Evaluating Short and Long-Term Toxicity Response of Models Comprised of Fully Differentiated Primary Bronchial Tracheal Epithelial Cells to Either Cadmium Chloride or Pentamidine

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Abstract

Respiratory tract diseases stemming from toxic compound exposure significantly contribute to the global health burden. Traditional in vitro airway models, due to their lack of physiological relevance, are often unable to provide meaningful and accurate toxicological assessments. Advanced in vitro airway models, however, promise to provide more predictive information for use in human airway health. Here, we constructed mature airway models comprising fully differentiated primary bronchial tracheal epithelial cells incubated in 24-well plate inserts and cultured under air-liquid interface for 4 weeks. The toxicological response to short-term (24 hours) exposure to either cadmium chloride ($CdCl_2$) or pentamidine were evaluated and compared in both differentiated and undifferentiated cells. The toxicological response to long-term exposure (1, 2 weeks) to either compound in differentiated airway models was also explored. Changes in viability and cytokine expression were quantified and compared in both models. Additionally, histological imaging (H&E, alcian blue, IHC) was conducted on mature airway models to visually assess model disruption, inflammation, and tight junction disruption. We observed that all airway models expressed dose-dependent response to both $CdCl_2$ and pentamidine exposure, with increased cell death corresponding with increased compound concentrations. Additionally, differentiated models demonstrated higher resistivity to cell death compared to undifferentiated counterparts. Moreover, exposure to low concentrations of the compound resulted in increased cytokine expression relative to untreated controls. Finally, long-term exposure to $CdCl_2$ resulted in model disruption and death, whereas pentamidine exposure demonstrated limited model disruption. These results suggest that these airway models may serve as useful tools future airway toxicity research.

Background

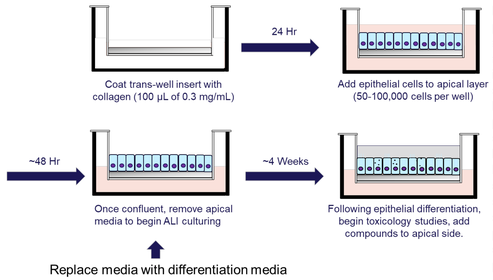


Figure 1. Schematic of airway model fabrication process. Trans-well inserts with PET membranes are added to interior wells of 24-well plates. Membranes are apically coated with 0.3 mg/mL collagen solution and incubated overnight at 4˚C. Membranes are rinsed and outer-wells filled using PBS prior to cell seeding. 1˚ bronchial epithelial cells are apically seeded at 10^5 cells per well and incubated for 48-72 hours to ensure full confluency. Afterwards, apical media is removed, and basal media replaced with STEMCELL Technologies PneumaCult™-ALI maintenance media. Cells are cultured under ALI for at least 4 weeks to ensure epithelial differentiation. Once cells are fully differentiated, selected concentrations of either $CdCl_2$ or pentamidine are added to the mature models. In contrast, undifferentiated cells are seeded in 96-well plates 24 hours prior to testing.

Results

Microscopy images of bronchial epithelial cell differentiation and airway model disruption from short-term administration of $CdCl_2$

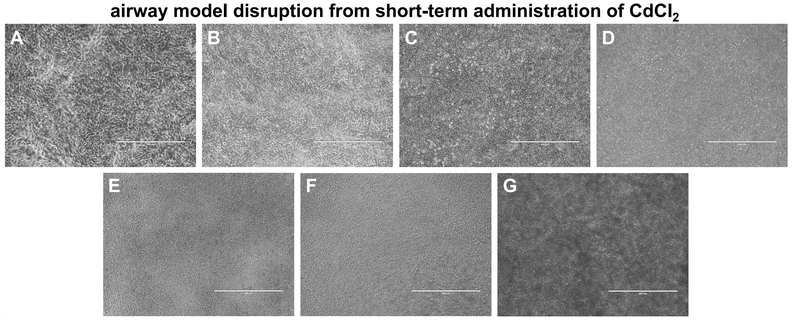
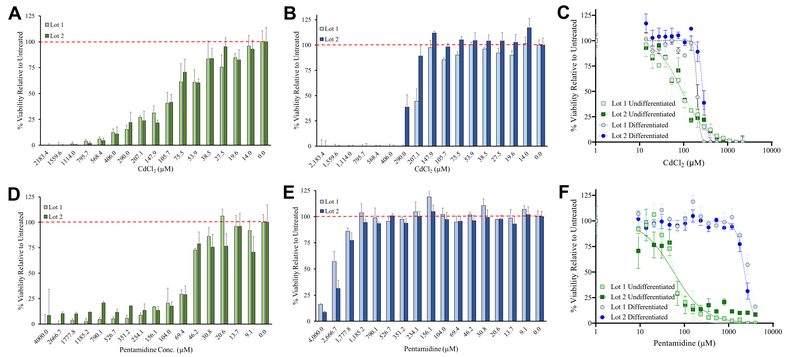


