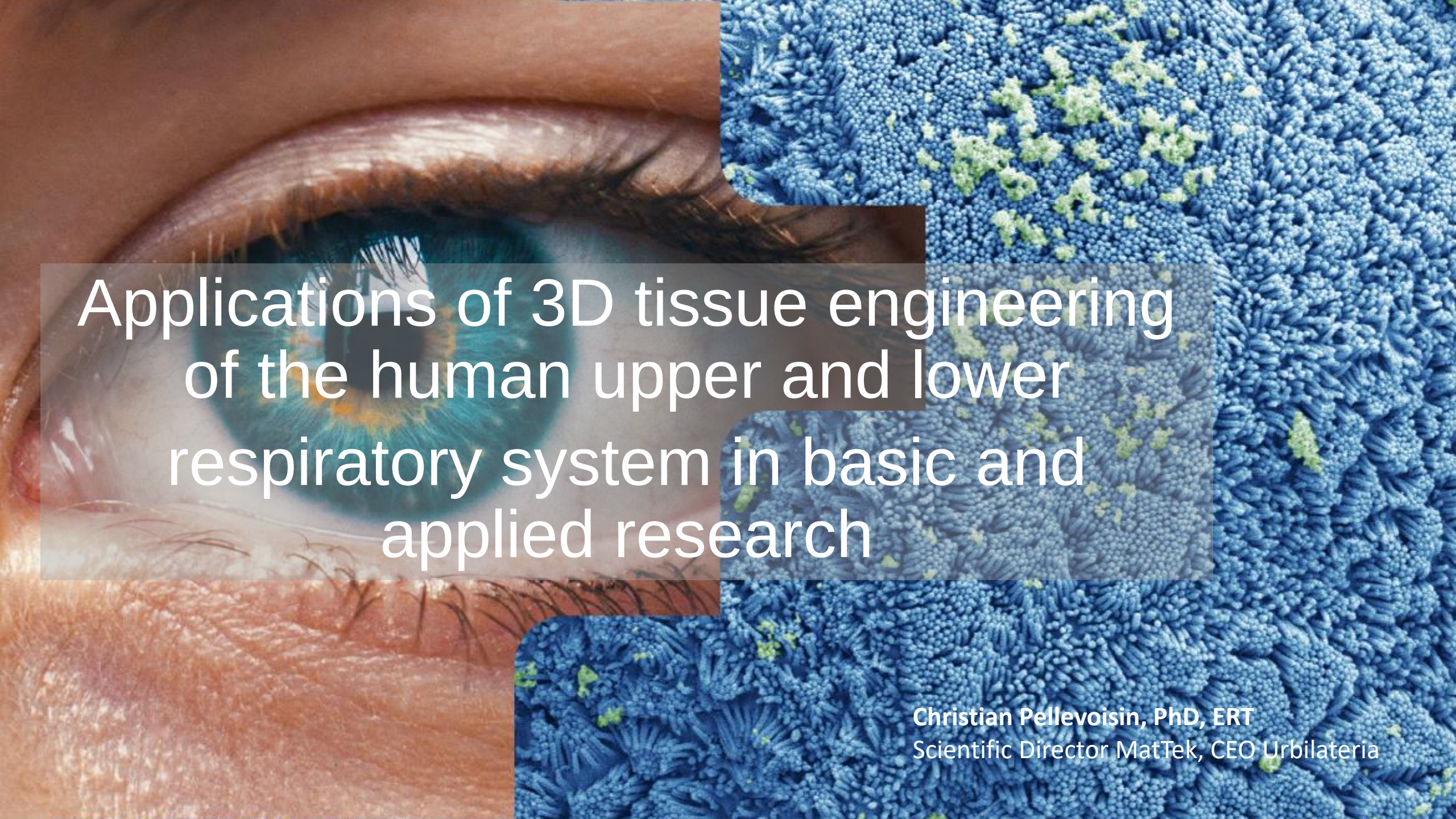
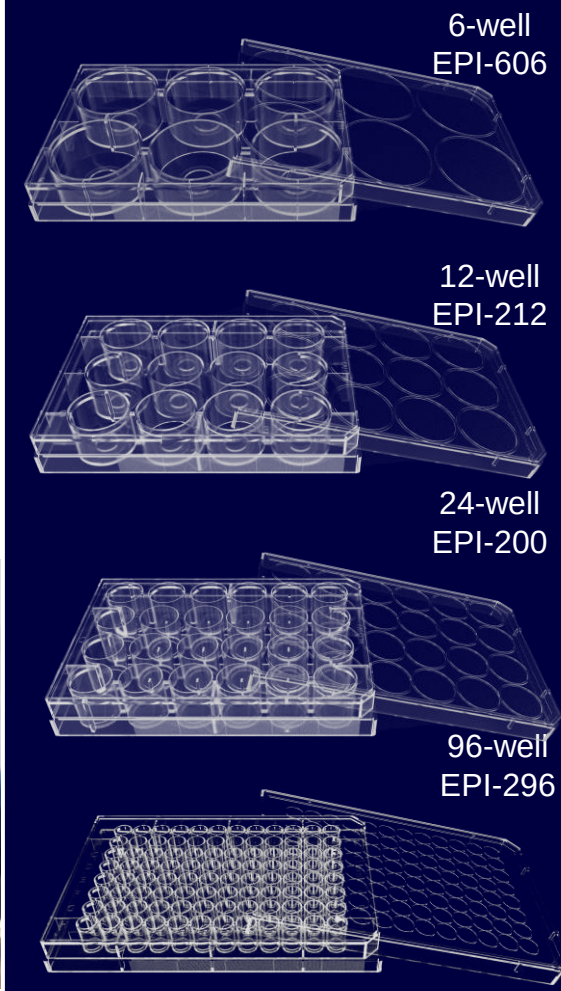


Applications of 3D tissue engineering of the human upper and lower respiratory system in basic and applied research

Christian Pellevoisin, PhD, ERT
Scientific Director MatTek, CEO Urbilateria

Principle of Reconstruction of Organotypic Models



Toxicological inhalation studies

Acute tox
(4h-14d)

OECD TG 403
(1981-2009) → Mortality

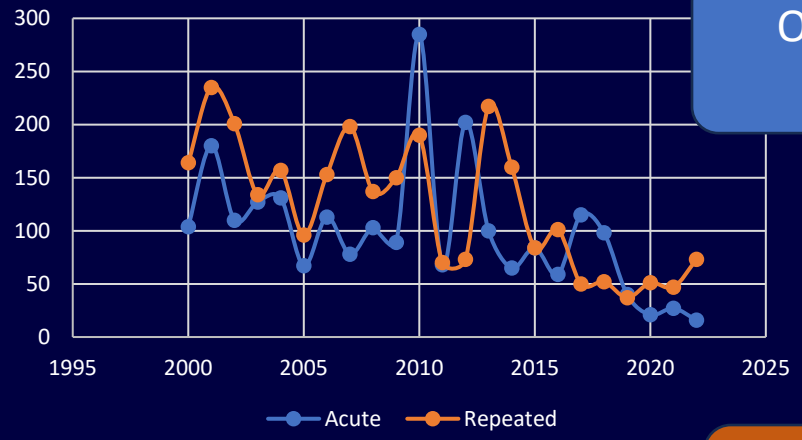
OECD TG 436
(2009) → Mortality → LC_{50} →

OECD TG 433
(2017) → Evident signs of toxicity*

GHS category

Globally Harmonized System (GHS) – acute toxicity					
Category 1	Category 2	Category 3	Category 3 Respiratory System	Category 4	Category 5
Danger		Danger	Danger	Warning	No pictogram Warning
330 Fatal if inhaled		331 Toxic if inhaled	335 May cause respiratory irritation	332 Harmful if inhaled	333 May be harmful if inhaled

Inhalation studies according to OECD TG



Subacute
/subchronic tox

OECD TG 412 (28d)
(2017) →
Bronchoalveolar lavage (BALF)
lung histopathology
Clinical pathology (haematology, chemistry)
Body weight, food consumption
Gross pathology/necropsy
→ BMC/LOAEC/NOAEC

OECD TG 413 (90d)
(2017) →
Optional investigations, such as toxicokinetics, and/or systemic toxicity evaluations (e.g., immune, hepatic, neurologic and/or cardiovascular effects evaluations)

* Evident toxicity has been reached if one or more animals display any one of the listed signs: tremors, hypoactivity, irregular respiration or bodyweight loss (>10% prestudy value).

Differences between rat and human respiratory tracts

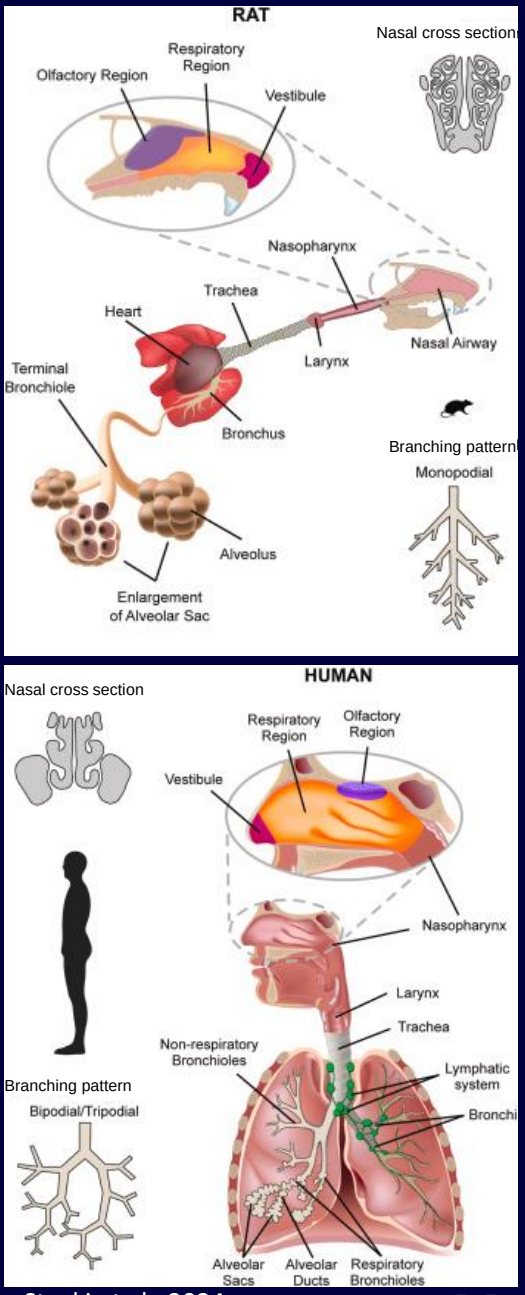
1. Nasal Anatomy: Rats have more complex nasal turbinates compared to humans, affecting airflow dynamics and particle filtration (Schreider J and Raabe O, 1981).

2. **Airway Structure:** Rats have a monopodial branching system, while humans have a symmetric branching pattern (Parent P, 2015). This affects particle deposition patterns, with humans experiencing deposition mainly at airway bifurcation points, which cannot be replicated in rat models (Hofmann W et al., 2018).

