- Lung Model Edition -

Introduction

Whereas animal models to study lung physiology have provided invaluable information on the understanding of certain conditions such as cystic fibrosis and asthma [1, 2], there is a growing demand for more physiologically relevant human *in vitro* models according to the 3Rs principle of reducing, replacing and refining the involvement of animals in scientific procedures.

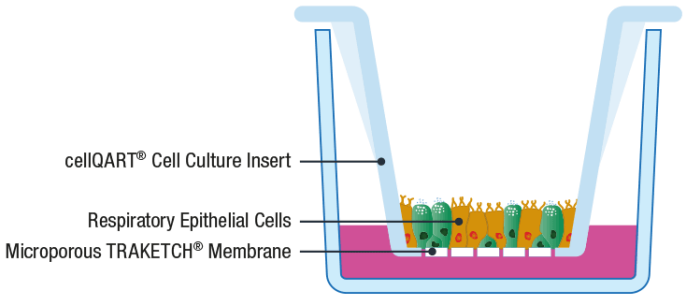
For this purpose, the precise *in vitro* modelling of the complexity of the airway epithelium is crucial. It is of especial interest the use of adequate, reliable and reproducible models that mimic the pseudostratification of the epithelium, as well as the ability of the airway cells to properly differentiate. In this regard, Air-Liquid-Interface cultures (ALI) offer an exceptional option to model and study the respiratory tract.

What is the respiratory epithelium?

The respiratory epithelium is the lining of the airway tract.

The respiratory epithelium is in charge of [3]:

- Protecting from pathogens and debris
- Moisten
- Facilitate gas exchange



The respiratory epithelium is:

- Ciliated: formed by cells with hair-like projections in the apical side [4]
- Polarized: apical and basolateral membrane domains are distinct [5]
- Pseudostratified: appears to be stratified, however it is a cell monolayer where nuclei are positioned at different levels [5]

The respiratory epithelium contains the following cell types [6]:

- Secretory club cells: produce and secrete mucous
- Ciliated cells: located in the apical side, move mucous across the track
- Basal progenitor cells: bear the capacity to differentiate and restore the epithelial layer as a response to injury
- Rare cell types: tuft cells, solitary neuroendocrine cells, pulmonary ionocytes and goblet cells. There is compelling evidence showing that these cells might be implicated in lung disease [6]

Thus, it is of fundamental significance the use of in vitro models that allow the growth and differentiation of common and rare subtypes, as differentiated cells reflect the lung environment in a more reliable way.

What is ALI culture?

In vitro ALI cultures are pivotal and widely used to simulate the respiratory tract.

In lung ALI cultures, airway epithelial cells are grown on permeable membranes at the air-liquid interface. Under this conditions the cells differentiate, polarise and form a pseudostratified epithelium, closely resembling *in vivo* conditions [6, 7]. Airway cells cultivated in this way predict more accurately the normal biology and physiology than cells in submerged culture, where they fail to display the essential mucociliary phenotype.

How to do an ALI culture?

The general procedure to set up an ALI culture starts with the seeding of epithelial cells on a porous support, such as SABEU's tissue cultured treated TRAKETCH® membranes of cellQART® inserts. The typically used inserts/membranes have a pore size of 0.4 µm. The high porosity-translucent membrane is ideal for fast barrier formation, whereas the low porosity-clear membrane has superior optical properties in phase contrast microscopy.

Following cell propagation and confluency achievement, the culture medium from the apical compartment is discarded. By doing this, the apical side of the cells is exposed to the air while only the basal side is supplied with nutrients through the lower compartment. By providing an *in vivo*-like environment, cells differentiate and polarize, creating a physiological relevant *in vitro* model [8].

Detailed experimental protocols to perform ALI cultures can be found in the following references [9, 10].

What are some applications of ALI cultures?

- Test aerosolized drugs [11]
- Measure the health effect of certain air pollutants [12]
- Tobacco research [13, 14]
- Perform studies on both normal and disease states [15]
- Study of respiratory viral infections e.g. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [16]

The *in vitro* study of the respiratory system is of cardinal importance to increase our knowledge on the basic mechanisms in normal and altered states. The investigation on the different respiratory epithelium cell subtypes will contribute to underscore the role of each subpopulation in disease and normal airway-surface regulation. This will accelerate the development of potent and targeted therapeutic approaches.

It is important to mention that ALI cultures have many other uses such as *in vitro* skin models, organoids and biofilm assays. Stay tuned for many more interesting ALI applications.

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