Does Differentiation Matter? Comparing the Toxicological Response Between Airway Epithelial Models

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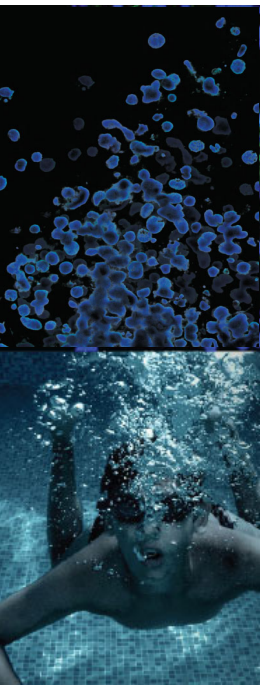
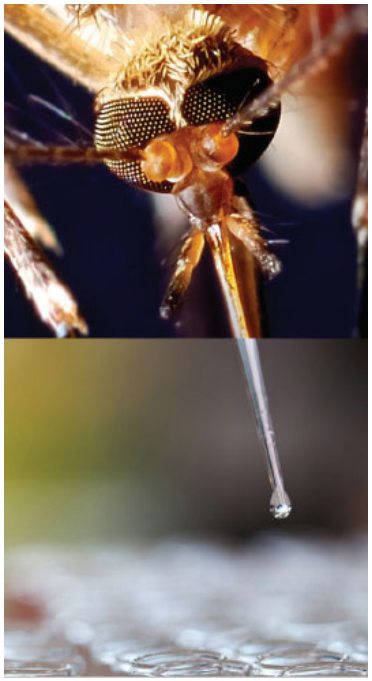
Scientist, ATCC

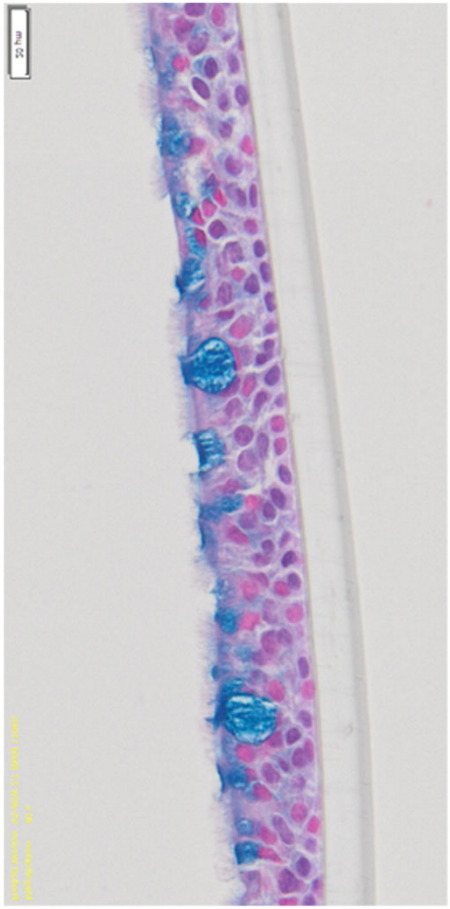




Credible Leads to Incredible™

Talk: agenda

- Background: provide rationale on the usage of advanced 3D airway models.
- Overview of studies: discuss experiment setup and review of the 3D airway model fabrication process.
- Short-term testing: evaluate model response to 24-hour exposure of selected toxins.
- Long-term testing: compare 1- and 2-week response of models to selected toxins exposure.



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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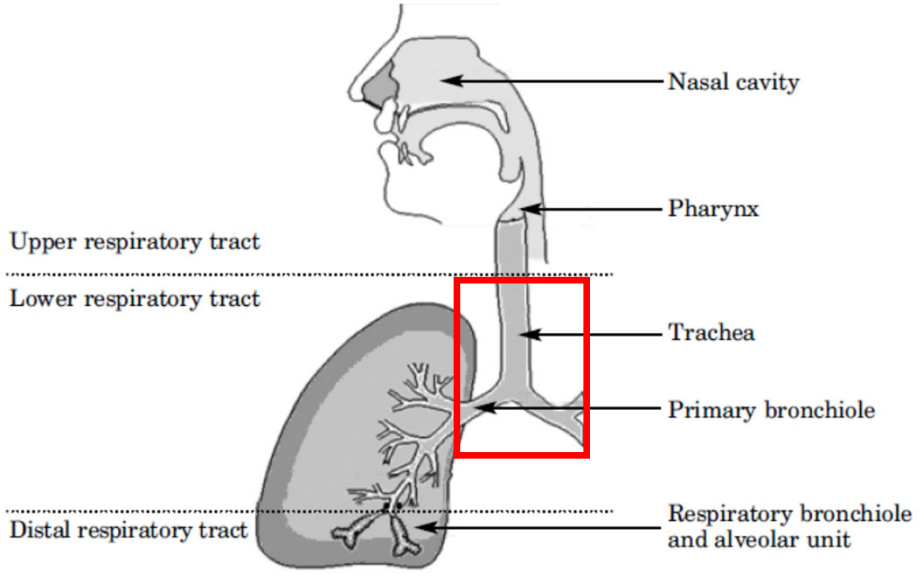
Background: human respiratory tract

Overview of the human airway

- Comprised of 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, whereas small airways also include club cells. In contrast, the 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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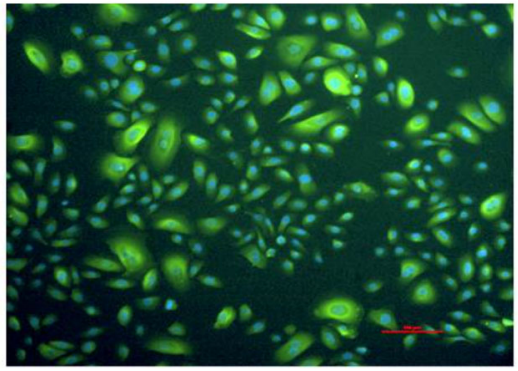
Background: ATCC products for airway models

- Primary cells
  - Bronchial/tracheal epithelial cells
  - 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

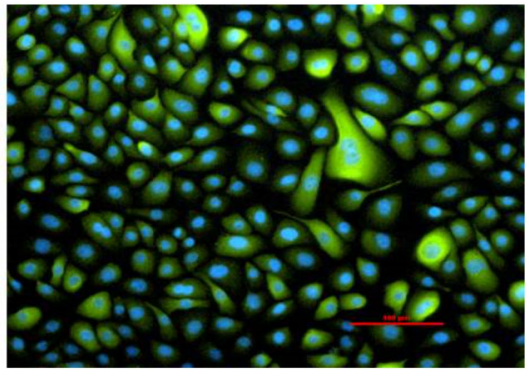
- hTERT-immortalized primary cells
  - HBEC-3KT (Bronchial epithelial cells)
  - NuLi-1 (Bronchial epithelial cells)
  - HSAEC1-KT (Small airway epithelial cells)
  - HTERT Lung Fibroblast

CCSP + DAPI

Primary Bronchial/Tracheal Epithelial Cells

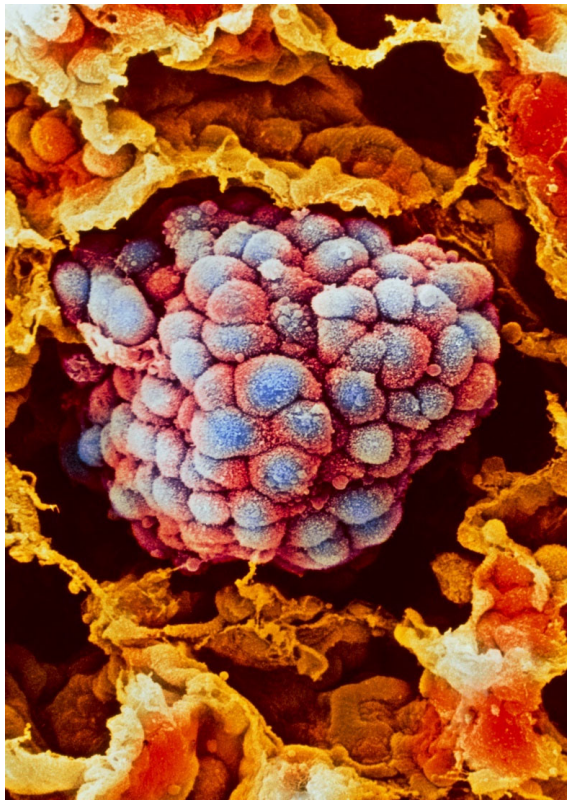


HSAEC1-KT



Background: airway models

- Respiratory infections represent the most common form of infection and act as a significant focus in disease research.
- Inhalation toxicity is the most prominent route of toxicity exposure, causing an estimated 7 million early deaths worldwide each year.
- Inhalation serves as a viable of route of therapeutic administration, bypassing the first pass metabolism.
- Utilizing relevant models able to recapitulate the human respiratory tract for these diverse scientific fields is of critical importance for global health.



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Background: selected toxins

- Cadmium is highly ranked on the Disease Registry substance priority list as well as categorized as a substance of very high concern by the European Chemical Agency due to its toxicity and risk of inhalation exposure.

- Pentamidine is a potent antimicrobial agent that can be administrated via inhalation and is listed in the World Health Organization list of essential medicines. However adverse side effects from its use are commonly reported.

- Both compounds were chosen to serve as representative substances in the fields of environmental/industrial monitoring and pharmaceutical safety.

Image taken from https://www.cdc.gov/niosh/topics/cadmium

Overview of toxicology studies

Objectives of these studies

- Here, we compared the response of undifferentiated and differentiated bronchial airway models to short term (24 hours) exposure to either to cadmium chloride ($CdCl_2$) or pentamidine.
- Moreover, we assessed long-term exposure (1 and 2 weeks) of mature airway models to these compounds as well.

Experimental parameters.

- Two different ATCC primary cell lots were tested.
- Undifferentiated cell models were comprised of freshly seeded cells grown in 96-well plates for 24 hours.
- Airway models were fabricated based on the optimal method devised in our previous study, with bronchial epithelial cells incubating 24-well plates under ALI and Stemcell Technology's differentiation media for 4 weeks.
- Tests including assessing changes in viability and inflammation using either apical or basal media extracts from samples.
- During viability measurements, samples are lysed to assess total ATP values, separate sample sets were used to generate histological microscopy images.