Figure 2. Airway model maturation and short-term assessment of $CdCl_2$. Representative microscopy images of primary bronchial epithelial cells under (A) 0, (B) 1, (C) 2, and (D) 3 weeks of ALI. Prolonged incubation under ALI conditions induces fully epithelial differentiation in airway models. Microscopy images of airway models apically treated with either (E) PBS, (F) low, or (G) intermediate concentrations of $CdCl_2$. No observable differences were seen between untreated airway model controls and models administrated with low concentrations of $CdCl_2$. In contrast, intermediate $CdCl_2$ exposure results in high levels of cell death and model disruption (floating black spots). Scale bars represent 400 µm.

Short-term viability measurements



G

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

H

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

Figure 3. Short-term viability studies comparing 3-D airway models to undifferentiated cells. Changes in viability between (A) undifferentiated HBECs and (B) 3-D airway models, (C) as well as IC_{50} curves from 24-hour exposure to $CdCl_2$. Changes in viability were also measured on in both (D) undifferentiated cells and (E) 3-D airway models, (F) as well as IC_{50} curves generated from 24-hour exposure to pentamidine. IC_{50} values were calculated for models exposed to both (G) $CdCl_2$ and (H) pentamidine. Viability measurements were conducted using the CellTiter-Glo® 3-D Cell Viability Assay kits (Promega™ Corporation).

Short-term histology

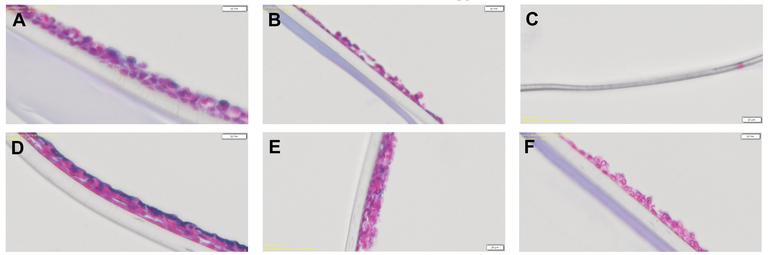


Figure 4. Increased model disruption from increased compound administration. Representative alcian blue stained images of airway models apically treated with 24-hour exposure to either (A) 53.9, (B) 147.9, or (C) 2183.4 µM $CdCl_2$, as well as models treated with either (D) 46.2, (E) 1,185, or (F) 4,000 µM pentamidine for 24 hours. Increased $CdCl_2$ administration results in greater airway model disruption and cell death. Compared to $CdCl_2$ counterparts, airway models demonstrated no observable model degradation from short-term pentamidine exposure, except for the highest administered dosage of 4,000 µM, which exhibited moderate model disintegration. Scale bars represent 20 µm.

Measuring model inflammation

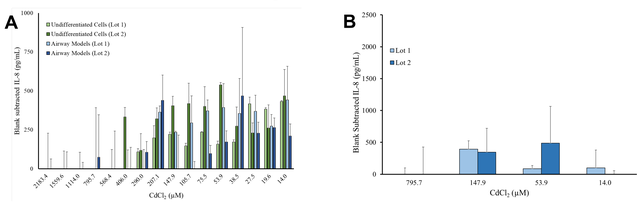


Figure 5. IL-8 cytokine measurements via ELISA. Analysis of IL-8 cytokine expression from (A) 24-hour and (B) 1-week administration of $CdCl_2$ to both undifferentiated HBECs and mature airway models. Values are shown as blank subtracted. Low concentrations of $CdCl_2$ results in increased proinflammatory cytokine expression, relative to blank controls. In contrast, intermediate to high administration, results in decreased expression, due to cell death. Pentamidine administration inhibits IL-8 production in airway models.

