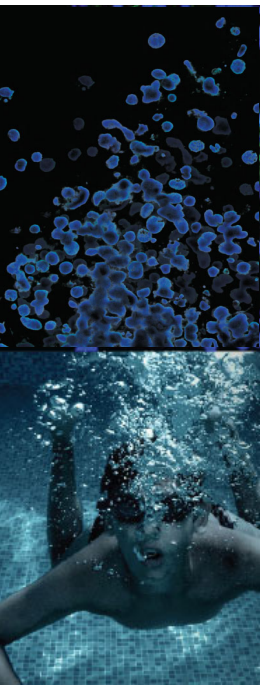
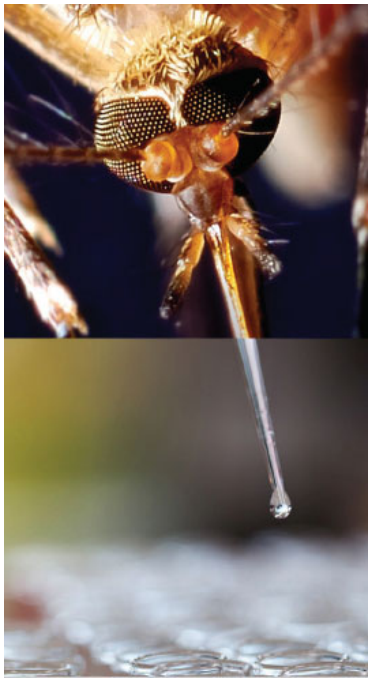
Evaluating the Differentiation Potential of Primary Airway Cells in 3-D Models

Kevin Tyo, PhD
Scientist, ATCC





Credible Leads to Incredible™

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

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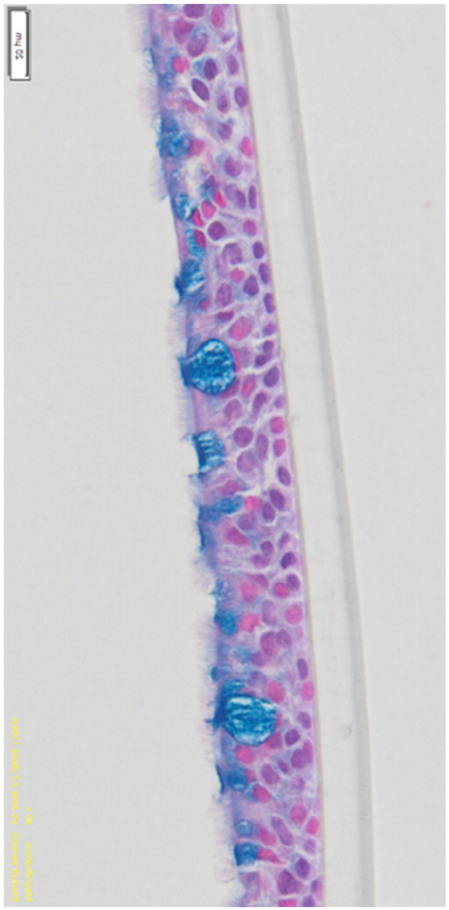
Overview

- 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



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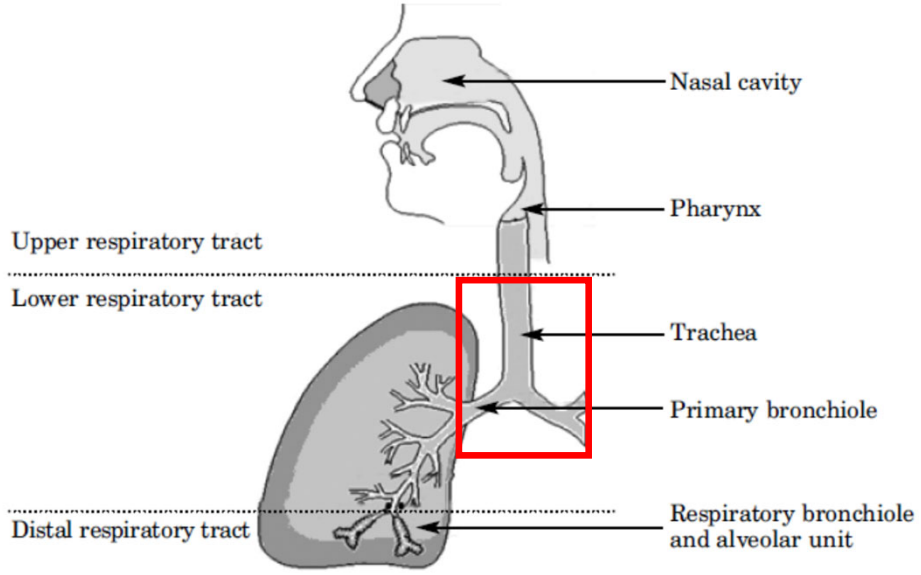
Human respiratory tract

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.



BéruBé, K. et al. In Vitro Models of Inhalation Toxicity and Disease. ATLA 37, 89–141, 2009

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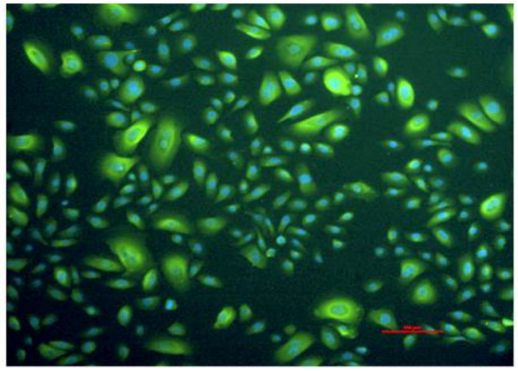
ATCC products for airway models

- 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

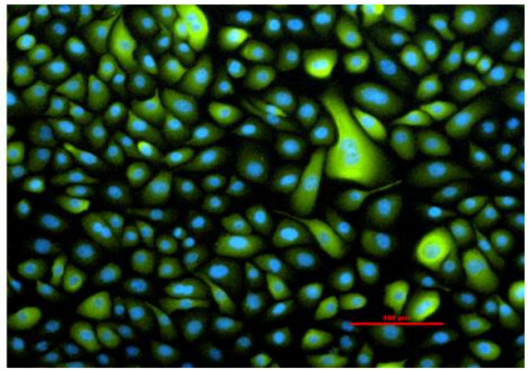
- hTERT-immortalized primary cells
  - HBEC-3KT (Bronchial epithelial cells)
  - NuLi-1 (Bronchial epithelial cells)
  - HSAEC1-KT (Small airway epithelial cells)
  - HTERT Lung Fibroblast

CCSP + DAPI

Primary Bronchial/Tracheal Epithelial Cells



HSAEC1-KT



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.

Media Differentiation Test	
Primary Lot #	**Differentiation Media**
1	100% Bronchial Growth 80:20: Bronchial:Fibroblast Growth Lifeline Cell Technology Media Stemcell Technologies Media
2	
3	
4	
NuLi-1 Control	