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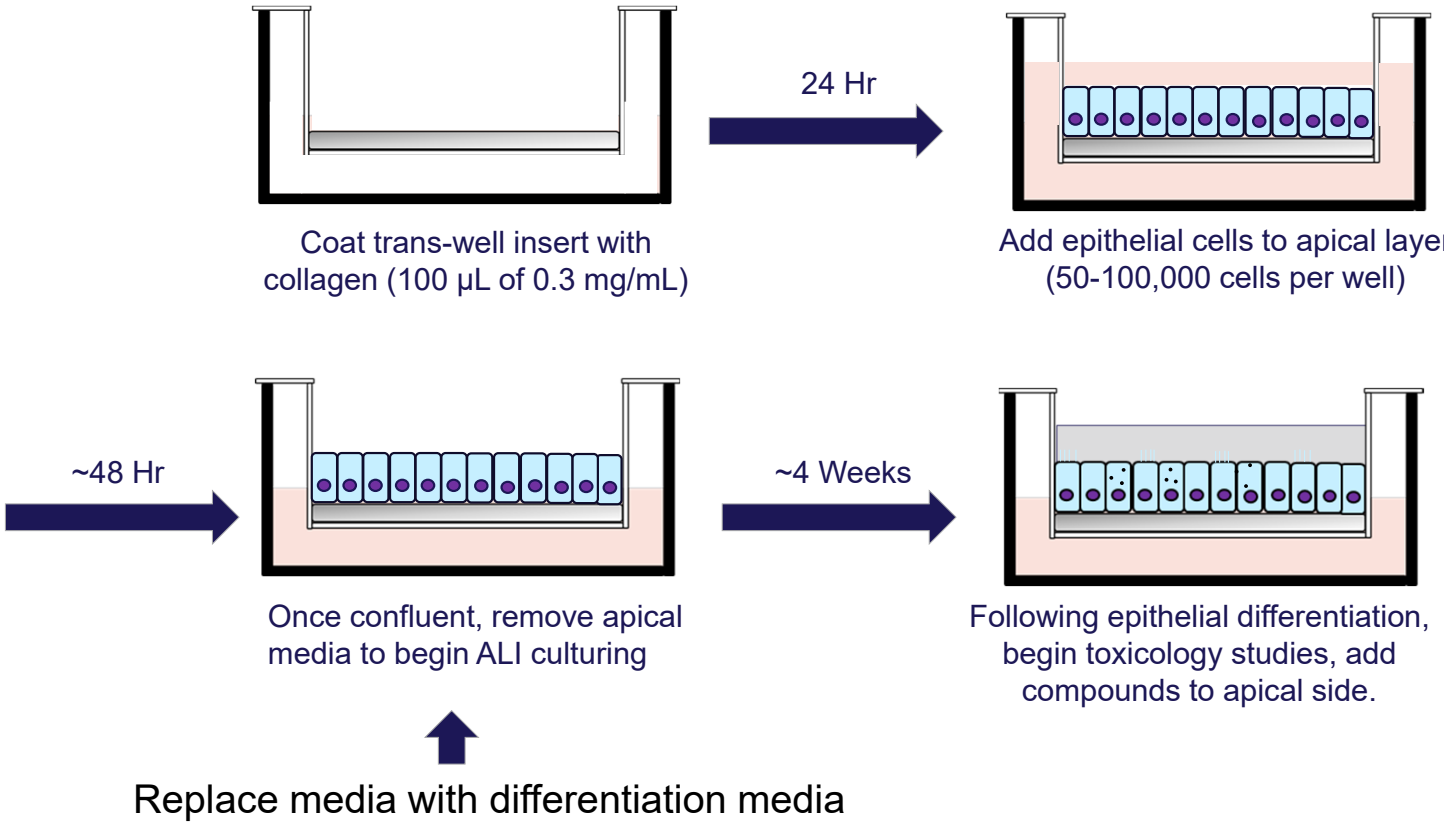
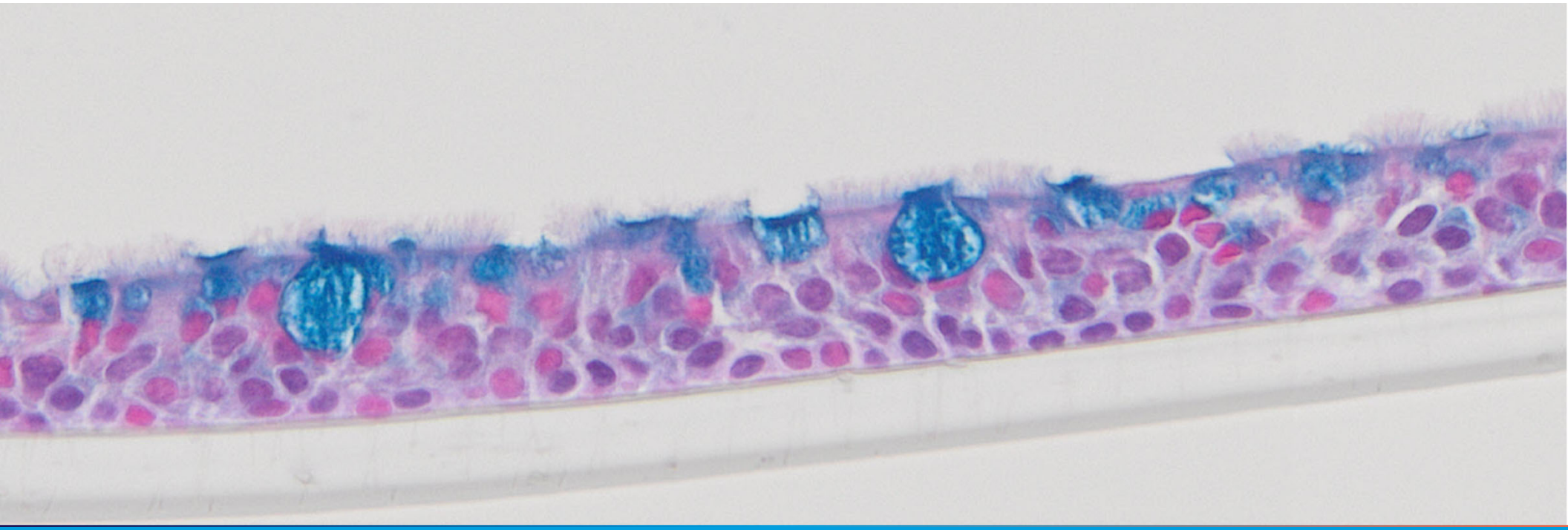
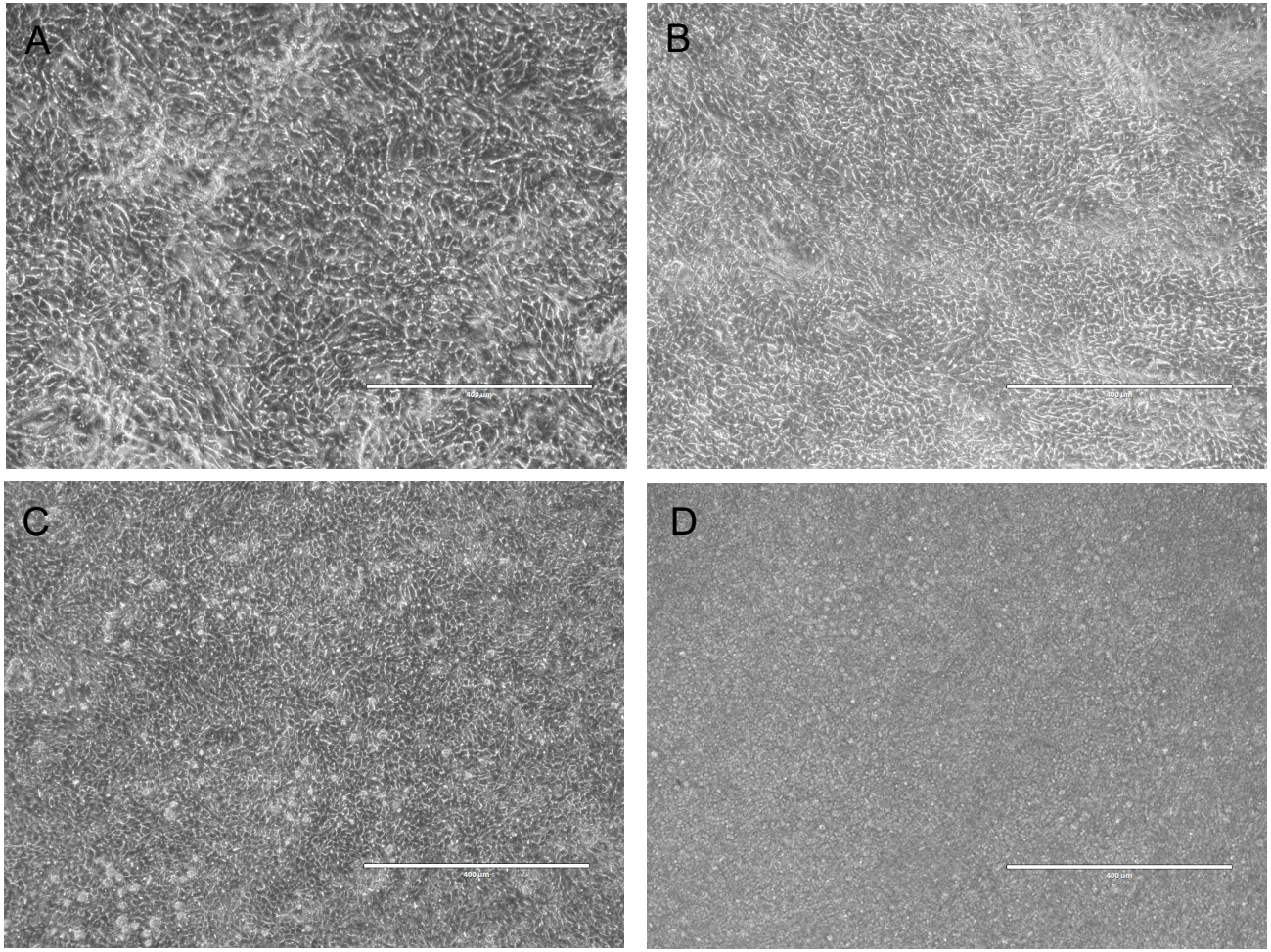
Overview of airway model fabrication process