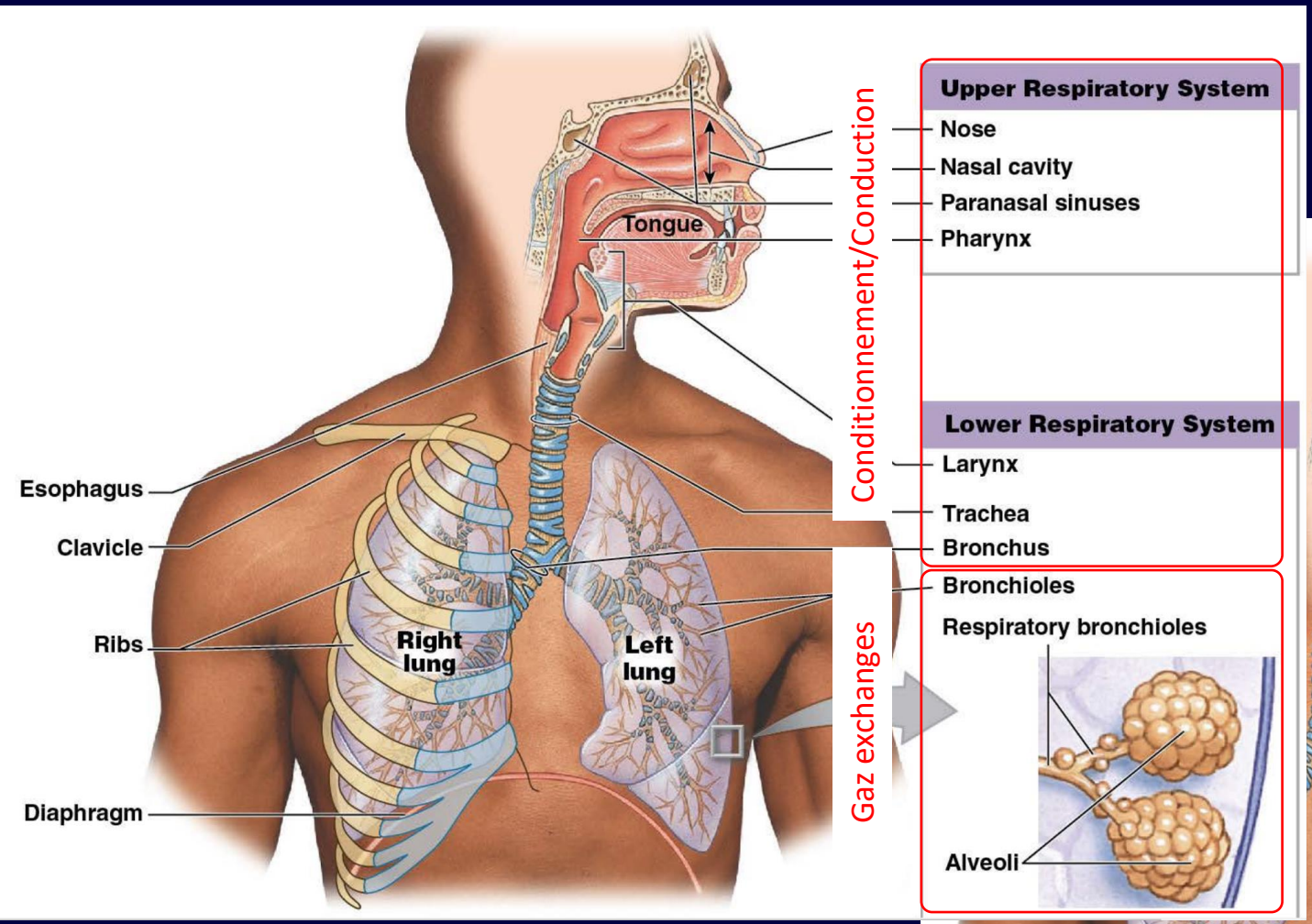
3.Breathing Mode: Rats are obligate nose breathers, while humans are oronasal breathers. This strongly influences how inhaled particles and gases deposit in the respiratory tract (Schlesinger R, 1985).

4.Airway Dimensions: Rat airways have smaller diameters than human airways, potentially leading to airway obstruction at high doses of insoluble aerosols, even when the compound is non-toxic to humans (Hofmann W et al., 2018).

5.Metabolic Variations: Cytochrome P450 activity in the nasal mucosa and lower respiratory tract is less efficient in humans compared to rats. Conversely, phase II enzymes are more active in humans than in rats (Pauluhn J, 2003).



Stucki et al., 2024



Conditionnement/Conduction

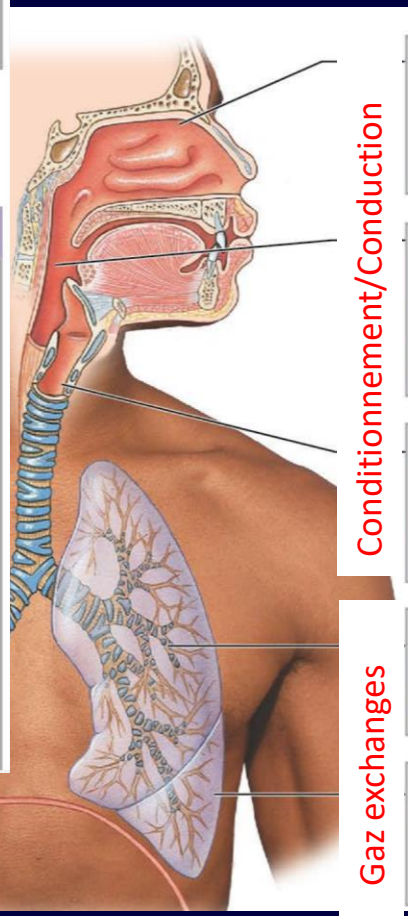
Upper Respiratory System

- Nose
- Nasal cavity
- Paranasal sinuses
- Pharynx

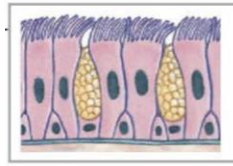
Lower Respiratory System

- Larynx
- Trachea
- Bronchus
- Bronchioles
- Respiratory bronchioles

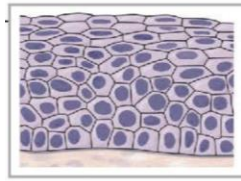
Gaz exchanges



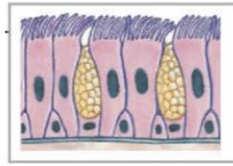
Conditionnement/Conduction



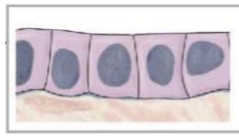
Respiratory mucosa, with mucous cells



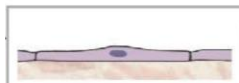
Stratified squamous epithelium



Respiratory mucosa with mucosa cells

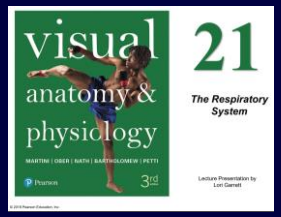


Cuboidal epithelium

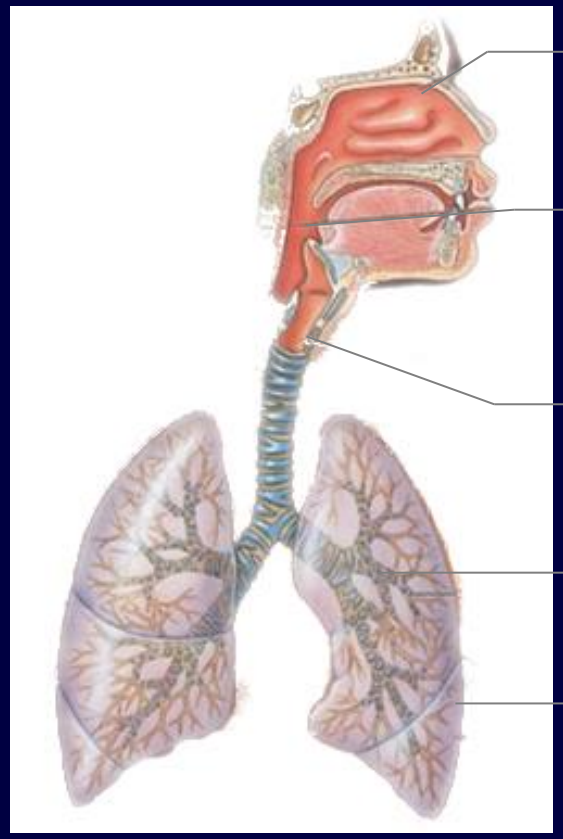


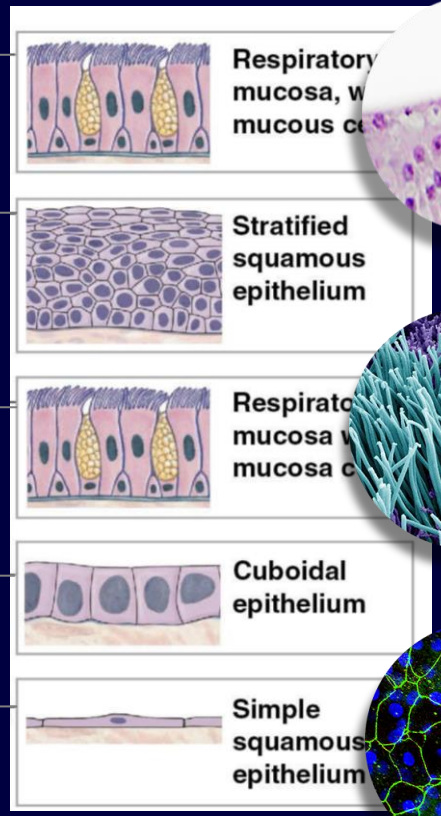
Simple squamous epithelium

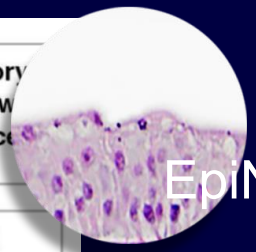
Gaz exchanges



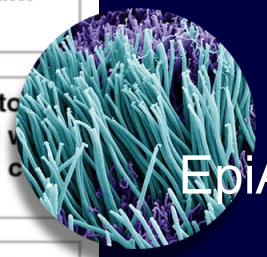
https://www.palmbeachstate.edu/slc/Documents/AandPch21LecturePearson.pdf



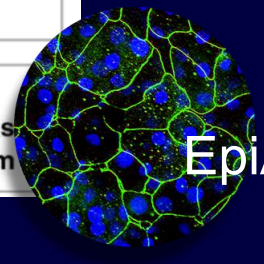




EpiNasal



EpiAirway



EpiAlveolar

Figure 2: H&E staining of the in vitro 3D human nasal epithelial tissue model.

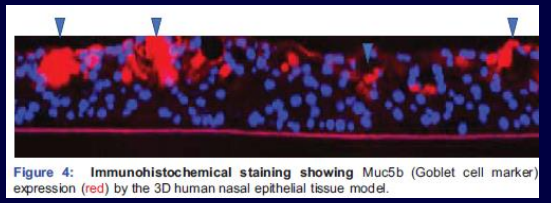


Figure 4: **Immunohistochemical staining showing** Muc5b (Goblet cell marker) expression (red) by the 3D human nasal epithelial tissue model.

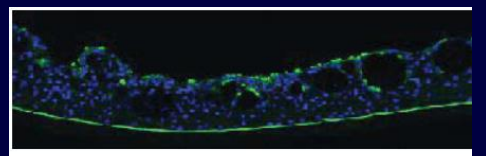
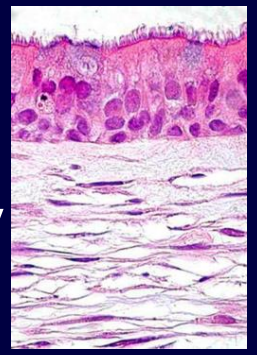
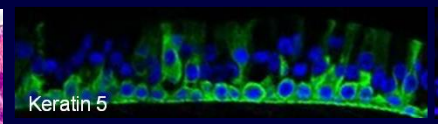
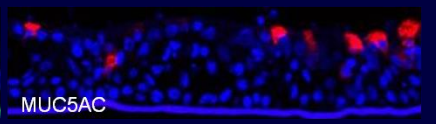
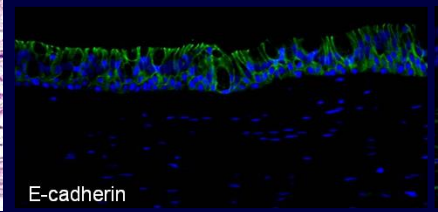


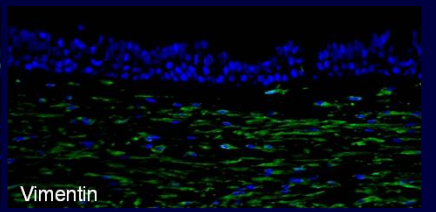
Figure 3: **Immunohistochemical staining showing** α-Tubulin (marker expression on the apical surface of the 3D human nasal epithelial tissue mode

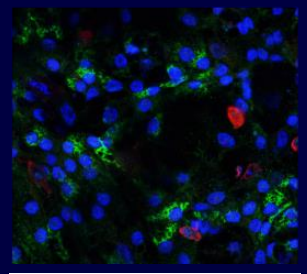




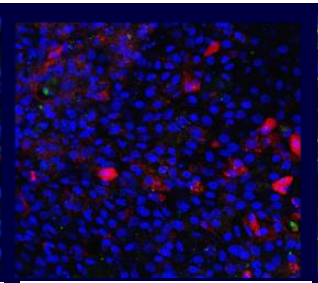




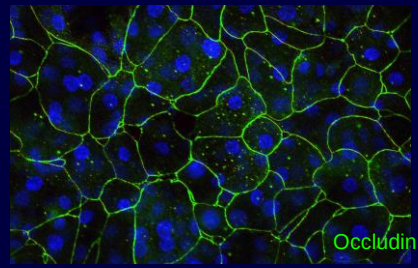


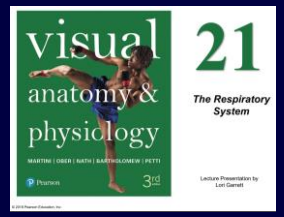


Confocal image of tissue stained for **Type I** cells (water channel protein Aquaporin 5, green), **Type II** cells (ABCA3, red), and nuclei (dapi, blue).