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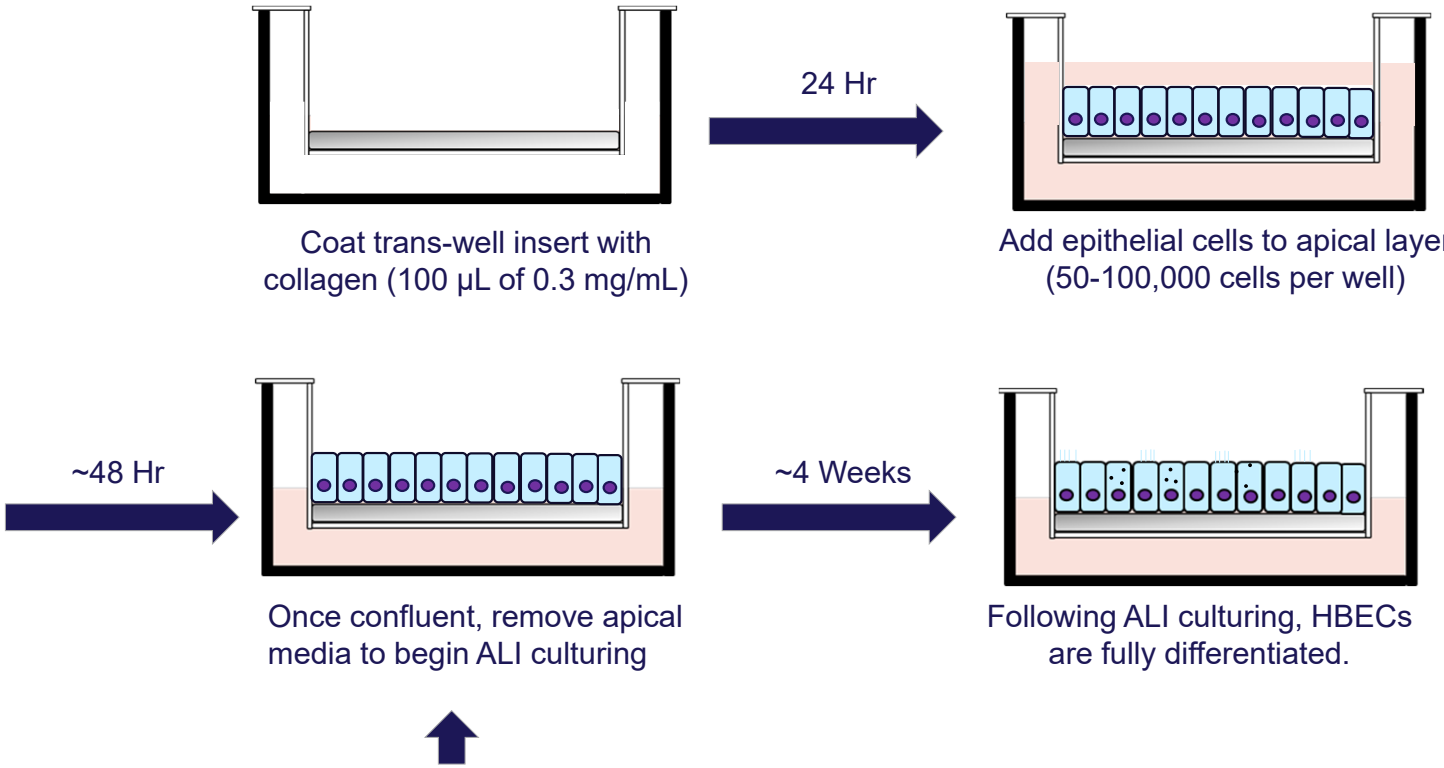
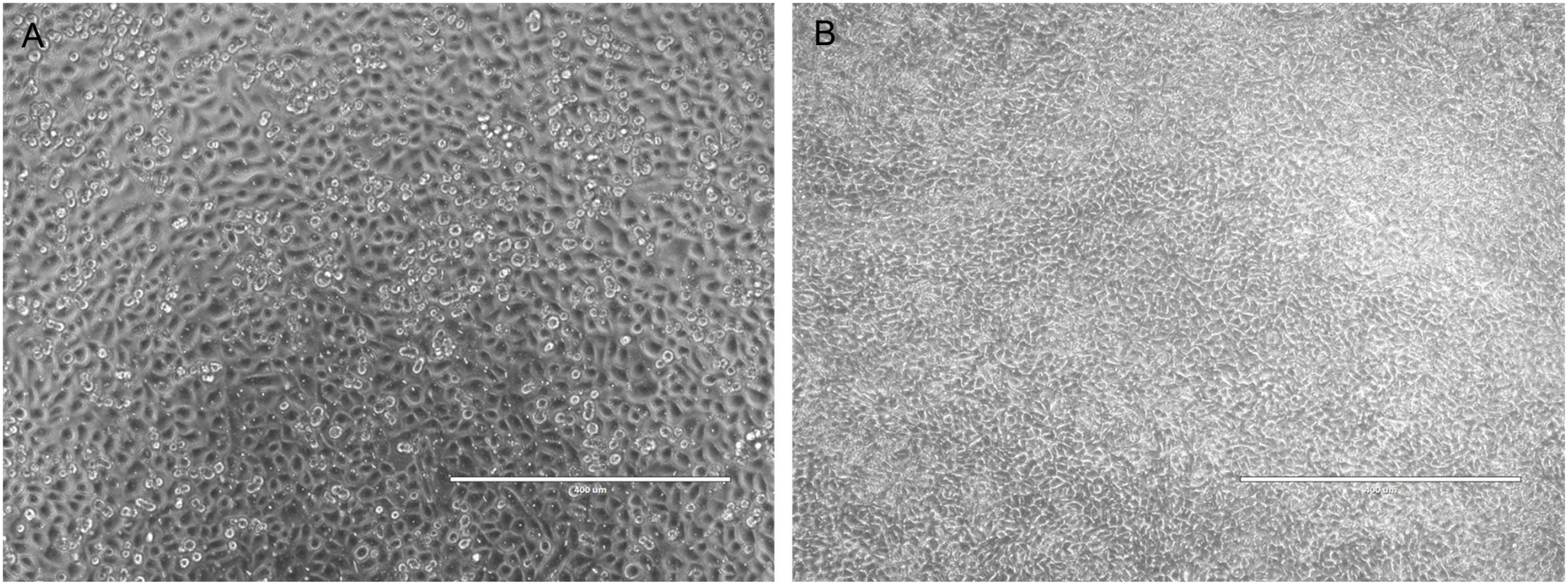
Overview of current model fabrication method