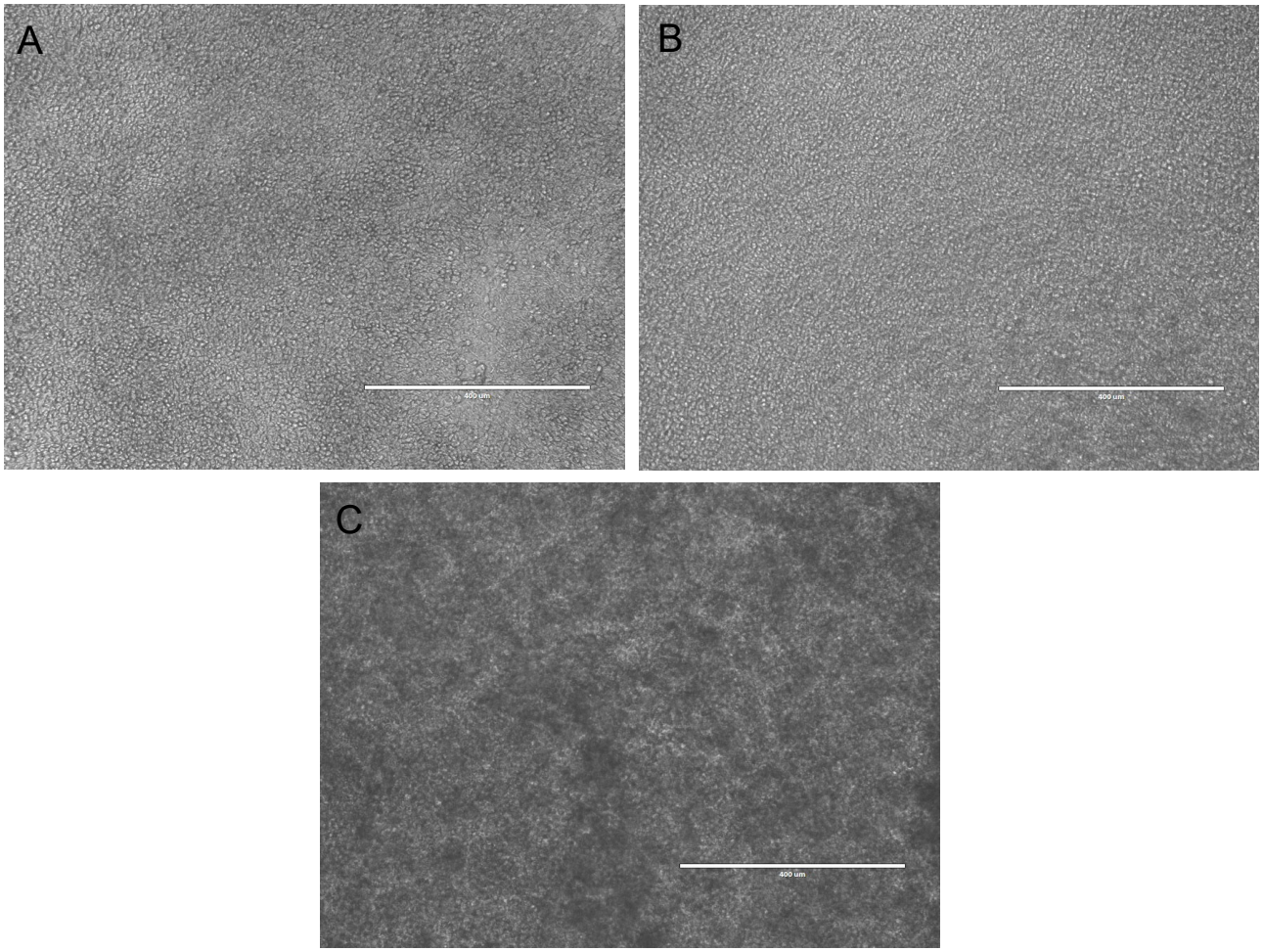
24-hour CdCl2 testing

Microscopy: images from ALI incubation



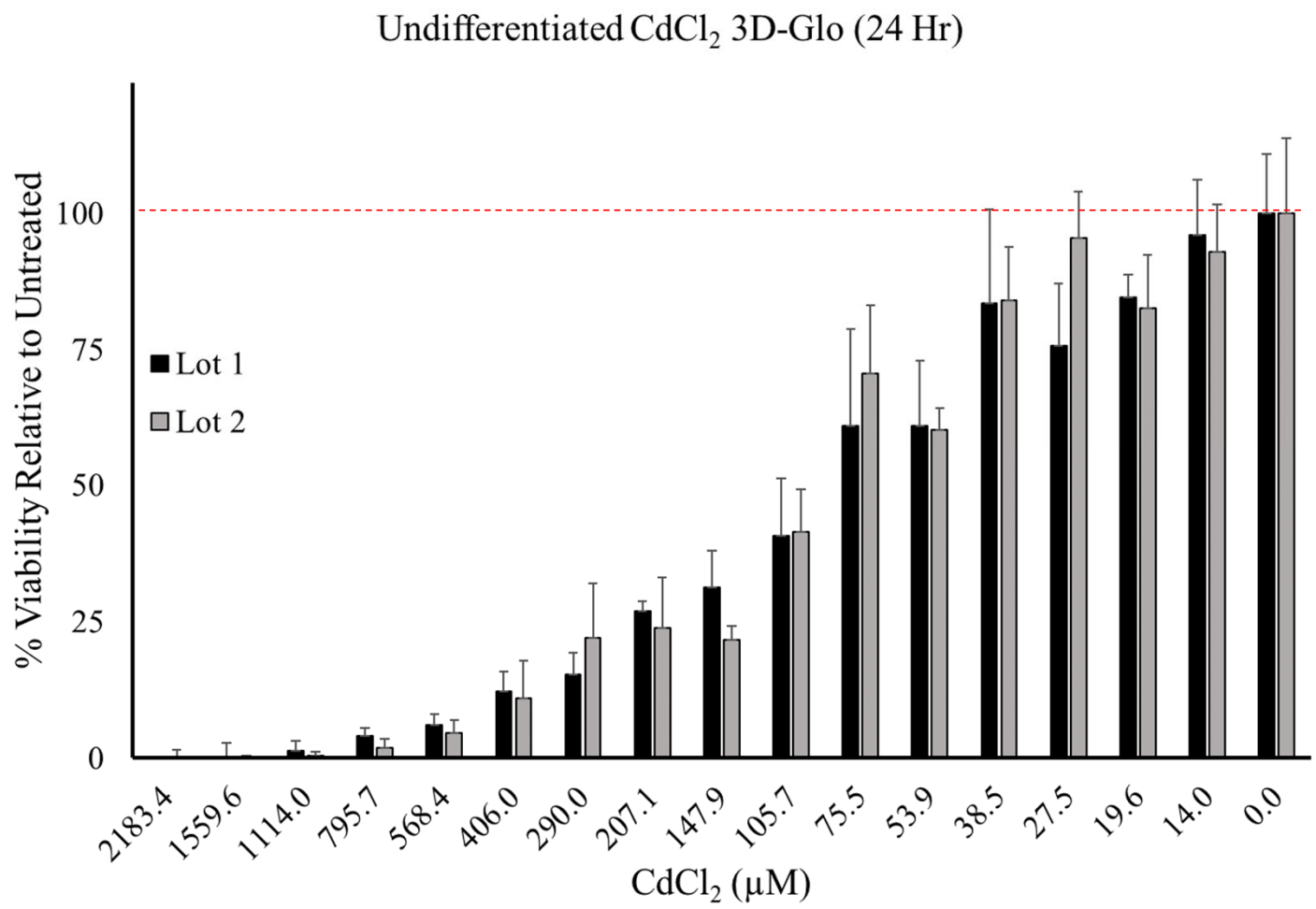
Representative microscopy images of primary bronchial epithelial cells under (A) 0, (B) 1, (C) 2 and (D) 3 weeks of ALI. Scale bars represent 400 µm.

24 Hr $CdCl_2$: microscopy images

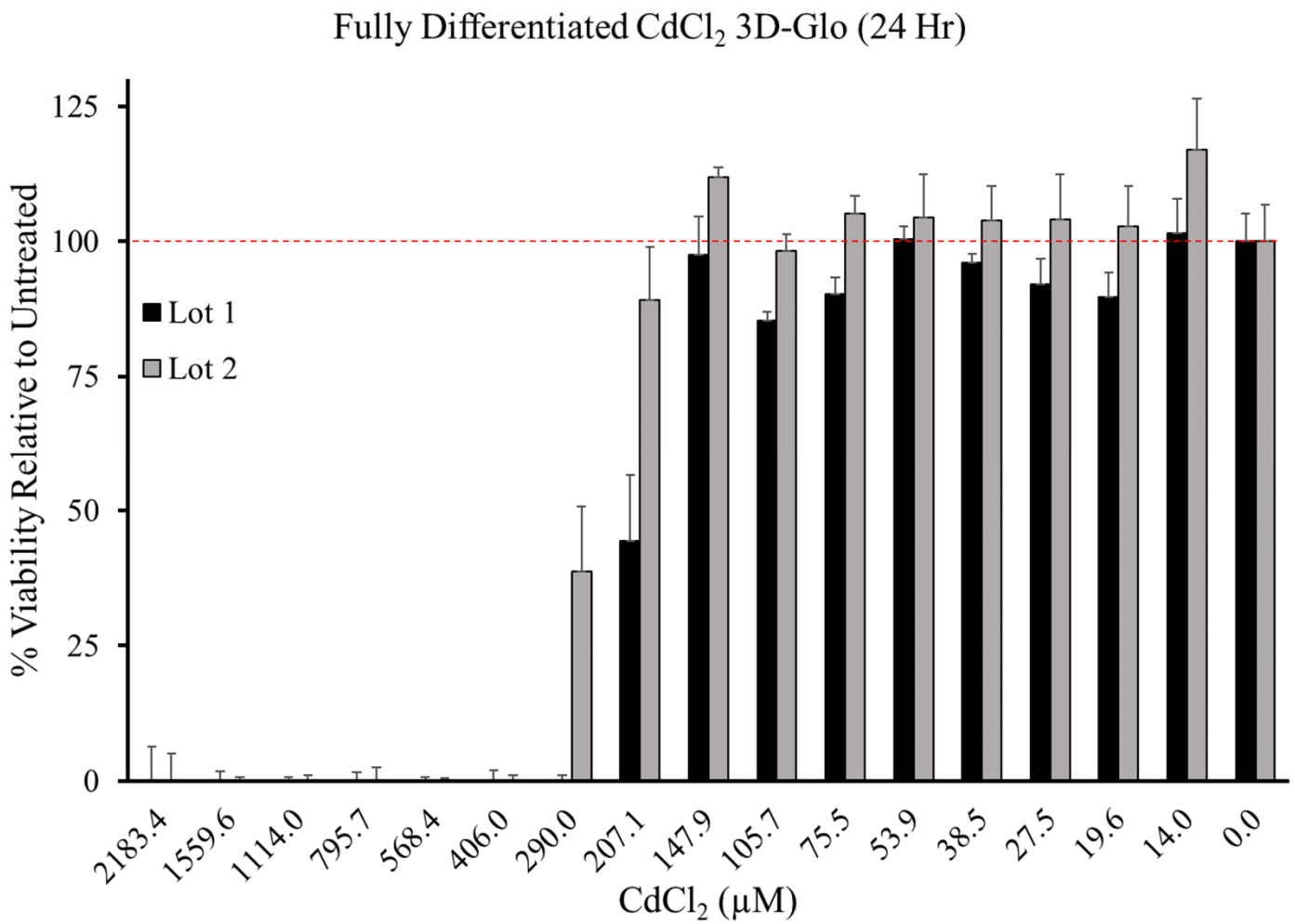


Representative microscopy images of primary bronchial epithelial cells apically treated with either (A) 0, (B) 53.9, or (C) 795.6 µM $CdCl_2$ for 24 Hr. Scale bars represent 400 µm.

