Epithelix

***in vitro* Solutions for Respiratory Diseases and Chemical Testing**

The SupAir project: A long term culture of nasal-tracheal-bronchial and bronchiolar human airway epithelia at interconnected and dynamic liquid flow conditions

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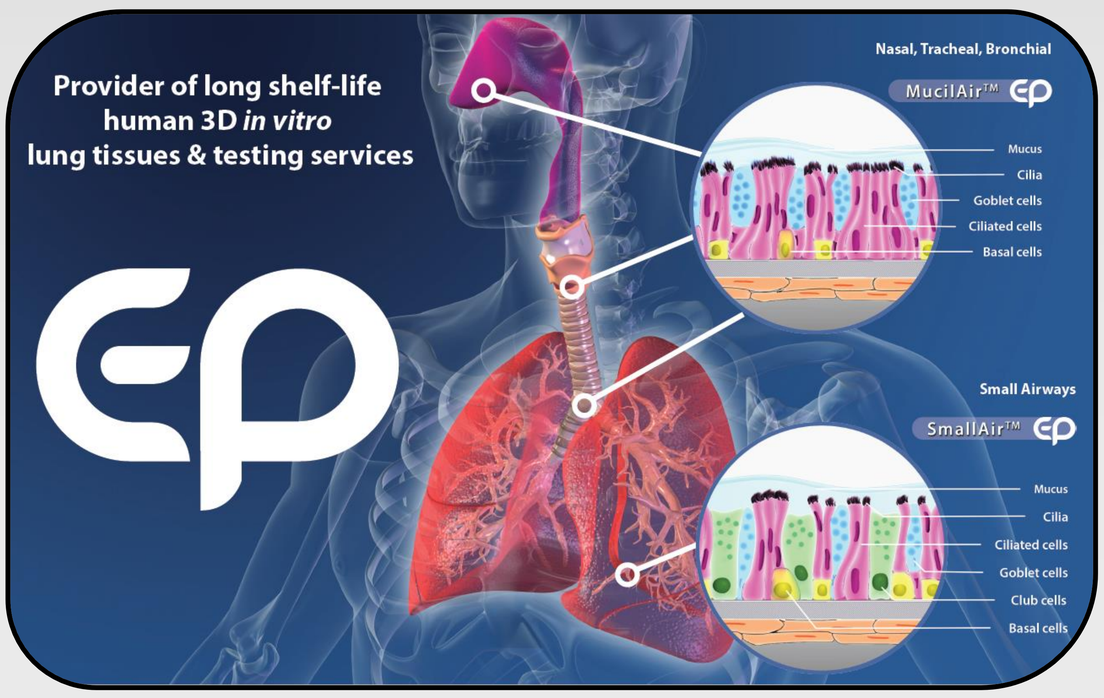
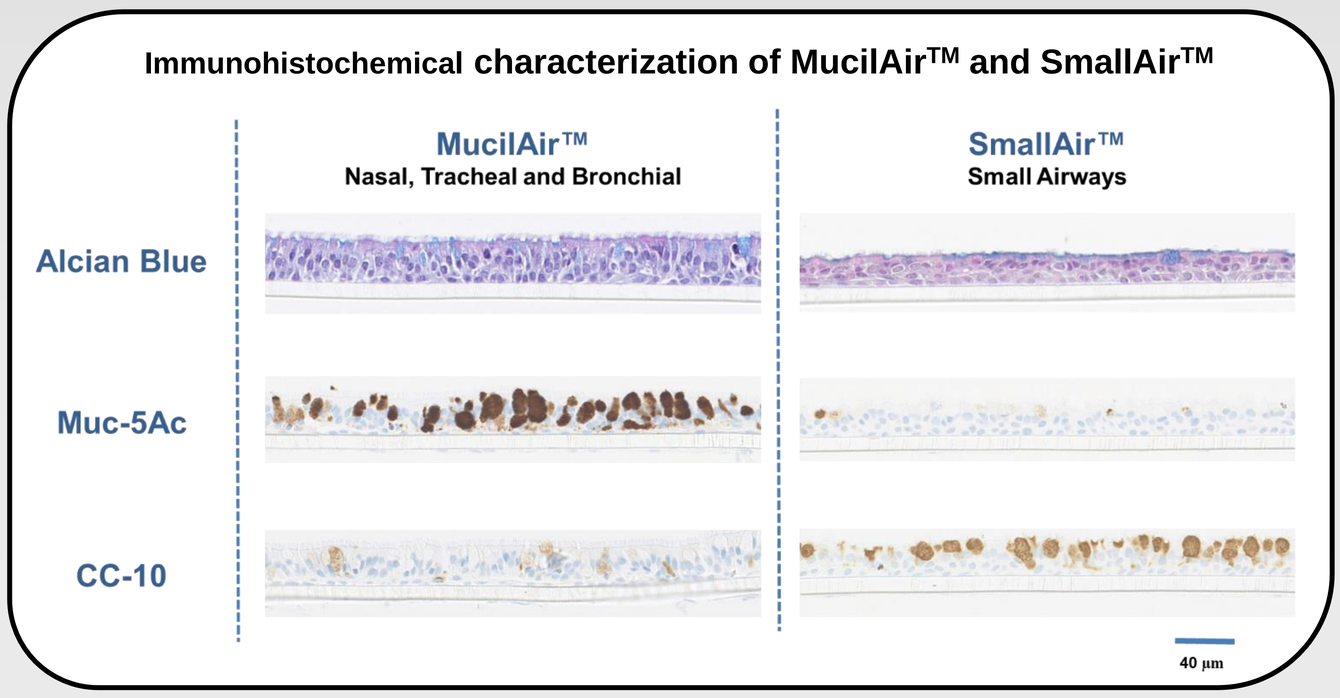
In vitro Inhalation Toxicology is an emerging and fast growing field. Until now only animal models are used in OECD test guidelines for inhalation toxicity testing. With new legal reinforcement in EU (REACH) and the paradigm shift in US (21st TOX program), *in vitro* alternatives based on human cells and tissues are urgently needed. The airway epithelium is the first line of defense of the respiratory tract against external insults while breathing. In the human lung, the cellular composition as well as the function varies at different anatomical regions: namely upper and lower airways, small airways and alveolar acini.

