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in vitro Solutions for Respiratory Diseases and Chemical Testing

Establishment and Characterization of an *in vitro* Human Small Airway Model (SmallAir™)

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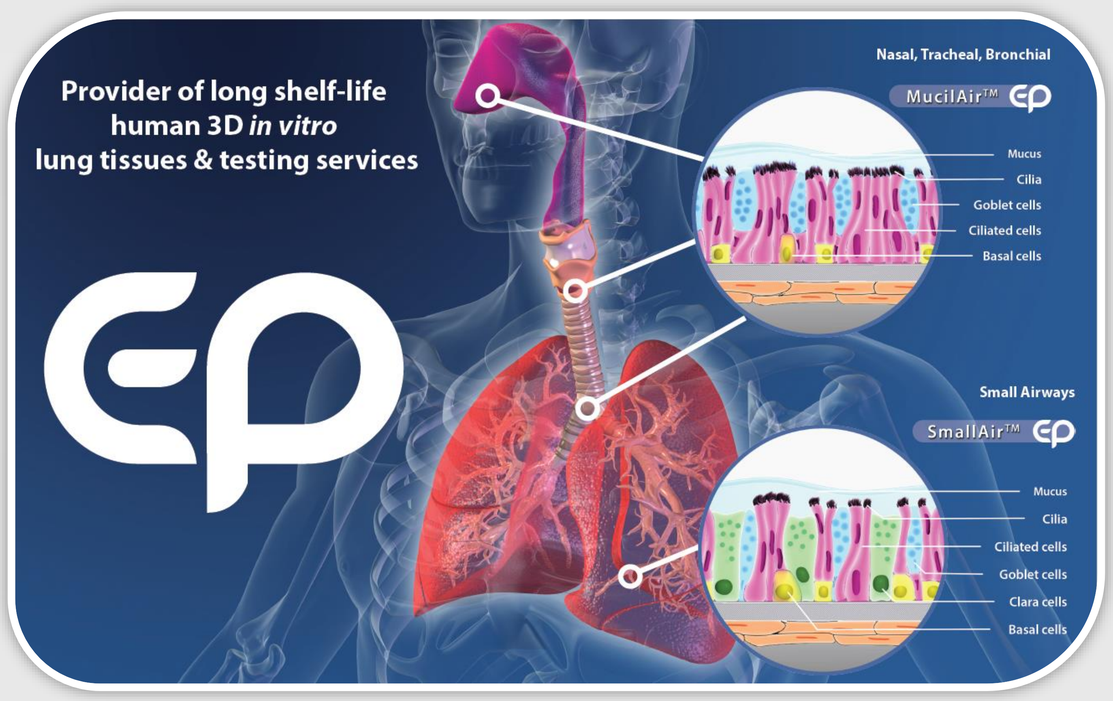
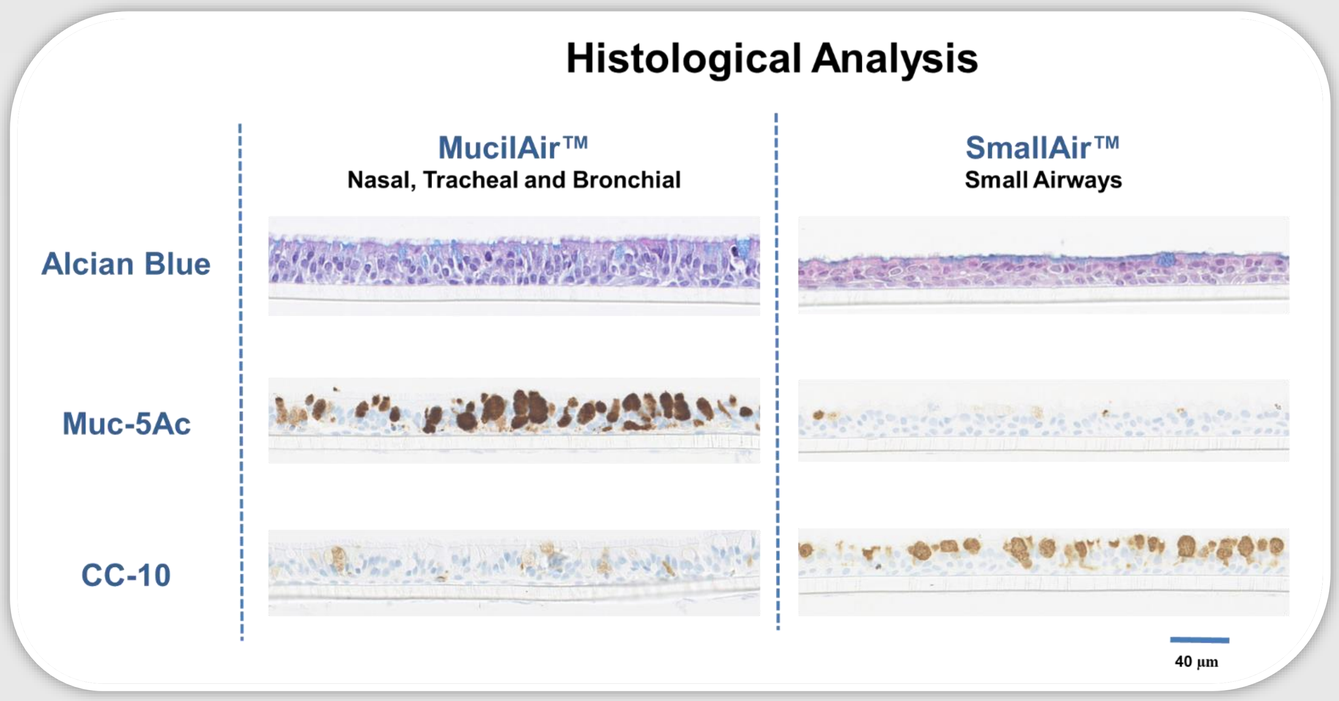
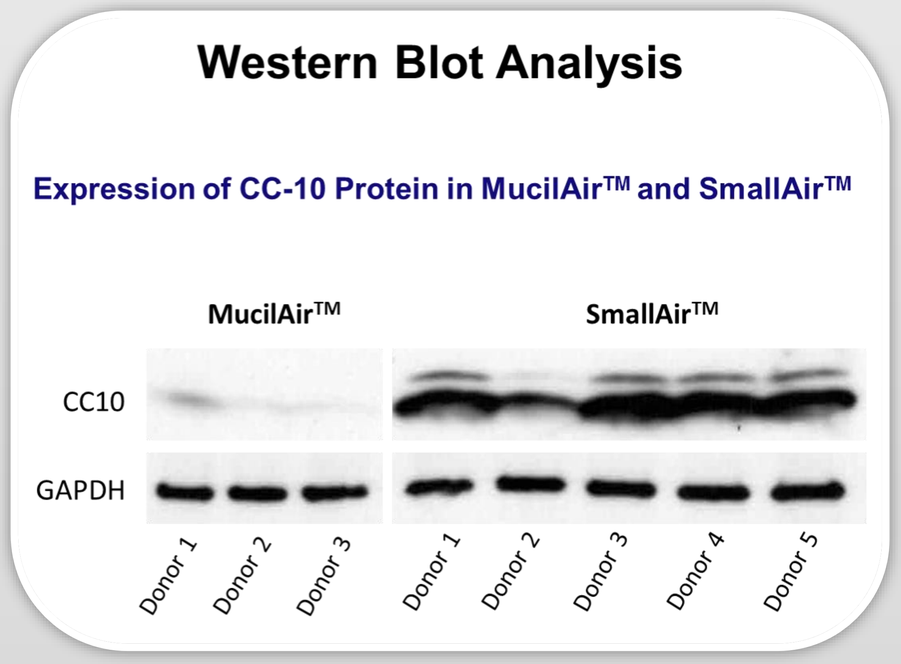
The small airways are non cartilaginous airways with a diameter < 2mm, which are extremely venerable to external insults such as tobacco smoke, mineral dust, air-pollutants, allergens, drugs, bacterial and viral infections. They play an important role in many lung diseases including chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), sarcoidosis and obliterative bronchiolitis (OB). However, the small airways constitute one of the least understood areas of the lungs due to the inaccessibility *in vivo*. Therefore, an *in vitro* model of the human small airway model would be tremendously valuable for toxicity testing of chemical substances and for studying various respiratory diseases.

