EPITHELIX
In vitro respiratory solutions

Evaluation of the local tolerance of flagellin aerosol therapy (FLAMOD) on primary human cell-based 3D *in vitro* nasal, bronchial, small-airway and alveolar models

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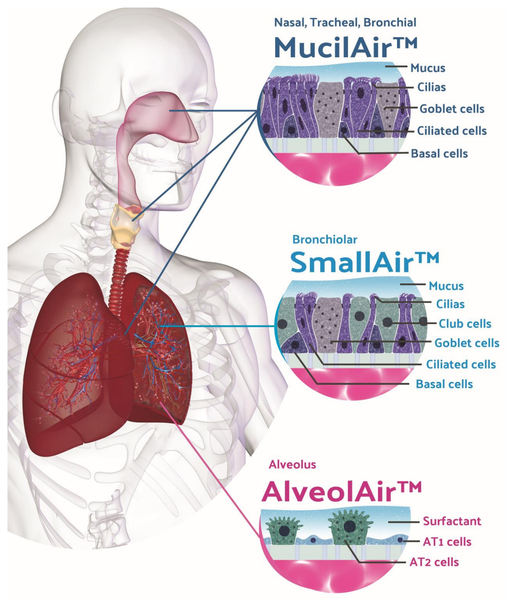
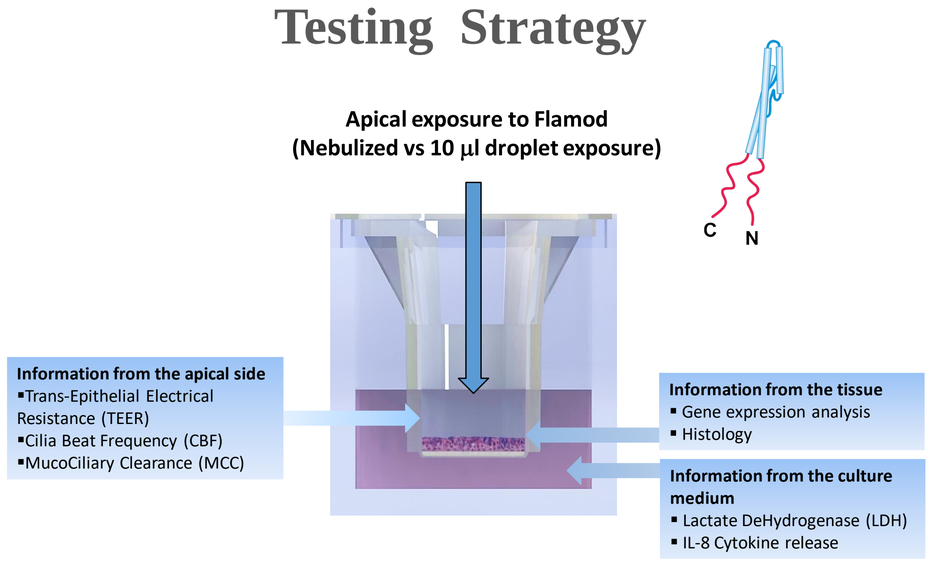
Bacterial pneumonia is a major cause of morbidity and mortality in humans. To counter this, the European consortium FAIR aims to develop more efficient therapies, based on recombinant flagellin FliCΔ174-400, to treat pneumonia with or without a concomitant uptake of antibiotics. Recombinant flagellin works as an immune-modulator which boosts the innate immunity of airway epithelia via the activation of TLR-5. Delivery of recombinant flagellin to the lung via nebulization has the advantage of directly targeting the airway epithelial cells while conferring minimal systemic immune activation.

We herein describe the local tolerance evaluation of a flagellin-based formulation (FLAMOD) for mesh-nebulization on primary human airway and lung epithelial models. Regional effects on fully differentiated nasal, bronchial (MucilAir™), small airways (SmallAir™) and alveolar (AlveolAir™) epithelial function were evaluated using a multi-parametric approach and a dynamic analysis.



MucilAir™, SmallAir™ and AlveolAir™ are fully differentiated 3D in vitro cell model of the human upper, small airway and alveolus epithelia. The cells were freshly isolated from biopsies. At air-liquid interface, the epithelia were used fully differentiated, both morphologically and functionally.