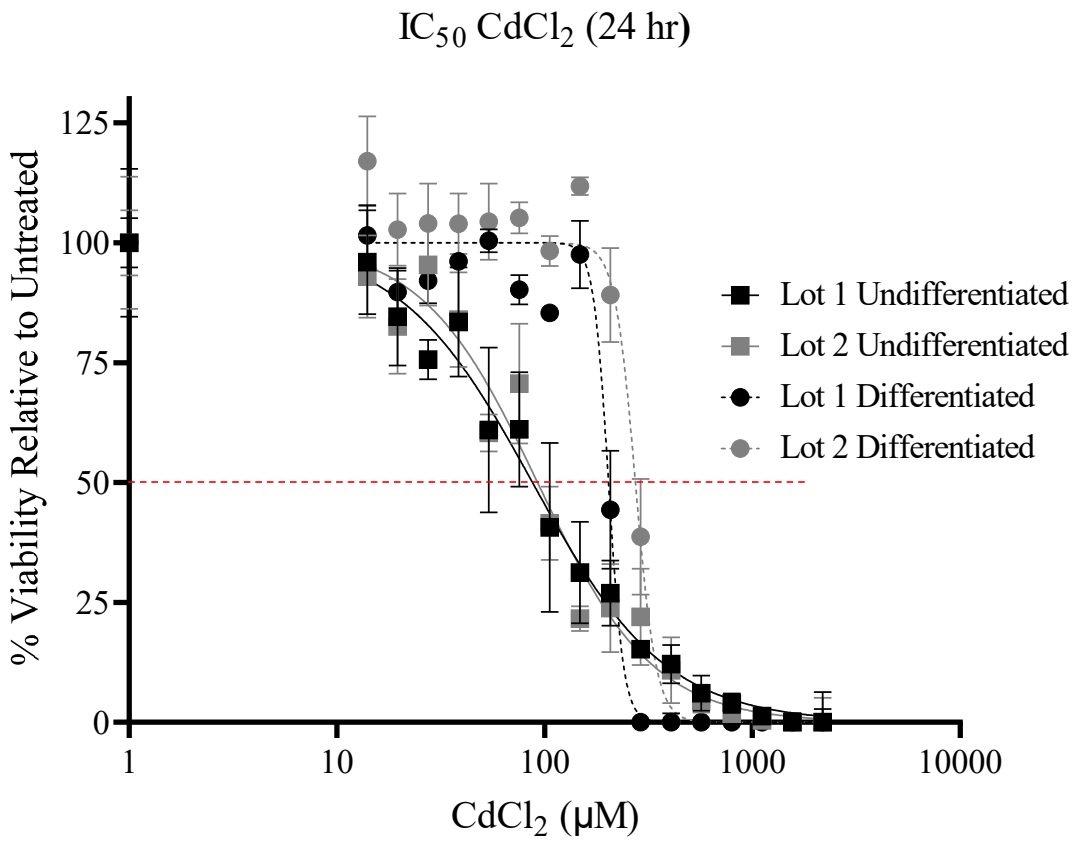
24 Hr $CdCl_2$: 96-well plate viability assay



24 Hr $CdCl_2$: 24-well plate viability assay



24 Hr $CdCl_2$: IC_{50} curves



24 Hr $CdCl_2$: IC_{50} value comparison

Cadmium Chloride IC_{50} values (µM)	
Sample Name	**IC_{50} Value (µM)**
Undifferentiated Lot 1	87.47 ± 10.8
Undifferentiated Lot 2	92.49 ± 10.8
Differentiated Lot 1	203.1 ± 7.2
Differentiated Lot 2	273.7 ± 12.3

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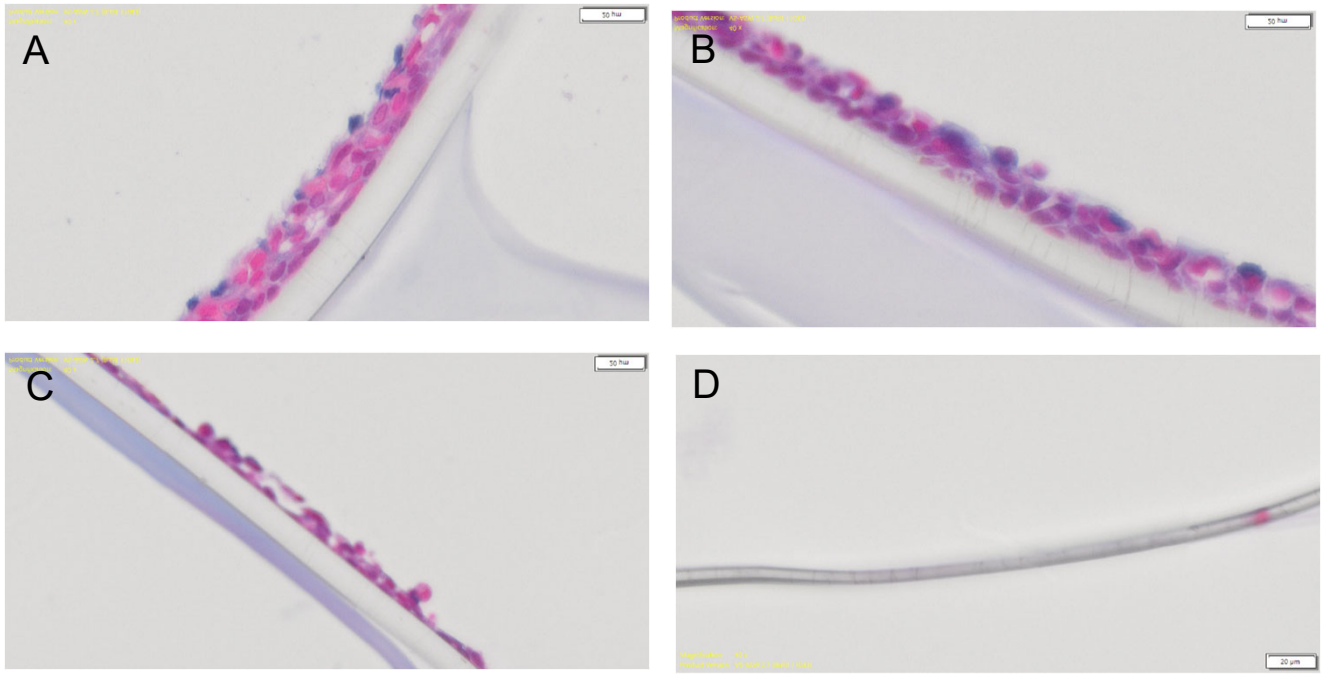
24 Hr $CdCl_2$: histology images



Representative alcian blue stained images of primary bronchial epithelial cells apically treated with (A) 0, (B) 53.9, (C) 147.9, or (D) 2183.4 µM $CdCl_2$ for 24 Hr. Scale bars represent 400 µm.

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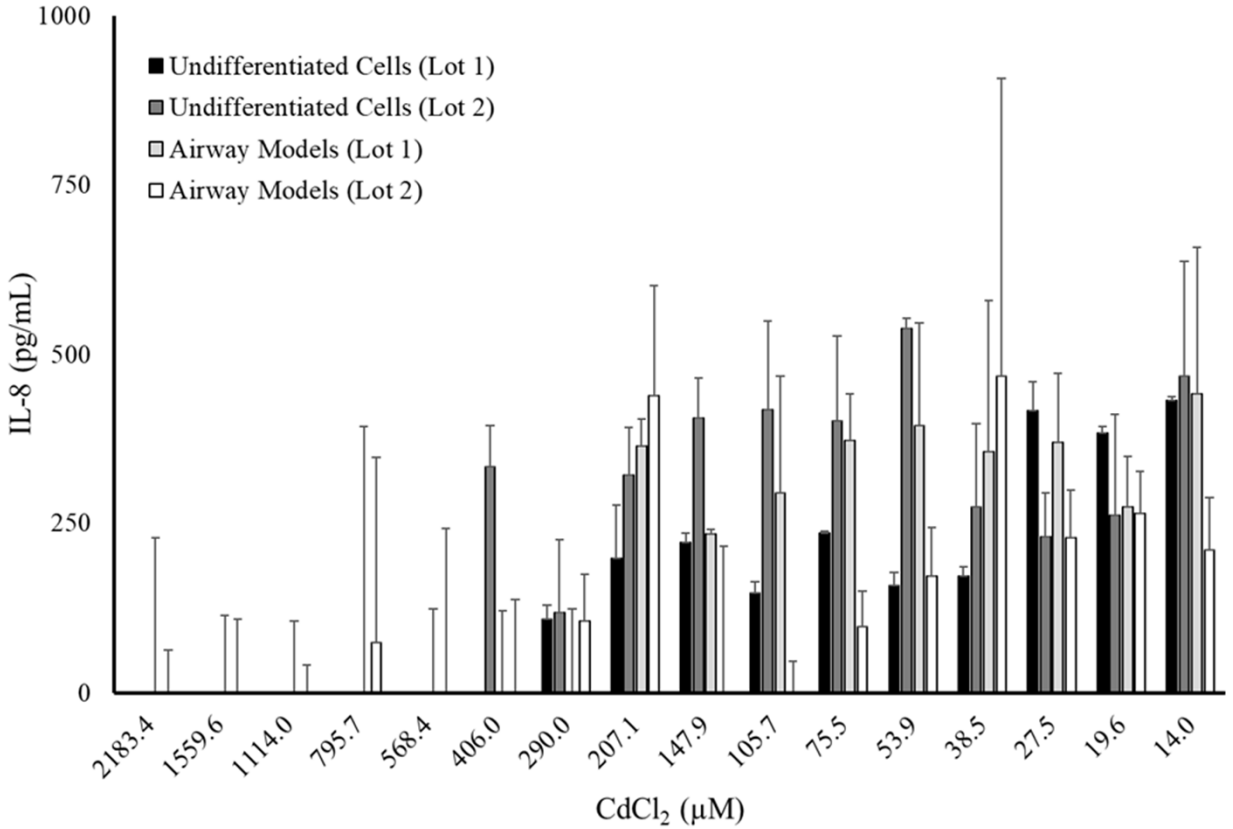
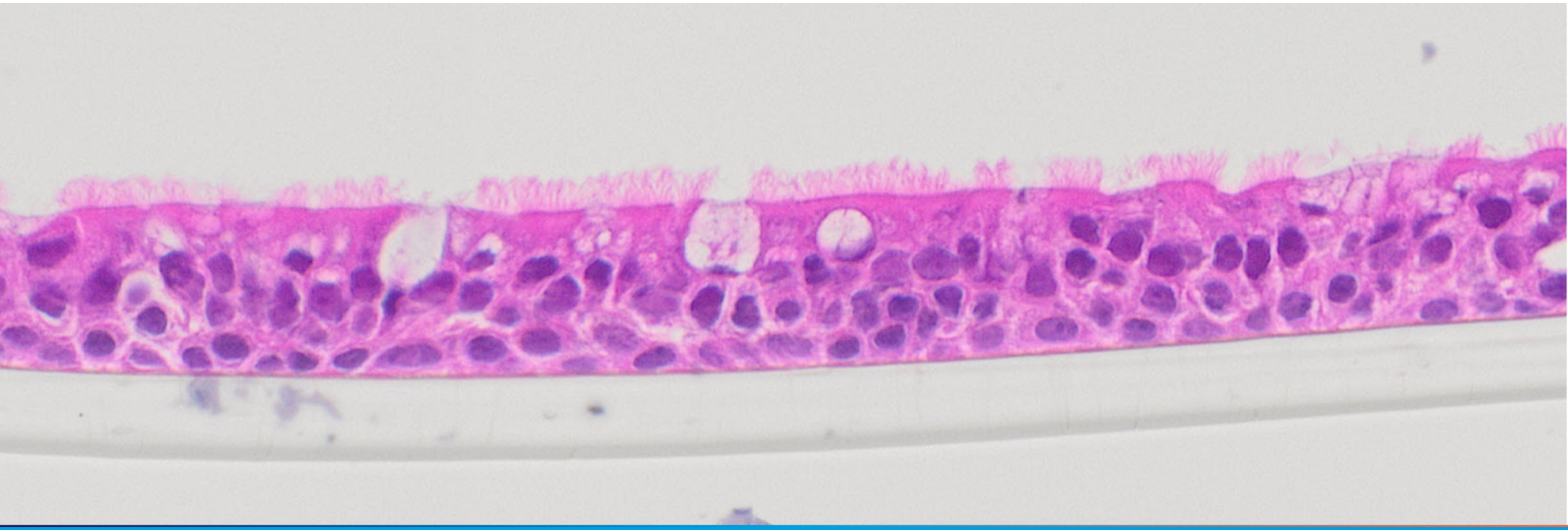
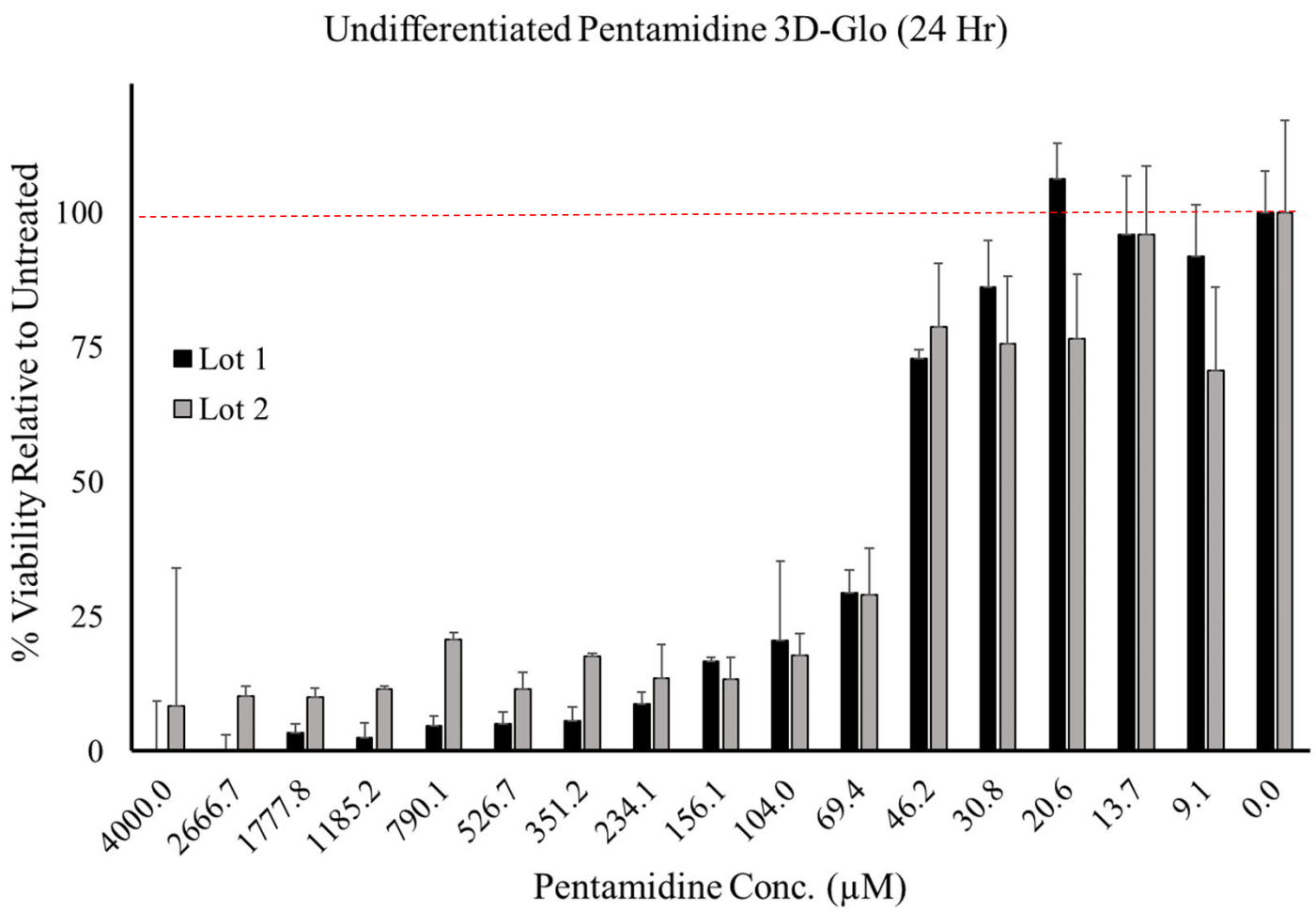
24 Hr $CdCl_2$: 24-well plate cytokine IL-8



