Multi Species 3D Airway Tissue Models for Translational Inhalation Toxicity Studies

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Abstract

Background and Purpose: *In vitro* models of the respiratory tract are highly differentiated and have been commercially available since 2000. These models have been widely used for toxicological, respiratory infection, tobacco safety, and inhaled drug delivery studies. Availability of tissue models representing the different segments of the respiratory tract were instrumental in gaining insight into the underlying mechanisms of SARS-CoV-2 infection and for screening of anti-viral compounds. Despite these useful applications, there are large databases of animal toxicity data which are not directly translatable to data obtained from the human *in vitro* airway tissue models due to species differences.

Methods: To close this translational gap, cells harvested from both rat and non-human primate (rhesus monkey) tissues were utilized to develop models similar to EpiAirway, the tracheobronchial tissue model offered by MatTek that is cultured with normal human cells. The tissues were characterized for structure (histology), epithelial cell markers (IHC), barrier integrity (transepithelial electrical resistance, TEER measurement), and functionality (inhalation toxicological studies). To verify the reliability of these models worldwide, quality control (QC) data for tissue lots produced in the US and Europe were compared.

Results: The animal cell-derived 3D tissues exhibited similar characteristics to human tissues including: well polarized epithelia with physiological TEER values of >300 Ω*cm^2, cilia formation on the apical surfaces, and mucin production mimicking the airway microenvironment. Acute exposure to 4 test articles (TA) showed species-specific changes in tissue viability and membrane integrity as measured by MTT and TEER assays, respectively. The effective dose concentration that reduces tissue viability by 50% (ED-50) for vinyl acetate (VA) and chloroacetaldehyde (CA) were both <2 mg/tissue and the ED-50 for propylene glycol (PG) was >20 mg/tissue for all species. However, the ED-50 values for toluene (T) showed differences between the species: human >20 mg, primate 16.2±1.7 mg, and rat 13.8±0.1mg. Based on the MTT viability and TEER values, the test chemicals were rank ordered from high to minimal toxicity: CA > VA > T > PG and the vehicle controls (water and corn oil). TEER values from standardized QC tests averaged 1094 ± 325 (n=141 lots) in the US vs. 913 ± 238 (n=64 lots) in Europe. TEER values were not statistically different ($p < 0.001$).

Conclusions: Although more chemicals need to be tested, the multispecies 3D airway tissue models will be vital translational tools to predict airway inhalation toxicity and to bridge the *in vitro* - *in vivo* knowledge gap to reliably predict human responses, while providing a worldwide alternative approach to animal experimentation.

Methods

Tissue Preparation: Airway tracheobronchial cells were isolated from excised airway tissues including lungs, mainstem bronchi and trachea obtained from 8-week-old male Crl:CD(SD) rats (Charles River Laboratories, Wilmington, MA), rhesus monkeys, and humans following institutional/organizational ethical guidelines. Airway cells from the three species were seeded onto collagen coated cell culture inserts (MatTek Corporation) and then raised to the air liquid interface and cultured for approximately 20 days. Tissues that passed standard quality control test were used in the various experiments.

Histology & Immuno-Staining: Reconstructed airway tissue cultures were fixed in 10% formalin (overnight, room temperature), paraffin embedded, sectioned, and stained with hematoxylin and eosin (H & E) according to standard procedures (**Figure 1**). Slides were immuno-stained for cilia (β-tubulin), tight junction (E-cadherin), epithelial cells (CK5), and mucus producing goblet cells (MUC 5B) (**Figure 2**). H&E cross sections were also used to monitor structural damage to the tissues following exposure to test articles (**Figure 4**).

Test Article (TAs) Exposure: For inhalation toxicity experiments, three irritants (chloroacetaldehyde, vinyl acetate, and toluene) and a non-irritant control (propylene glycol) were used. Chemical exposure was performed by adding 100 uL of each test article onto the apical surface, followed by sealing tissue inserts with insert caps (MILICEL-MTK-CAP, MatTek Life Sciences). Exposure to TAs proceeded for 4 hr to mimic in vivo rat exposure experiments. After 4 hr, dosed tissues were washed with PBS and allowed to recover for 20 hr at 37°C and 5% CO_2.

MTT Viability Assay: Following treatment with the test articles, tissue viability was determined using the MTT assay. % viability was determined using the equation: % viability = OD (treated tissue)/OD (control tissue)*100. MTT results are shown in **Figure 3**.

Transepithelial Electrical Resistance (TEER): To examine barrier function, TEER measurements were made using the EVOM2 volt-ohmmeter equipped with an Endohm-12 electrode chamber (World Precision Instruments, Sarasota, FL). %TEER was calculated as TEER (Ohms*cm^2) of treated tissues (TTT) divided by the TEER of untreated tissues (TUT) times 100 (% TEER = (TTT/TUT*100). As shown, the TEER values parallel the MTT results (**Figure 3**).