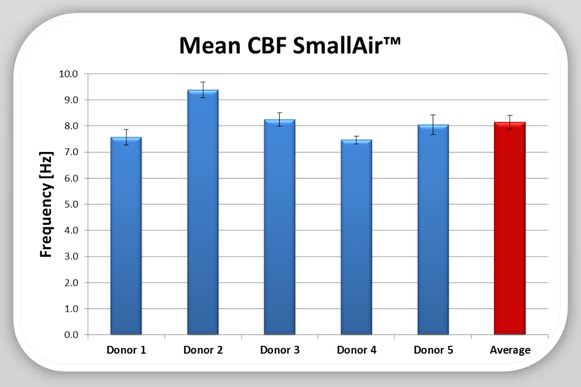
We report here the establishment and characterization of an *in vitro* human small airway model (SmallAir™). The epithelial cells were isolated from the distal lungs by enzymatic digestion. After amplification, the cells were seeded on the microporous membrane of Transwell inserts. Once confluent, the cultures were switched into air-liquid interface. After 3 weeks of culture, the epithelium became fully differentiated, with morphology of columnar epithelium, and a thickness of 10-15 µm. Most significantly, CC-10, a specific marker of Clara cells, was highly expressed in SmallAir™. CC-10 was detected by both immune-cytochemisty and Western Blot. As expected, SmallAir™ contained few Muc5-Ac positive cells (goblet cells). In contrast, CC-10 was not detected in MucilAir™, an *in vitro* model of the human nasal, tracheal and bronchial epithelial model. Instead, Muc-5Ac was highly expressed in MucilAir™. However, both MucilAir™ and SmallAir™ contain basal cells and ciliated cells, showing cilia beating and mucociliary clearance. Clearly, MucilAir™ and SmallAir™ are two distinct airway epithelial models.

