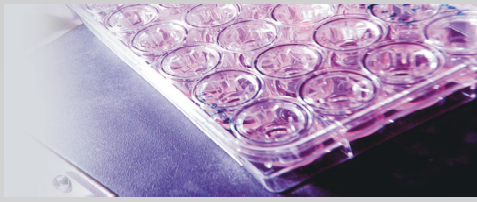
Epithelix

in vitro Solutions for Respiratory Diseases and Chemical Testing



POTENTIAL OF 3D HUMAN AIRWAY EPITHELIA RECONSTITUTED IN VITRO (MUCILAIR™) FOR IDENTIFYING RESPIRATORY SENSITIZERS

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Respiratory sensitizers and irritants are considered as substances of higher risk. However, until now there is no validated animal model/method, nor *in vitro* cell model for identifying the respiratory chemical sensitizers. The aim of this study is to develop an in vitro cellular assay for identification of respiratory chemical sensitizers based on a novel *in vitro* human 3D airway epithelium model (MucilAir™).

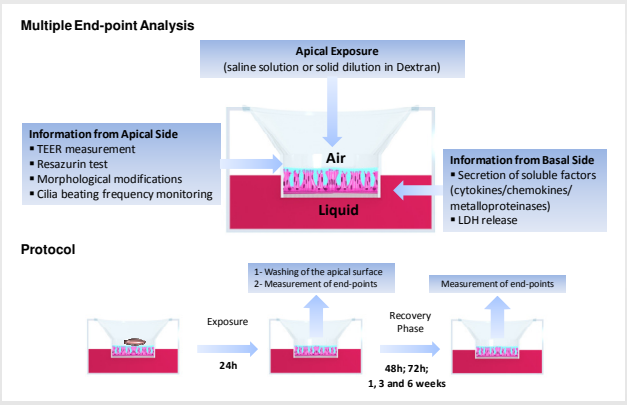
To evaluate the potential of MucilAir™ as a tool for identifying the respiratory chemical sensitizers, 9 chemical compounds belonging to 3 classes (irritants, dermal sensitizers and respiratory sensitizers) were tested. The cytotoxic effects of these chemicals were assessed by several endpoints: TEER measurement, Resazurin test, cilia beating monitoring, morphological observation, etc... The cytokines, such as IL-8, IL-6, Rantes, Gro-α, etc… were used as biomarkers for discriminating these molecules. Interestingly, the respiratory irritants and respiratory sensitizers greatly increased the amount of cytokines released into the culture media, whereas the dermal sensitizers showed no obvious effect. The stimulating effects the respiratory sensitizers and irritants are observable at concentrations several folds below the toxic doses. Moreover, some long term effects of the respiratory sensitizers on cytokine release, 4 weeks after the exposure, have been observed.

Further studies on a large number of donors (36), using two reference compounds (TMI and DNCB), highlighted the importance of the genetic predisposition of the donors: only the allergic subgroup reacted differentially to the respiratory chemical sensitizers and the dermal sensitizers. Our results are in line with the epidemiological and genetic studies of the asthma diseases.

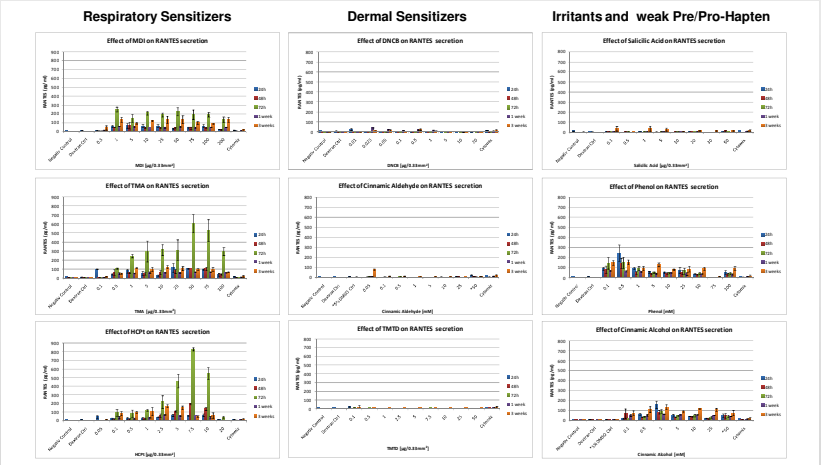
The advantages of MucilAir™

- It is composed of **primary human respiratory cells.**
- It **mimics** the morphology and functions of the native human airway epithelium.
- It has a **unique shelf-life of 12 months**.
- Epithelia from **different pathologies** are available (asthma, COPD, CF, allergic rhinitis).
- It is **ready and easy to use**.

Testing Strategy



Long Term Effect of Respiratory Sensitizers



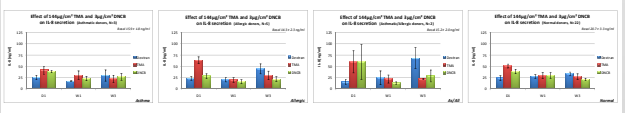
The figures show some examples of the results obtained on RANTES secretion at non cytotoxic concentrations. Interestingly, two days after removal of the compounds (e.g. at 72h-time point), RANTES was highly up-regulated when exposed to respiratory chemical sensitizers. This up-regulation was not observed for respiratory irritants and for dermal sensitizers.

Summary

Potential biomarkers for the discrimination of Respiratory Sensitizers

Discrimination potential	Recommended Cytokine and Recovery Time
Respiratory sensitizers *vs* Dermal Sensitizers	RANTES (72 h) IL-6 (24h, 48h, 72h; 1, 3 and 6 weeks) MCP-1 (3 and 6 weeks)
Respiratory sensitizers *vs* Respiratory Irritants	RANTES (72 h) Gro-α (24 h)

Genetic Predisposition



Statistically, after 24 hours exposure to TMA, the amount of IL-8 released at D1 was significantly increased for all pathologies. It is also the case for DNCB, except for allergic group, where DNCB seems to have no significant stimulatory effect on IL-8 release. After 6 and 20 days recovery (week 1 and 3), Il-8 secretion returned to the normal level for both sensitizers and for all pathologies.

Conclusions

1: It may be possible to discriminate the respiratory sensitizers from dermal sensitizers or respiratory irritants using a right panel of biomarkers (cytokines) and recovery time, based on MucilAir™.
2: Dextran Carrier Method is an easy and powerful method for delivering non soluble or non water compatible insoluble material on apical surface ALI cultures.
3: Standard Operating procedures are accessible.

Acknowledgements

FENRIV
Ligue suisse contre la vivisection

More Information