HUMIMIC–InHALES—A Human-Relevant Aerosol Test Platform for Systemic Exposure Studies

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Background

- The delivery kinetics of volatile particles and those of different sizes vary among distinct regions of the human respiratory tract *[1, 2, 3]*. Available *in vitro* aerosol exposure systems fail to capture this complexity *[4, 5]*. Therefore, the aerosol fractions they deliver to cell cultures are not, or only partly, representative of the *in vivo* situation.
- This might decrease the relevance of *in vitro* aerosol exposure experiments, especially when using complex cell cultures that are able to respond to physical and chemical stimuli in a highly differentiated manner.
- The logical combination of aerosol delivery testing for advanced cell culture systems and cutting-edge microfluidics microphysiological systems (MPS) enables physical mimicry of interconnections among cell types and systemic delivery of aerosols.
- The technology allows for flexible and customized combinations of different tissue constructs or organ equivalents on a chip-based MPS.
- We developed an *in vitro* aerosol exposure system that mimics the structural and functional aspects of the human respiratory tract—the **In**dependent **H**olistic **A**ir–**L**iquid **E**xposure **S**ystem (InHALES) [6].
  - *Independent:* It is capable of operating and actively inhaling aerosols and smoking cigarettes.
  - *Holistic:* It consists of modules that represent the relevant regions of the respiratory tract; the prototype consists of the oral cavity, laryngopharynx, trachea, bronchi, bronchioles, and lung lumen.
  - *Air-liquid exposure system:* It is designed for aerosol exposures at the air-liquid interface.
- Recently, a prototype of the system was built (Figure 1) to implement TissUse's proprietary commercial HUMIMIC microfluidic platform. The HUMIMIC MPS technology can maintain and culture miniaturized organs, emulating the biological function of their respective full-size counterparts over long periods.

The HUMIMIC-InHALES system

Figure 1: Total view of the HUMIMIC-InHALES system. The system is modular; individual parts (mouth pump, trachea, main bronchi, bronchial tree, and lung pumps) can be altered without changing the overall system structure or function.

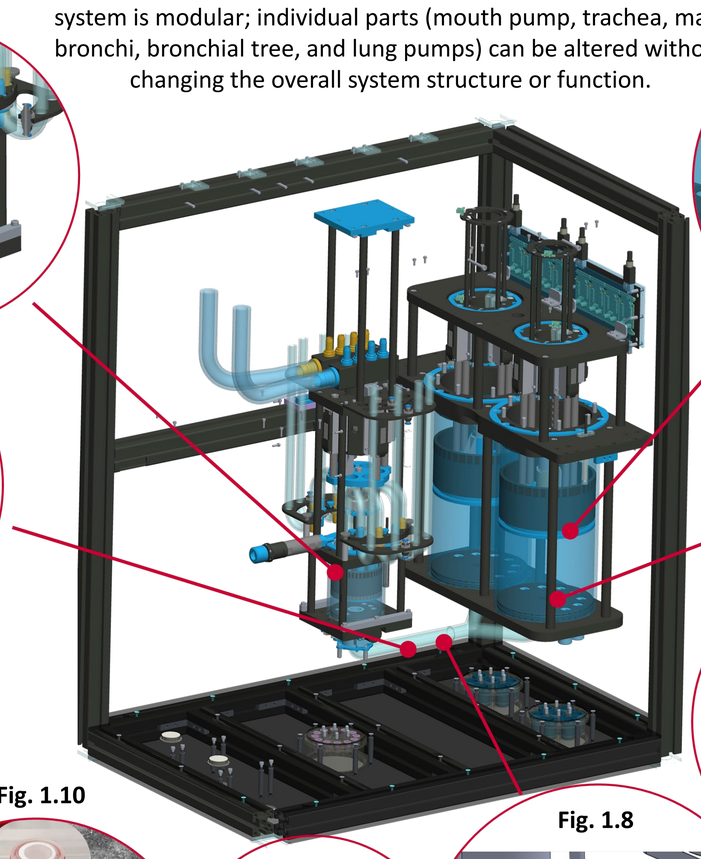


Fig. 1.1

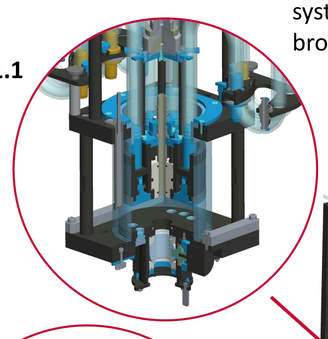


Figure 1.1: Mouth pump
- 0–140 mL; maximum flexibility for puff generation
- Connection to the tracheal model is sealed
- Five slots for placing cell cultures

