An air-liquid interface culture system for small airway epithelial cells

By Sorin Damian, Marjorie R. Smithhisler and George J. Klarmann, Lonza Walkersville, Inc., Walkersville, MD, USA

Introduction

Availability of primary normal human small airway epithelial cells facilitates the *in vitro* research of complex aspects of the airway morphology, functionality and physiology. Here we present a functional system consisting of small airway epithelial cells established in a 3-D model of air-liquid interface. The model reproduces the epithelial inner lining of small airways where the cells are attached by their basal pole, and faces the lumen with the apical pole. Our model is highly functional *in vitro* after an induced process of differentiation that necessitates the usage of particular media formulations, a special filter membrane attachment support and an optimized differentiating protocol. The existence of such a model opens the door to a very large number of relevant experimental approaches, like study of disease of small airways resulting from exposure to tobacco smoke, mineral dust, air-pollutants, viral infections etc., and study of inflammatory mediators as well as physiological changes of bronchioles, small bronchi or alveolar area. The model can be used in a more relevant way than standard submerged culture for the research of nearly any aspect of the biology of the small airway epithelium. Macrophage cell lines or normal human macrophages can be added and the interactions with small airway epithelial cells can be addressed experimentally from a new perspective in 3-D airlifted cultures.

This 3-D model system more directly resembles the human airway compared to traditional bronchial epithelial cell culture methods, which should facilitate better experimental design and results[1].

Methods and materials

In this study we grew small airway epithelial cells (Clonetics™ SAEC, Cat. No. CC-2547) from normal, human donor lung tissues in an air-liquid interface (ALI) model to assess potential morphological and functional changes *in vitro* toward the differentiated phenotype.

Cell isolation: Small airway epithelial cells (SAEC) were isolated through an enzymatic digestion protocol from normal cadaveric lung tissue samples located at the lower end of either left or right lung (small airway sections). Isolated cells were expanded in standard submerged culture in SAGM™ small airway growth medium then passaged once and cryopreserved at 500,000 cells/vial with a specific computer cryopreservation program. Vials were stored in liquid nitrogen until further use.

Cell culture: For airlifted cultures, normal SAEC were expanded for 3 days in S-ALI™ growth media (Lonza, Cat. No. CC-3281) then seeded in collagen-coated Corning PET Transwells® 0.4 µm filter inserts at a density of 60,000 live cells. Media was removed from the apical chamber at day 4 and S-ALI™ differentiation media (Lonza, Cat. No. CC-3282) was added to the basal chamber to initiate the air-liquid interface (ALI) culture. Trans-epithelial electrical resistance (TEER) was measured with EVOM equipped with STX2 electrodes. Tight junction proteins were assessed through immunostaining with ZO-1 (Life Technologies, Cat. No. 33-9100) and detected with ALEXA Fluor 488 (Life Technologies, Cat. No. A11017).Cytokeratin 19 was detected with mouse anti-human CK-19 (Sigma) and developed with a goat anti-mouse-IgG FITC conjugate (Sigma). Secreted mucin was directly stained with Alcian Blue, and cilia formation was assessed through immunostaining with anti-ß-tubulin FITC conjugate (Sigma clone TUB 2.1, Cat. No. F-2043).

Figure 1
SAEC differentiation process using S-ALI™ small airway ALI media kit.