In order to assess the effect of a chemical compound on the lung, it would be much more informative if it could be tested in an *in vitro* model comprises all these anatomical regions.

We herein report 6 weeks interconnection of several components of upper and lower human respiratory tract. Indeed we successfully interconnected four fully differentiated epithelia reconstituted from primary human cells from different anatomical origin namely from the nose the trachea and the bronchi (three versions of the MucilAir™ system) and small airways (SmallAir™).



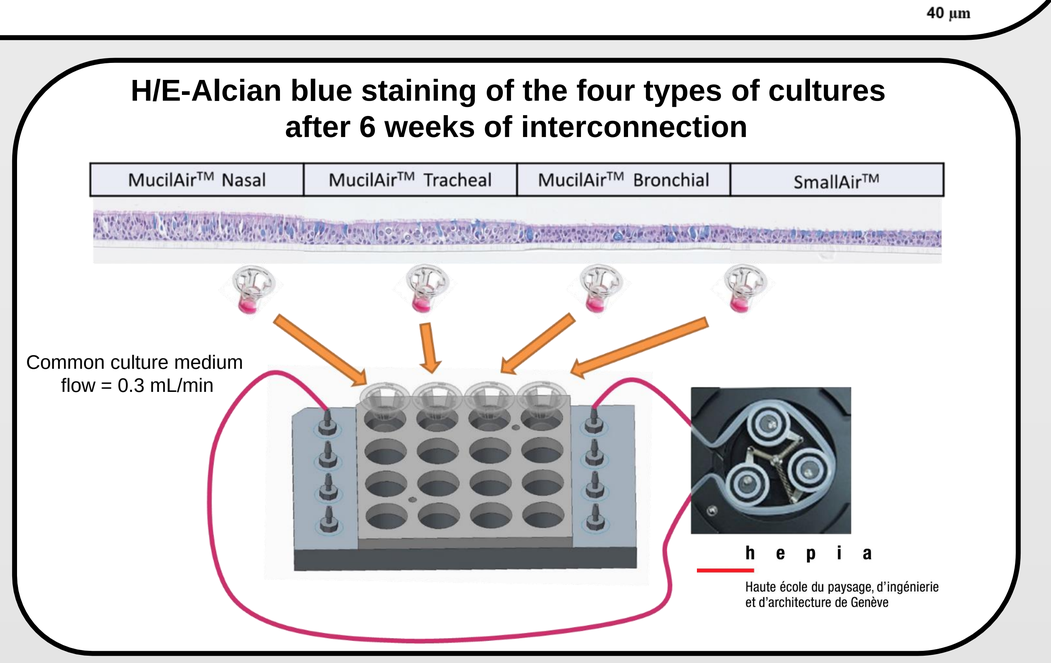


The system is composed of a culture plate allowing 3D models grown in Transwell to be (i) interconnected via the basal compartment through meso-fluidics (0.3 ml/min of a common culture medium) and (ii) maintained at the Air-Liquid Interface.