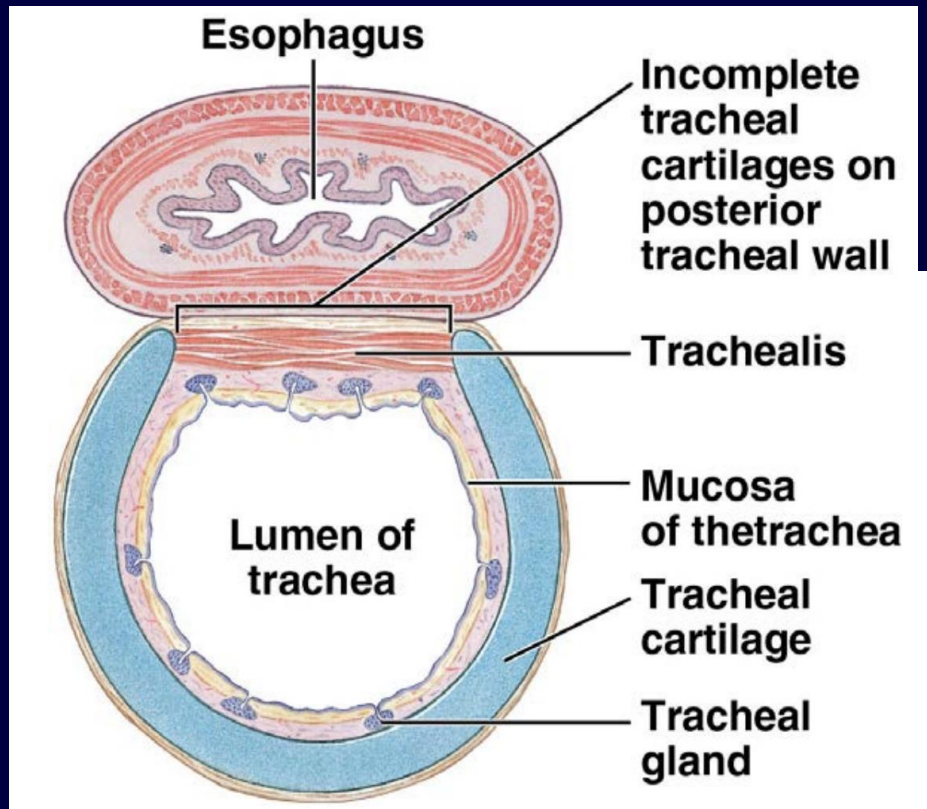
Confocal image of tissue stained for **Type II** marker Pro-surfactant protein C (red) and nuclei (dapi, blue)

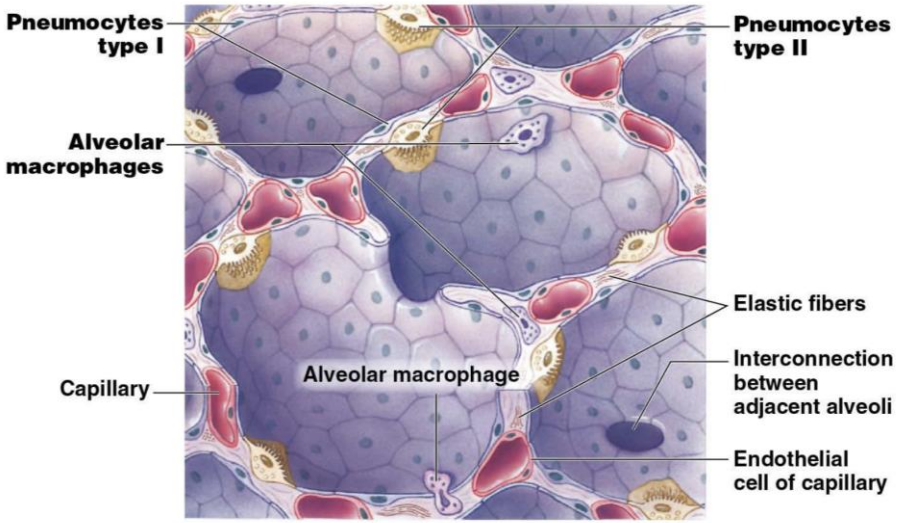


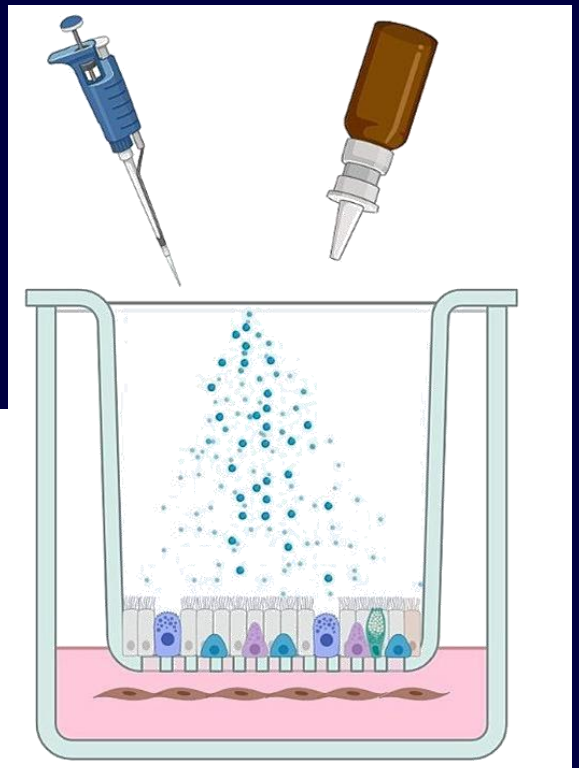


https://www.palmbeachstate.edu/slc/Documents/AandPch21LecturePearson.pdf

A.L.I. models

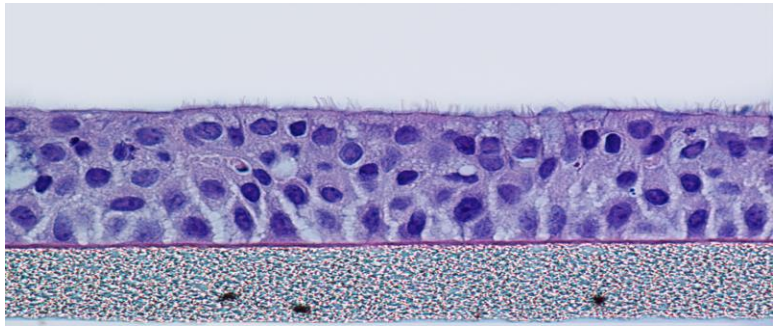




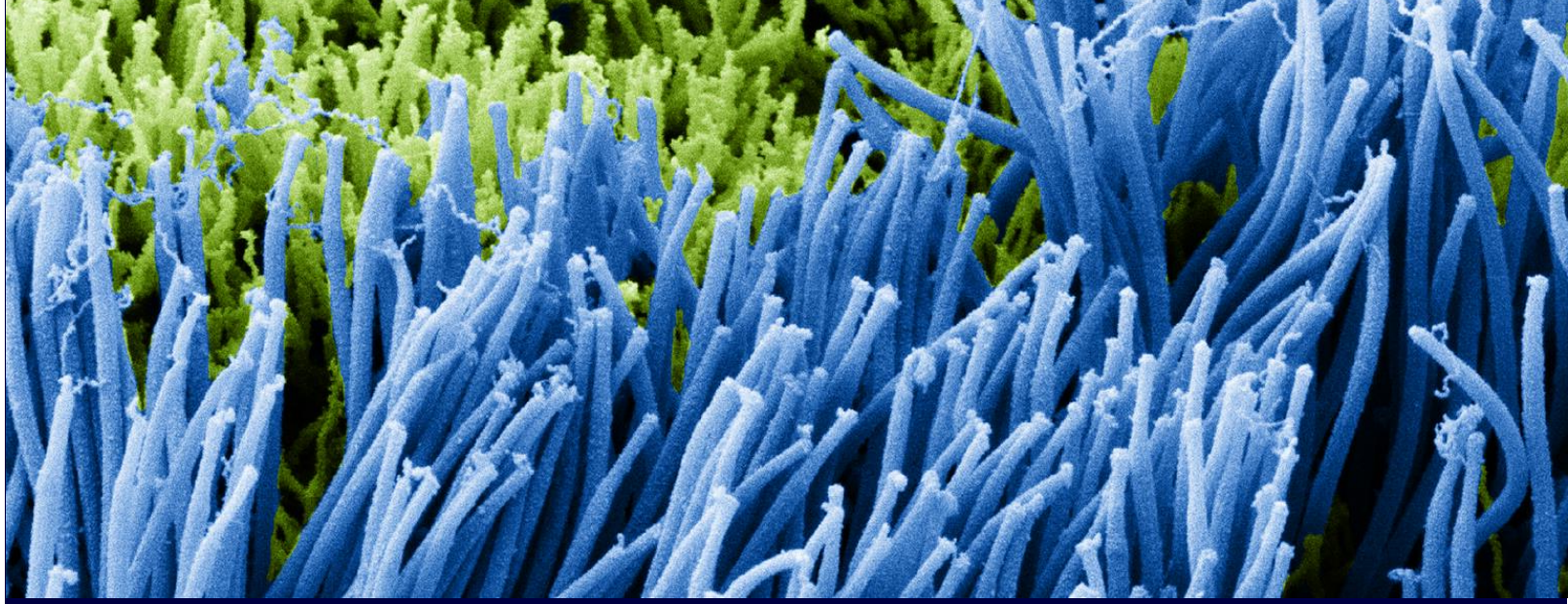


Air-liquid interface (ALI) conditions

EpiAirway



MatTek's EpiAirway is advancing in vitro respiratory research worldwide. Allowing for physiological exposures to pathogens, chemicals or therapeutics, EpiAirway's human-relevant biological responses are changing the way scientists research respiratory diseases and drug development.



30+ DONORS AVAILABLE

COPD ASTHMA SMOKER

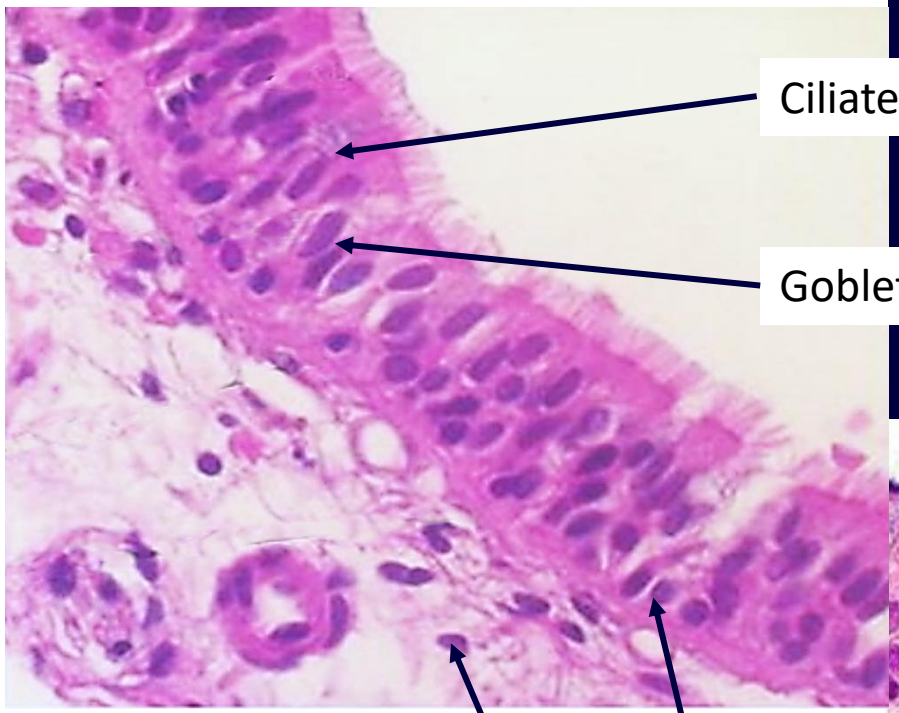
ADULT CHILD PATIENT HISTORIES

- **TOBACCO AND VAPE STUDIES**
- **VIRAL/BACTERIAL INFECTION**
- **DRUG DELIVERY**
- **INHALATION TOXICOLOGY**
- **INFLAMMATION AND FIBROSIS**
- **DISEASE MODELING (ASTHMA, COPD)**

