EPITHELIX
In vitro respiratory solutions

Development of an in vitro *model of Dry Nose*

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Introduction

When the nasal cavities do not contain enough moisture, dry nose symptoms can manifest such as pain and swelling, nosebleeds, and even airway infections. Nasal dryness can often degrade the quality of life, therefore, novel topical treatments formulated as nasal sprays are needed to treat the severe cases. To test simultaneously efficacy and toxicity effect of novel formulations, we developed an in vitro "Dry Nose" model based on a fully differentiated human nasal epithelium cultured at the air-liquid interface.

Material and Method

Epithelia (MucilAir™-Pool – *EP02MD*) were reconstituted with a mixture of primary human nasal epithelial cells isolated from 14 different healthy donors. Dry air was applied onto the apical surface of the epithelia using the Vitrocell 12/6 CF gas dispenser module (figure 1) at a speed of 6 L/min from 1 to 10 minutes.

To measure the effect of dry air on nasal epithelial cells, the following end-points were evaluated (figure 2): tissue integrity (TEER) – (*EVOMX (Epithelial VoltohmMeter*) and *electrode (STX2)*); cytotoxicity (LDH) – (*Dojindo ref: CK12-20*) ; ciliopathic effect with the Cilia Beating Frequency (CBF) – (*Cilia-x software*) and pro-inflammation (IL-8 release – *ELISA kit BD Biosciences 555244*). Proof-of-Conception for treatment as well as prevention of Dry Nose symptoms was provided by application of saline solution on the apical side of the tissue to mimic nasal spray exposure (0.9% NaCl).

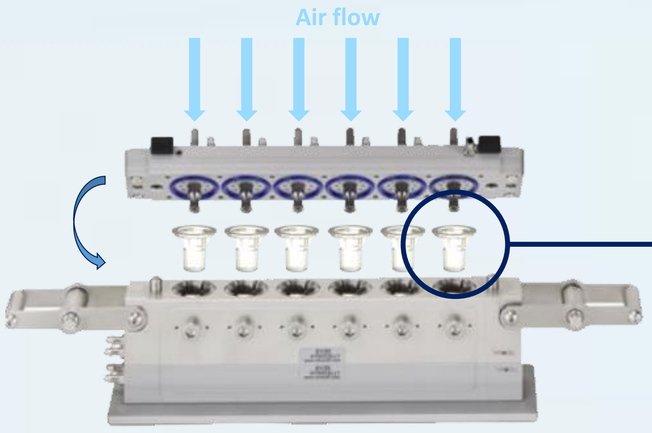

Figure 1: schematic representation of the opened Vitrocell 12/6 module.

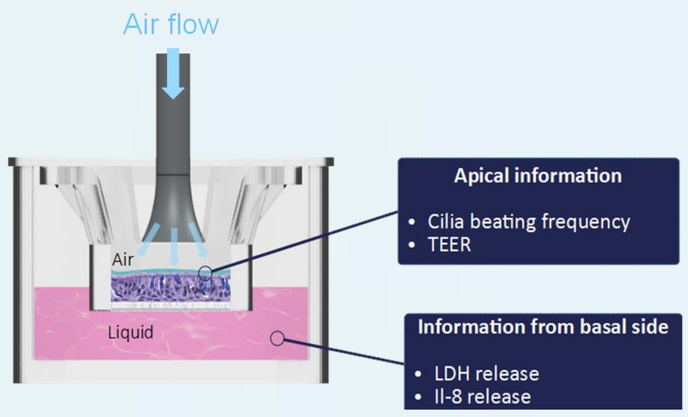

Figure 2: schematic representation of the MucilAir™, air exposure trumpet and endpoints measurements.