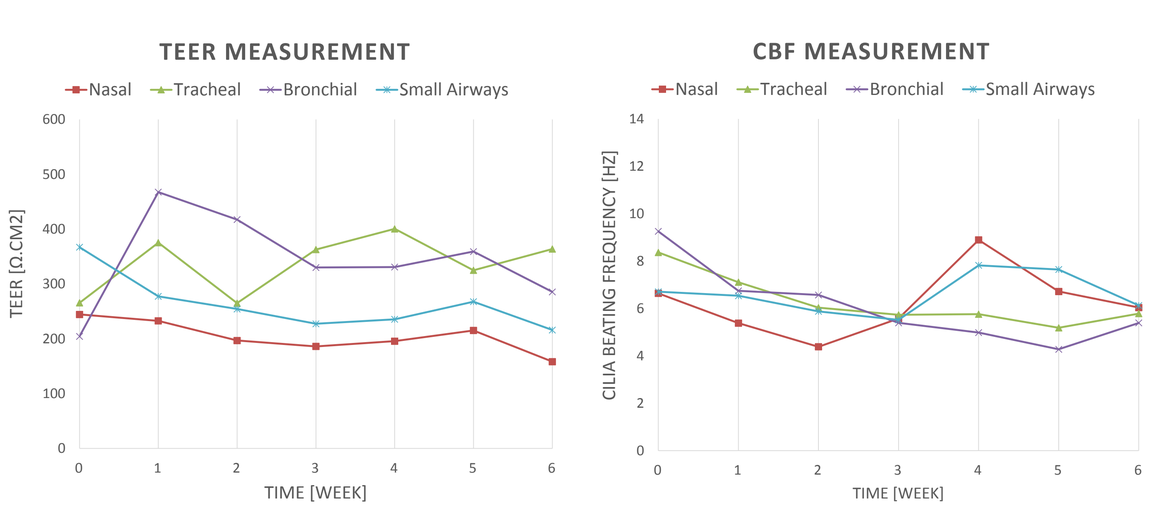
Stability in term of morphology and function of the four epithelia was evaluated. Fortunately, most of these changes can be easily monitored on the fully differentiated human airway epithelia.

End points measurement included

1. Longitudinal tissue integrity assessment (TEER)
2. Cilia activity (Cilia Beating Frequency)
3. Histological (H/E-Alcian blue staining)
4. Immunochemistry (ki67 and Fox J1) evaluation

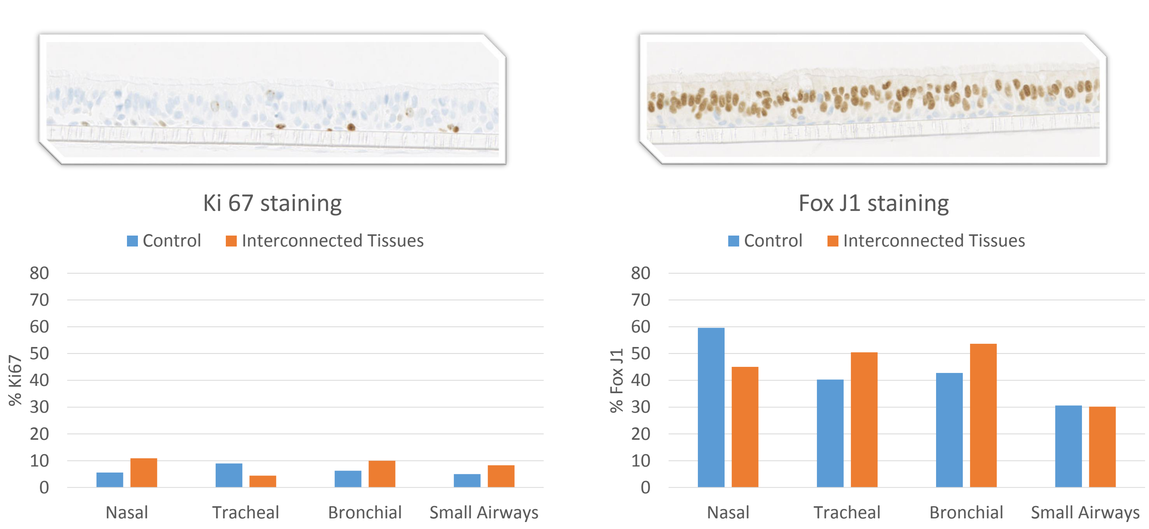


Evaluation of barrier and CBF function over 6 weeks Interconnection



- Epithelia are tight over 6 weeks interconnection (11 weeks old cultures)
- Cilia Beating Frequency is not impaired by interconnection.

Immunochemistry after 6 weeks interconnection



- No differential trend in proliferation (Ki67) or ciliation (FoxJ1) were observed between interconnected and static conditions

Conclusion

- While interconnected in dynamic liquid flow conditions (0.3 ml/min) with common a culture medium Nasal, Tracheal, Bronchial and Bronchiolar fully differentiated human epithelia are stable over 6 weeks.
- This easy set-up might find applications to test simultaneous toxicity and efficacy effects of inhaled or systemically transported compounds.