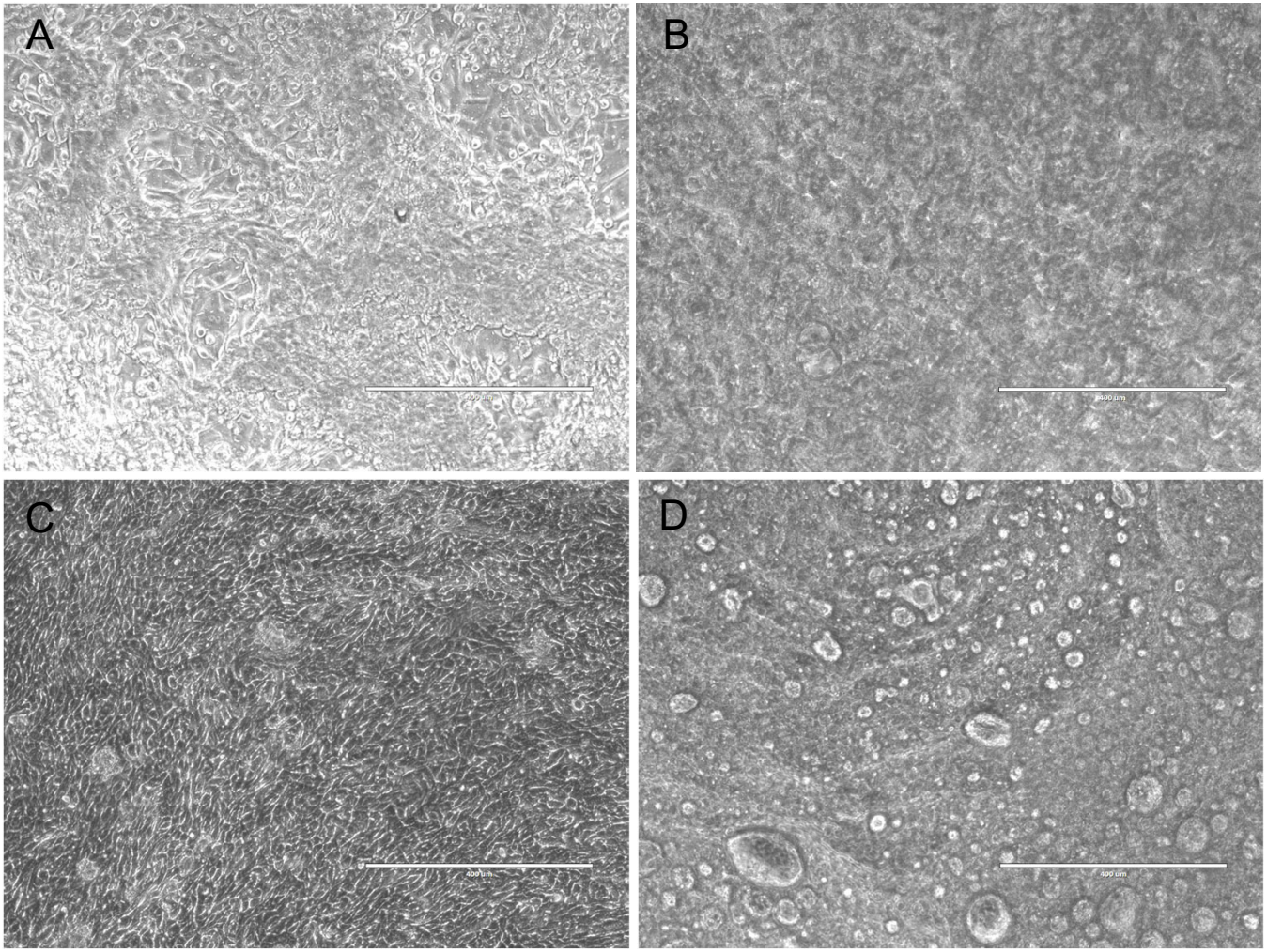


Microscopy images: week 0 and 1



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

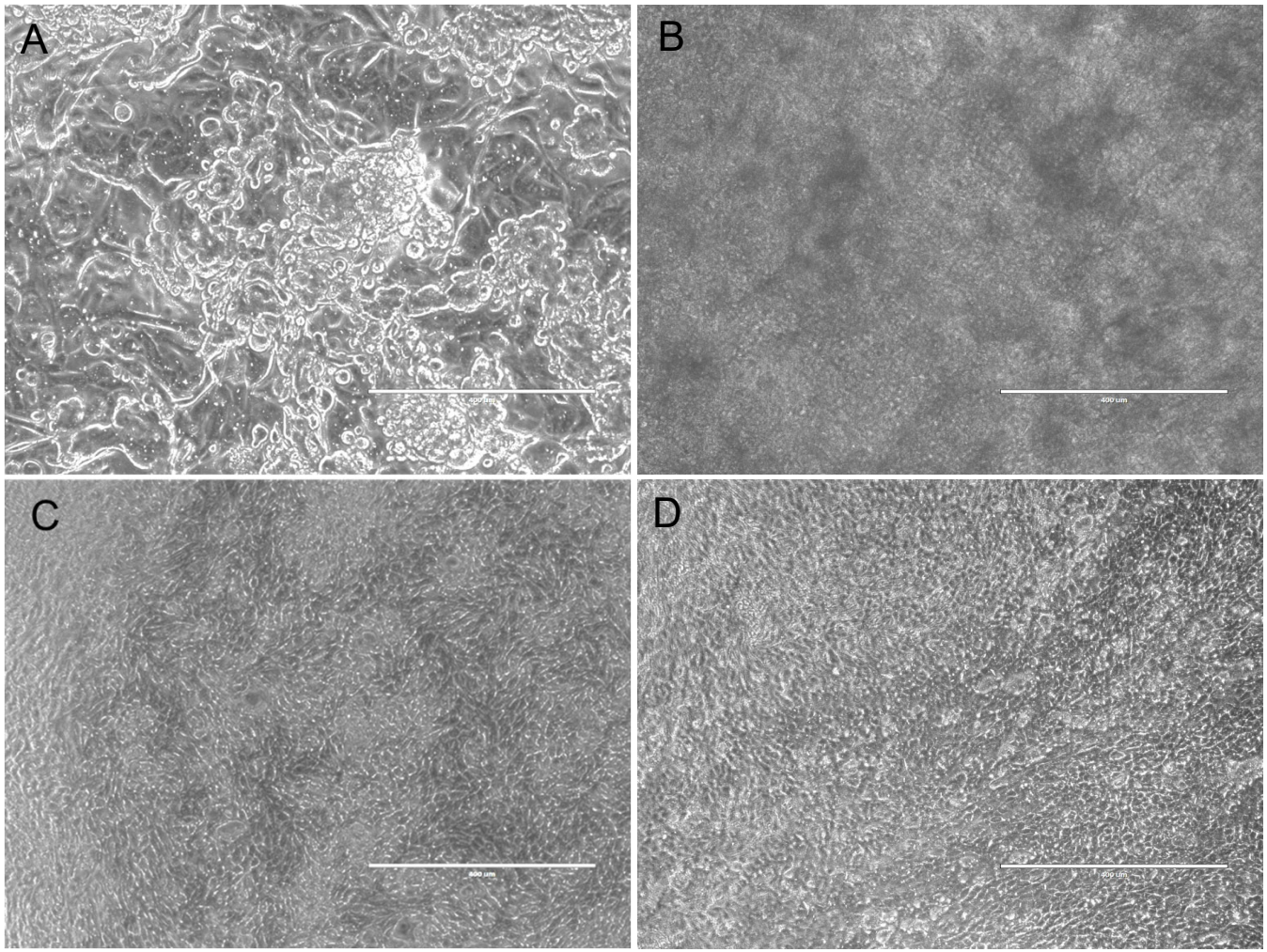
Microscopy images: week 2



Representative images of HBECs incubated under ALI for 2 weeks with:

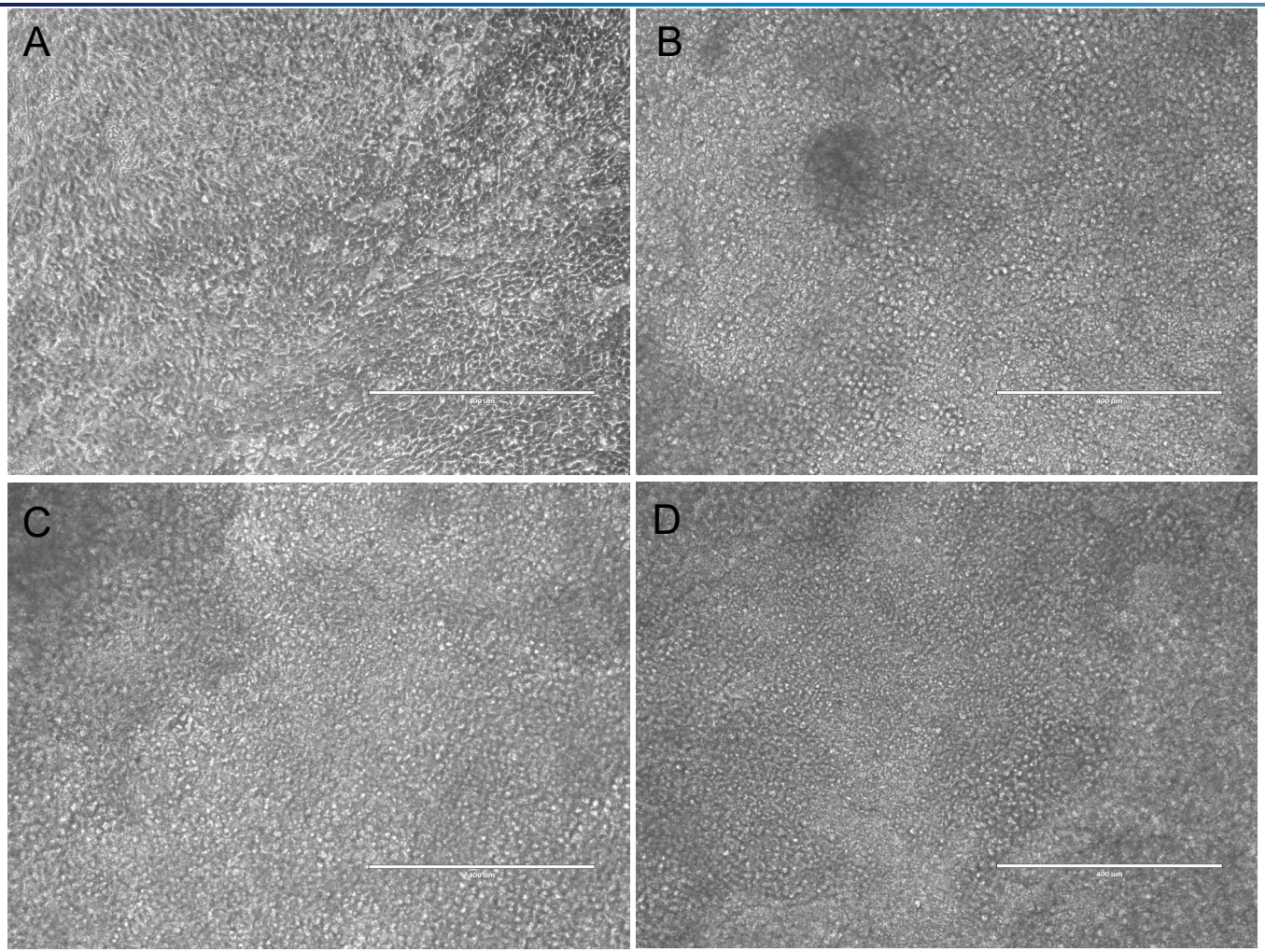
(A) Bronchial media only
(B) 80:20 bronchial: fibroblast media
(C) Lifeline media
(D) Stemcell media

Microscopy images: week 5



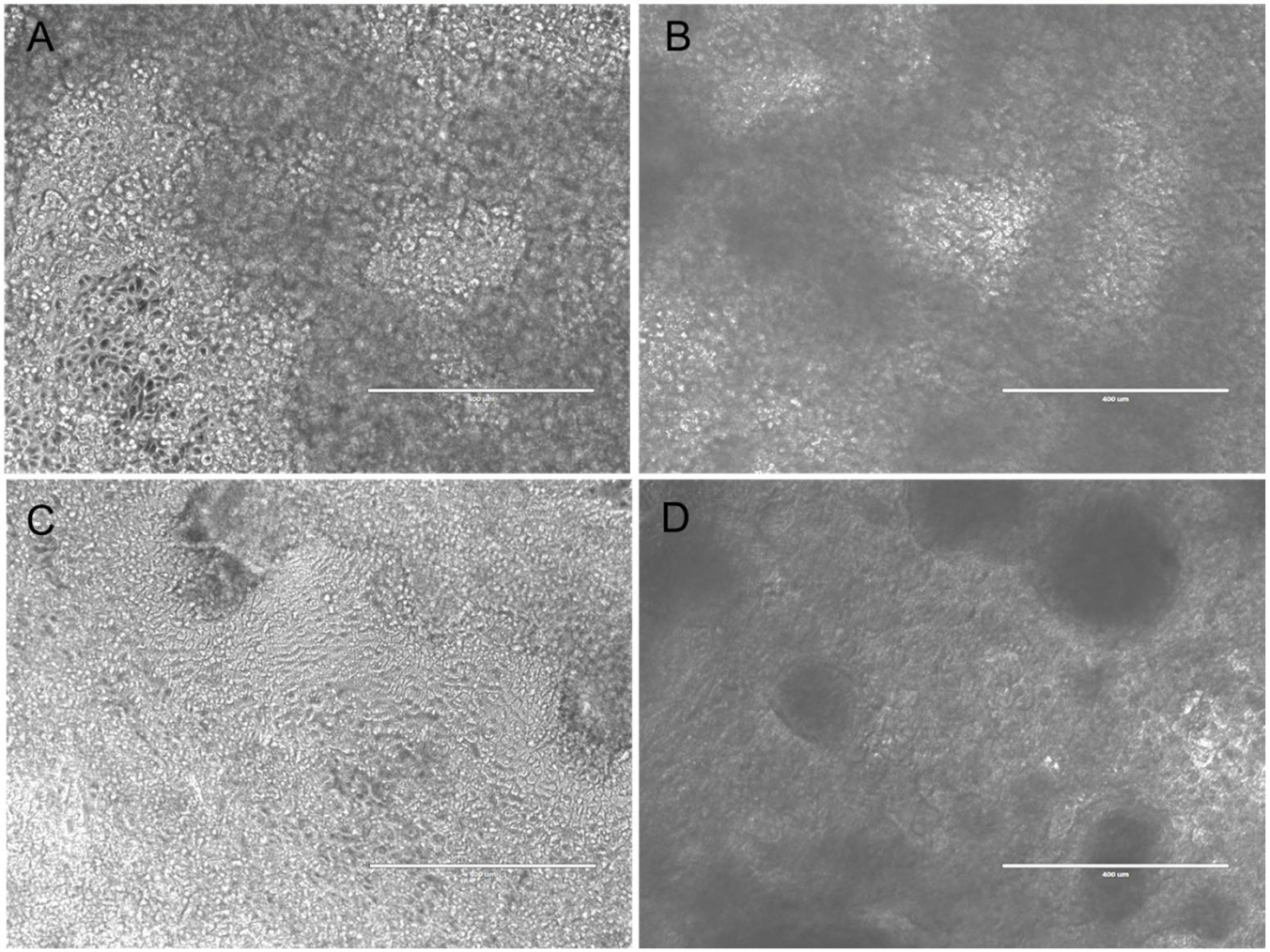
Representative images of HBECs incubated under ALI for 5 weeks with:
(A) Bronchial media only
(B) 80:20 bronchial: fibroblast media
(C) Lifeline media
(D) Stemcell media