Testing Strategy



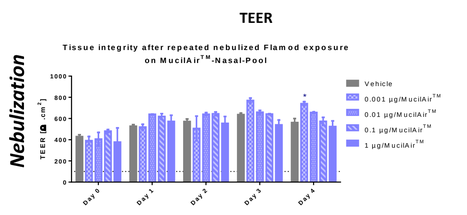
Flamod was applied on the apical surface of MucilAir™, SmallAir™ and AlveolAir™ either by nebulization (Vitrocell®-Cloud) or via 10 µl droplet exposure (diluted in saline solution – 0.9% NaCl). For all models, trans-epithelial electric resistance (TEER), cytotoxicity (LDH), pro-inflammatory cytokines (IL-8) and a panel of genes were evaluated. Additionally, on upper respiratory tract models, Cilia beating frequency (CBF) and Mucociliary clearance (MCC) were assessed.

Standard protocol and schedule of end point measurement, N= 3 per condition and per time point. The tissues were exposed to 0.0001, 0.001; 0.01; 0.1 or 1 µg/0.33 cm² of FLAMOD apically 2 h/day, for 5 days.

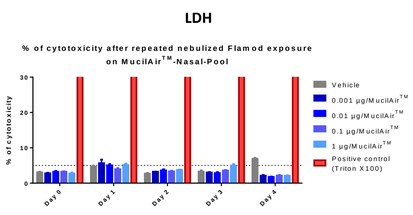
Effect of nebulized vs droplet exposure to Flamod on nasal epithelium

A) Vitrocell-Cloud with Aerogen solo nebulizer B) 10 µl droplet exposure via pipetting

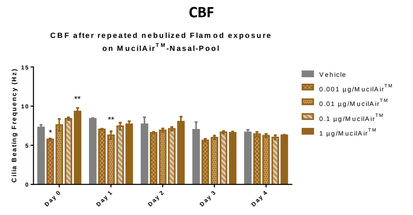
Nebulization



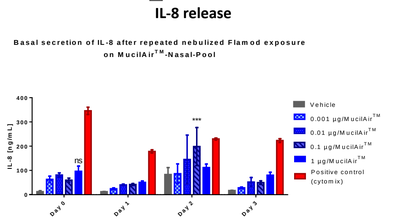
No loss of TEER was observed



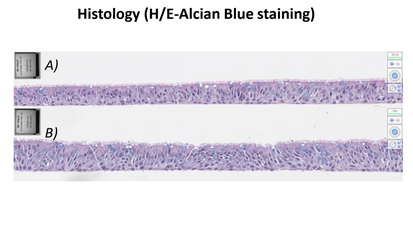
No cytotoxicity was observed



No ciliopathic effect was detected

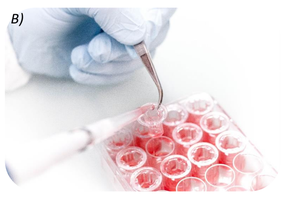


Consistent dose dependent increase of IL-8 release was quantified

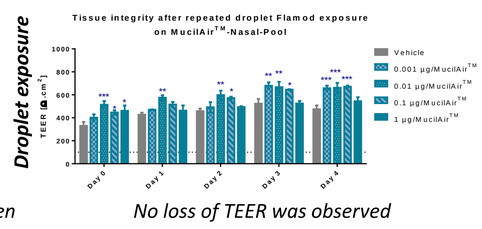


Normal pseudo-stratified architecture was observed for A) Untreated tissues or B) Flamod exposure at 1 µg/0.33 cm²

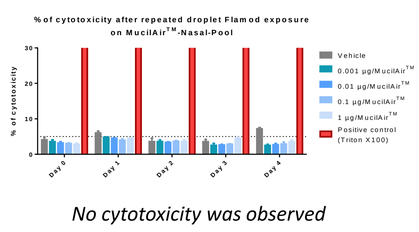
Droplet exposure



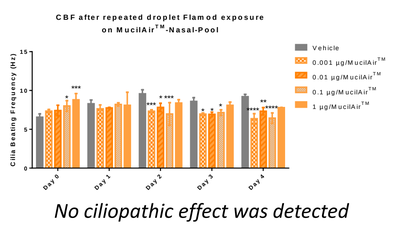
A) Vitrocell-Cloud with Aerogen solo nebulizer B) 10 µl droplet exposure via pipetting