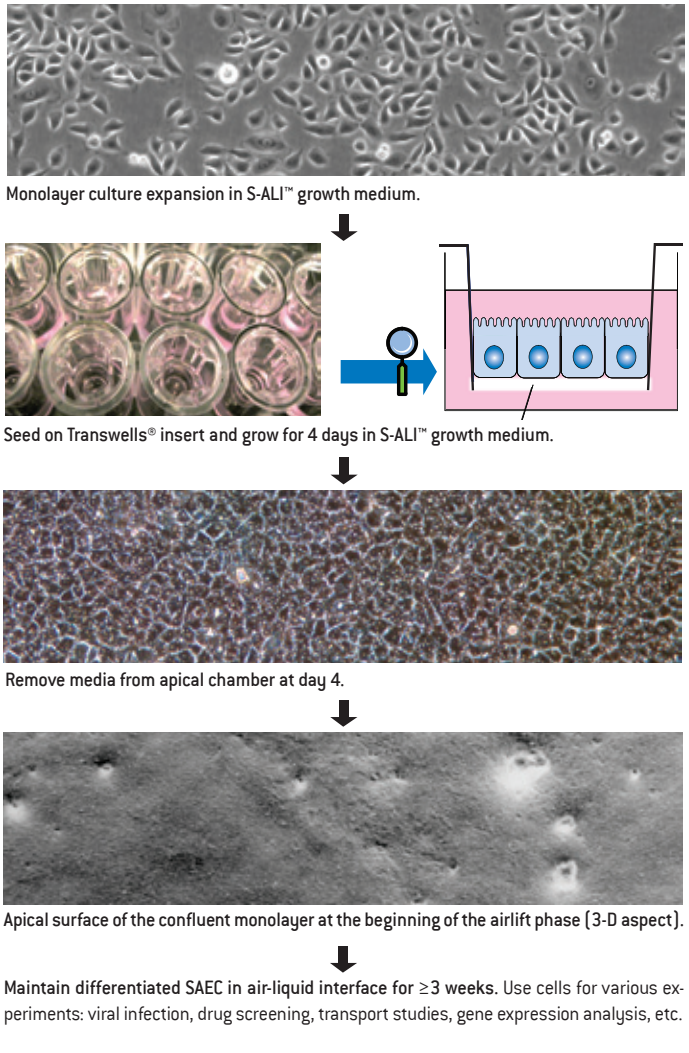
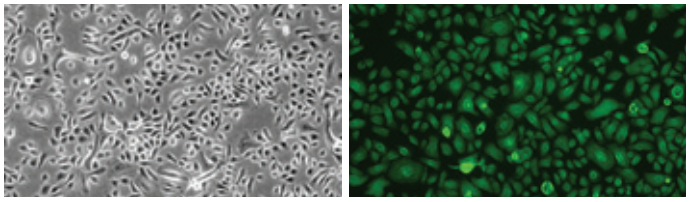
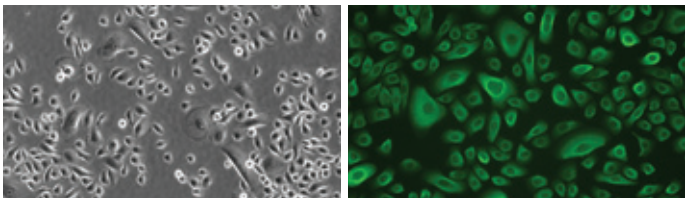


Figure 2
In submerged culture SAEC express a typical proliferative epithelial phenotype (left column) and express cytokeratin 19, a specific marker (right column).

Donor 1



Donor 2



Donor 3

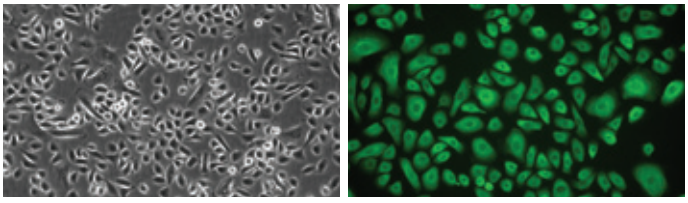
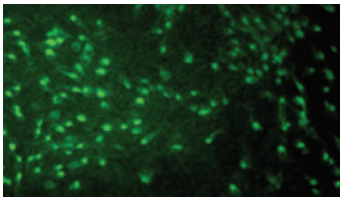
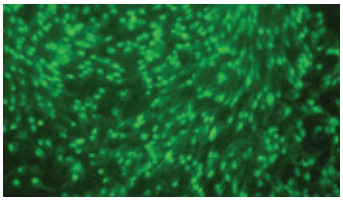


Figure 4
SAEC in air-liquid interface culture exhibit a ciliated phenotype (ß-tubulin staining by day 20). Control is day 0 in airlifted conditions.