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

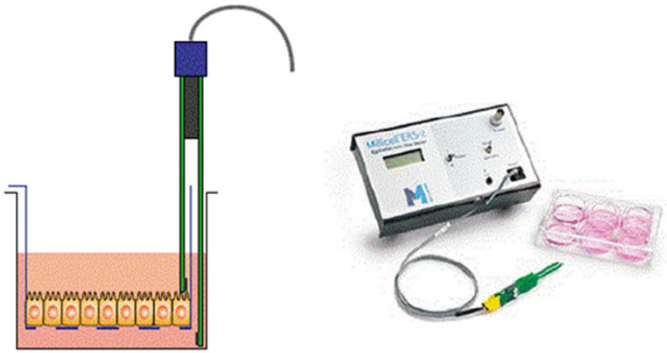


Representative images of NuLi-1 cells incubated under ALI for 5 weeks with:

(A) Bronchial media only
(B) 80:20 bronchial: fibroblast media
(C) Lifeline media
(D) Stemcell media

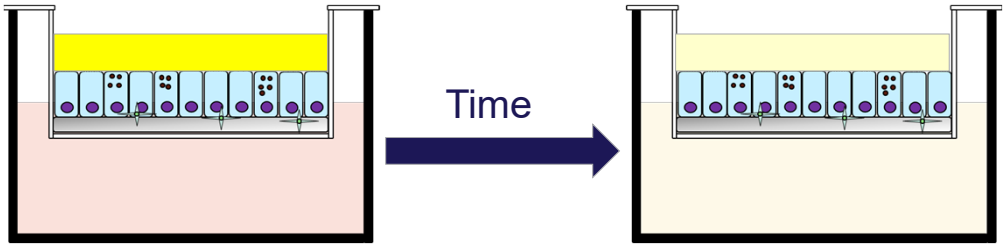
Overview of tight junction studies

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



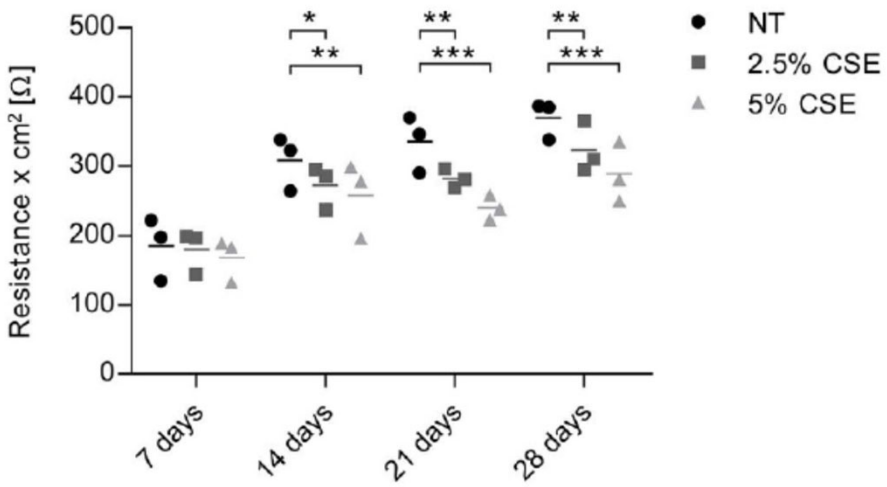
Verhoeckx K. The Impact of Food Bioactives on Health: in vitro and ex vivo models [Internet]. Cham (CH): Springer; 2015.

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

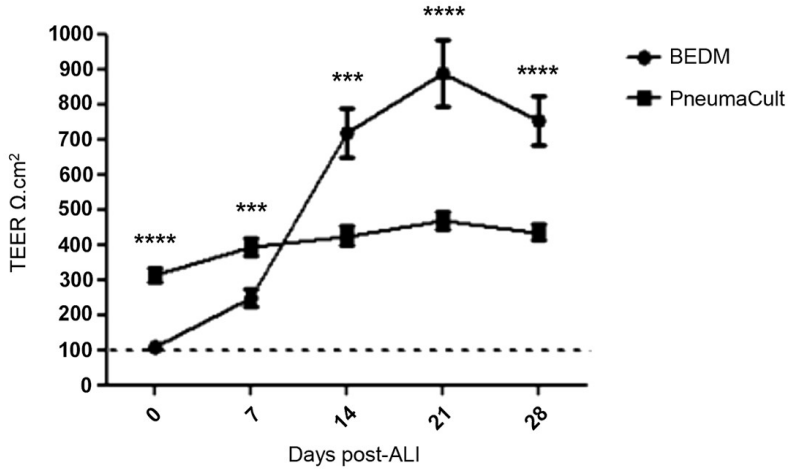


Other studies show high TEER variance

"Bronchial primary cells generally lead to TEER values of 400–4,000 ΩXcm^2."
Papazian D, Würtzen P, A, Hansen S, W, K: Polarized Airway Epithelial Models for Immunological Co-Culture Studies. Int Arch Allergy Immunol 2016;170:1-21. doi: 10.1159/000445833

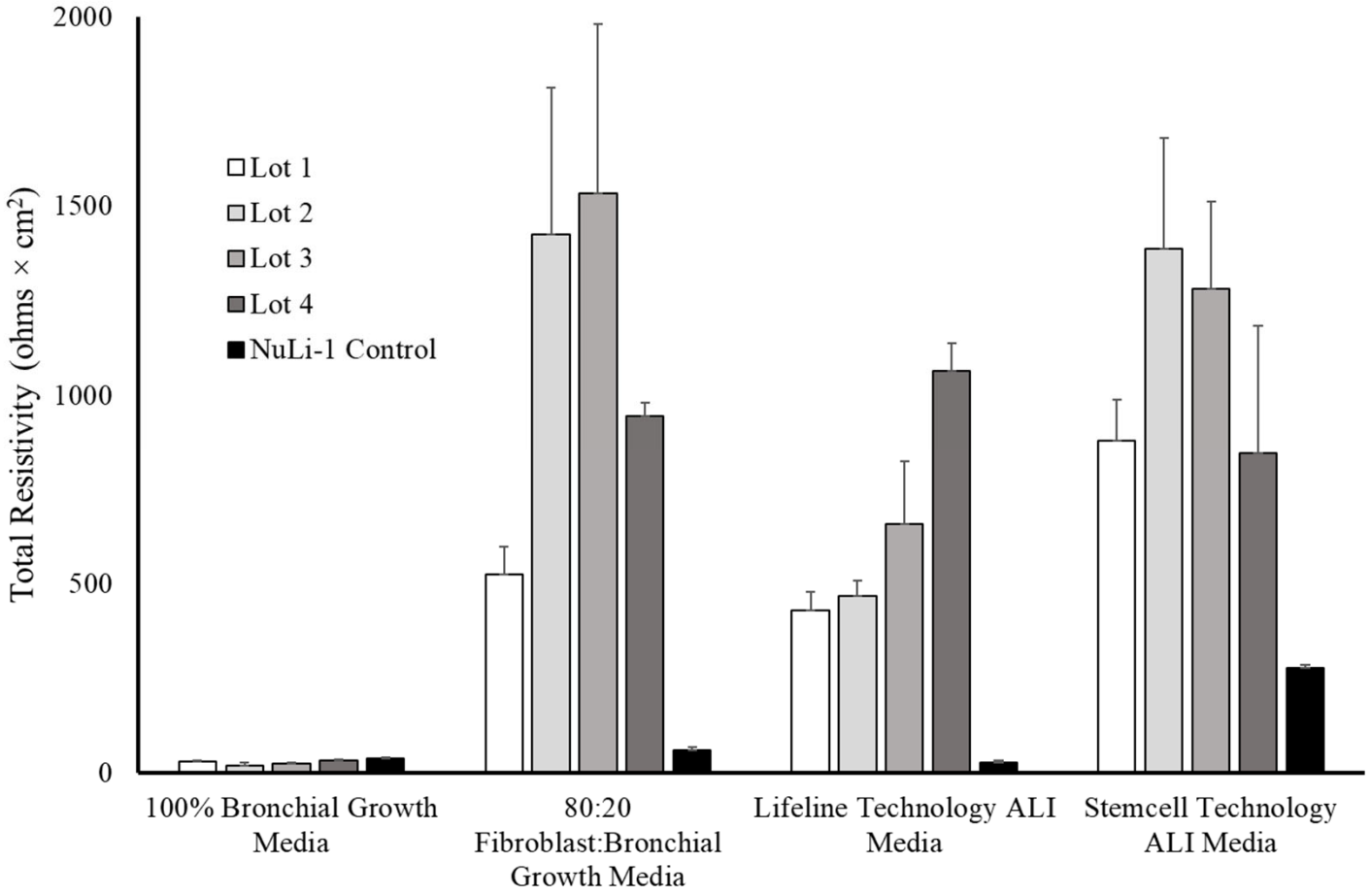


Schamberger AC. Cigarette smoke alters primary human bronchial epithelial cell differentiation at the air-liquid interface. Sci Rep. 2015;5 8163.

