems specifically designed for delivering aerosols generated from liquid test matrices to cell cultures under controlled and reproducible conditions. It allows exposing 24 cell cultures of the 24-well format simultaneously and in addition provides a feature for sequential dosing, which decreases the need for cell culture handling, and thereby increases system efficacy and throughput.
Whereas the Vitrocell® Cloud 6 has been characterized in detail (Lenz et al 2014), little is known about how aerosols are delivered within the Cloud SEQ 24 and how cell cultures respond to exposures in the system. We therefore conducted system characterization in cell free experiments and by exposing three-dimensional organotypic tissue cultures of the human bronchial epithelium to a non steroidal anti-inflammatory drug (Ibuprofen) and the proteasome inhibitor Bortezomib. Special attention was thereby put on the system's ability to deliver the test aerosols in a repeatable and uniform manner and to trigger test compound specific biological responses.

System Description and System Testing

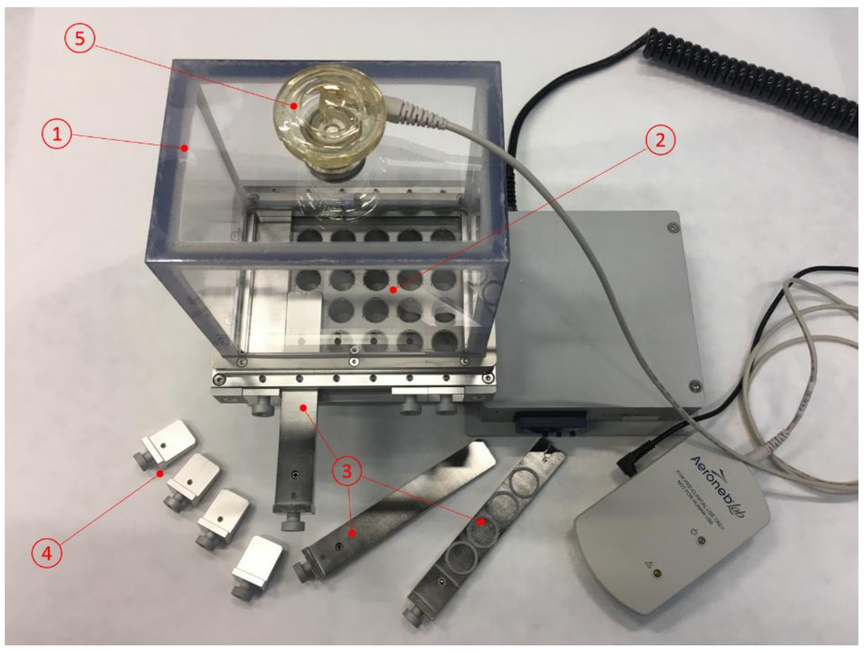
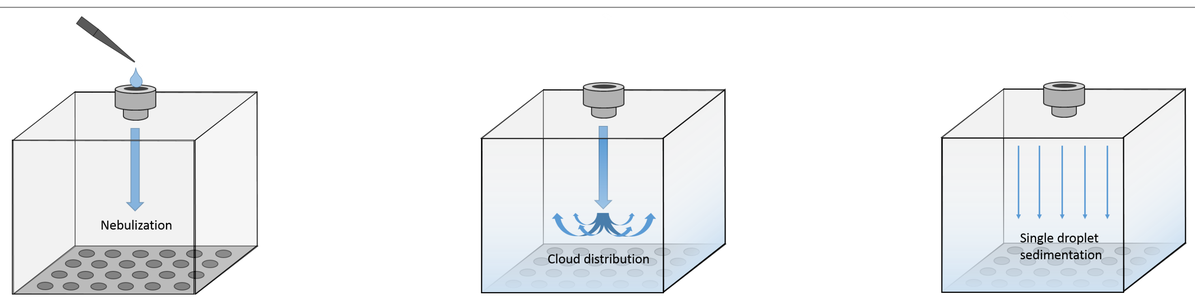


Figure 1: The Vitrocell® Cloud SEQ 24. The aerosol chamber (1) is located on top of the heated multi-well base module (2), which holds 24 wells fitted for receiving 24-well-format cell culture inserts. For sequential dosing, groups of four wells can be covered by cover slides (3), which by the means of seals on their lower side close the wells tightly and keep aerosol from entering. The openings toward the environment at rows where no cover slides are inserted can be sealed by steel plugs (4). Aerosols are generated by a vibrating mesh nebulizer (5), installed at a centrally located opening in the cover of the aerosol chamber.

