Using cloud motion for fast, efficient and realistic *in vitro* delivery of inhaled drugs to pulmonary cells

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Chronic lung diseases such as chronic obstructive pulmonary disease, lung cancer, and asthma are the second leading cause of death worldwide (WHO). Currently, there is no causal cure for any of these diseases. Hence, research in this field is expected to intensify significantly in the upcoming years. In addition, recent technological advances in inhalation technology have provided highly efficient, breath-controlled inhalation devices which are making inhaled drugs increasingly more attractive not only for the treatment of lung disease, but also for non-invasive systemic drug delivery. However, the preclinical development of aerosolized drugs for inhalation therapy is hampered by the lack of easy-to-use and yet efficient *in vitro* methods for aerosol-to-cell drug delivery.

Our study describes an innovative droplet-to-cell delivery system for Air-Liquid Interface Cell Exposure (ALICE-CLOUD), which utilizes the principles of cloud physics to close this gap (Lenz et al., 2009). The ALICE-CLOUD is designed for preclinical testing of aerosolized liquids/drugs at physiologically realistic (air-liquid interface) cell culture conditions. The underlying principle of cloud motion and the physical performance of the system are described. As a biological proof-of-concept study we apply this technology to test the inflammatory efficacy of aerosolized Bortezomib - a FDA-approved proteasome inhibitor for systemic cancer treatment – which has not been used for inhalation therapy, yet.

An aqueous fluoresceine solution was used to characterize the physical performance of the ALICE-CLOUD. Figure 1 shows that nebulization of 200 µl of aqueous fluoresceine (5 µg/ml) with an AeronebPro vibrating membrane nebulizer (MMD ~ 5µm; Aerogen Inc.) is completed within 180 s, where 95% of the final (asymptotic) dose is delivered already 100 s after initiating nebulization. In this period of time 88+/-12% (95% CL) of the nebulized liquid is deposited onto the bottom plate of the ALICE-CLOUD, where a standard multi-well plate for cell culturing is placed (here: 6-well plate with transwell insert for air-liquid interface cell cultures). This corresponds to a cell delivery efficiency of 19% (6-well insert) due to limited cell coverage in standard multi-well plates. The dose variability between different transwell inserts is <15% (95% CL) and the aerosol-to-cell delivery rate is 0.5 µl drug per cm^2 cell area per minute.

In a biological proof-of-concept study we stimulated human alveolar lung cells (A549) with TNF-α to induce an inflammatory response (here: 8-fold activation of the IL- promoter after 24h). Simultaneous application of a single dose of aerosolized Bortezomib with the ALICE-CLOUD reduced proteasome activity by up to 80% without impairing cell viability (WST-1 and LDH assays). A significant reduction of the IL-8 level and hence a therapeutic effect was observed after nebulising 200 µl of >100µM Bortezomib solution. Comparative dose-response measurements using submerged cell cultures and non-nebulized Bortezomib revealed that the cell-specific efficacy of nebulized and non-nebulized Bortezomib is similar.

The ALICE-CLOUD technology is a compact, efficient and simple to operate system for screening for inhalation drugs. Our data suggest that Bortezomib can be aerosolized without loss of efficacy to mediate potent anti-inflammatory effects on a human lung cells. Hence, Bortezomib is a promising candidate drug for inhalation therapy.

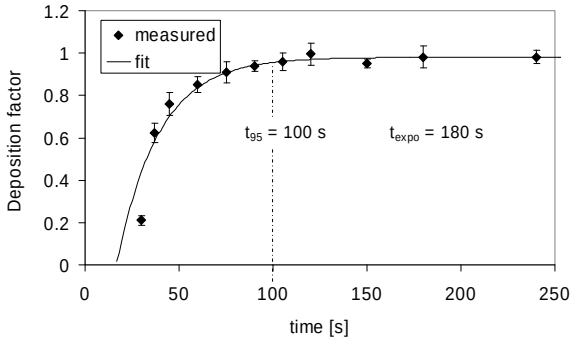

Figure 1. Time course of aerosol deposition onto cells in the ALICE-CLOUD (unity corresponds to 100% of the invested liquid is evenly distributed onto the bottom plate of the ALICE-CLOUD)

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