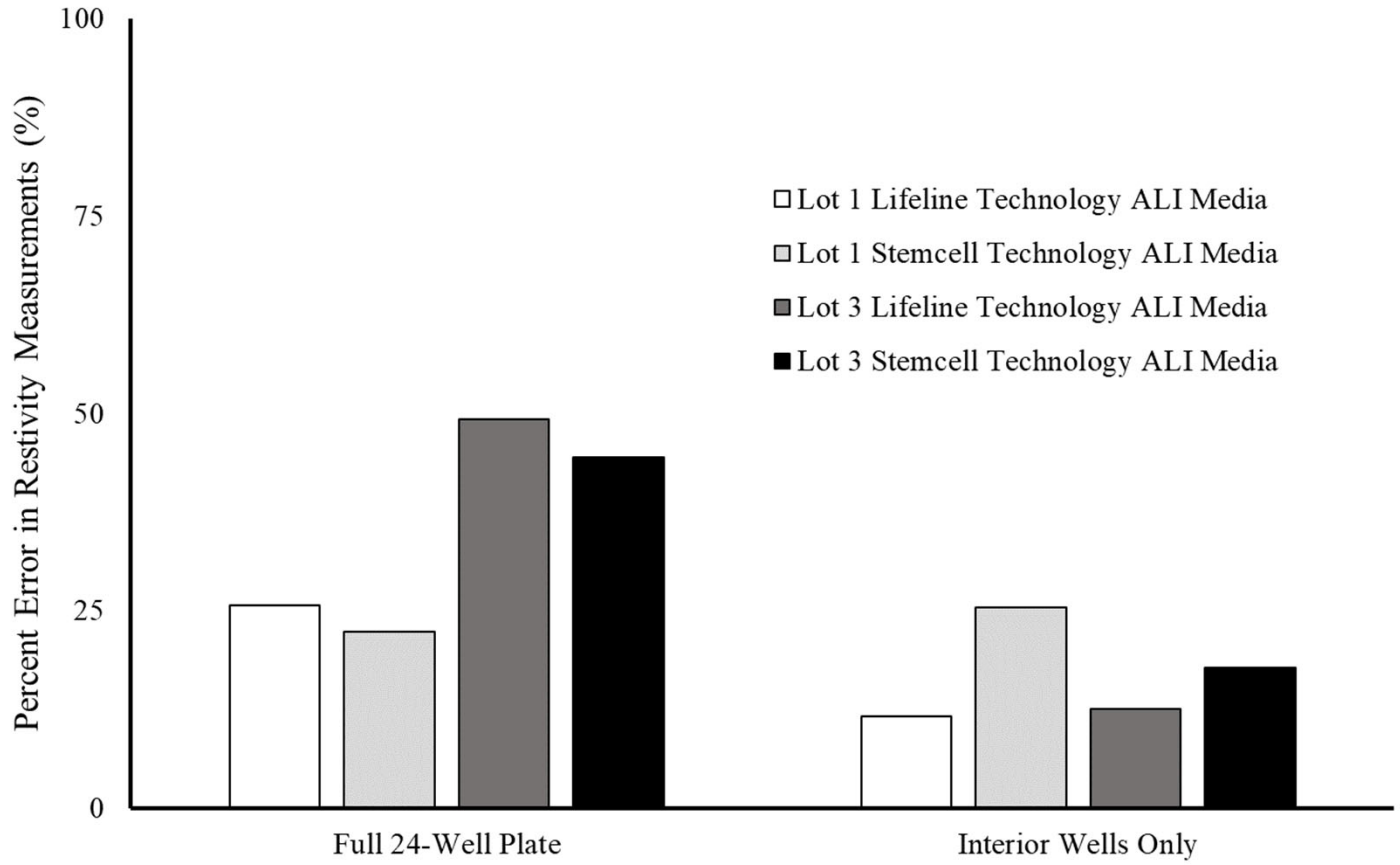


Clarus Leung. Structural and functional variations in human bronchial epithelial cells cultured in air-liquid interface using different growth media. American Journal of Physiology-Lung Cellular and Molecular Physiology 2020 318:5

TEER data: media comparison

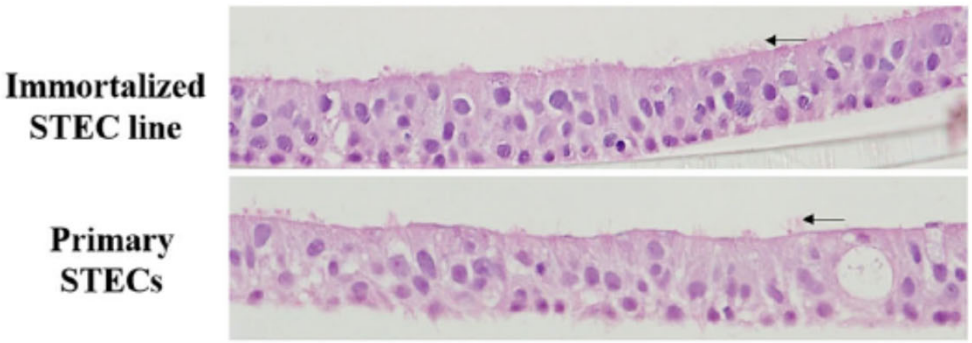


TEER data: full plate vs. interior wells only

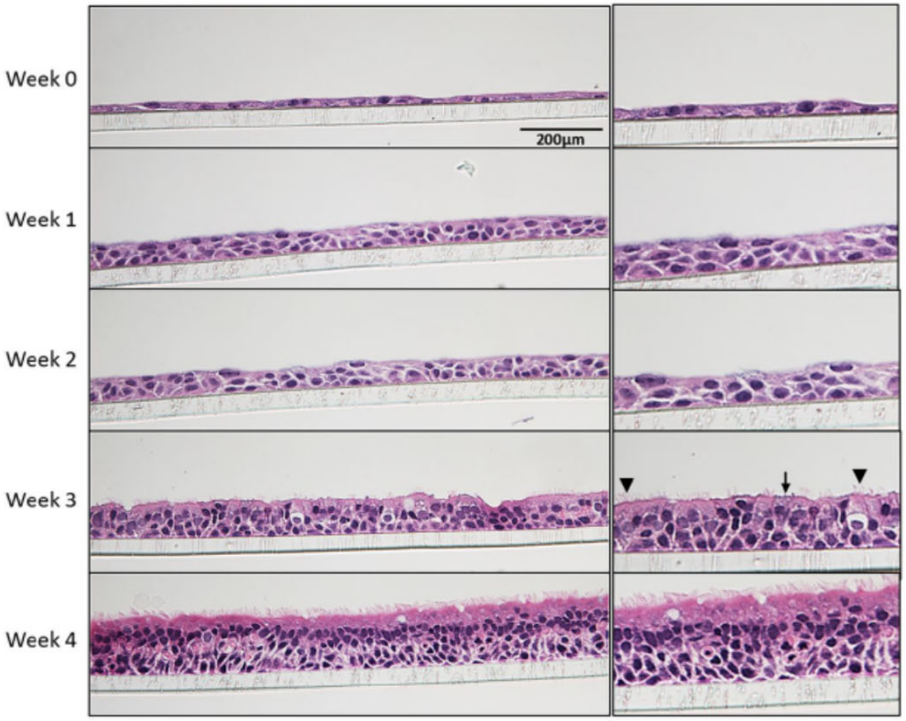


Histology images from scientific literature

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.



