Evaluating the Differentiation Potential of Primary Airway Cells in 3-D Models

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Scientist, ATCC
About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and a center of scientific excellence in Gaithersburg, MD
- We have the world’s largest, most diverse biological materials and information resource for cell culture – the “gold standard”
- Innovative R&D company featuring gene editing, differentiated stem cells, advanced models
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 550+ employees, over one-third with advanced degrees
Overview

- Overview of the methods used to generate 3-D airway models
- Comparison of primary and hTERT immortalized airway models
- Investigation of variation in primary cell lots
- Effects of model formation from different differentiation media
- Comparison of airway models comprised of cells from ATCC or suppliers
- Review of common pitfalls in airway model fabrication
Overview of the human airway

- Comprise different regions, each consisting of different specialized cells or ratios of different cell types.
- The primary bronchus is populated by differentiated epithelial cells including goblet, ciliated, and basal cells.
- Small airways also include club cells.
- Alveoli epithelium consists of types I and II alveolar cells.
ATCC products for airway models

- Primary cells
  - Bronchial/tracheal epithelial cells (HBECs)
  - Small airway epithelial cells
  - Lobar epithelial cells
  - Lung smooth muscle cells
  - Bronchial/tracheal smooth muscle cells
  - Lung fibroblasts
  - Disease airway cells
    - Asthma, COPD, Cystic Fibrosis, Fibrosis

- hTERT-immortalized primary cells
  - HBEC-3KT (Bronchial epithelial cells)
  - NuLi-1 (Bronchial epithelial cells)
  - HSAEC1-KT (Small airway epithelial cells)
  - HTERT Lung Fibroblast
Overview of 1st study, media differentiation test

Objectives in this study

- Compare the epithelial differentiation potential of airway models generated from four separate lots of primary HBECs.
- Compare hTERT-immortalized HBECs to primary HBECs.
- Generate airway models utilizing different medias.
- Investigate how plate layout (full or partial plates) will affect epithelial differentiation and replicate variability in airway models.

Other considerations

- After 2 weeks of ALI culturing, weekly TEER measurements as well as apical washings were conducted.
- Outer wells of partial plates were filled with 2 mL DPBS.

<table>
<thead>
<tr>
<th>Media Differentiation Test</th>
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<tbody>
<tr>
<td>Primary Lot #</td>
<td>Differentiation Media</td>
</tr>
<tr>
<td>1</td>
<td>100% Bronchial Growth</td>
</tr>
<tr>
<td>2</td>
<td>80:20: Bronchial:Fibroblast Growth</td>
</tr>
<tr>
<td>3</td>
<td>Lifeline Cell Technology Media</td>
</tr>
<tr>
<td>4</td>
<td>Stemcell Technologies Media</td>
</tr>
<tr>
<td>NuLi-1 Control</td>
<td></td>
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</tbody>
</table>
Overview of current model fabrication method

Coat trans-well insert with collagen (100 µL of 0.3 mg/mL)

Add epithelial cells to apical layer (50-100,000 cells per well)

Once confluent, remove apical media to begin ALI culturing

Following ALI culturing, HBECs are fully differentiated.

Replace media with differentiation media
Microscopy images: week 0 and 1

Representative images of cells (A) prior to ALI culturing, and (B) 1 week of ALI.
Microscopy images: week 2

Representative images of HBECs incubated under ALI for 2 weeks with:
(A) Bronchial media only
(B) 80:20 bronchial: fibroblast media
(C) Lifeline media
(D) Stemcell media
Microscopy images: week 5

Representative images of HBECs incubated under ALI for 5 weeks with:
(A) Bronchial media only
(B) 80:20 bronchial: fibroblast media
(C) Lifeline media
(D) Stemcell media
Microscopy images: Stemcell media cell lots

Representative images of cell Lots (A) 1, (B) 2, (C) 3, and (D) 4 incubated with Stemcell media under ALI for 5 weeks.
Microscopy: hTERT controls

Representative images of NuLi-1 cells incubated under ALI for 5 weeks with:
(A) Bronchial media only
(B) 80:20 bronchial: fibroblast media
(C) Lifeline media
(D) Stemcell media
Overview of tight junction studies

Utilize EVOM ohmmeter to calculate transepithelial/transendothelial electrical resistance (TEER) of these models.

Assess FITC dextran (40 kDa) movement from apical side of trans-well insert into the basal side. This will determine cellular tight junction formation.

Verhoeckx K. The Impact of Food Bioactives on Health: in vitro and ex vivo models [Internet]. Cham (CH): Springer; 2015.
Other studies show high TEER variance

“Bronchial primary cells generally lead to TEER values of \(400-4,000 \, \Omega \text{Xcm}^2\).”


TEER data: media comparison

![Graph showing total resistivity (ohms x cm²) for different media and lots.