Working principle

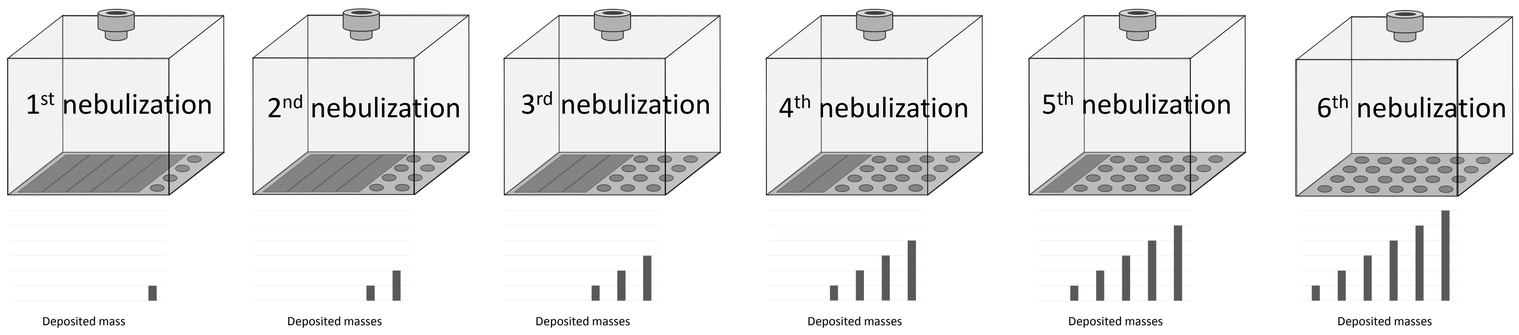


1) The test liquid is nebulized into the aerosol chamber

2) Due to its kinetic energy and convection, the aerosol distributes inside the chamber

3) The aerosol deposits by sedimentation

Sequential mode exposures



Repeated runs of nebulization with cover slides being sequentially removed result in differential dosing in the six rows of the base module. This mode allows exposing cell cultures to different doses without opening the system and handling cell cultures and therefore decreases the risk of introducing artifacts and increases efficacy and throughput.

Experimental procedures

Cell-free experiments

- Test solution was phosphate buffered saline (PBS) spiked with 0.5 g/L disodium fluorescein (DSF)
- 200 µL of the solution were nebulized per exposure
- 100 µL PBS, present in cell culture inserts in the base-module, served as surrogates for cell cultures
- Exposures were conducted in the non-sequential and the sequential mode
- Upon exposures, the PBS samples were retrieved from the cell culture inserts and the DSF was quantified flourometrically and used for characterization of the aerosol deposition in the system.
  - Targeted system characteristics in the non-sequential mode were the kinetics of aerosol deposition, the uniformity of deposition across the 24 positions and the aerosol delivery efficiency
  - Targeted system characteristics in the sequential mode were the functionality of the covers and the accuracy of dosing

Exposure of three dimensional organotypic tissue cultures of the human bronchial epithelium

- Organotypic tissues were obtained from Epithelix SarL, (Geneva, Switzerland) and cultivated according to the suppliers instructions
- The cultures were exposed in the non-sequential mode to nebulized Ibuprofen and Bortezomib. The composition of the nebulized solutions is summarized in **Table 1**.
- 1 hour after exposure, a pro-inflammatory stimulus (15ng TNF-α and 15ng IL-1b per mL) was applied to to the tissues basolaterally
- 24 hours after exposure, biological endpoints were measured:
  - Cytotoxicity: by quantification of extracellular lactate dehydrogenase (Cytotoxicity Detection KitPLUS (LDH) (Roche, Basel, Switzerland, Ref# 04 744 926 001). Triton X-100 was used as a positive control)
  - The secretion of Interleukin 8 (IL8, IL-8 (human) AlphaLISA Detection Kit, Perkin Elmer, Seer Green, UK, Ref# AL224C) after exposure to Ibuprofen
  - The Proteasome activity (Proteasome-Glo™ Chymotrypsin-Like Cell-Based Assay (Promega, Madison, WI, USA, Ref# G8660)) after exposure to Bortezomib.