Normal human bronchiole
(cross sectionH&E stained)

EpiAirway FT
(full thickness)

EpiAirway

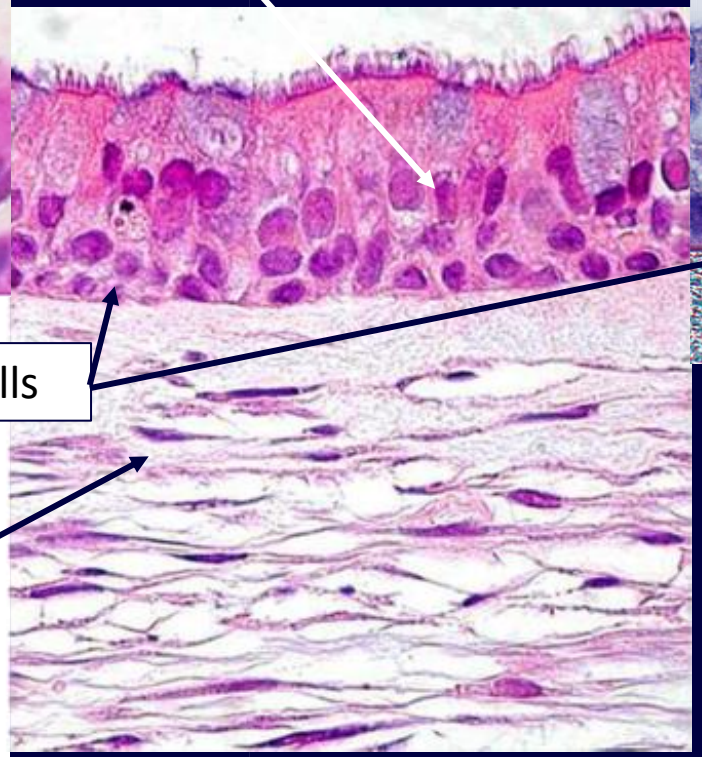


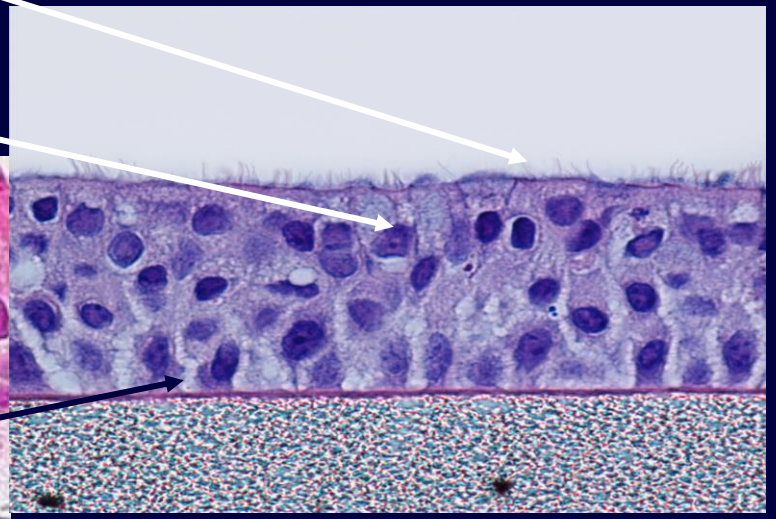
Ciliated cells

Goblet cells

Basal cells

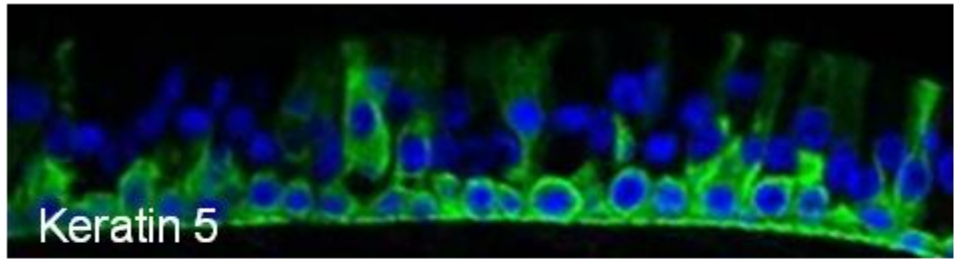
Stromal fibroblasts

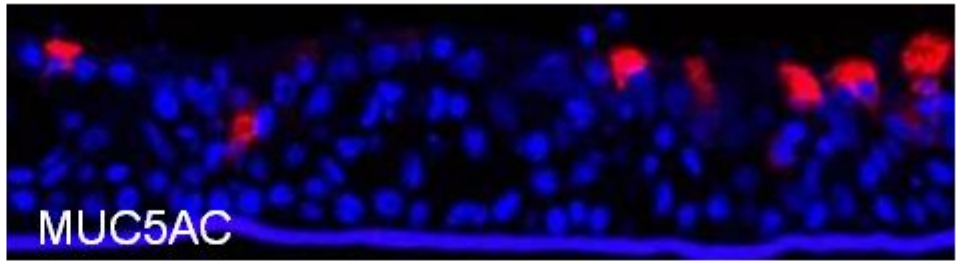




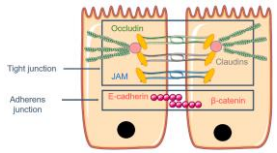
EpiAirway

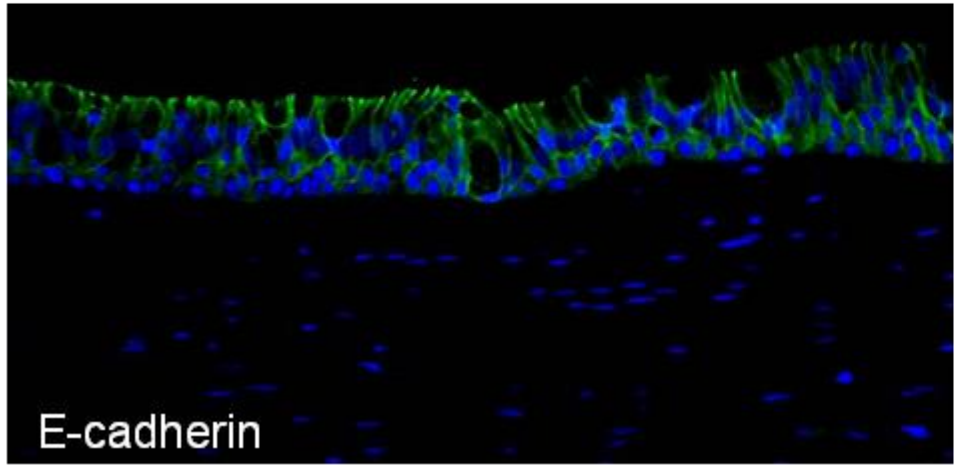
3D structure consists of organized Keratin 5+ basal cells mucus producing goblet cells functional tight junctions and beating cilia. EpiAirwayFT incorporates human fibroblasts in an extracellular stromal matrix ideal for inflammation and fibrosis research.

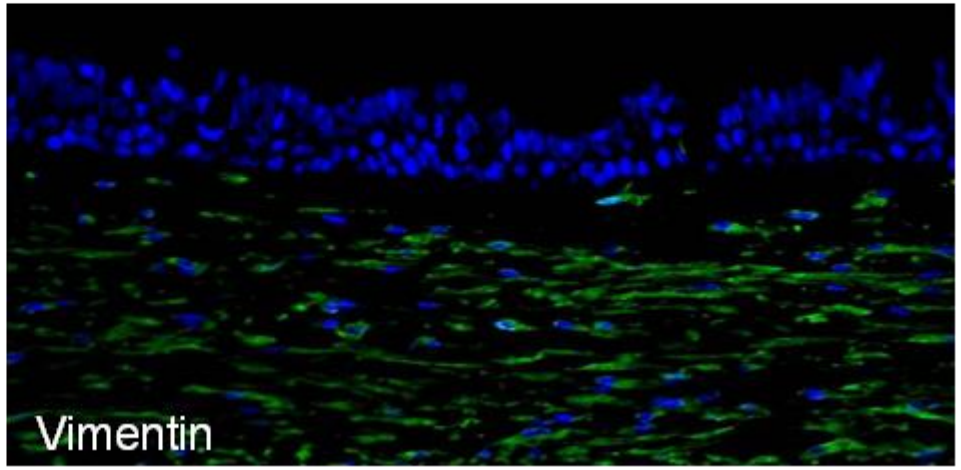




Muc5AC is a large gel-forming glycoprotein. In the respiratory tract it protects against infection by binding to inhaled pathogens that are subsequently removed by mucociliary clearance







Inhalation toxicology

APPLIED IN VITRO TOXICOLOGY
Volume 4, Number 2, 2018
Mary Ann Liebert, Inc.
DOI: 10.1089/aivt.2018.0004

Prevalidation of an Acute Inhalation Toxicity Test Using the EpiAirway *In Vitro* Human Airway Model

George R. Jackson, Jr., Anna G. Maione, Mitchell Klausner, and Patrick J. Hayden

Abstract

Introduction: Knowledge of acute inhalation toxicity potential is important for establishing safe use of chemicals and consumer products. Inhalation toxicity testing and classification procedures currently accepted within worldwide government regulatory systems rely primarily on tests conducted in animals. The goal of the current work was to develop and prevalidate a nonanimal (*in vitro*) test for determining acute inhalation toxicity using the EpiAirway™ *in vitro* human airway model as a potential alternative for currently accepted animal tests.
Materials and Methods: The *in vitro* test method exposes EpiAirway tissues to test chemicals for 3 hours, followed by measurement of tissue viability as the test endpoint. Fifty-nine chemicals covering a broad range of toxicity classes, chemical structures, and physical properties were evaluated. The *in vitro* toxicity data were utilized to establish a prediction model to classify the chemicals into categories corresponding to the currently accepted Globally Harmonized System (GHS) and the Environmental Protection Agency (EPA) system.
Results: The EpiAirway prediction model identified *in vivo* rat-based GHS Acute Inhalation Toxicity Category 1–2 and EPA Acute Inhalation Toxicity Category I–II chemicals with 100% sensitivity and specificity of 43.1% and 50.0%, for GHS and EPA acute inhalation toxicity systems, respectively. The sensitivity and specificity of the EpiAirway prediction model for identifying GHS specific target organ toxicity-single exposure (STOT-SE) Category 1 human toxicants were 75.0% and 56.5%, respectively. Corrosivity and electrophilic and oxidative reactivity appear to be the predominant mechanisms of toxicity for the most highly toxic chemicals.
Conclusions: These results indicate that the EpiAirway test is a promising alternative to the currently accepted animal tests for acute inhalation toxicity.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5994905/pdf/aivt.2018.0004.pdf