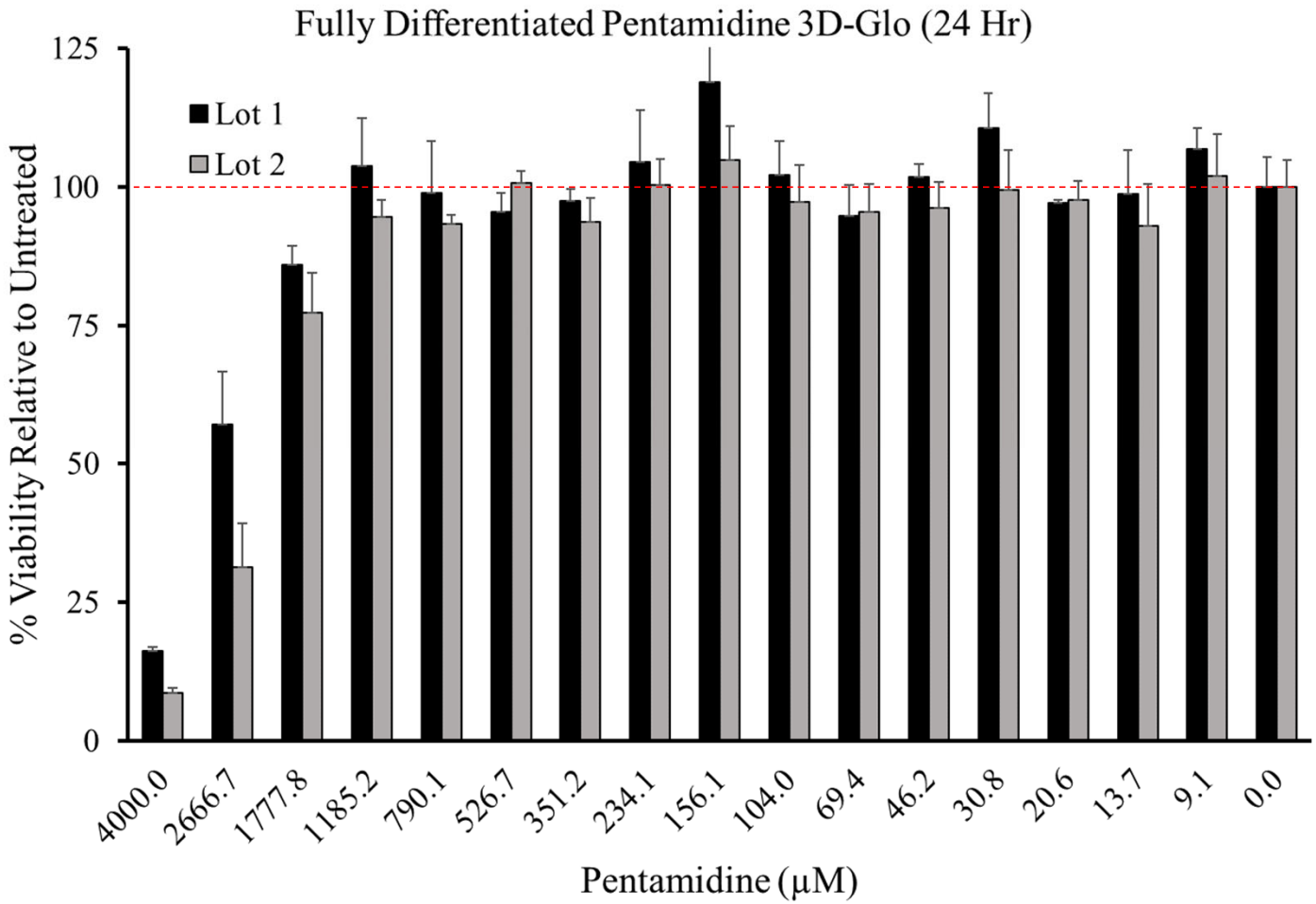


24-hour pentamidine testing

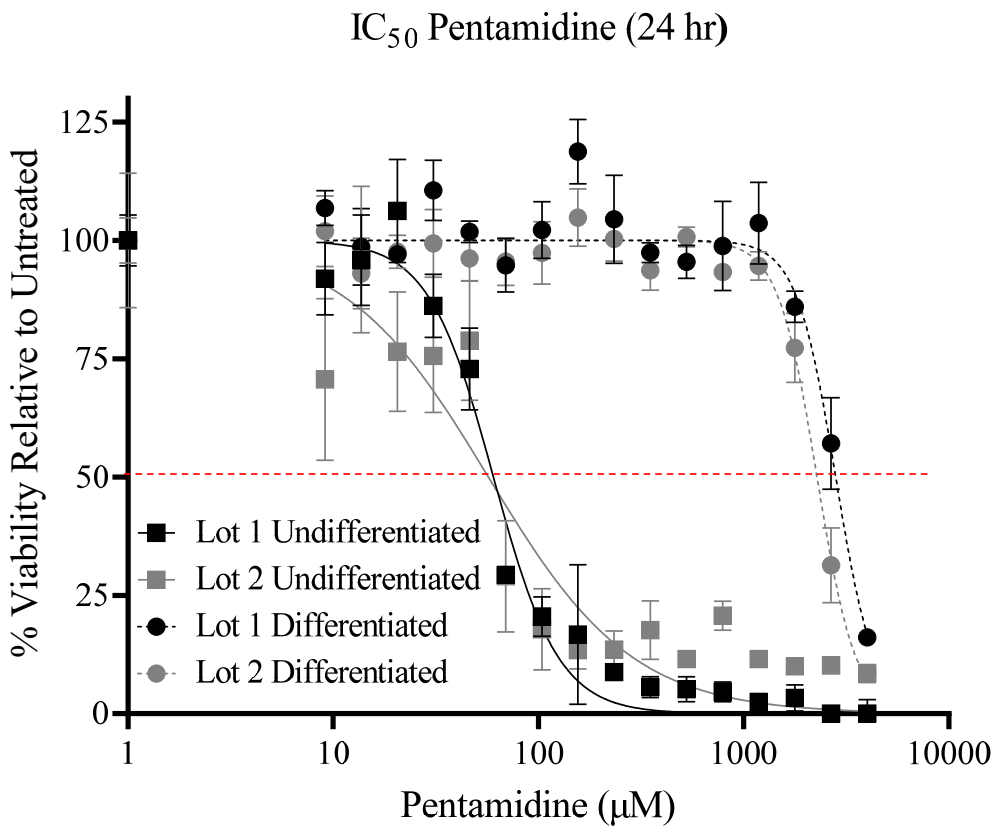
24 Hr pentamidine: 96-well plate viability