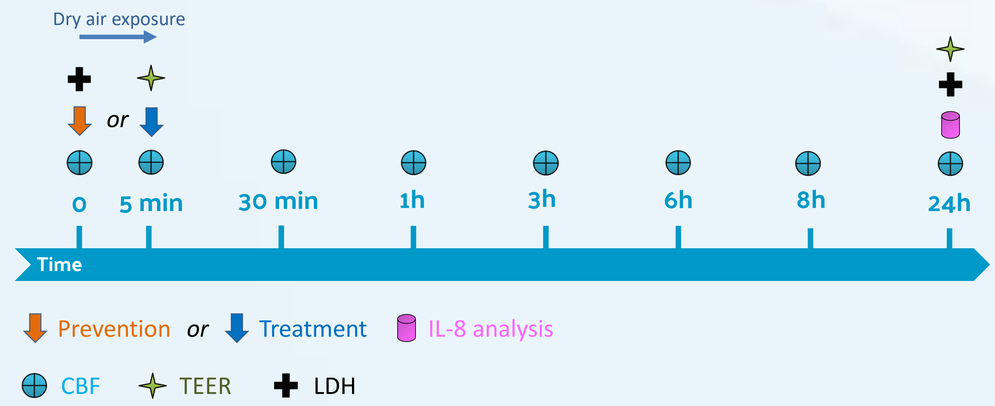

Figure 3: testing strategy sequence

Results and discussion

Data suggest that the optimal conditions to simulate dry nose were 5 minutes of exposure to dry air (6 L/min). The optimal condition is defined as the exposure time and flow rate sufficient to induce a system deregulation reflective of dry nose symptom without being irreversible of highly cytotoxic.
This exposure condition induces: A transient decrease of cilia beating frequency (Figure 4), an increase in LDH at 24h (Figure 5), a Transient decrease in TEER after air flow (Figure 6), an increase in IL-8 release (Figure 7).

Furthermore, preliminary data suggest improvement or protection of selected markers in presence of saline solution in prevention and/or as treatment. Kinetics of CBF could be considered as a primary endpoint for evaluation of compounds upon ciliary function restore. The saline solution also attenuated inflammatory effect of dry air measured by IL-8 release assay.