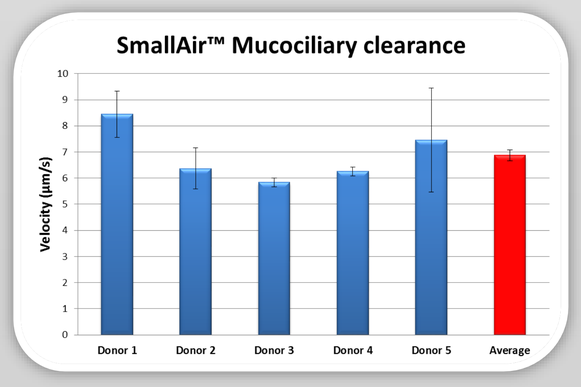


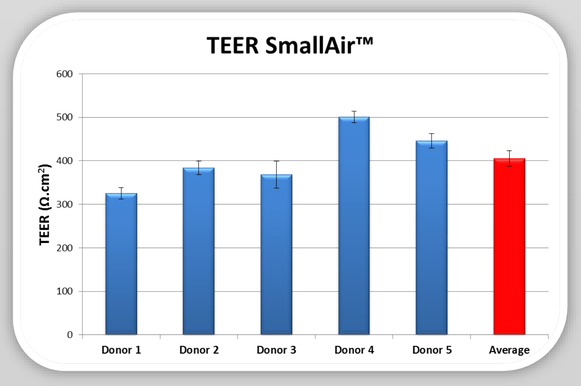


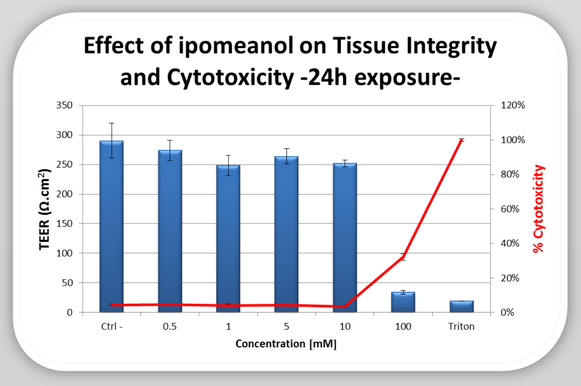
Materials and Methods: The epithelial cells were isolated from the distal lungs by enzymatic digestion. After amplification, the cells were seeded on the microporous membrane of Transwell inserts (24 well format, Cat# 3470, Corning). Once confluent, the cultures were switched into air-liquid interface. After 3-4 weeks of culture, the epithelium became fully differentiated. The epithelium was characterized by **Immunocytochemical analysis** on paraffin-sections: HE/Alcian blue staining, anti-CC10 antibody (Santa Cruz Biotechnology, CA, USA), anti-Muc5Ac antibody (Abcam, UK). The same anti-CC10 antibody was used in **Western blot** experiments. **TEER** was measured by EVOMX (WPI, UK).
Cilia Beating Frequency is measured by a dedicated setup for this purpose. The system consists of three parts: a camera (Sony XCD V60 Firewire), a PCI card and a specific package of software. The Cilia Beating Frequency is expressed as Hz.
The mucociliary clearance is monitored using a high speed acquisition camera (Sony) connected to an Axiovert 200M microscope (Zeiss). 30 µm Microbeads are added onto the apical surface of the MucilAir™. Then, 1 minute movies (4 movies/insert) showing the movement of the small beads will be taken and analyzed using the imaging software Image Pro Plus (Mediacy). The movement of the beads is tracked and velocity of each particle is calculated in order to determine the speed of the mucociliary clearance.











SmallAir™ represents a unique and powerful tool for studying the physiology and function of small airways and it should provide new insights into this major area of lung diseases.