Table 1: dosing applied in the cell culture exposures

Ibuprofen	
Vehicle	PBS
Concentrations in the nebulized liquid (µM)	3125, 6250, 12500, 25000, 50000, 100000
Bortezomib	
Vehicle	1:3 DMSO:PBS (25% DMSO)
Concentrations in the nebulized liquid (µM)	50, 200, 800, 3200

Results and Discussion

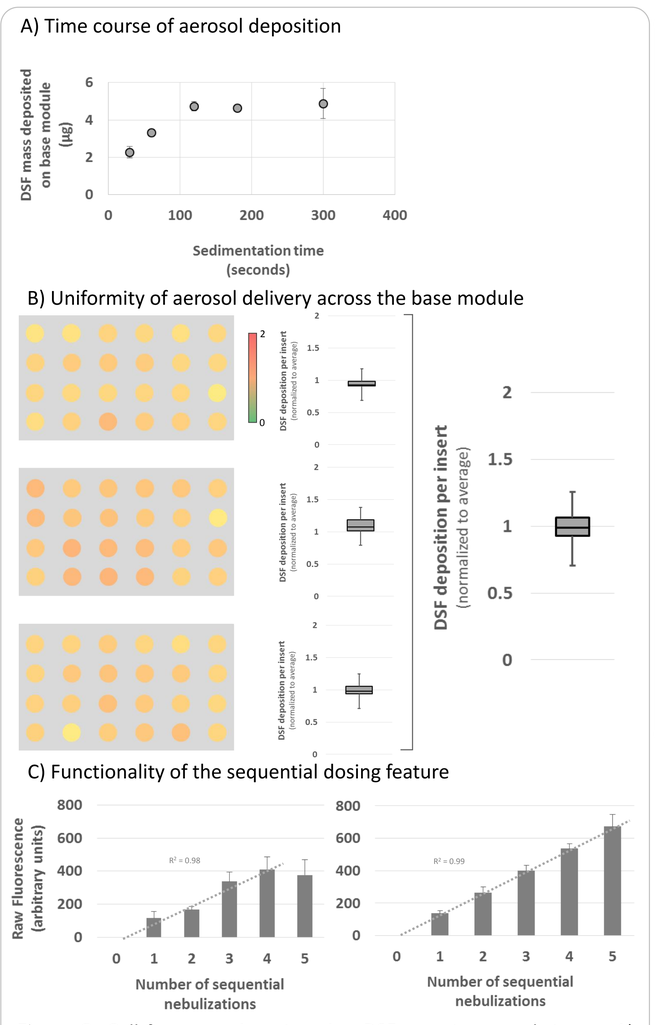


Figure 2: Cell-free experiments using DSF as a tracer substance. A) time course of the aerosol deposition inside the aerosol chamber (n=3, the average total mass deposition ± SEM is shown). B) uniformity of aerosol deposition across the base-module in three independent exposures (five minutes deposition time). C) dosing in the sequential exposure mode in three independent repetitions, without (left) and with (right) cleaning the aerosol chamber between nebulizations. The columns represent the total DSF mass deposited per row ± SEM.

Table 2: key parameters of exposure in the Vitrocell® Cloud SEQ 24. Data were generated in three independent repetitions of cell-free exposures (72 cell culture inserts)

	Average	±	Standard deviation	Relative standard deviation (%)
Delivery efficiency* to all 24 positions (in %)	4.0	±	1.6	41
Delivery efficiency* per single position (in %)	0.17	±	0.09	56
Deposition factor** (in %)	69	±	28	41

* mass % of the DSF added to the nebulizer detected in the expsoed PBS
** observed aerosol deposition in % of the aerosol deposition that would be expected under ideal conditions, i.e. in absence of losses at the walls or in the nebulizer