Fig. 1.2

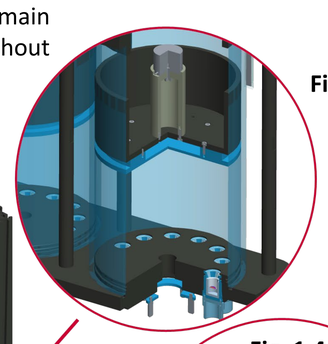


Figure 1.2: Lung pumps
- 0–6000 mL; full flexibility with respect to timing, profile, and residual volume
- Five slots for cell cultures per pump

Fig. 1.3

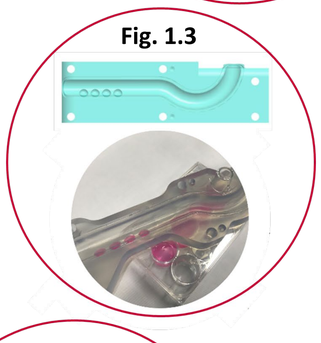


Figure 1.3: Trachea and main bronchi
- Size and geometry based on human anatomy
- Cell cultures on hydrogels, maximizing the flexibility of positioning and minimizing culture size

Fig. 1.4

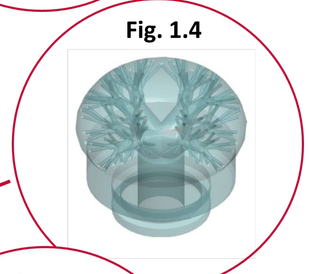


Figure 1.4: Bronchial tree
Plugged inside the lung pumps
- 5 generations of bifurcations realized in the prototype
- Key geometrical parameters based on Weibel's model *[8]*

Fig. 1.5

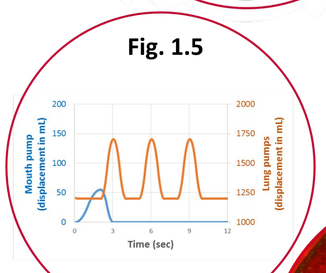


Figure 1.5: System operation
- Orchestrated operation of valves and pumps
- Simulation of virtually any breathing pattern is possible

Fig. 1.6

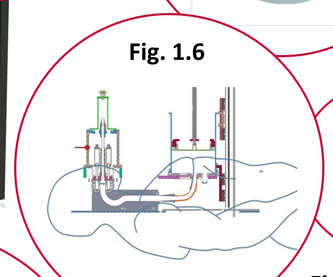


Fig. 1.7

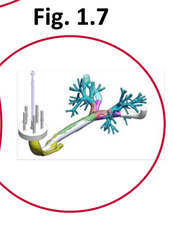


Figure 1.6: Arrangement of the mouth pump, trachea, main bronchi, and lung lumen mimics human physiology.

Figure 1.7: Trachea and main bronchi are based on human anatomy in terms of size and geometry:
- Brno lung cast model *[7]*
- Weibel's model *[8]*

Fig. 1.8

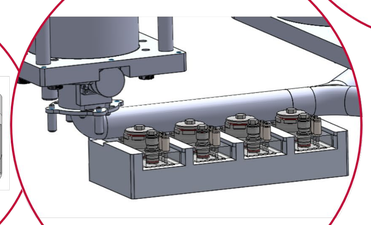


Figure 1.8: Arrangement of the cell culture insert along the trachea. Each module can be plugged-in without a tool.

Fig. 1.9

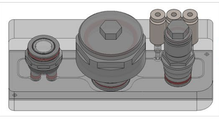


Figure 1.9: HUMIMIC-Chip3: Cell culture chip adapted to InHALES with an internal flow circuit. Each chip has an independent pump.

Fig. 1.10

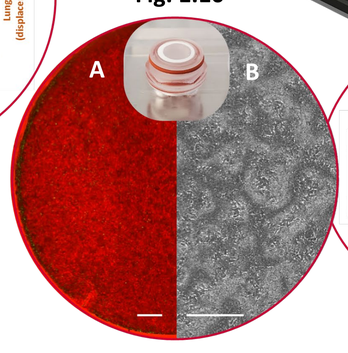


Figure 1.10: HUMIMIC-InHALES Chip3 Cell culture insert feasibility test: The newly developed insert is tested for hAELVi cell viability as a proof of concept. **A)** CellTox/CalceinAM staining of hAELVi after transfer to InHALES-Chip following 6 days (3 days in air-liquid interphase) culture. **B)** Light microscopy images of the hAELVi on the cell culture insert on day 6. Cell culture insert is depicted in the middle. Scale bar: 100 µm.