OECD Test Guideline 403/436:
Acute Inhalation Toxicity Test: Rat LD50 test

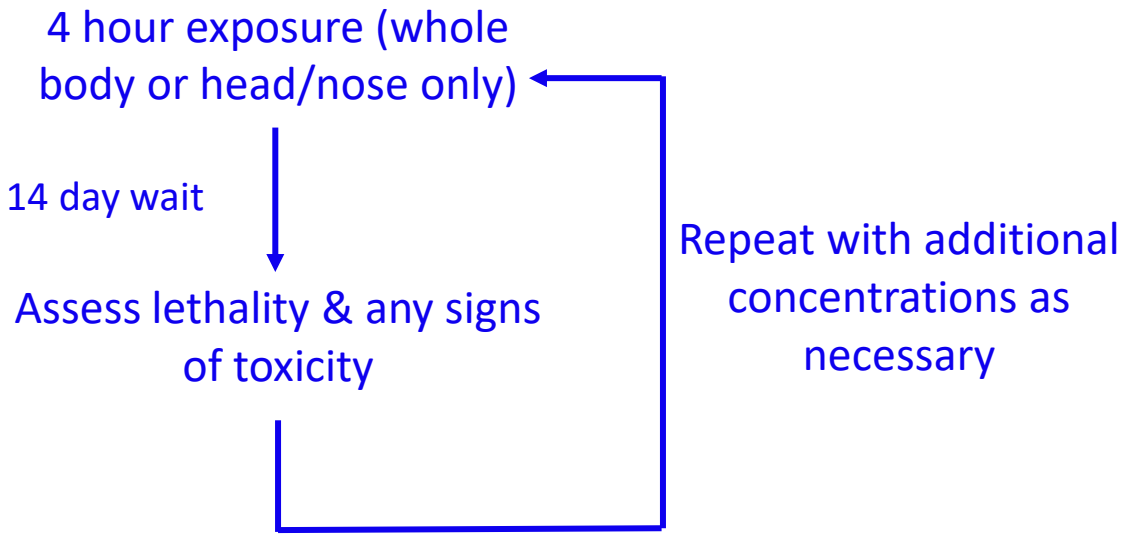
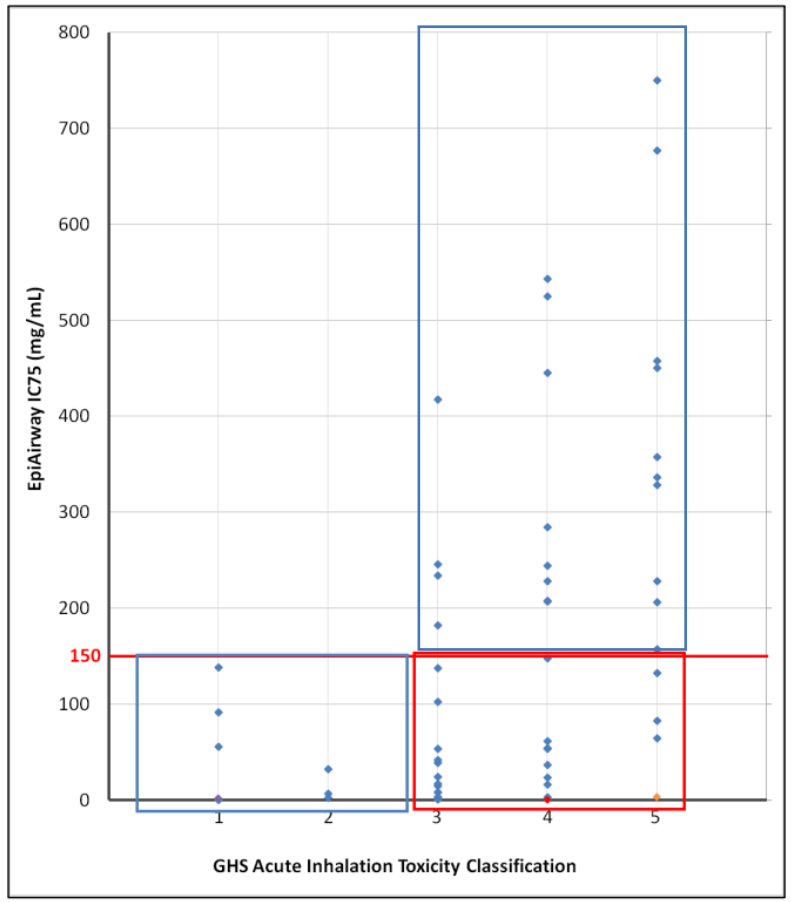
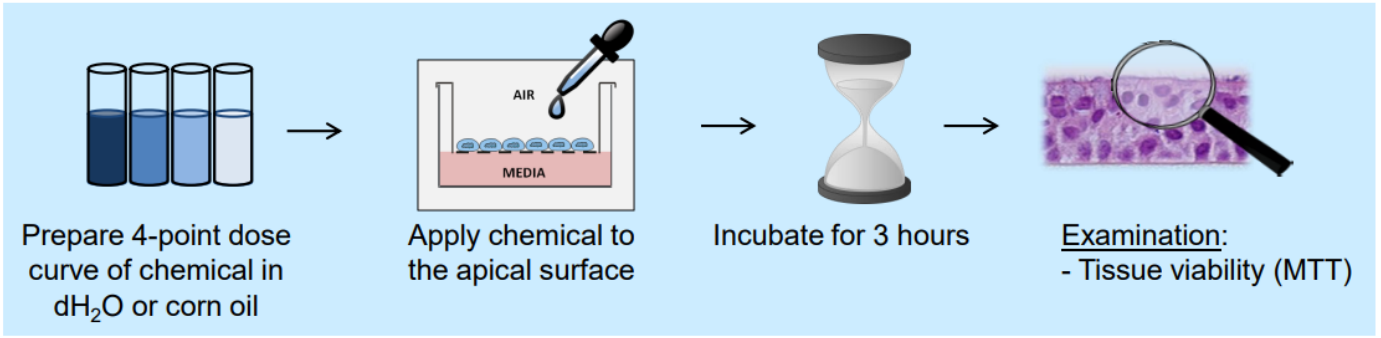


Figure 1A. Globally Harmonized System (GHS): Acute Toxicity

Category 1	Category 2	Category 3	Category 4	Category 5
				No pictogram
Danger	Danger	Danger	Warning	Warning
330 Fatal if inhaled	**330** Fatal if inhaled	**331** Toxic if inhaled	**332** Harmful if inhaled	**333** May be harmful if inhaled





-Tested 59 chemicals with a range of inhalation toxicities

-Determined IC75 (dose at which tissues are 75% viable)

-Correlated in vitro data to in vivo rat LD50 data (GHS category) to develop a prediction model

EpiAirway IC75	*GHS Acute Inhalation Toxicity Category*		
	1–2	*≥3*	*Total*
≤150 mg/mL	8	29	37
>150 mg/mL	0	22	22
Total	8	51	58

Compared to GHS Rat Data	
Sensitivity	8/8 = 100%
Specificity	22/51 = 43%
Overall Accuracy	30/59 = 51%

Highly toxic (GHS Category 1-2); moderately toxic (GHS Categorie 3); mildly toxic (GHS Categories 4–5) and nontoxic or nonhazardous.

P16-46

Development and Validation of in vitro Human Inhalation Toxicity Tests for Volatile Liquids, Mists, and Sprays (#718)

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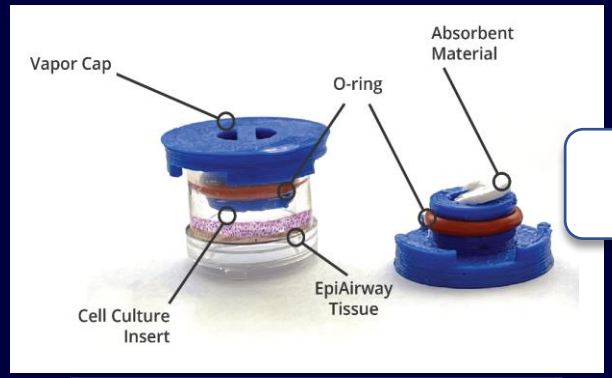


2 laboratories
53 chemicals
EpiAirway

Vapor Cap
Neat or diluted in oil
0.5, 2, 10, 20 mg/tissue

Direct Application
Diluted in oil or water
0.5, 2, 10, 20, 100 mg/tissue

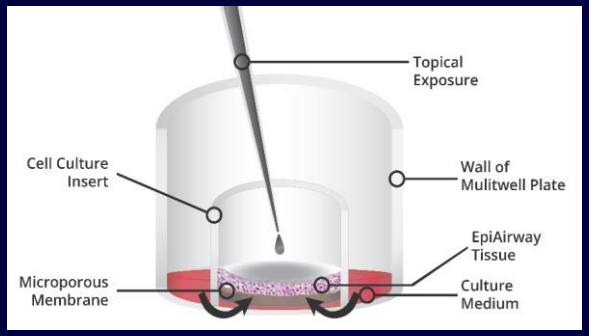
Exposure 4h
Covered with Vapor Cap
Wash with PBS

Post-Exposure 20h

MTT Assay

Measure TEER

Effective doses (ED) which reduced tissue viability by 25% (ED-25) or by 75% (ED-75)



Prediction Model ED-75

GHS CLASSIFICATION	
Cat.1&2	≤ 5 mg/tissue
Cat.3&4	5 to 15 mg/tissue
Cat.5&NC	≥ 15 mg/tissue

Prediction Model ED-25

GHS CLASSIFICATION	
Cat.1&2	≤ 5 mg/tissue
Cat.3&4	5 to 20 mg/tissue
Cat.5&NC	≥ 15 mg/tissue

Performances

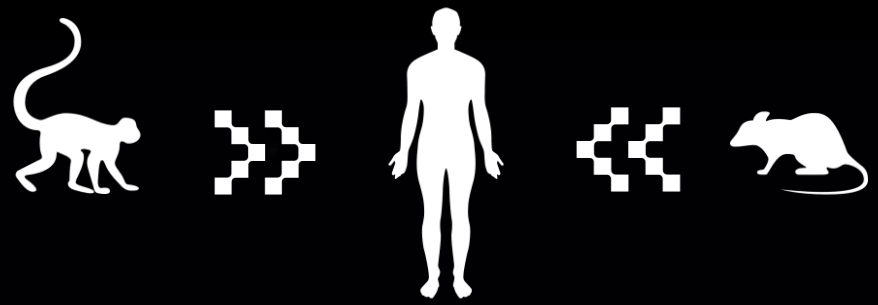
Vapor Cap	GHS hazard classification			
	MTT		TEER	
	MatTek	IVLSL	MatTek	IVLSL
Sensitivity (%)	70,8	71,9	64,4	67,1
Specificity (%)	83,2	83,2	78,5	80,1
Accuracy (%)	77	77,5	71,5	73,6

Performances

Direct Application	GHS hazard classification			
	MTT		TEER	
	MatTek	IVLSL	MatTek	IVLSL
Sensitivity (%)	63,5	63,8	65,9	64,1
Specificity (%)	76,1	76,1	76,7	76,6
Accuracy (%)	69,8	70	71,3	70,3

Bridging the gap.

MatTek's lab-grown airway tissue models of rats and non-human primates provide translatability, further reduce the use of live animal experiments, and encourage the adoption of NAMs in testing pipelines.



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

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Abstract ID # 4626 Poster # P516

Abstract

Scalable, reproducible in vitro 3-dimensional organotypic models from different species are needed for translational studies to develop reliable alternatives to traditional in vivo animal inhalation toxicity tests. The aim of this study is to compare toxicity responses of 3D airway tissue models constructed using primary tracheobronchial epithelial cells harvested from rat, primate, and human tissues. Primary cells from the different species were expanded in monolayer culture and seeded onto microporous membrane inserts to reconstruct 3D organotypic tissue models. Tissues were characterized for polarity of epithelial cells (histology), epithelial cell markers (IHC), barrier integrity (transepithelial electrical resistance, TEER measurements), and functionality in inhalation toxicological studies by testing 3 well-characterized chemical toxicants (CT). Polyethylene glycol was used as the positive control and water, or corn oil was used as the negative control. CT (100 µL) were applied to the apical surface, and tissue inserts were sealed with insert caps (MILICEL-MTK-CAP, MatTek Life Sciences) for 4 hr to mimic in vivo rat exposure experiments. After 4 hr, dosed tissues were washed and allowed to recover for 20 hr at 37°C. Analysis of the 3D tissues from the different species showed a well polarized epithelium with a physiological TEER values of >300 Ω*cm², cilia formation on the apical surface, and mucin production mimicking the airway microenvironment. Acute exposure to CT for 4 hr showed varying levels of tissue viability and membrane integrity by MTT and TEER assays, respectively. The effective dose concentration that reduced tissue viability by 50% (ED-50) for vinyl acetate and chloroacetaldehyde were comparable (<2 mg/tissue) for all species, but the ED-50 value for toluene showed differences: 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: chloroacetaldehyde > vinyl acetate > toluene > propylene glycol and the vehicle controls. While the human and primate airway models showed comparable MTT values, the rat airway tissue was more sensitive to the higher concentration of toluene. 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 an 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-polyethylene terephthalate 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: Tissues were characterized for polarity of epithelial cells (histology), barrier integrity (transepithelial electrical resistance, TEER measurement), epithelial cell markers (IHC), and functionality in inhalation toxicological studies by testing 3 well-characterized chemical toxicants (CT). 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**). Unstained slides were used show cilia (β-tubulin), tight junction (E-cadherin), epithelium (CK5), and mucus producing goblet cells (MUC 5B) (**Figure 2**).