24 Hr pentamidine: 24-well plate viability



24 Hr pentamidine: IC_{50} curves



24 Hr pentamidine: IC_{50} value comparison

Pentamidine IC_{50} values (µM)	
Sample Name	**IC_{50} Value (µM)**
Undifferentiated Lot 1	60.4 ± 5.5
Undifferentiated Lot 2	57.1 ± 13.5
Differentiated Lot 1	2811 ± 200.5
Differentiated Lot 2	2279 ± 113

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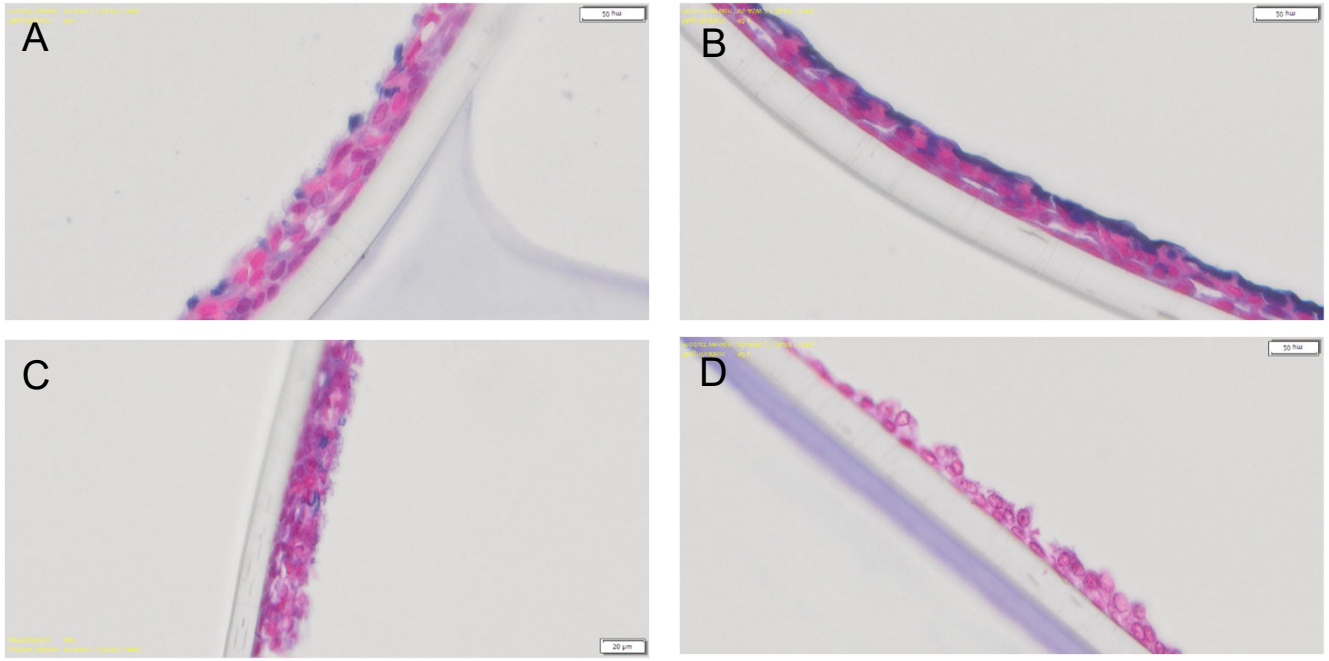
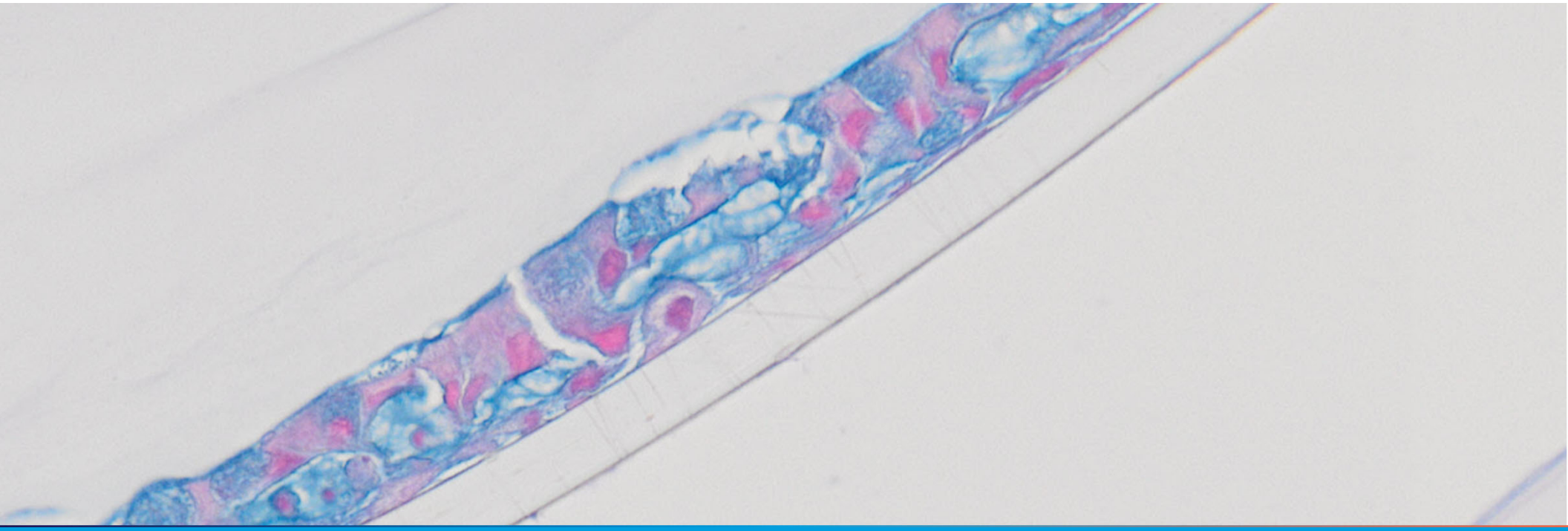
24 Hr pentamidine: histology images

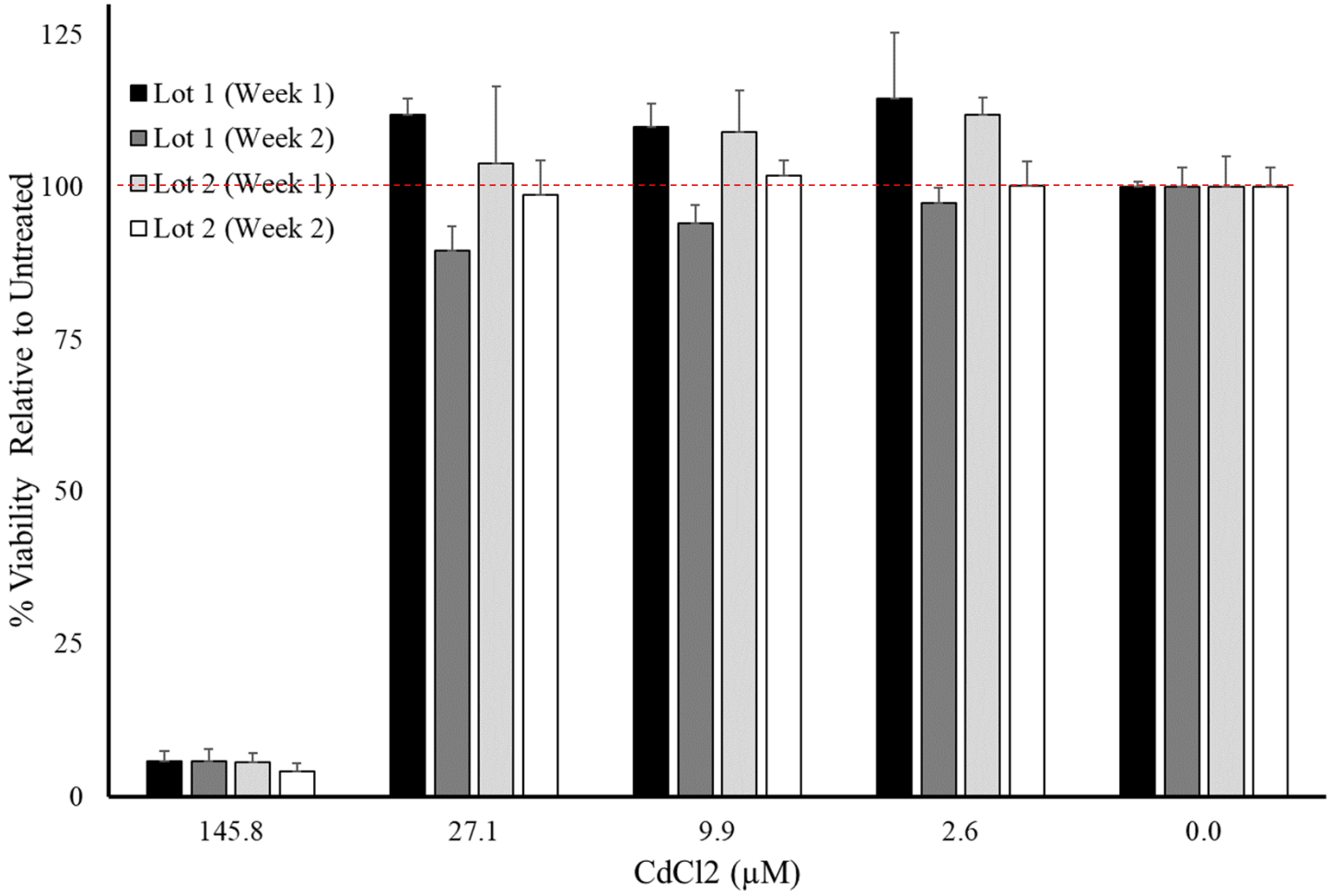


Representative alcian blue stained images of primary bronchial epithelial cells apically treated with either (A) 0, (B) 46.2, (C) 1,185, or (D) 4,000 µM pentamidine for 24 Hr. Scale bars represent 400 µm.