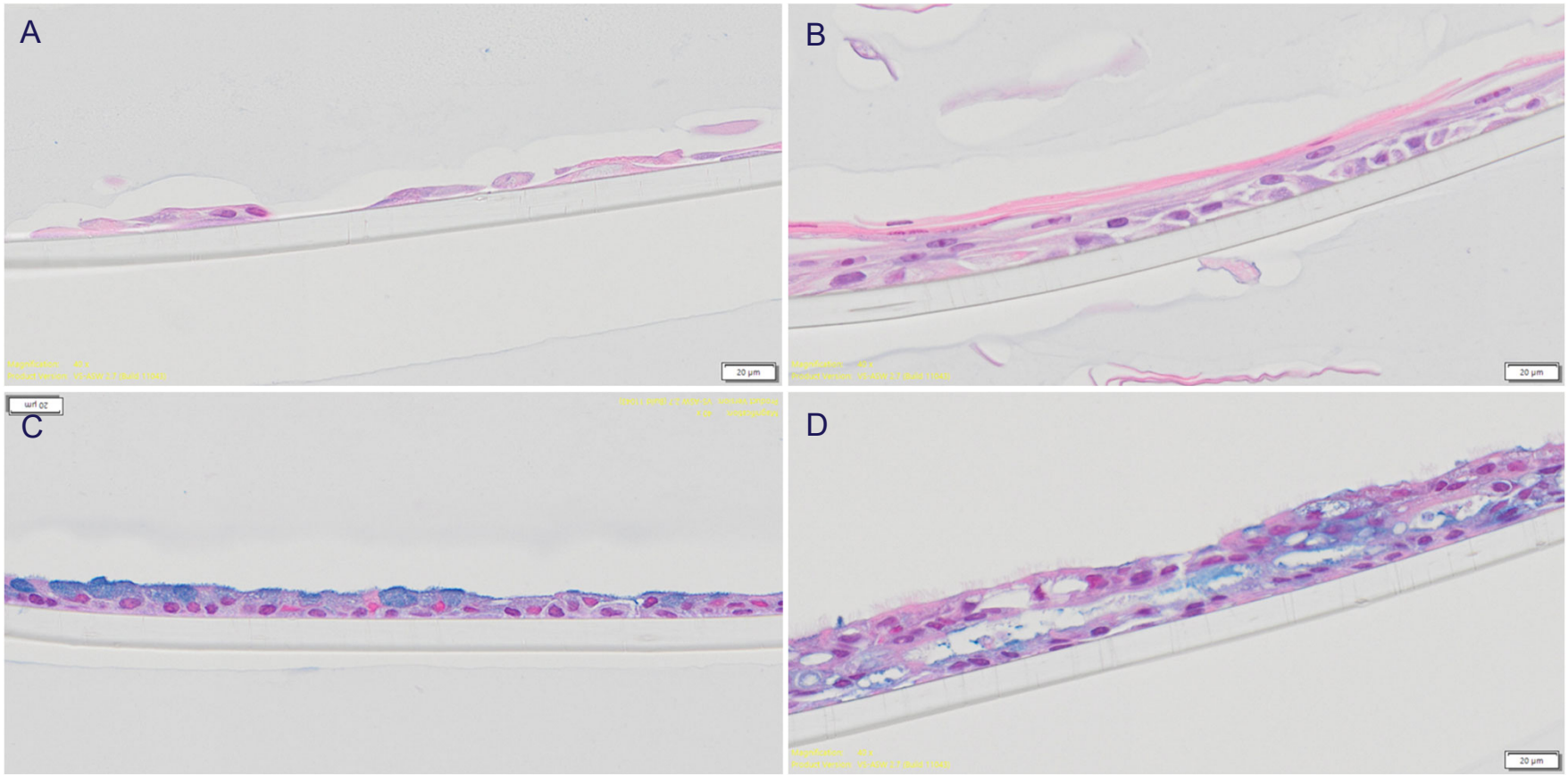
Wang H. Establishment and comparison of air-liquid interface culture systems for primary and immortalized swine tracheal epithelial cells. BMC Cell Biol. 2018 Jun 28;19(1):10.



Rayner RE. Optimization of Normal Human Bronchial Epithelial (NHBE) Cell 3D Cultures for in vitro Lung Model Studies. Sci Rep. 2019 Jan 24;9(1):500.

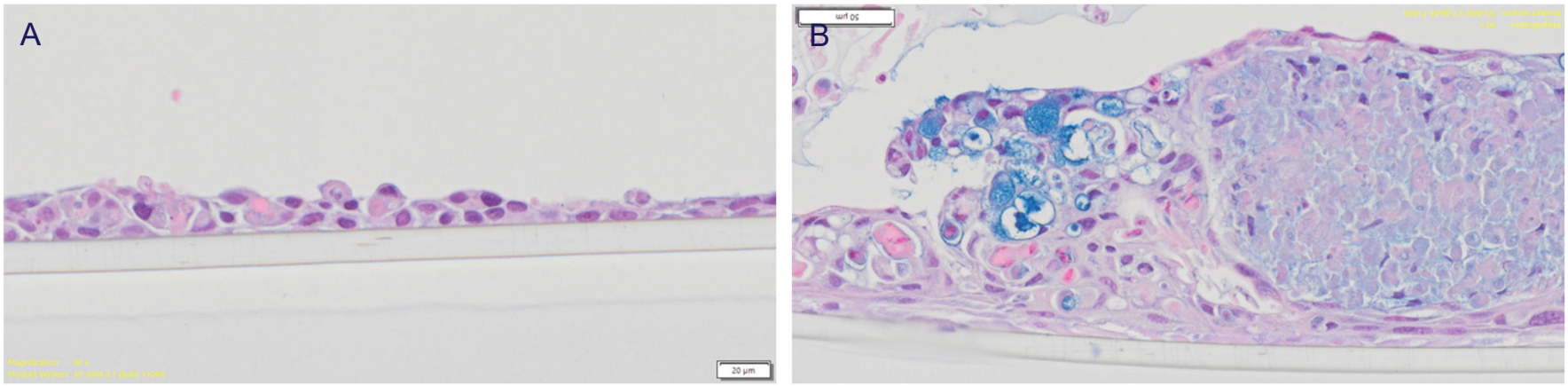
ATCC

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.

Histology: hTERT Controls



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.

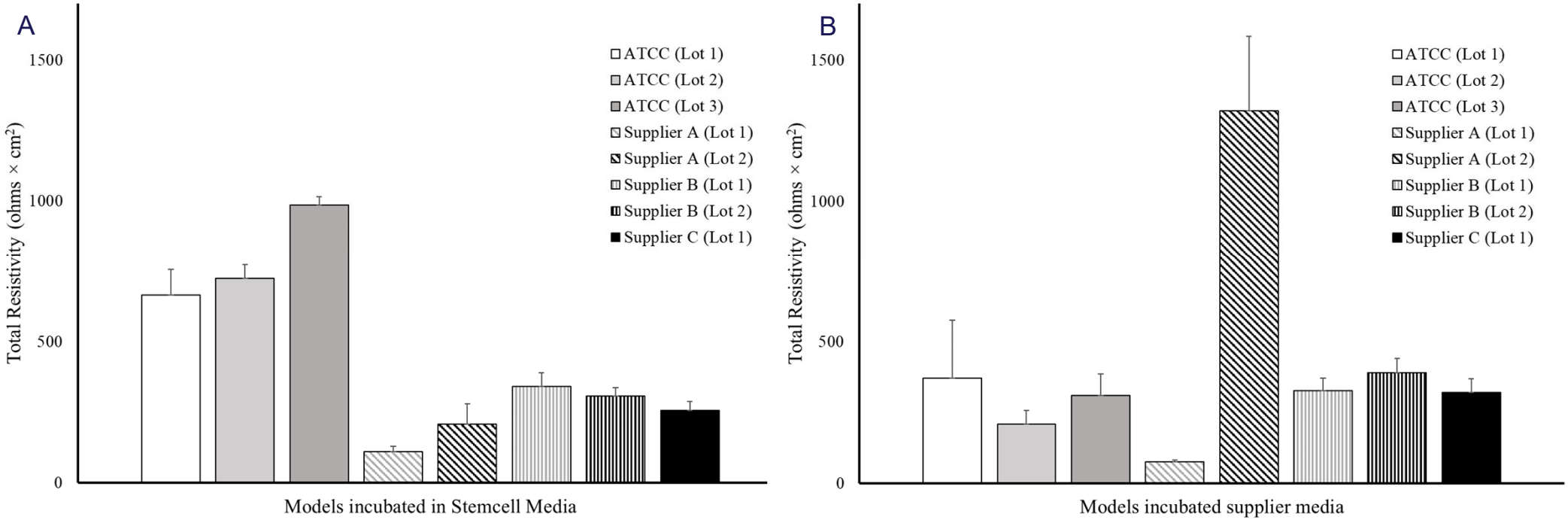
Overview of 2nd study, comparing primary cells

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.