Quality Control (QC) Comparison: Human cell derived airway tissues (EpiAirway) were produced in the US (MatTek Headquarters) and in Slovakia (MatTek Europe). Standardized QC tests (TEER measurements on 6+ randomly chosen tissues from the tissue lot) were conducted on each tissue lot produced during the period September 2020 – September 2023.

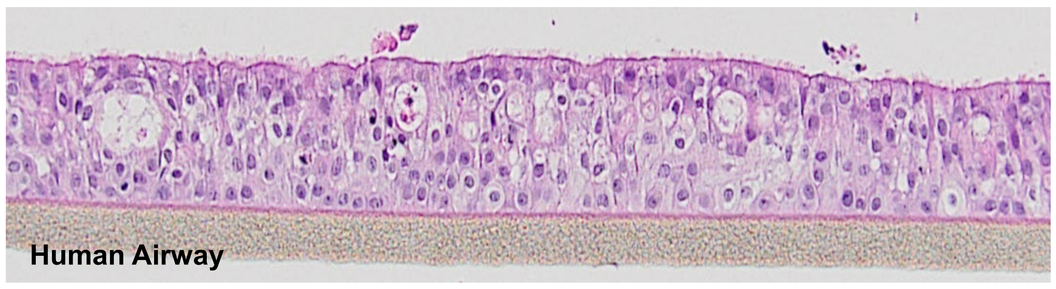
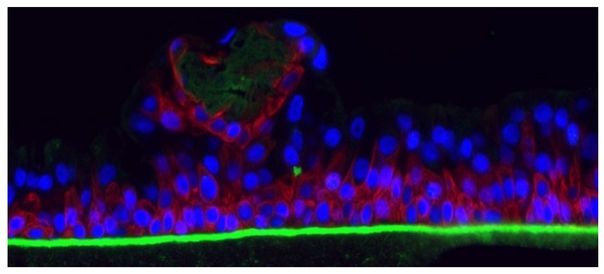
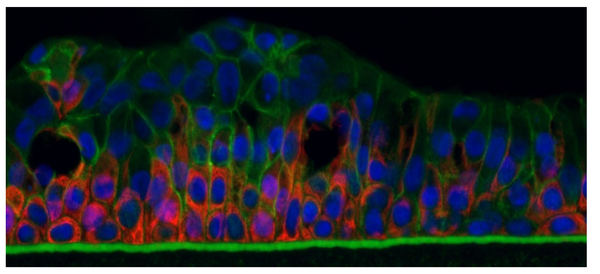


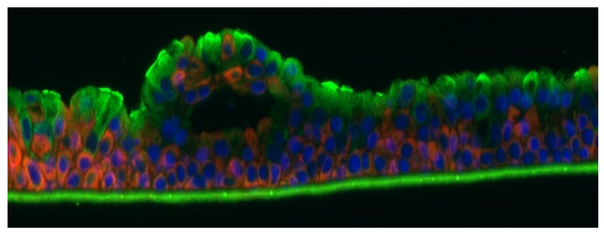
Figure 1: H&E-stained histological cross-sections of in vitro reconstructed airway tissue models reconstructed using cells from multiple species (human, rhesus monkey, and rat). Tissues show well developed cilia on the apical tissue surface.



Overlap: MUC 5B (green) and CK5 (red)



Overlap: E-cadherin (green) and CK5 (red)



Overlap: β-tubulin (green) and CK5 (red)

Figure 2: Immuno-stained histological cross-sections of in vitro reconstructed rhesus monkey airway tissue model. The tissue model shows well a developed epithelium (CK5), cilia (β-tubulin), tight junctions (E-cadherin), and mucus producing goblet cells (MUC 5B).