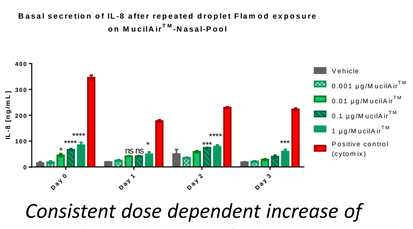
No loss of TEER was observed



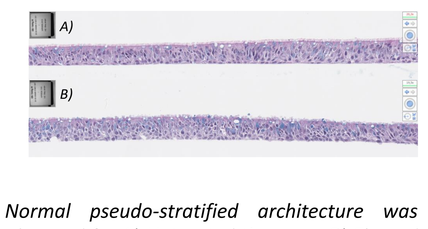
No cytotoxicity was observed



No ciliopathic effect was detected



Consistent dose dependent increase of IL-8 release was quantified

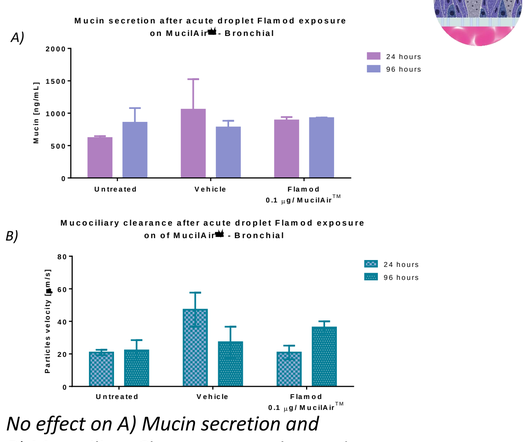


Normal pseudo-stratified architecture was observed for A) Untreated tissues or B) Flamod exposure at 1 µg/0.33 cm²

Overall no differences were observed between repeated dose exposure to Flamod via nebulization or droplet exposure

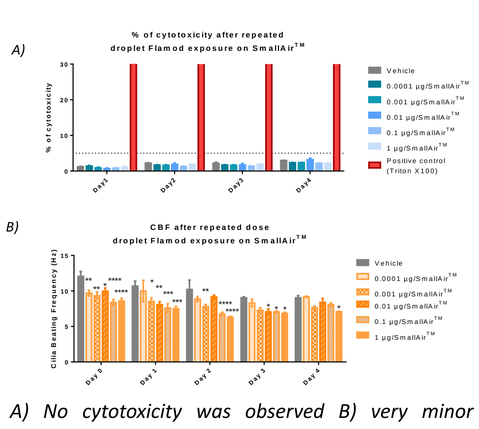
Droplet exposure to Flamod on bronchial, small-airways and alveolar epithelia

MucilAir™-Bronchial



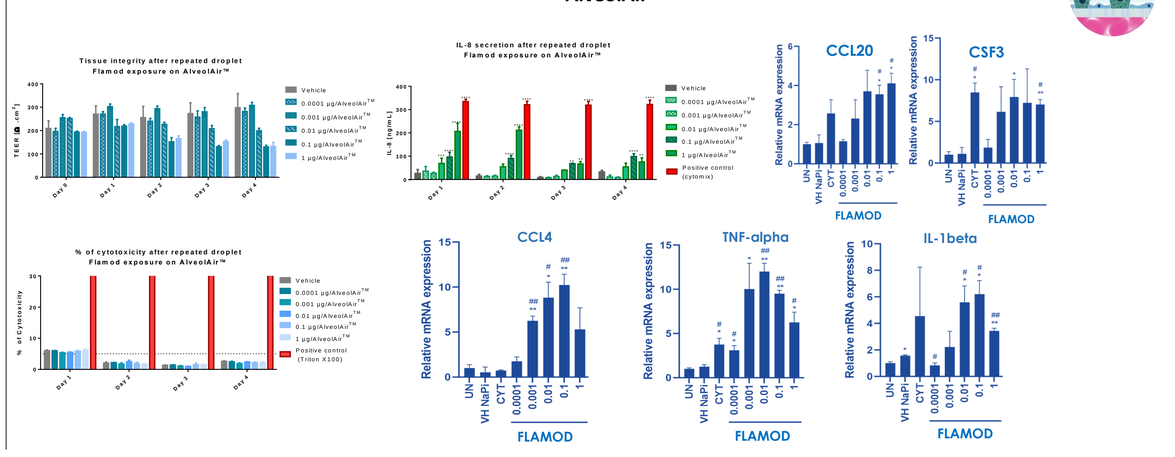
No effect on A) Mucin secretion and B) Mucociliary Clearance was observed

SmallAir™



A) No cytotoxicity was observed B) very minor dose dependent decrease of CBF was observed

AlveolAir™



Although no effect on TEER, CBF, MCC and cytotoxicity was observed for all tested conditions, FLAMOD did induce a dose dependent (i) mild increase of the cytokine IL-8 starting at 0.03 µg/cm² and (ii) upregulation of expression of genes coding *CCL4*, *TNF-α*, *IL-1β*, *CSF3*, or *CCL20* with a plateau obtained at 0.01 µg/0.33cm².

Conclusion

Altogether, FLAMOD was well tolerated by nasal, bronchial, small airway and alveolar epithelia. Apical exposure induced biomarkers upregulation, thus highlighting FLAMOD's immunomodulation potential all along the respiratory and lung mucosa.

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FAIR FLAGELLIN AEROSOL THERAPY