Test Article 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) 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 chemicals, 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 EVOM volt-ohmmeter equipped with an Endohm electrode chamber (World Precision Instruments, Sarasota, FL). %TEER was calculated as TEER (Ohms*cm²) 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**).

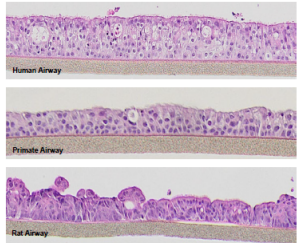


Figure 1: H&E-stained histological cross-sections of in vitro reconstructed airway tissue models reconstructed using cells from multiple species (human, rhesus monkey, and rat). Tissues show well developed cilia on the apical tissue surface.

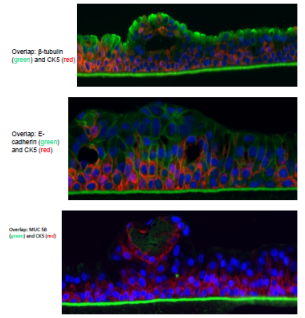
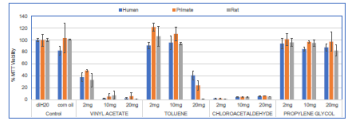


Figure 2: Imuno-stained histological cross-sections of in vitro reconstructed rhesus monkey airway tissue model. The tissues model show well developed epithelium (CK5) and cilia (β-tubulin), tight junction (E-cadherin), and mucus producing goblet cells (MUC 5B.



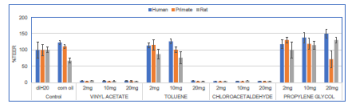


Figure 3: Tissue viability (MTT) and barrier integrity (TEER) of in vitro 3D airway tissue models reconstructed using cells from multiple species (human, rhesus monkey, and rat) following exposure to different concentrations of test chemicals.

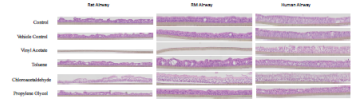
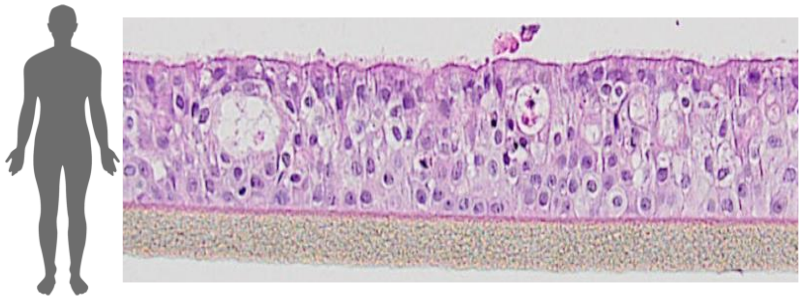
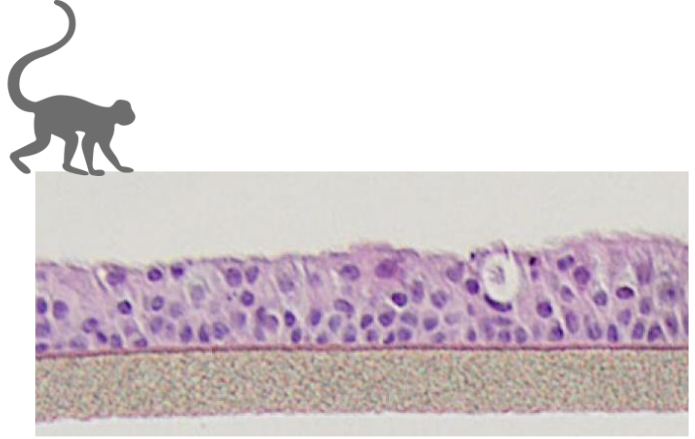


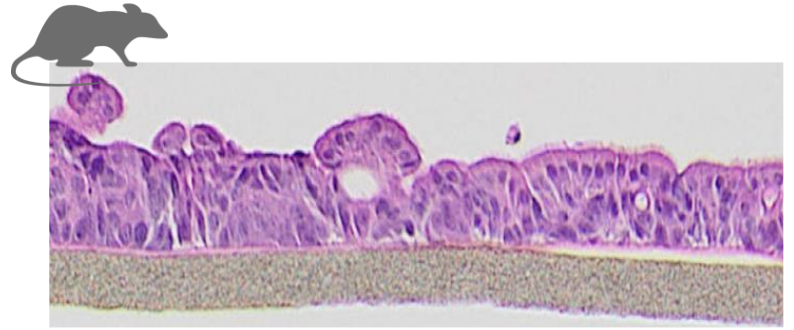
Figure 4: H&E-stained histological cross-sections of in vitro reconstructed airway tissue models from multiple species (rat, rhesus monkey, and human) exposed to chemical irritants (10 mg/ml) for 4 hr followed by a 20 hr recovery period.

Conclusions

- Histological evaluation showed a well polarized, stratified, and differentiated 3D tissue structure 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.







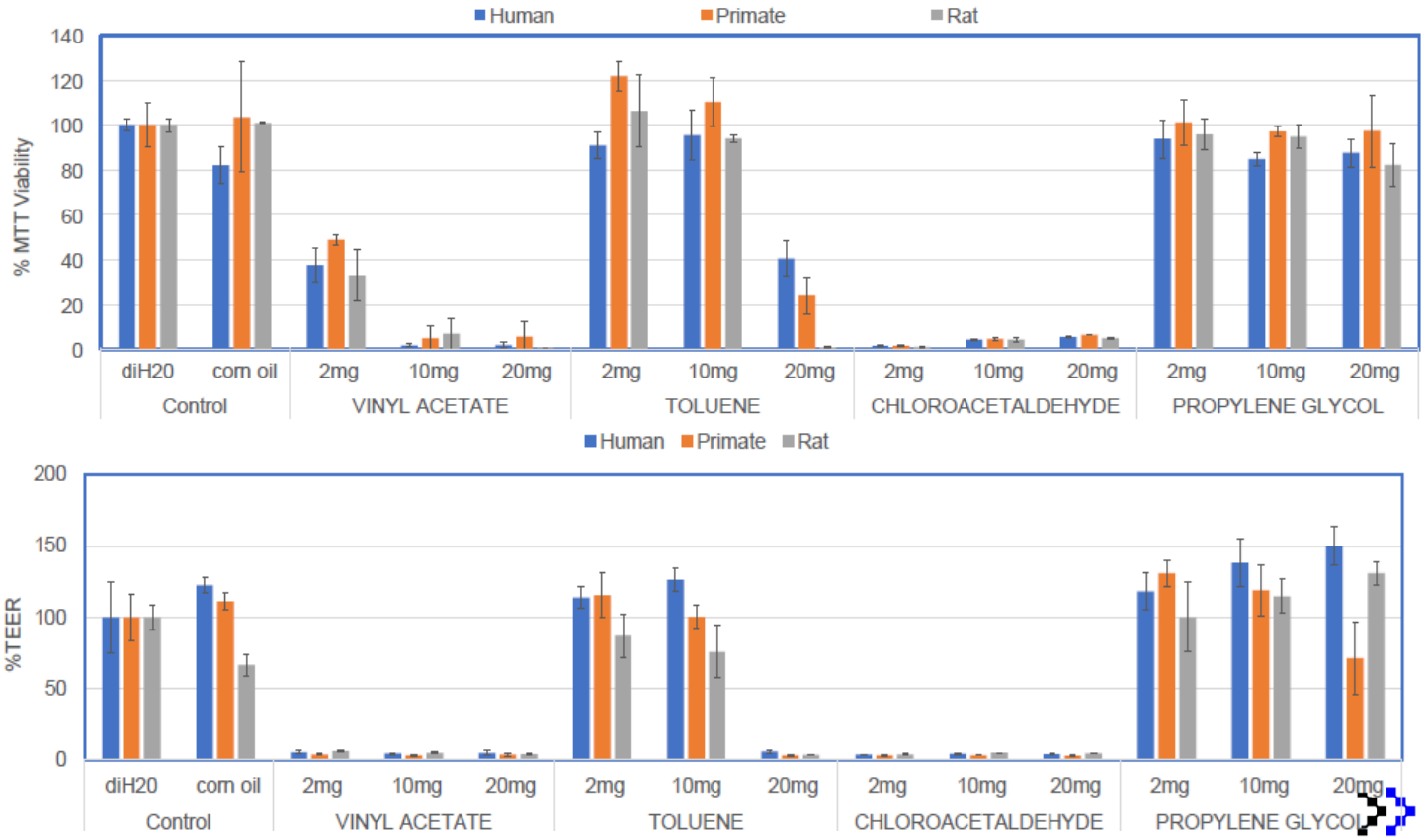
The animal cell-derived 3D tissues exhibited similar characteristics to human tissues including: well polarized epithelia with physiological TEER values of >300 Ω/cm2,, cilia formation on the apical surfaces, and mucin production mimicking the airway microenvironment.

Test articles, chloroacetaldehyde, vinyl acetate, and toluene, were identified as respiratory irritant in the 3 species

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.



INHALATION TOXICOLOGY

OECD

1. **TG 403** – Acute toxicity (LC50)
2. **TG 436** – new Acute Toxicicty (fewer animals)
3. **TG 412** – 28-day inhalation guideline
4. **TG 413** – 90-day inhalation guideline
5. **TG 433** – fixed concentration procedure

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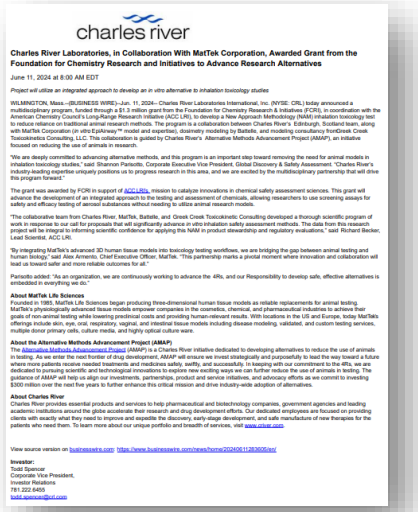
$1.3 million grant from the Foundation for Chemistry Research & Initiatives (FCRI)



charles river

Charles River Laboratories, in Collaboration With MatTek Corporation, Awarded Grant from the Foundation for Chemistry Research and Initiatives to Advance Research Alternatives

June 11, 2024 at 8:00 AM EDT

Project will utilize an integrated approach to develop an in vitro alternative to inhalation toxicology studies

WILMINGTON, Mass.--(BUSINESS WIRE)--Jun. 11, 2024-- Charles River Laboratories International, Inc. (NYSE: CRL) today announced a multidisciplinary program, funded through a $1.3 million grant from the Foundation for Chemistry Research & Initiatives (FCRI), in coordination with the American Chemistry Council's Long-Range Research Initiative (ACC LRI), to develop a New Approach Methodology (NAM) inhalation toxicology test to reduce reliance on traditional animal research methods. The program is a collaboration between Charles River's Edinburgh, Scotland team, along with MatTek Corporation (*in vitro* EpiAirway™ model and expertise), dosimetry modeling by Battelle, and modeling consultancy fromGreek Creek Toxicokinetics Consulting, LLC. This collaboration is guided by Charles River's Alternative Methods Advancement Project (AMAP), an initiative focused on reducing the use of animals in research.

"We are deeply committed to advancing alternative methods, and this program is an important step toward removing the need for animal models in inhalation toxicology studies," said Shannon Parisotto, Corporate Executive Vice President, Global Discovery & Safety Assessment. "Charles River's industry-leading expertise uniquely positions us to progress research in this area, and we are excited by the multidisciplinary partnership that will drive this program forward."

The grant was awarded by FCRI in support of ACC LRI's mission to catalyze innovations in chemical safety assessment sciences. This grant will advance the development of an integrated approach to the testing and assessment of chemicals, allowing researchers to use screening assays for safety and efficacy testing of aerosol substances without needing to utilize animal research models.

"The collaborative team from Charles River, MatTek, Battelle, and Greek Creek Toxicokinetic Consulting developed a thorough scientific program of work in response to our call for proposals that will significantly advance *in vitro* inhalation safety assessment methods. The data from this research project will be integral to informing scientific confidence for applying this NAM in product stewardship and regulatory evaluations," said Richard Becker, Lead Scientist, ACC LRI.