Long-term viability measurements

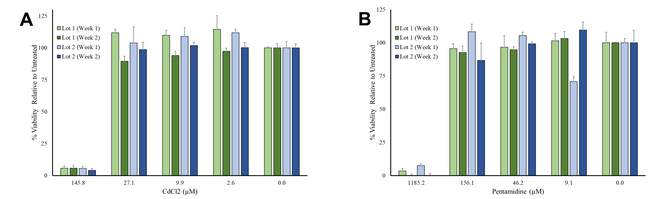


Figure 6. Long-term viability studies on 3-D airway models. Changes in viability in airway models exposed to either (A) $CdCl_2$ or (B) pentamidine for 1 and 2 weeks.

Long-term histology

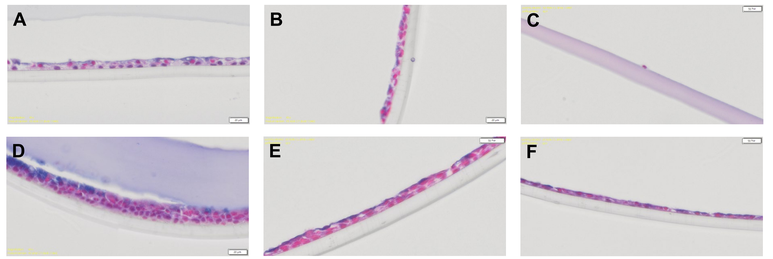


Figure 7. Long-term exposure of airway models from either $CdCl_2$ or pentamidine. Representative alcian blue stained images of airway models apically treated with either (A) 14.0, (B) 53.9, (C) 795.6 µM $CdCl_2$, or (D) 46.2, (E) 156, (F) 1185 µM pentamidine for 1 week. Similar to short-term administration, long-term exposure to progressively higher $CdCl_2$ administrations results in increased airway model disruption. In contrast, airway model integrity is better preserved from long-term pentamidine exposure. Scale bars represent 20 µm.

Immunohistochemistry microscopy imaging

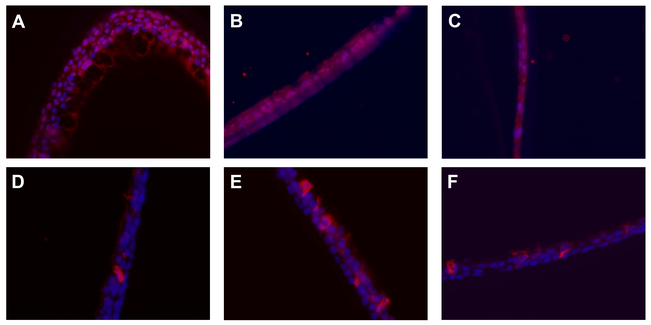


Figure 8. IHC images of 3-D airway models. Representative IHC-stained images of (A) untreated control airway models, airway models exposed to (B) 14.0, or (C) 147.9 µM $CdCl_2$ for 2 weeks. Here, ZO-1 protein expression is shown as red with DAPI control in blue, with increased ZO-1 protein disruption associated with increased $CdCl_2$ exposure. Representative IHC-stained images of airway models treated with (D) 0, (E) 9.1, (F) 46 µM pentamidine for 24 Hr. MUC5AC expression shown as red with DAPI control in blue. There was no correlation with increased MUC5AC expression with increasing pentamidine administration.

Conclusion

- Self-fabricated 3-D airway models can be utilized to provide more physiologically relevant data relative to traditional 2D in vitro models.
- Differentiated airway models showed higher tolerance to selected compounds, relative to freshly seeded undifferentiated counterparts.
- Cadmium administration results in greater decreases in airway model viability compared to pentamidine exposure.
- Histology images of airway models corresponded to viability data.
- Inflammation assays revealed the relationship between $CdCl_2$ dosage and cytokine production, with low to intermediate dosage exhibiting higher cytokine production, whereas high $CdCl_2$ administration results little or no cytokine expression due to cell death.
- Trends from long-term testing correlated with the results from short-terms studies.
- In all studies, both primary cell lots demonstrated dose-dependent response to selected compounds.

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