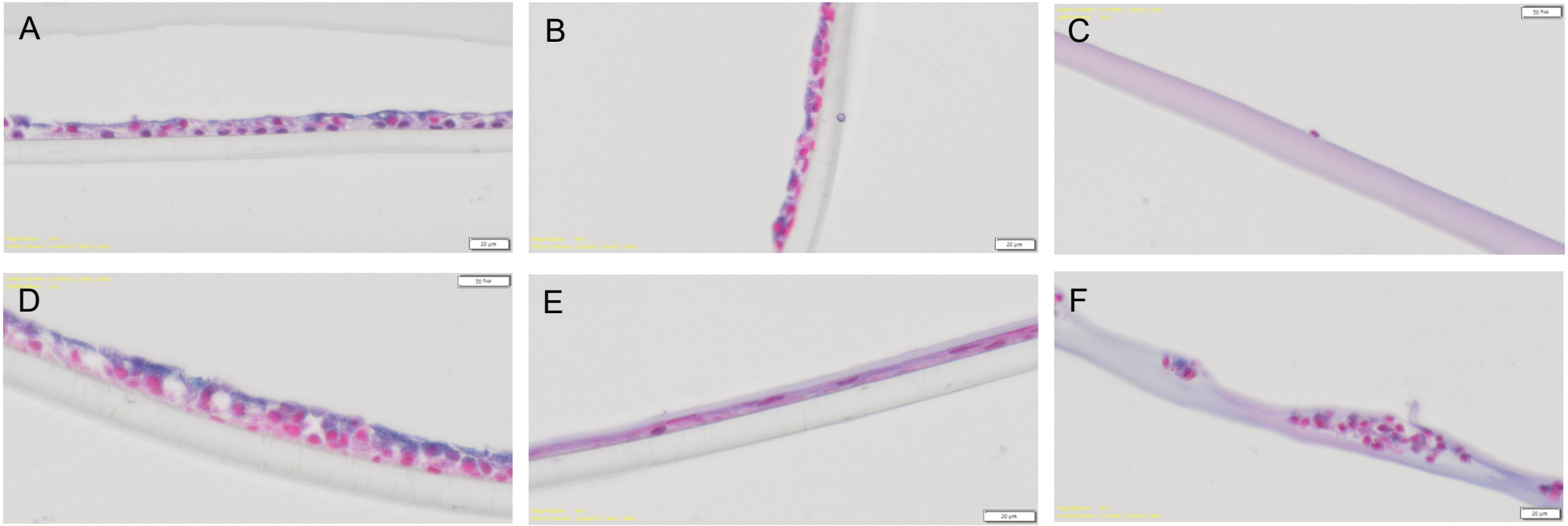


Long-term CdCl2 testing

Long-term $CdCl_2$: viability

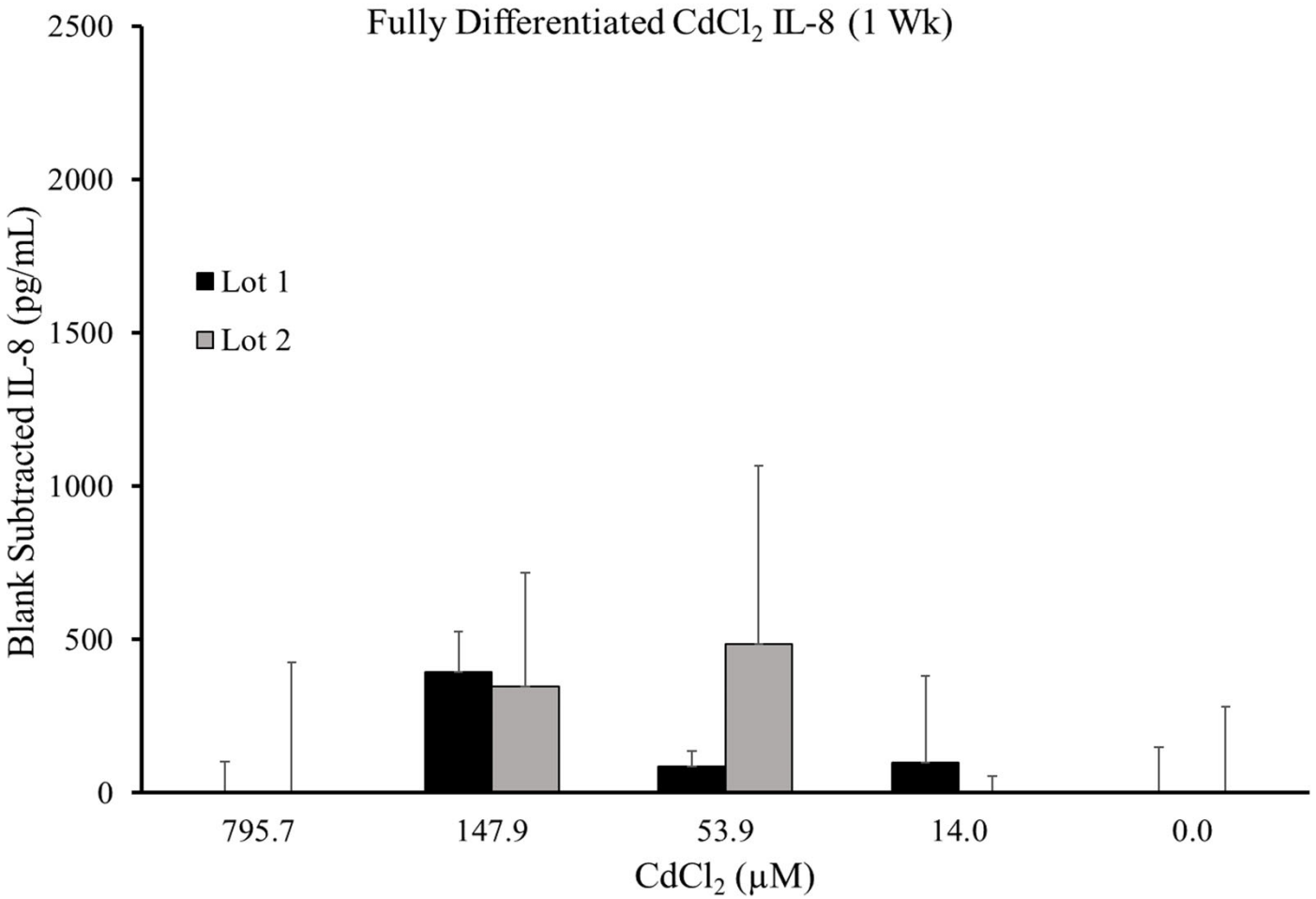


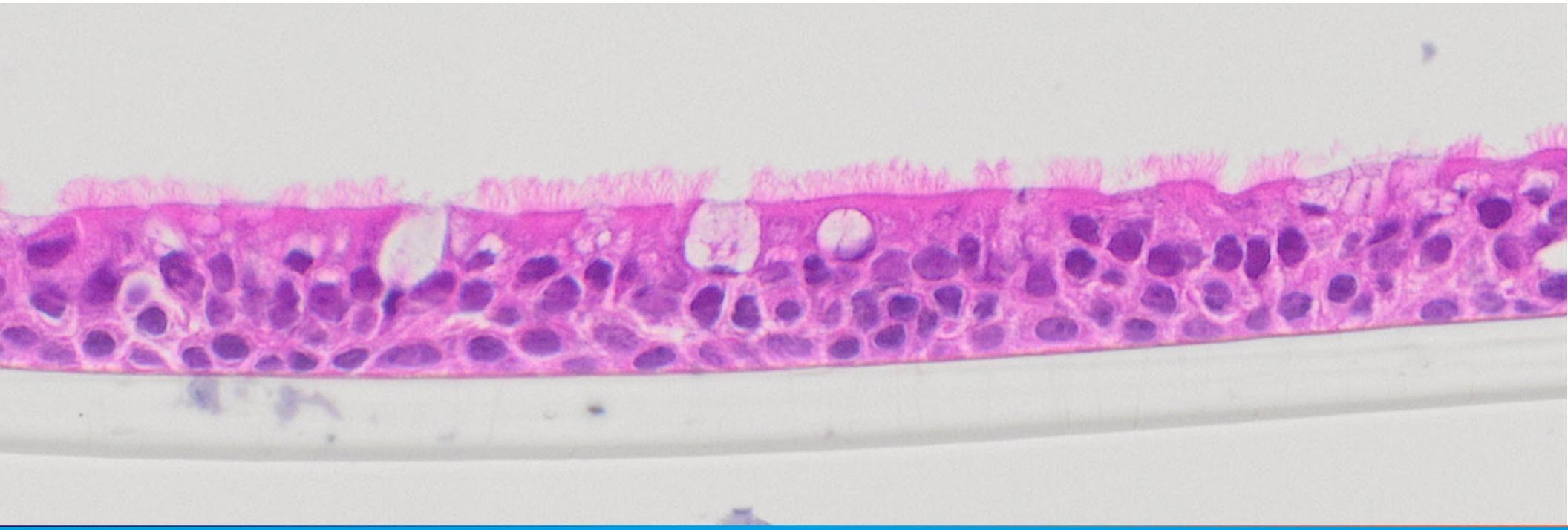
Long-term $CdCl_2$: histology images



Representative alcian blue stained images of primary bronchial epithelial cells apically treated with (A) 14.0, (B) 53.9, or (C) 795.6 µM $CdCl_2$ for 1 weeks, and (D) 14.0, (E) 53.9, or (F) 795.8 µM $CdCl_2$ for 2 weeks. Scale bars represent 400 µm.