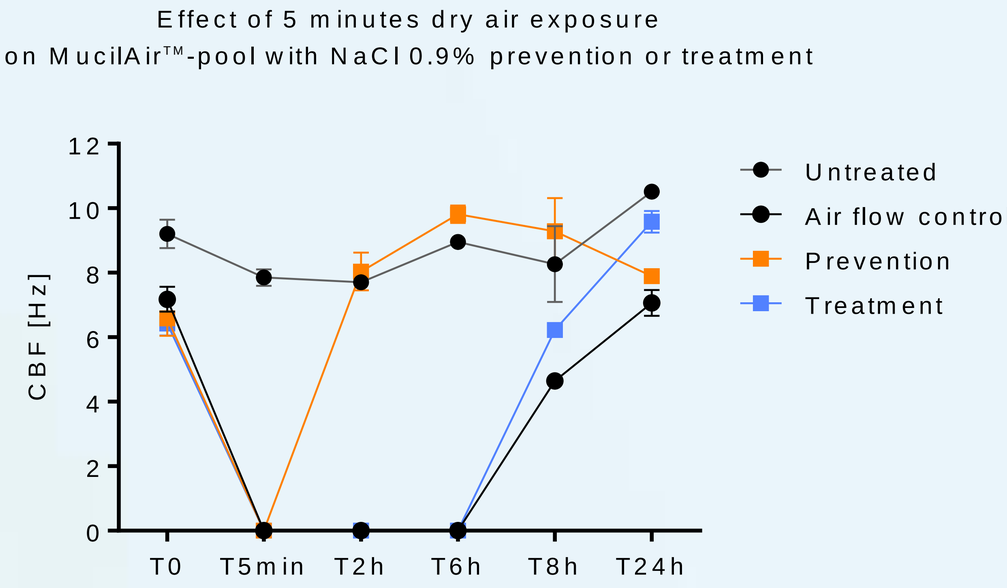

Figure 4: cilia beating frequency monitoring

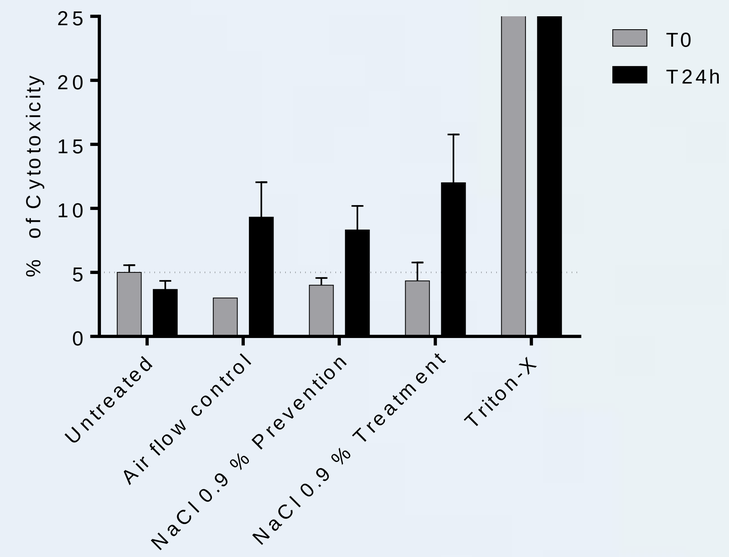

Figure 5: LDH release measurement

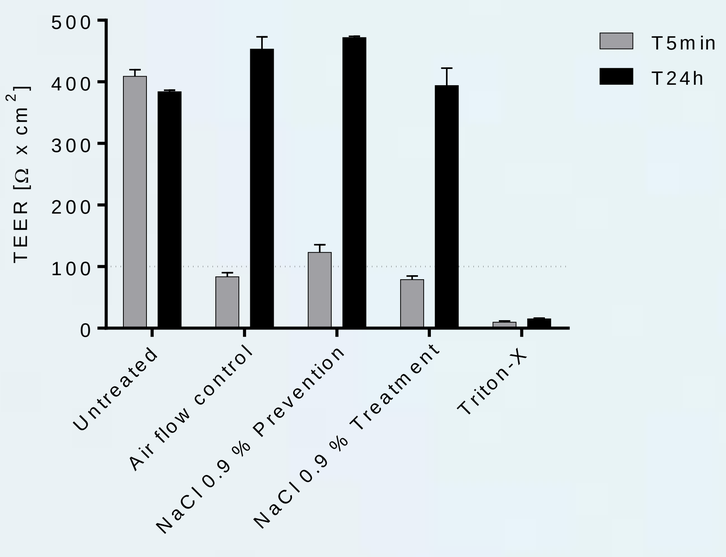

Figure 6: TEER measurement

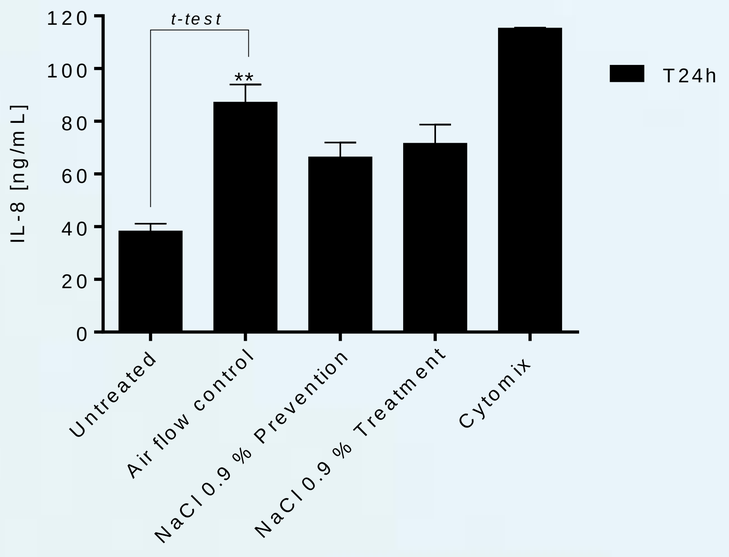

Figure 7: IL-8 release measurement

Conclusions This set of data suggest that the human nasal epithelia is a versatile and convenient tool for assessing the efficacy of moisturizing formulations designed to treat dry nose. Earlier time point for toxicity assessment would be interesting to investigate.