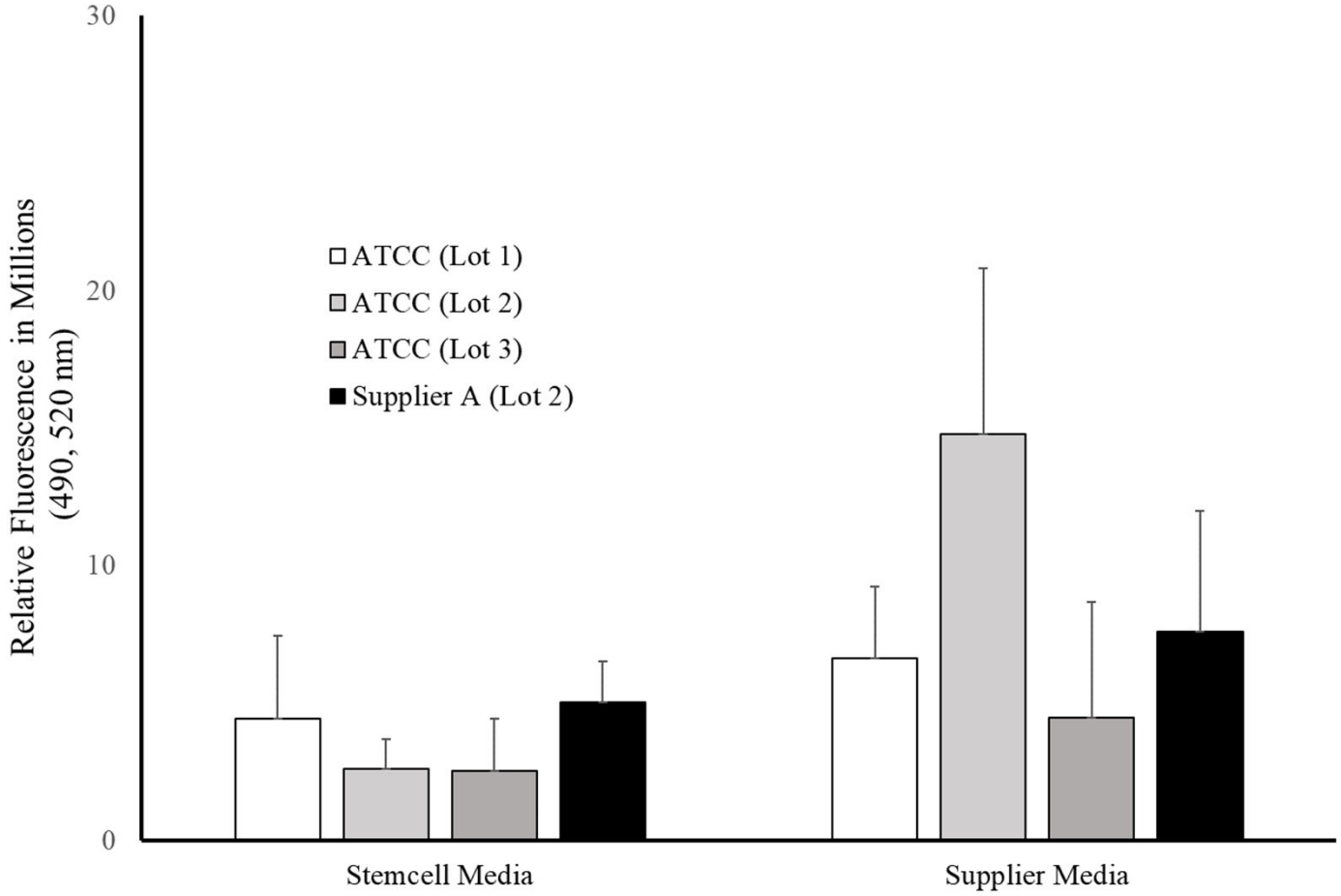
Primary Cell Comparison		
Cell Type	**Study Designation**	**Differentiation Media Used**
ATCC 1˚ Bronchial Tracheal Epithelial Cells (3 Lots)	ATCC Lot 1	Stemcell Media
	ATCC Lot 2	
	ATCC Lot 3	Lifeline Media
Supplier A 1˚ Bronchial Tracheal Epithelial Cells (2 Lots)	Supplier A (Lot 1)	Stemcell Media
	Supplier A (Lot 2)	Supplier A Media
Supplier B 1˚ Bronchial Tracheal Epithelial Cells (2 Lots)	Supplier B (Lot 1)	Stemcell Media
	Supplier B (Lot 2)	Supplier B Media
Supplier C 1˚ Bronchial Tracheal Epithelial Cells (1 Lot)	Supplier C (Lot 1)	Stemcell Media
		Supplier C Media

TEER data: ATCC and other suppliers

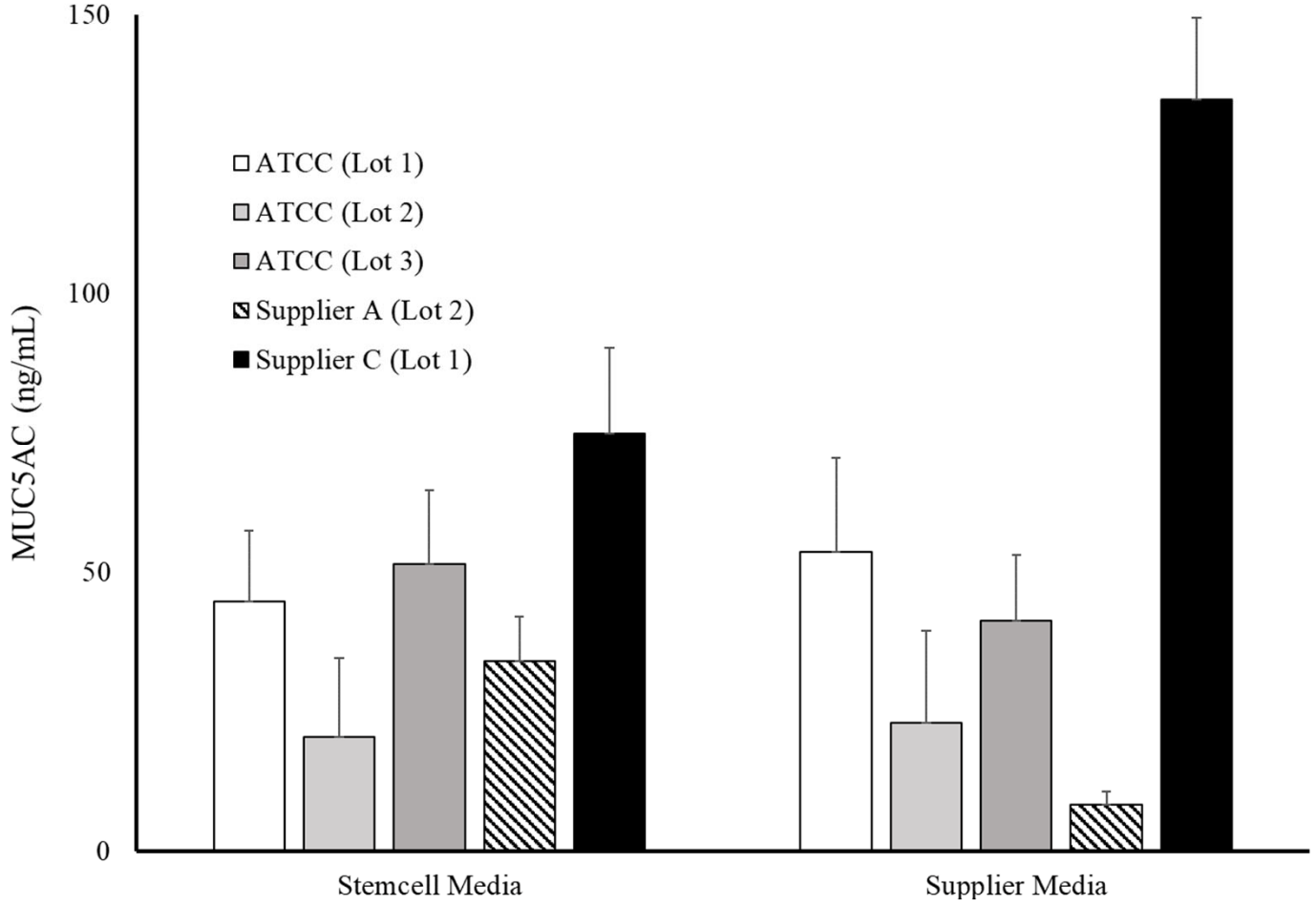


TEER values from airway models comprised of primary HBECs from either ATCC or other commercial vendors incubated in either (A) Stemcell Technologies or (B) respective supplier ALI differentiation media.

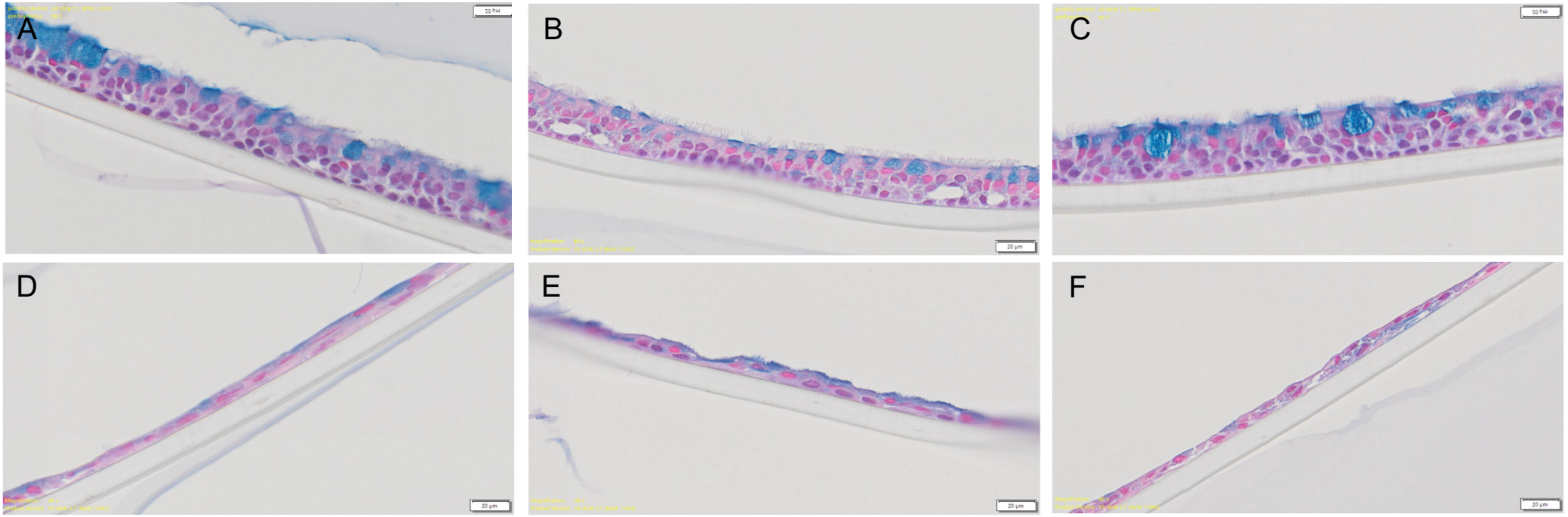
Dextran testing: ATCC and other suppliers