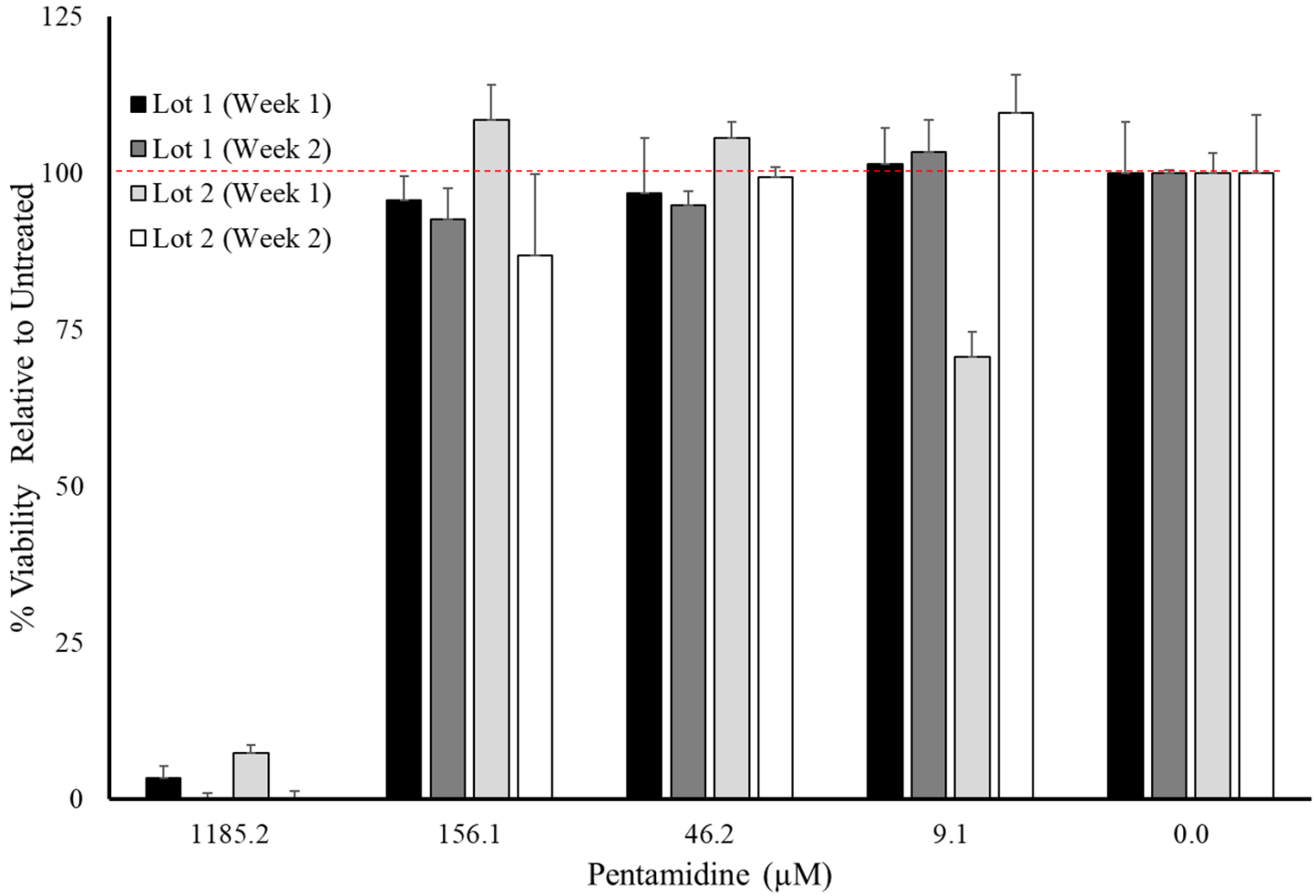
Long-term $CdCl_2$: cytokine IL-8



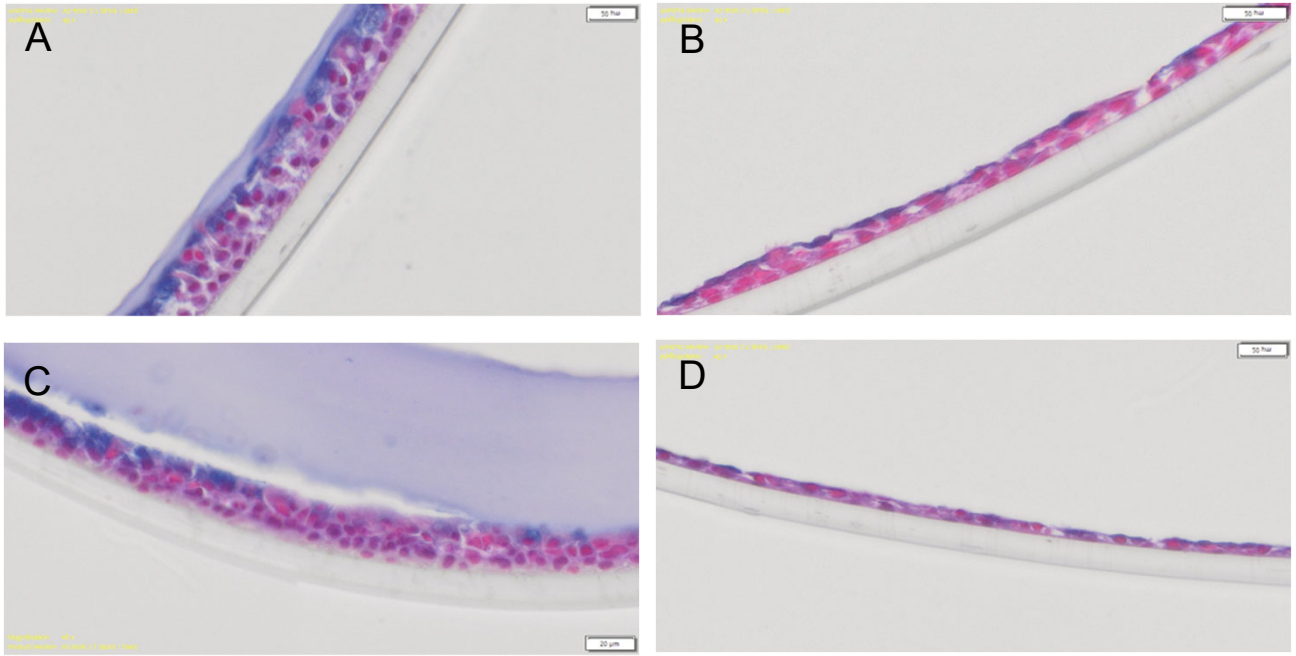


Long-term pentamidine testing

Long-term pentamidine: viability



Long-term pentamidine: histology images



Representative alcian blue stained images of primary bronchial epithelial cells apically treated with (A) 0, (B) 46.2, (C) 156, or (D) 1185 µM pentamidine for 1 week. Scale bars represent 400 µm.

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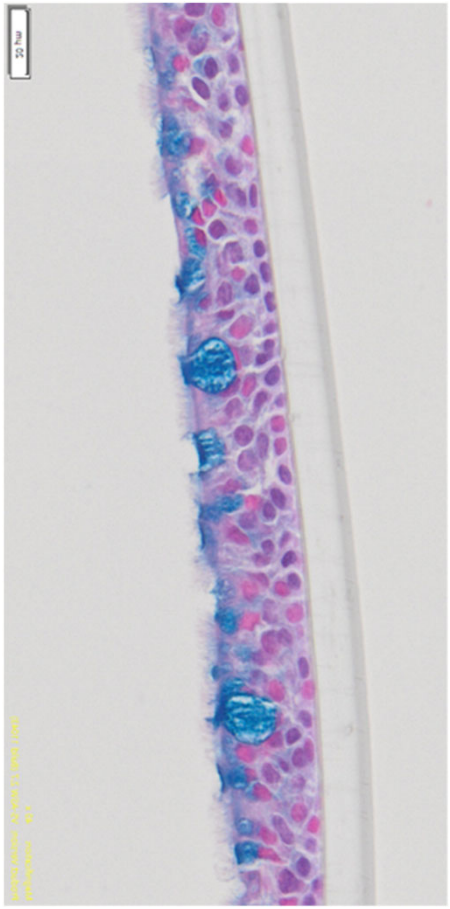
Summary of results: short-term studies

Short-term exposure

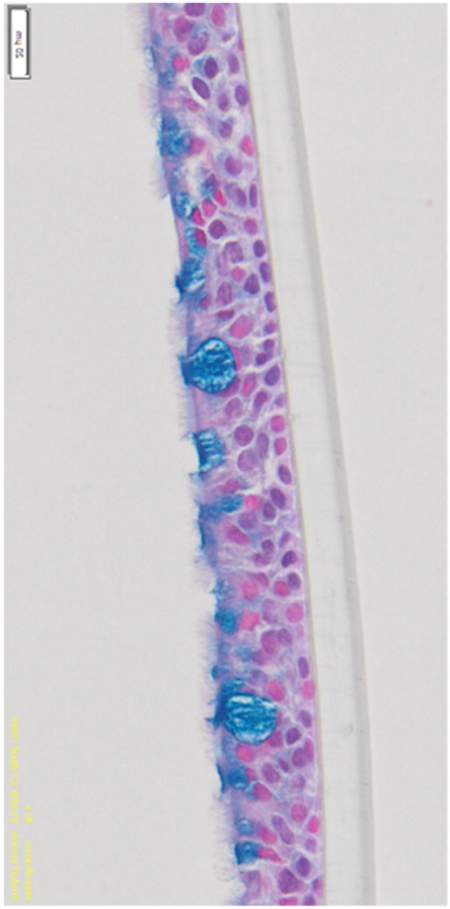
- Both primary lots demonstrated dose-dependent response to selected compounds.
- Differentiated airway models showed higher tolerance to selected compounds, relative to freshly plated counterparts.
- Inflammation assays reveal correlation between compound dosage and cytokine production, with high concentrations demonstrating little or no cytokine expression due to cell death. Pentamidine inhibits IL-8 production.
- Intermediate and low concentrations of $CdCl_2$ cause elevated cytokine expression relative to untreated controls.
- Images of models correspond to viability data. Higher cadmium exposure results in greater disruption of model integrity.

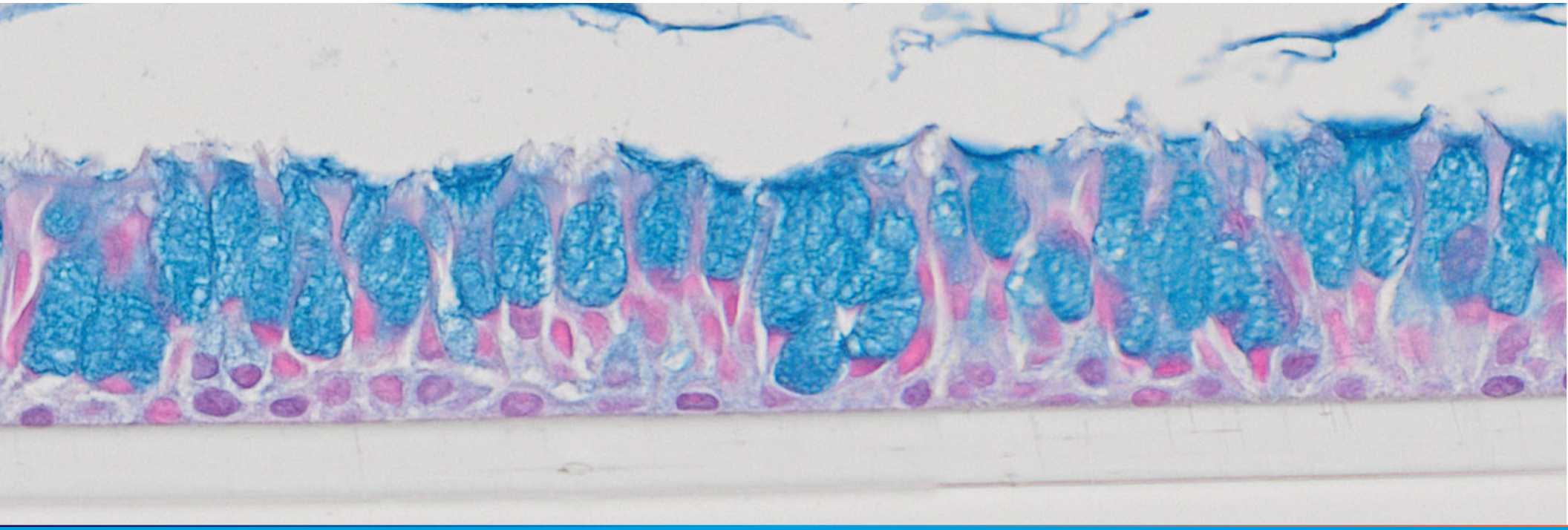


Summary of results: long-term studies

Long-term exposure

- Long-term cadmium exposure resulted in higher cell death, compared to samples administered with pentamidine.
- Inflammation data was similar to short-term testing, with increased cytokine expression in samples administered with low concentrations, relative to untreated controls. Again, pentamidine suppresses IL-8 expression in samples.
- Histology images of models correspond to viability data. Higher cadmium exposure results in greater disruption of model integrity.
- Pentamidine samples demonstrated long-term resistance against model disruption.





Thank You

Questions?

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

Going to the 43rd Annual Meeting of the American College of Toxicology?

Stop by our booth #112, or visit our scientific posters:

Development of hTERT-immortalized Neonatal Melanocytes for Toxicity Studies and Melanogenesis Regulation
November 14, 2022
5:00 PM - 6:30 PM

Evaluating Short- and Long-Term Toxicity Response of Models Comprised of Fully Differentiated Primary Bronchial Tracheal Epithelial Cells to Either Cadmium Chloride or Pentamidine
November 14, 2022
5:00 PM - 6:30 PM