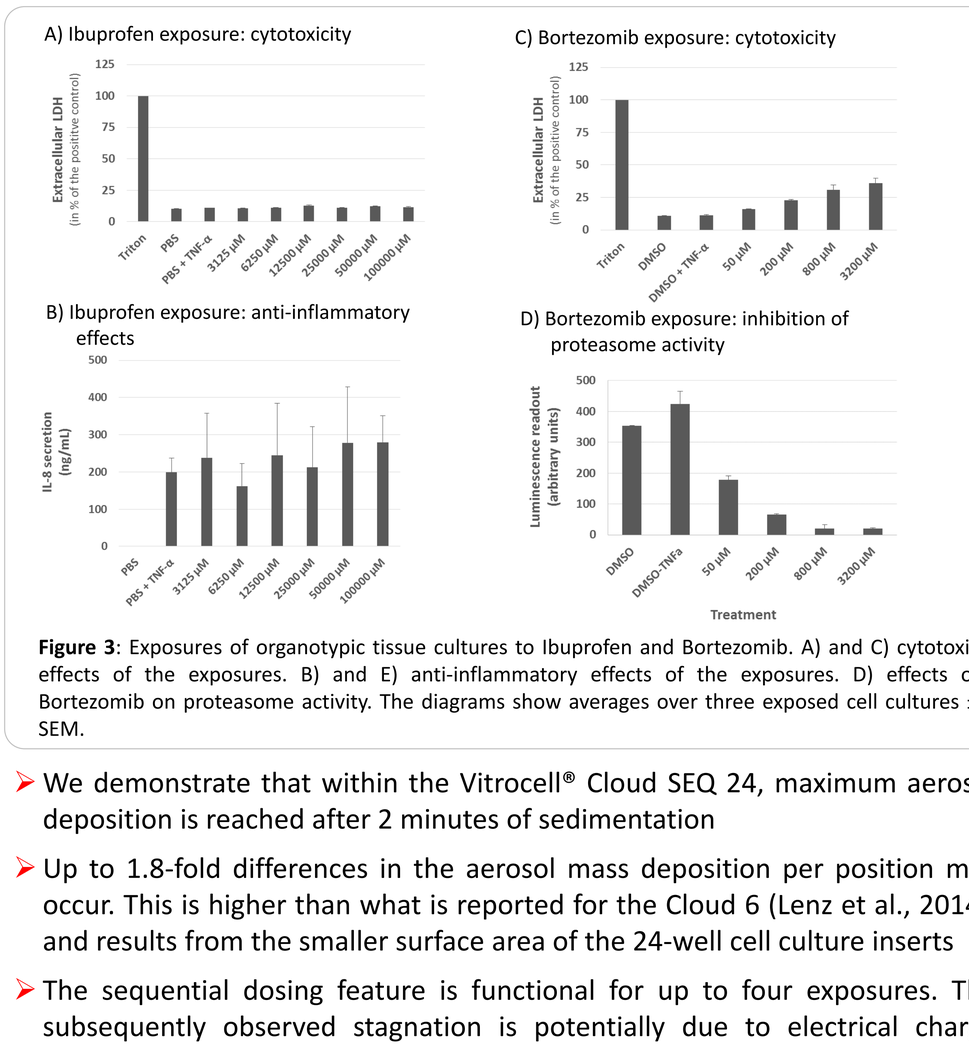


Figure 3: Exposures of organotypic tissue cultures to Ibuprofen and Bortezomib. A) and C) cytotoxic effects of the exposures. B) and E) anti-inflammatory effects of the exposures. D) effects of Bortezomib on proteasome activity. The diagrams show averages over three exposed cell cultures ± SEM.

- We demonstrate that within the Vitrocell® Cloud SEQ 24, maximum aerosol deposition is reached after 2 minutes of sedimentation
- Up to 1.8-fold differences in the aerosol mass deposition per position may occur. This is higher than what is reported for the Cloud 6 (Lenz et al., 2014), and results from the smaller surface area of the 24-well cell culture inserts
- The sequential dosing feature is functional for up to four exposures. The subsequently observed stagnation is potentially due to electrical charge deposited on the chamber walls and can be eliminated by cleaning the aerosol chamber between repeated nebulizations
- The delivery efficiency of the system is in the range of 0.2%, i.e. roughly 0.4 µL of the nebulized liquid is deposited per cell culture. The inter- and intra-run variation in aerosol deposition translates into large variations in the delivery efficiencies (relative standard deviations up to 56%)
- The system per se does not induce cytotoxic effects
- Pharmaceutically active compounds induce the expected biological responses in a dose dependent manner, as demonstrated by the inhibition of proteasome activity upon exposure to Bortezomib. The used 3D organotypic cell cultures were not responsive to Ibuprofen, which can however not be attributed to the Cloud SEQ 24.

References: Lenz et al. 2014. *Efficient Bioactive Delivery of Aerosolized Drugs to Human Pulmonary Epithelial Cells Cultured in Air–Liquid Interface Conditions*. American Journal of Respiratory Cell and Molecular Biology Volume 51 Number 4 :

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Competing Financial Interest
The research described in this poster was sponsored by Philip Morris Products & Manufacturing SA