MUC5AC testing: ATCC and other suppliers

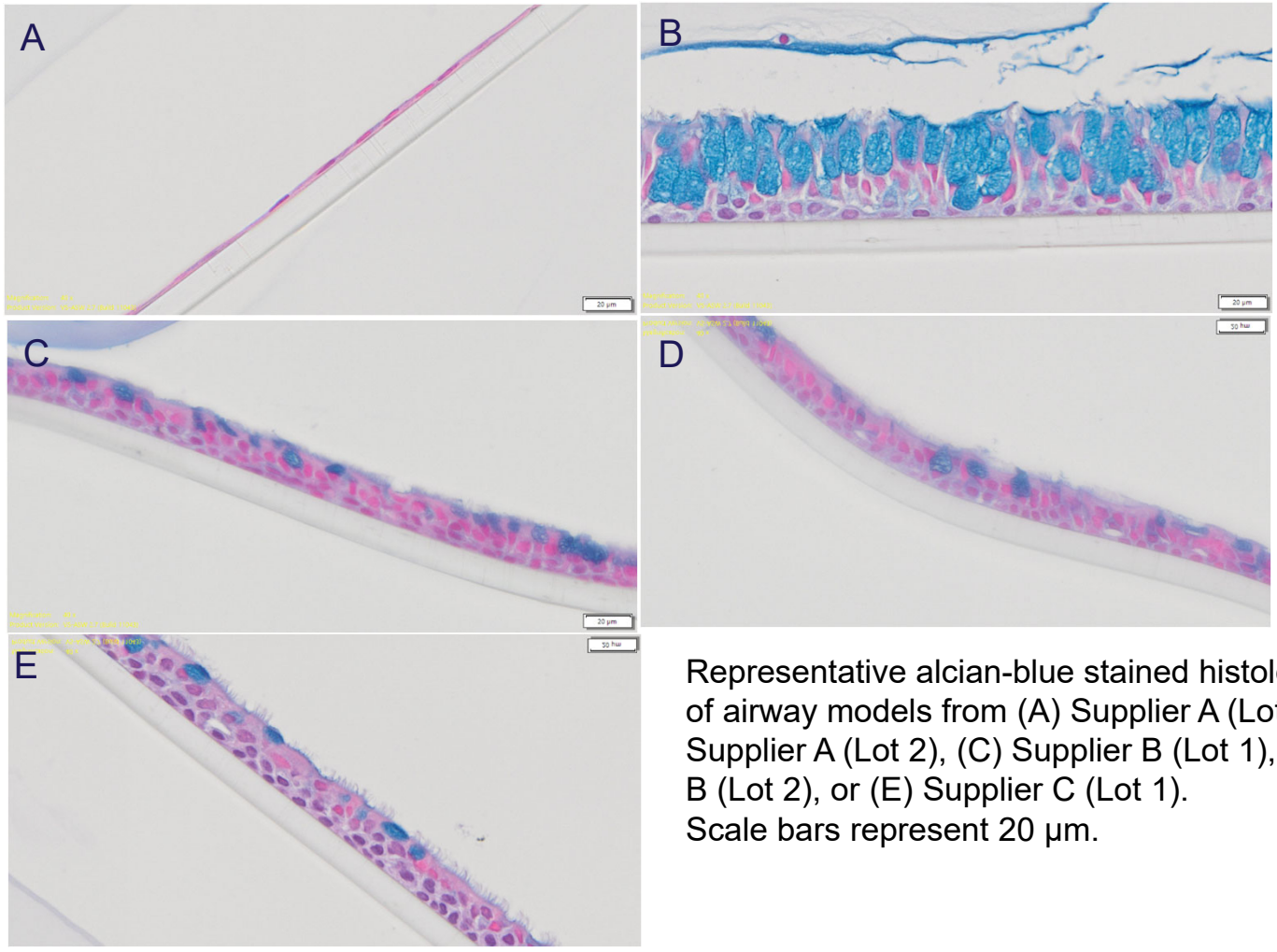


Histology: ATCC cell lots



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.

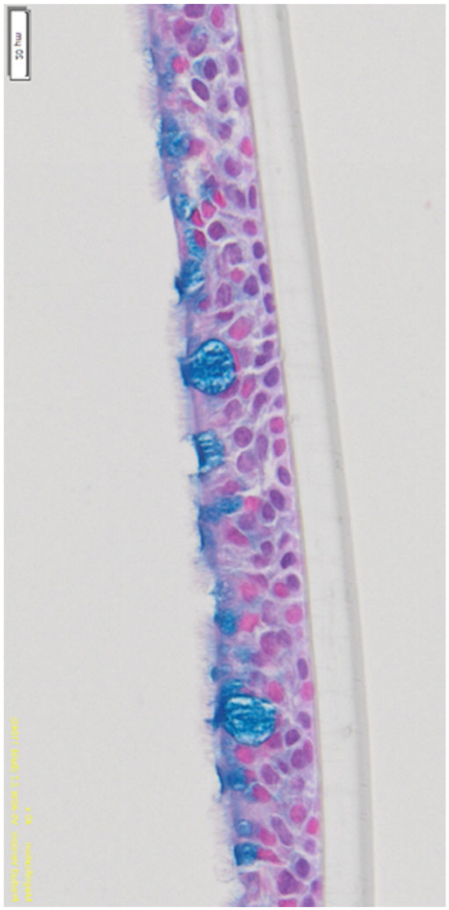
Histology: other supplier lots

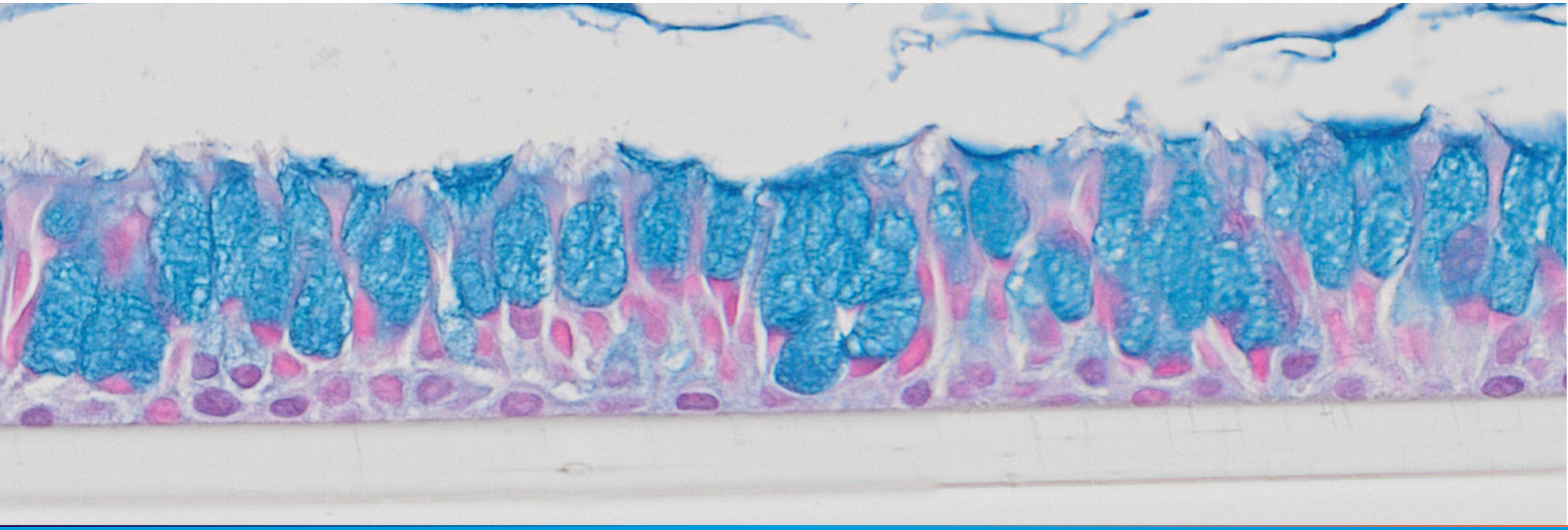


Representative alcian-blue stained histological images of airway models from (A) Supplier A (Lot 1), (B) Supplier A (Lot 2), (C) Supplier B (Lot 1), (D) Supplier B (Lot 2), or (E) Supplier C (Lot 1).
Scale bars represent 20 µm.

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?

Learn more: **www.atcc.org/tox**

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