- 100% Bronchial Growth Media
- 80:20 Fibroblast:Bronchial Growth Media
- Lifeline Technology ALI Media
- Stemcell Technology ALI Media

Legend:
- Lot 1
- Lot 2
- Lot 3
- Lot 4
- NuLi-1 Control]
TEER data: full plate vs. interior wells only

![Graph showing percent error in resistivity measurements for different plate configurations and media lots.](image-url)
Histology images from scientific literature

Imaging is conducted using both alcian blue and hematoxylin eosin (H&E) staining on histological sections of airway models. This allows for the visual confirmation of the presence of basal, goblet, and ciliated cells.


Histology: media differences

Representative alcian-blue stained histological images of airway models comprised of primary HBECs incubated with (A) complete bronchial growth media, (B) 80:20 bronchial: fibroblast growth media, (C) Lifeline media, or (D) Stemcell media. Scale bars represent 20 µm.
Images of airway models comprised of hTERT-immortalized cell NuLI-1 controls cultured in either (A) Lifeline media or (B) Stemcell media. Scale bars represent 20 µm.
Objectives in this study

- Compare epithelial differentiation and model morphology in airway models.
- Airway models were generated from either ATCC’s or other supplier’s primary HBECs.
- Models were generated using optimized processes validated during the first phase of studies.

### Primary Cell Comparison

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Study Designation</th>
<th>Differentiation Media Used</th>
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</thead>
<tbody>
<tr>
<td>ATCC 1° Bronchial Tracheal Epithelial Cells (3 Lots)</td>
<td>ATCC Lot 1</td>
<td>Stemcell Media</td>
</tr>
<tr>
<td></td>
<td>ATCC Lot 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ATCC Lot 3</td>
<td>Lifeline Media</td>
</tr>
<tr>
<td>Supplier A 1° Bronchial Tracheal Epithelial Cells (2 Lots)</td>
<td>Supplier A (Lot 1)</td>
<td>Stemcell Media</td>
</tr>
<tr>
<td></td>
<td>Supplier A (Lot 2)</td>
<td>Supplier A Media</td>
</tr>
<tr>
<td>Supplier B 1° Bronchial Tracheal Epithelial Cells (2 Lots)</td>
<td>Supplier B (Lot 1)</td>
<td>Stemcell Media</td>
</tr>
<tr>
<td></td>
<td>Supplier B (Lot 2)</td>
<td>Supplier B Media</td>
</tr>
<tr>
<td>Supplier C 1° Bronchial Tracheal Epithelial Cells (1 Lot)</td>
<td>Supplier C (Lot 1)</td>
<td>Stemcell Media</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Supplier C Media</td>
</tr>
</tbody>
</table>
TEER data: ATCC and other suppliers

TEER values from airway models comprised of primary HBECs from either ATCC or other commercial vendors incubated in either (A) Stemcell Technologies or (B) respective supplier ALI differentiation media.
Dextran testing: ATCC and other suppliers

- ATCC (Lot 1)
- ATCC (Lot 2)
- ATCC (Lot 3)
- Supplier A (Lot 2)

Relative Fluorescence in Millions (490, 520 nm)

- Stemcell Media
- Supplier Media
MUC5AC testing: ATCC and other suppliers

![Graph showing MUC5AC testing results for ATCC and other suppliers.](image-url)
Representative alcian-blue stained histological images of airway models generated from ATCC primary cell lots 1-3 in either (A-C) Stemcell media or (D-F) Lifeline media. Scale bar represents 20 µm.
Representative alcian-blue stained histological images of airway models from (A) Supplier A (Lot 1), (B) Supplier A (Lot 2), (C) Supplier B (Lot 1), (D) Supplier B (Lot 2), or (E) Supplier C (Lot 1). Scale bars represent 20 µm.
Summary of results

- Airway models comprising ATCC or commercial vendor cell lines were successfully generated.
- Differences between primary cell lots were present; media choice played a much larger role in model maturation.
- Both commercial differentiation medias provided the best levels of epithelial differentiation.
- Variability was minimized using partial plates with DPBS in the outer wells.
- Despite the presence of goblet cells using Stemcell Technologies ALI maintenance media, hTERT NuLi-1 cells lines are not an appropriate substitute for primary HBECs in airway model fabrication.
- These results demonstrate that ATCC primary HBECs are an effective tool to generate airway models with appropriate epithelial differentiation, model morphology, and mature functionality.
Thank You

Questions?
Coming soon!

Luciferase Reporter Cancer Cell Lines: Facilitate Your CAR-T Development  
Presenter: John Foulke, MS  
October 13, 12:00 ET  

Does Differentiation Matter? Comparing the Toxicological Response Between Airway Epithelial Models  
Presenter: Kevin Tyo, PhD  
November 3, 12:00 ET