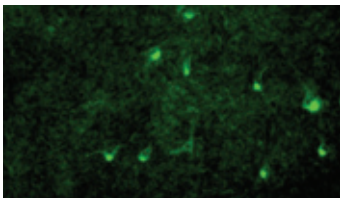
Donor 1



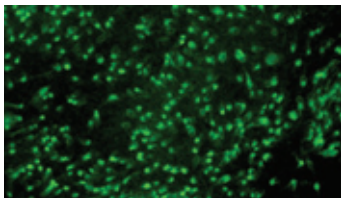
Donor 4



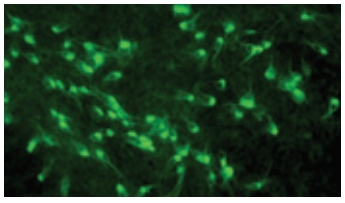
Donor 2



Donor 5



Donor 3



Sample control

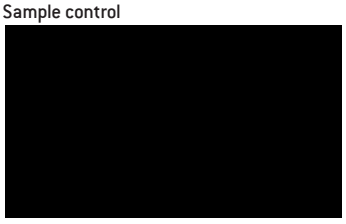
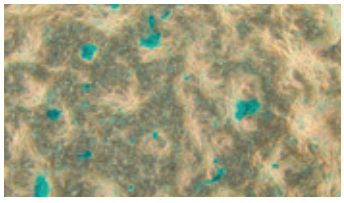


Figure 3
SAEC in air-liquid interface culture exhibit a secretory phenotype (mucin secretion by day 20). Control is day 0 in airlifted conditions.

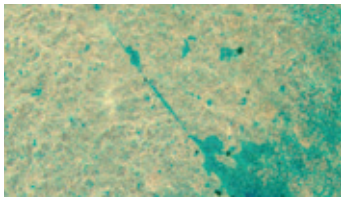
Donor 1

Donor 4

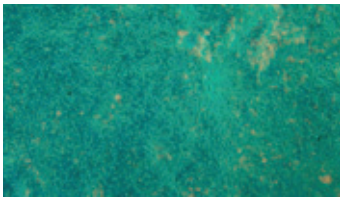
Donor 2



Donor 5



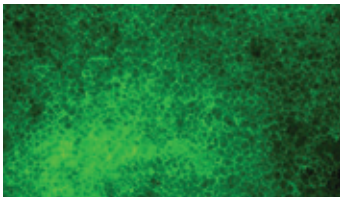
Donor 3



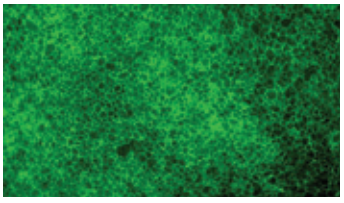
Sample control

Figure 5
SAEC in air-liquid interface culture have tight junctions. On day 20, cells were stained for ZO-1 directly into Transwells® inserts. Control is day 0 in airlifted conditions.

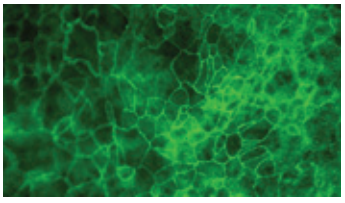
Donor 1



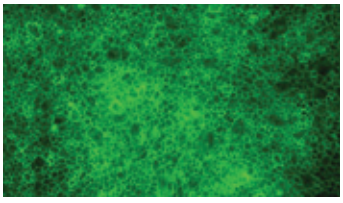
Donor 4



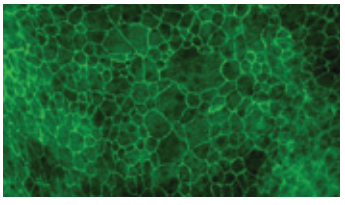
Donor 2



Donor 5

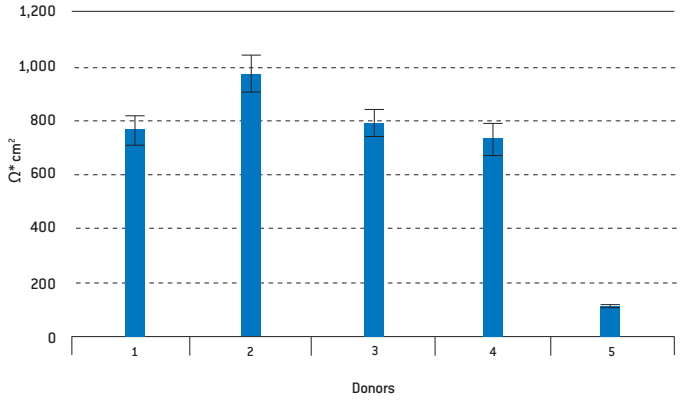


Donor 3



Sample control

Figure 6
SAEC in air-liquid interface culture exhibit a strong barrier, measured by TEER.