"By integrating MatTek's advanced 3D human tissue models into toxicology testing workflows, we are bridging the gap between animal testing and human biology," said Alex Armento, Chief Executive Officer, MatTek. "This partnership marks a pivotal moment where innovation and collaboration will lead us toward safer and more reliable outcomes for all."

Parisotto added: "As an organization, we are continuously working to advance the 4Rs, and our Responsibility to develop safe, effective alternatives is embedded in everything we do."

About MatTek Life Sciences
Founded in 1985, MatTek Life Sciences began producing three-dimensional human tissue models as reliable replacements for animal testing. MatTek's physiologically advanced tissue models empower companies in the cosmetics, chemical, and pharmaceutical industries to achieve their goals of non-animal testing while lowering preclinical costs and providing human-relevant results. With locations in the US and Europe, today MatTek's offerings include skin, eye, oral, respiratory, vaginal, and intestinal tissue models including disease modeling, validated, and custom testing services, multiple donor primary cells, culture media, and highly optical culture ware.

About the Alternative Methods Advancement Project (AMAP)
The Alternative Methods Advancement Project (AMAP) is a Charles River initiative dedicated to developing alternatives to reduce the use of animals in testing. As we enter the next frontier of drug development, AMAP will ensure we invest strategically and purposefully to lead the way toward a future where more patients receive needed treatments and medicines safely, swiftly, and successfully. In keeping with our commitment to the 4Rs, we are dedicated to pursuing scientific and technological innovations to explore new exciting ways we can further reduce the use of animals in testing. The guidance of AMAP will help us align our investments, partnerships, product and service initiatives, and advocacy efforts as we commit to investing $300 million over the next five years to further enhance this critical mission and drive industry-wide adoption of alternatives.

About Charles River
Charles River provides essential products and services to help pharmaceutical and biotechnology companies, government agencies and leading academic institutions around the globe accelerate their research and drug development efforts. Our dedicated employees are focused on providing clients with exactly what they need to improve and expedite the discovery, early-stage development, and safe manufacture of new therapies for the patients who need them. To learn more about our unique portfolio and breadth of services, visit www.criver.com.

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Charles River Laboratories, in Collaboration With MatTek Corporation, Awarded Grant from the Foundation for Chemistry Research and Initiatives to Advance Research Alternatives
June 11, **2024** at 8:00 AM EDT

<u>Project is to develop an in vitro integrated approach using 3D models as alternative to inhalation toxicology studies</u>

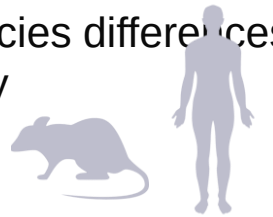
New Approach Methodology for Inhalation Tox

- Select **10 chemicals and 5 aerosols**

  With historical ***in vivo*** **and** ***in vitro*** **data**

- Experimental in vitro method:
  - **3D EpiAirway model**

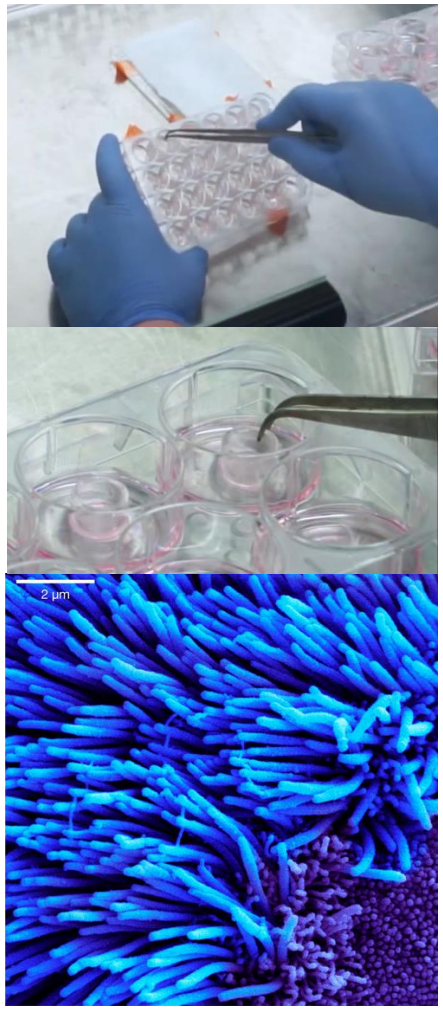
➔ Study translational species differences with same exposure using:
- **Human** EpiAirway
- **Rat** EpiAirway

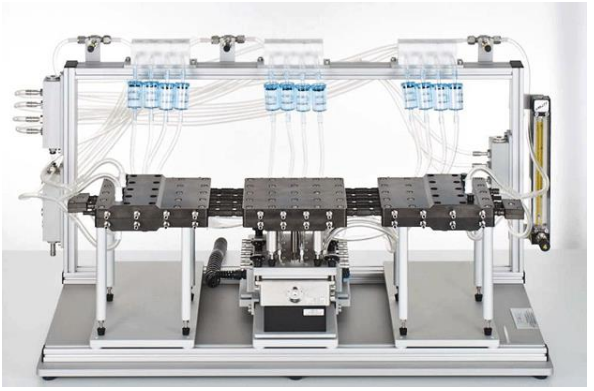


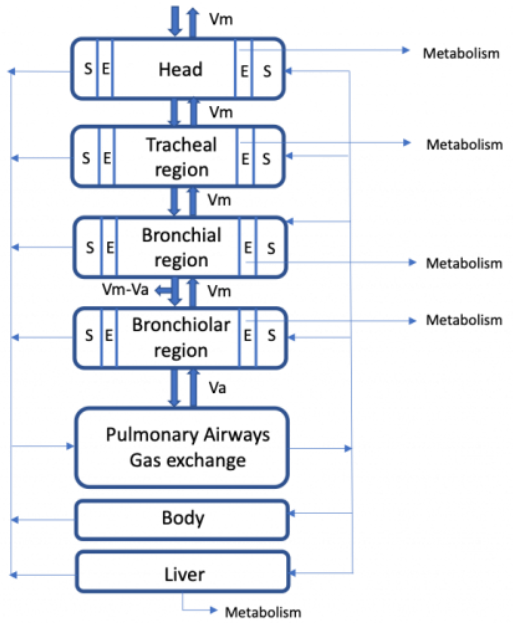
➔ Study **Acute/chronic** exposure
- Acute exposure: EpiAirway 4 exposure test
- Chronic exposure: EpiAirway 14-day scenario

➔ Compare exposure by applying test chemicals:
- by **direct application** with a pipette
- with **aerosol** application using our Vitrocell™ equipment.

➔ Study a **dosimetry model** to extrapolate the in vivo outcomes from the in vitro data.







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Diesel exhaust particle exposure exacerbates ciliary and epithelial barrier dysfunction in the multiciliated bronchial epithelium models

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ABSTRACT

Airway epithelium, the first defense barrier of the respiratory system, facilitates mucociliary clearance against inflammatory stimuli, such as pathogens and particulates inhaled into the airway and lung. Inhaled particulate matter 2.5 ($PM_{2.5}$) can penetrate the alveolar region of the lung, and it can develop and exacerbate respiratory diseases. Although the pathophysiological effects of $PM_{2.5}$ in the respiratory system are well known, its impact on mucociliary clearance of airway epithelium has yet to be clearly defined. In this study, we used two different 3D in vitro airway models, namely the EpiAirway-full-thickness (FT) model and a normal human bronchial epithelial cell (NHBE)-based air-liquid interface (ALI) system, to investigate the effect of diesel exhaust particles (DEPs) belonging to $PM_{2.5}$ on mucociliary clearance. RNA-sequencing (RNA-Seq) analyses of EpiAirway-FT exposed to DEPs indicated that DEP-induced differentially expressed genes (DEGs) are related to ciliary and microtubule function and inflammatory-related pathways. The exposure to DEPs significantly decreased the number of ciliated cells and shortened ciliary length. It reduced the expression of cilium-related genes such as acetylated α-tubulin, ARL13B, DNAH5, and DNAL1 in the NHBEs cultured in the ALI system. Furthermore, DEPs significantly increased the expression of MUC5AC, whereas they decreased the expression of epithelial junction proteins, namely, ZO1, Occludin, and E-cadherin. Impairment of mucociliary clearance by DEPs significantly improved the release of epithelial-derived inflammatory and fibrotic mediators such as IL-1β, IL-6, IL-8, GM-CSF, MMP-1, VEGF, and S100A9. Taken together, it can be speculated that DEPs can cause ciliary dysfunction, hyperplasia of goblet cells, and the disruption of the epithelial barrier, resulting in the hyperproduction of lung injury mediators. Our data strongly suggest that PM2.5 exposure is directly associated with ciliary and epithelial barrier dysfunction and may exacerbate lung injury.

1. Introduction

Airway epithelium, located in the trachea and brioche of the respiratory system, is the first defense barrier against inhaled pathogens, allergens, and particulates (Adivitiya et al., 2021; Knowles and Boucher, 2002). In healthy airway epithelium, inhaled pathogens and particles are removed, being trapped in the mucus secreted by goblet cells in the form of sputum by ciliary motility of ciliated cell particulates (Adivitiya et al., 2021; Knowles and Boucher, 2002). Ciliary dysfunction, such as abnormal motility, length, and structure, is associated with abnormal mucociliary clearance, such as hypersecretion of mucus, weakening of tight junction, and inflammatory response in the epithelium

Abbreviations: $PM_{2.5}$, Particulate matter 2.5; NHBE, normal human bronchial epithelial cell; ALI, air-liquid interface; DEP, diesel exhaust particle; DEG, differentially expressed gene; COPD, chronic obstructive pulmonary disease; CF, cystic fibrosis; PCD, primary ciliary dyskinesia; SRM, standard reference material; GO, gene ontology; IL, interleukin; TEER, transepithelial electrical resistance; ZOs, zona occludins.

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Diesel exhaust particle exposure exacerbates ciliary and epithelial barrier dysfunction in the multiciliated bronchial epithelium models

E.Park et al.

Ecotoxicology and Environmental Safety
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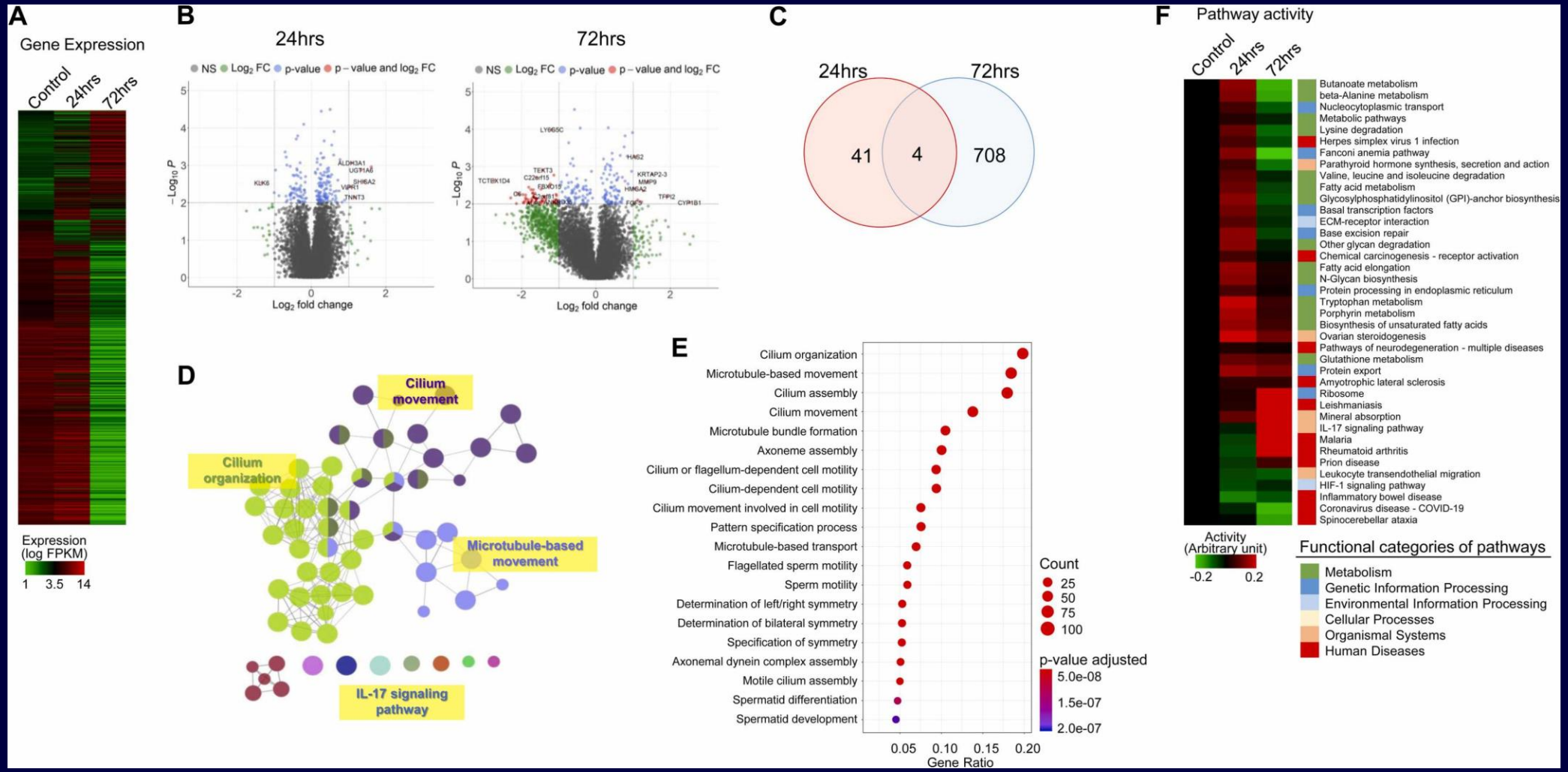
Airway epithelium, the first defense barrier of the respiratory system, facilitates mucociliary clearance against inflammatory stimuli, such as pathogens and particulates inhaled into the airway and lung. Inhaled particulate matter 2.5 ($PM_{2.5}$) can penetrate the alveolar region of the lung, and it can develop and exacerbate respiratory diseases. Although the pathophysiological effects of $PM_{2.5}$ in the respiratory system are well known, its impact on mucociliary clearance of airway epithelium has yet to be clearly defined.