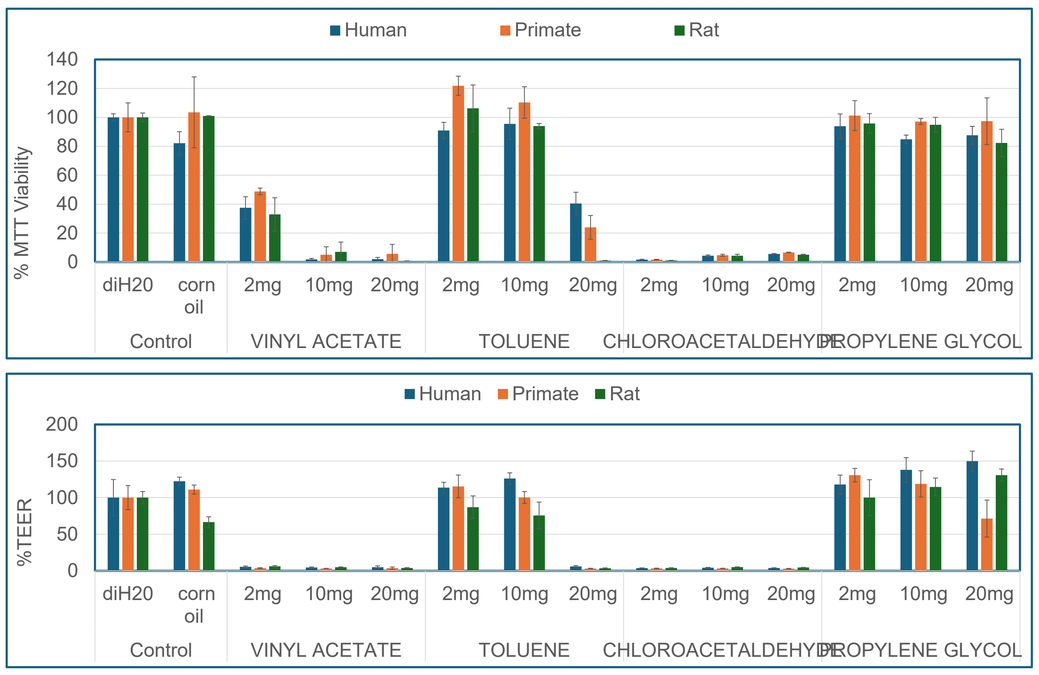


Figure 3: Tissue viability (MTT) and barrier integrity (TEER) of in vitro 3D airway tissue models reconstructed using cells from multiple species (human, rhesus monkey, and rat) following exposure to different concentrations of test chemicals.

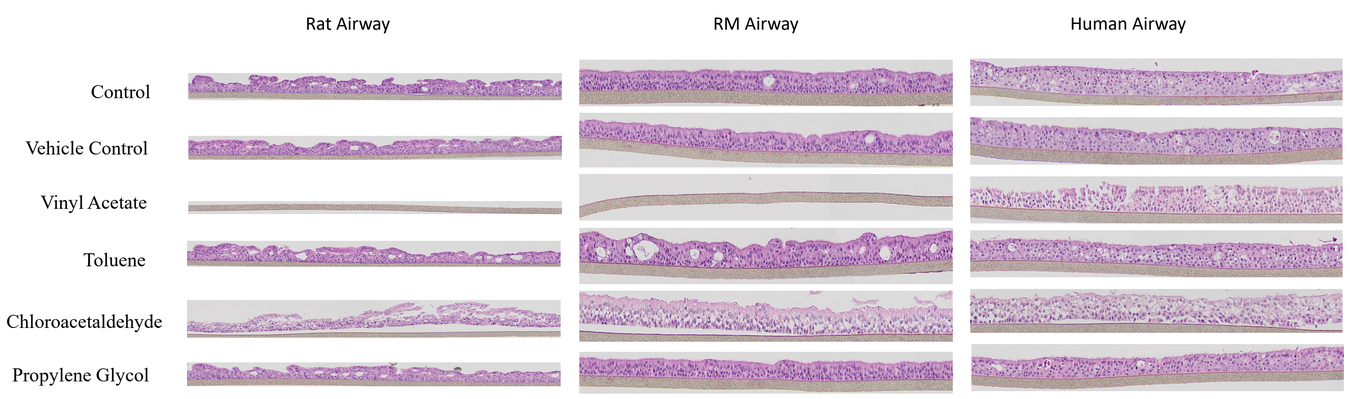


Figure 4: H&E-stained histological cross-sections of in vitro reconstructed airway tissue models from multiple species (rat, rhesus monkey, and human) exposed to chemical irritants (10 mg/ml) for 4 hr followed by a 20 hr recovery period.

	US	Slovakia
Avg (Ω*cm^2)	1094	913
St Dev	325	238
CV	29.7	26.0
Tissues lots	141	64
	ttest	0.00001

Table 1: QC results (TEER measurements) for EpiAirway tissue lots produced in the US at MatTek Headquarters (N=141) and in Slovakia at MatTek Europe (N=64). Tissues lots were produced during the period September 2020 – September 2023. A t-test comparison showed that the results were not statistically different ($p<0.0001$).

Conclusions

- Histological evaluation showed well polarized, stratified, and differentiated 3D tissue structures for each species donor (**Figure 1**).
- Immunohistochemical analysis showed the 3D airway tissues form epithelial layer (CK 5), cilia (β-tubulin), tight junction (E-cadherin), and mucus producing cells (goblet cells) as exemplified on rhesus monkey airway tissue model (**Figure 2**).
- Test articles, chloroacetaldehyde, vinyl acetate, and toluene, were identified as respiratory irritant in the 3 species (**Figure 3**).
- The MTT, TEER, and histology assays were found to be valuable endpoints identifying respiratory chemical irritants in tissues from the different species (**Figures 3 and 4**).
- Results were reproducible among the three species.
- Availability of airway tissue models from the three species most frequently used for screening of respiratory chemical irritation will have translational value and reduce animal use for experimentation.
- EpiAirway tissues produced in the US and in Europe were not statistically different (**Table 1**).

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