Results

TEER measurements, which exhibited donor-dependent response, were as high as 600Ω* cm^2 between 10 - 20 days in airlifted status, which indicates a solid barrier function. In most SAEC lots, ASL is visible by 10 days after airlift, but compared with similar 3-D cultures of tracheobronchial epithelial cells, SAEC secrete less mucin. Cilia were detected at 20 days after airlift, and indicated a donor dependency. Cells were positive for ZO-1 expression and exhibited a donor-dependent variation in the extent of tight junction networks.

- We successfully isolated highly pure cultures of small airway epithelial cells (SAEC) from normal donors. Those cells can be easily used in standard classical 2-D cultures for any study that requires a proliferative phenotype.
- All lots have a standard epithelial phenotype in submerged culture and express a specific epithelial marker, cytokeratin 19 (Figure 2; only three donors shown).
- We were able to differentiate SAEC in a 3-D environment by airlifting and differentiating the cells on PET filters and culturing in S-ALI™ media kit supplemented media.
- We screened multiple SAEC lots (from different donors) and found nearly 50% of the lots will be suitable for the airlifted model.
- The availability of guaranteed SAEC lots suitable for airlifted cultures, as well as specific media for the growth and differentiation of SAEC in filter plates, offers a biologically relevant *in vitro* model to the *in vivo*-like differentiated model.
- The differentiation process is characterized by consistent reproducibility and is based on the use of an original protocol and media formulation.
- The differentiated phenotype was validated through the most commonly recognized functional markers for small airway lining epithelium.
- Additional markers for cell junctions, like ß-catenin, desmoplakin, and occludin were also assessed at a later stage in the differentiation process. Distribution and abundance of all those markers were donor-dependent, but always present (data not shown).

Conclusion

Our data demonstrate that SAEC can be differentiated in an ALI culture system that is capable of forming tight junctions, displaying cilia and secreting ASL. This system should facilitate more physiologically relevant studies of both normal SAEC and those from diseases, such as COPD and asthma. The availability of SAEC for ALI culture from different donors with known health history increases our ability to perform more in-depth studies of apoptosis, cytokine secretion/stimulation. This 3-D model is extremely relevant for the *in vitro* study of airborne toxicants or epithelial injury by lethal toxicants (e.g., anthrax toxin) or bacterial/fungal/viral insults.

Research application examples

- Gene therapy studies i.e., delivery of genetically engineered cells or gene segments by way of specifically targeted adenoviral vector delivery
- Host defense mechanisms
- Gene expression analysis
- Preclinical drug development, disease models
- HTS using 96-well filter plates
- Airborne toxicant studies
- DOD bio-defense models
- *Bacillus anthracis* utilizing human lung epithelial cells[2]
- Thus, the cells and ALI culture system provide researchers with valuable tools in which to aid in their quest to understand the mechanisms of diseases.

References

1. Sorin Damian, George Klarmann, Marjorie Smithhisler, B-ALI™ Bronchial Air Liquid Interface Media Kit, a Guaranteed 3D *in vitro* Model for Respiratory Research, *Lonza Resource Notes™, Fall 2010, p. 13–17*.
2. Russell BH, Vasan R, Keene DR, Koehler TM, Xu Y., Potential dissemination of *Bacillus anthracis* utilizing human lung epithelial cells, *Cellular Microbiology, 2008, 10(4):945–57*.

www.lonza.com/research
www.lonza.com/airway-cells

Contact Information

North America
Customer Service: +1 800 638 8174 (toll free)
order.us@lonza.com
Scientific Support: +1 800 521 0390 (toll free)
scientific.support@lonza.com

Europe
Customer Service: +32 87 321 611
order.europe@lonza.com
Scientific Support: +32 87 321 611
scientific.support.eu@lonza.com

International
Contact your local Lonza distributor
Customer Service: +1 301 898 7025
Fax: +1 301 845 8291
scientific.support@lonza.com

International Offices

Australia	+ 61 3 9550 0883
Belgium	+ 32 87 321 611
Brazil	+ 55 11 2069 8800
France	0800 91 19 81 (toll free)
Germany	0800 182 52 87 (toll free)
India	+91 22 4342 4000
Japan	+ 81 3 6264 0660
Luxemburg	+32 87 321 611
Singapore	+ 65 6521 4379
The Netherlands	0800 022 4525 (toll free)
United Kingdom	0808 234 97 88 (toll free)

Lonza Walkersville, Inc. – Walkersville, MD 21793

AD-RNFall2011 07/11 GM-NE009