Test Exposure, Results, and Discussion

Test exposure settings: The system "puffed" and "inhaled" a fluorescent test aerosol (a nebulized mixture of propylene glycol [PG], glycerol [G], and water, labeled with disodium fluorescein [DSF]) The puffing process was repeated 4–5 times, and 60 puffs were delivered each repetition. Two exposure protocols were applied:

- **Shallow inhalation** (see Figure 1.5): 1200 mL residual volume in lung pumps; 2-second puff generation; immediate puff inhalation during 1 second, along with 500 mL clean air; 2 empty inhalations (no puffing) within 6 seconds.
- **Deep inhalation** (Figure 2): 1200 mL residual volume in lung pumps; 2-second puff generation; immediate puff inhalation during 1 second, along with 4600 mL clean air.

During exposure, the complete system was at 37 °C, and inhaled air was brought to a relative humidity of 95%.

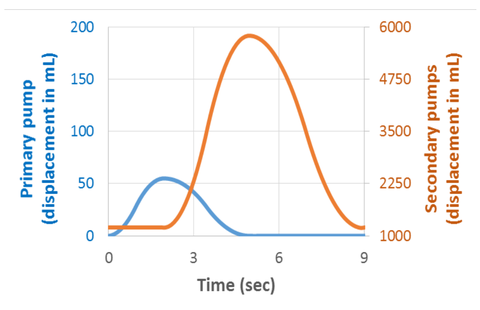
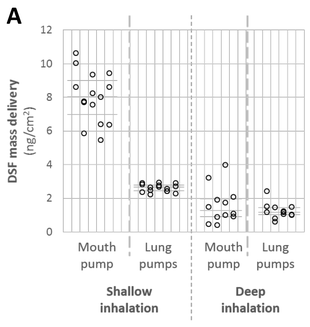


Figure 2: Deep inhalation exposure protocol

Aerosol delivery was investigated by exposing samples of 300 µL phosphate-buffered saline (PBS) followed by quantification of DSF by fluorometry (Figure 3). PBS was exposed in cell culture inserts (Figure 4) in the pumps only (and not in the trachea).



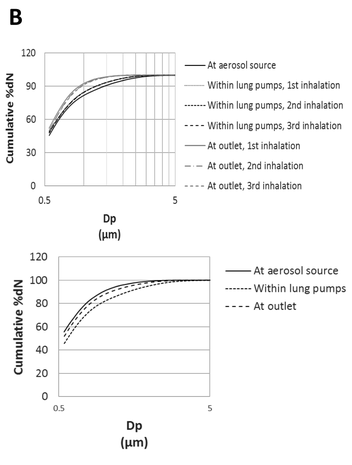


Figure 3: A) Delivery of DSF (PG/G exposure) within the mouth and lung pumps (4–5 repetitions, each with 3 samples exposed). **B)** Evolution of (cumulative) particle size distribution during the passage of aerosol through the system. This was measured at different locations in the system using an Aerodynamic Particle Sizer® 3321 from TSI.

Biological endpoints

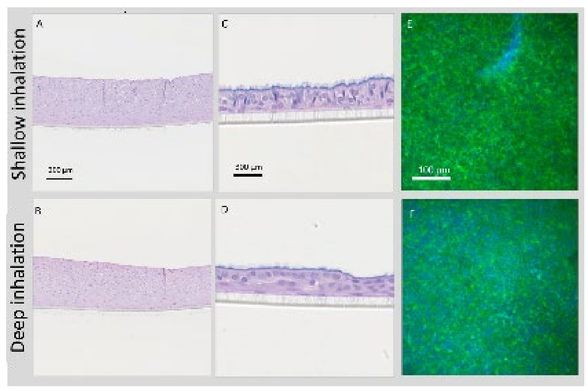


Figure 4: Biological responses to the test exposures: A) and **B)** H&E-stained EpiOral tissues exposed under shallow inhalation or deep inhalation settings, respectively. **C)** and **D)** H&E-stained MucilAir™ tissue patches. **E)** and **F)** A549 cultures stained for nucleic acids (Hoechst, blue) and F-actin (phalloidin, green). PG/G-exposed tissues were selected as an example.

Conclusions:

- The system's applicability for controlled aerosol delivery—especially in combination with biological test systems—was demonstrated.
- No system-related adverse effects of exposure were detected, and changes in system settings or test aerosols translate into differential biological responses
- Aerosol delivery within the system is stable and repeatable within the expected range. The geometry of the pump inlets is optimized, which is expected to increase the uniformity and repeatability of the aerosol delivery.
- The feasibility of air-liquid interface culture and transfer of the novel cell culture insert onto the chip is established.
- The next step is to test the newly developed tracheobronchial-airway HUMIMIC-Chip with InHALES.

The research described in this poster was sponsored by Philip Morris International.

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