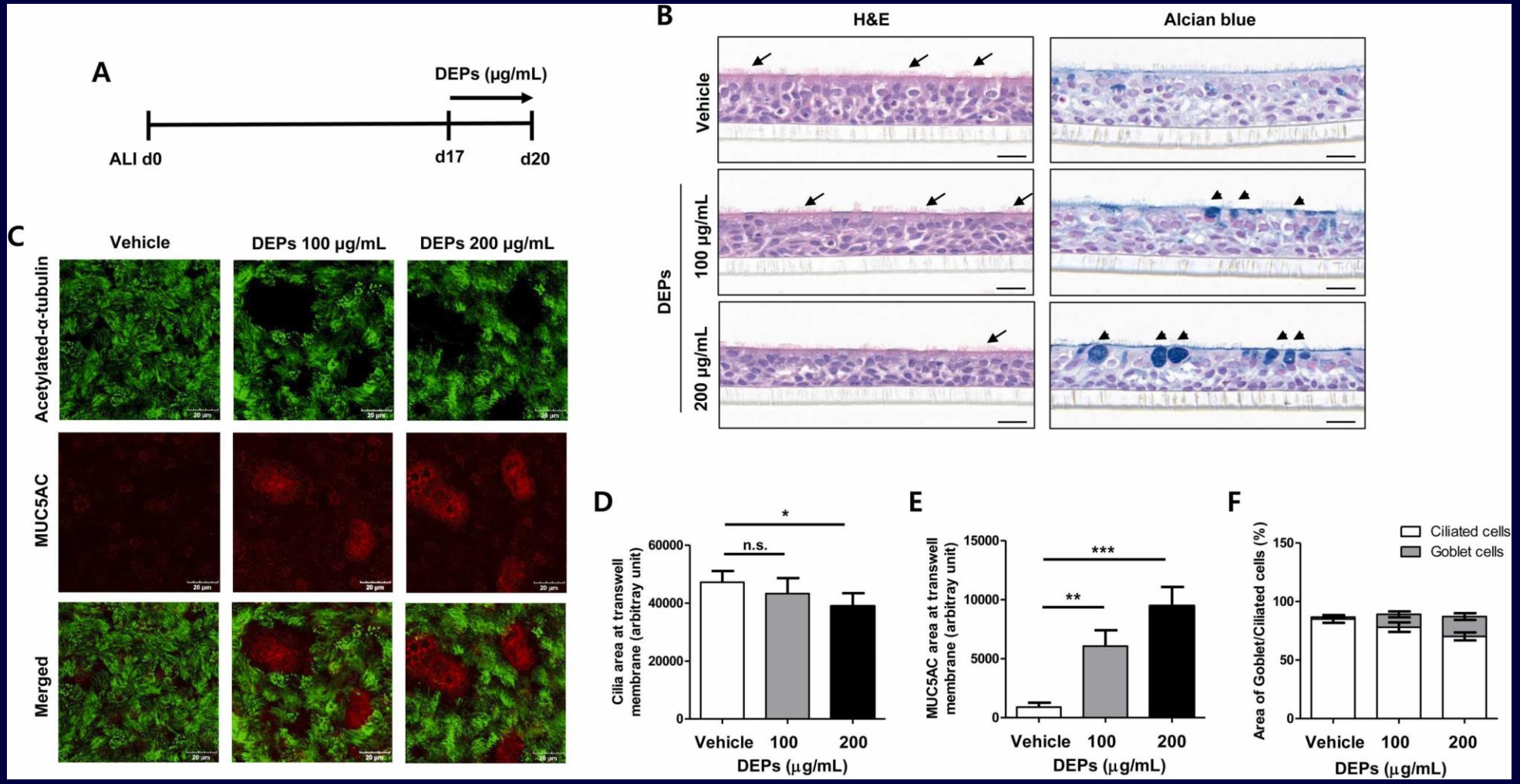
3.1. Expressional profile of genes, pathway activity, and functional enrichment



To investigate how DEPs (diesel exhaust particles) affect the airway defense machinery, the change of the gene expressions in **EpiAirwayFT apically exposed to DEPs for 24 or 72 h was accessed using RNA-sequencing analysis**. As shown in Fig. 1, **long-term exposure (72 h) to DEPs induced more dramatic gene expression changes than 24 h** exposure compared to that in the control (Fig. 1A and B). The distribution of the DEGs (increased more than 2-fold or decreased less than 0.5-fold compared to that of the control) upon DEP exposure **significantly differed between the two time points** (Fig. 1C). GO and KEGG pathway analysis **indicated that the DEGs at 72 h were significantly enriched in ciliary and microtubule function** ($p<0.001$) (Fig. 1D and E). **We evaluated the pathway activity altered by DEP exposure** using all genes involved in each pathway for a more general measure of biological function. Fig. 1F **shows that inflammatory-related pathways were upregulated, whereas metabolic-related pathways were downregulated following** 72 h exposure to DEPs compared to those in the control.

→ **These data suggest that the effect of DEPs against airway defense machinery may be associated with the regulation of ciliary function.**

3.3. DEP exposure affects ciliated to goblet cell ratio in fully differentiated NHBE cells



The results indicated that the DEP **treatment decreased the number of cilia**, whereas **increasing the mucus secretion** of the apical surface of the epithelium. However, the **thickness of epithelium was not significantly changed** by DEP treatment (Fig. 2B).
Additional immunofluorescence staining using antibodies against acetylated α-tubulin and MUC5AC also indicated that DEPs **decreased the area of ciliated cells** (acetylated α-tubulin-positive cells), but **increased the area of mucus-producing goblet cells** (MUC5AC-positive cells) (Fig. 2C–F).

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ARTICLE

Use of EpiAlveolar Lung Model to Predict Fibrotic Potential of Multiwalled Carbon Nanotubes

Hana Barosova, Anna G. Maione, Dedy Septiadi, Monita Sharma, Laetitia Haeni, Sandor Balog, Olivia O'Connell, George R. Jackson, David Brown, Amy J. Clippinger, Patrick Hayden, Alke Petri-Fink, Vicki Stone, and Barbara Rothen-Rutishauser*

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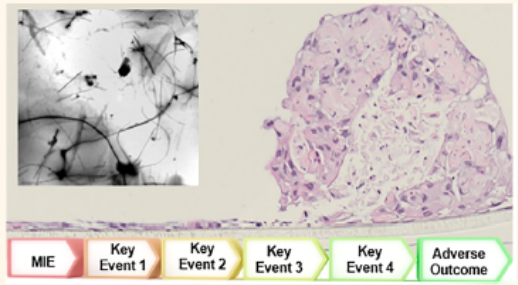
ABSTRACT: Expansion in production and commercial use of nanomaterials increases the potential human exposure during the lifecycle of these materials (production, use, and disposal). Inhalation is a primary route of exposure to nanomaterials; therefore it is critical to assess their potential respiratory hazard. Herein, we developed a three-dimensional alveolar model (EpiAlveolar) consisting of human primary alveolar epithelial cells, fibroblasts, and endothelial cells, with or without macrophages for predicting long-term responses to aerosols. Following thorough characterization of the model, proinflammatory and profibrotic responses based on the adverse outcome pathway concept for lung fibrosis were assessed upon repeated subchronic exposures (up to 21 days) to two types of multiwalled carbon nanotubes (MWCNTs) and silica quartz particles. We simulate occupational exposure doses for the MWCNTs (1−30 $\mu g/cm^2$) using an air−liquid interface exposure device (VITROCELL Cloud) with repeated exposures over 3 weeks. Specific key events leading to lung fibrosis, such as barrier integrity and release of proinflammatory and profibrotic markers, show the responsiveness of the model. Nanocyl induced, in general, a less pronounced reaction than Mitsui-7, and the cultures with human monocyte-derived macrophages (MDMs) showed the proinflammatory response at later time points than those without MDMs. In conclusion, we present a robust alveolar model to predict inflammatory and fibrotic responses upon exposure to MWCNTs.



KEYWORDS: *human primary cells, lung model, pulmonary fibrosis, multiwalled carbon nanotubes, air−liquid interface, long-term repeated exposures*

https://pubs.acs.org/doi/10.1021/acsnano.9b06860?fig=fig5&ref=pdf

Schematic depicting the adverse outcome pathway (AOP) for pulmonary fibrosis.

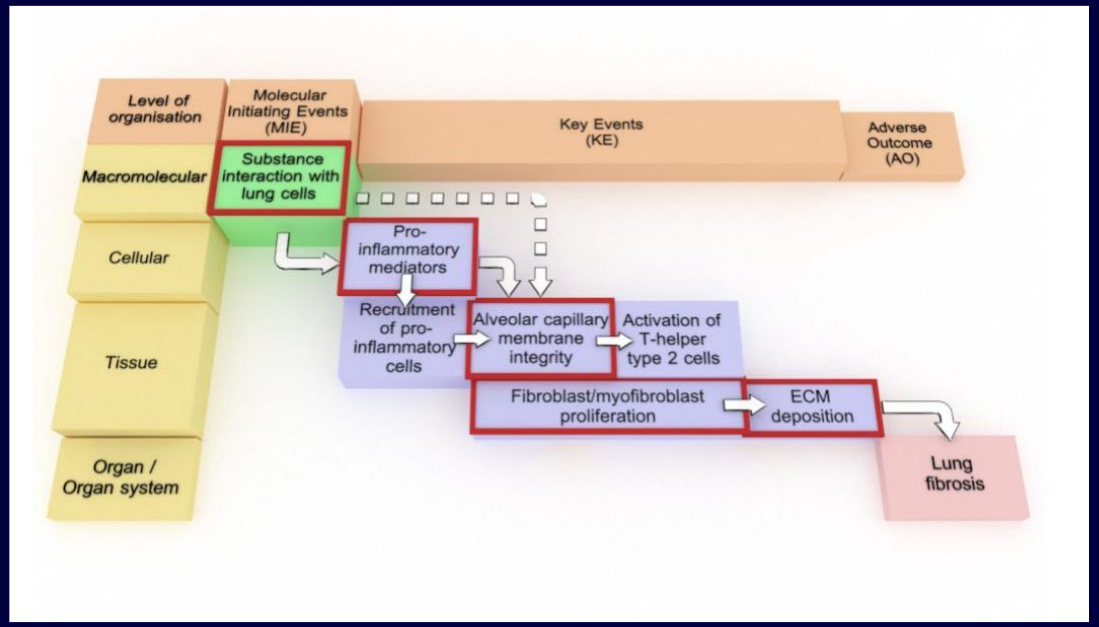
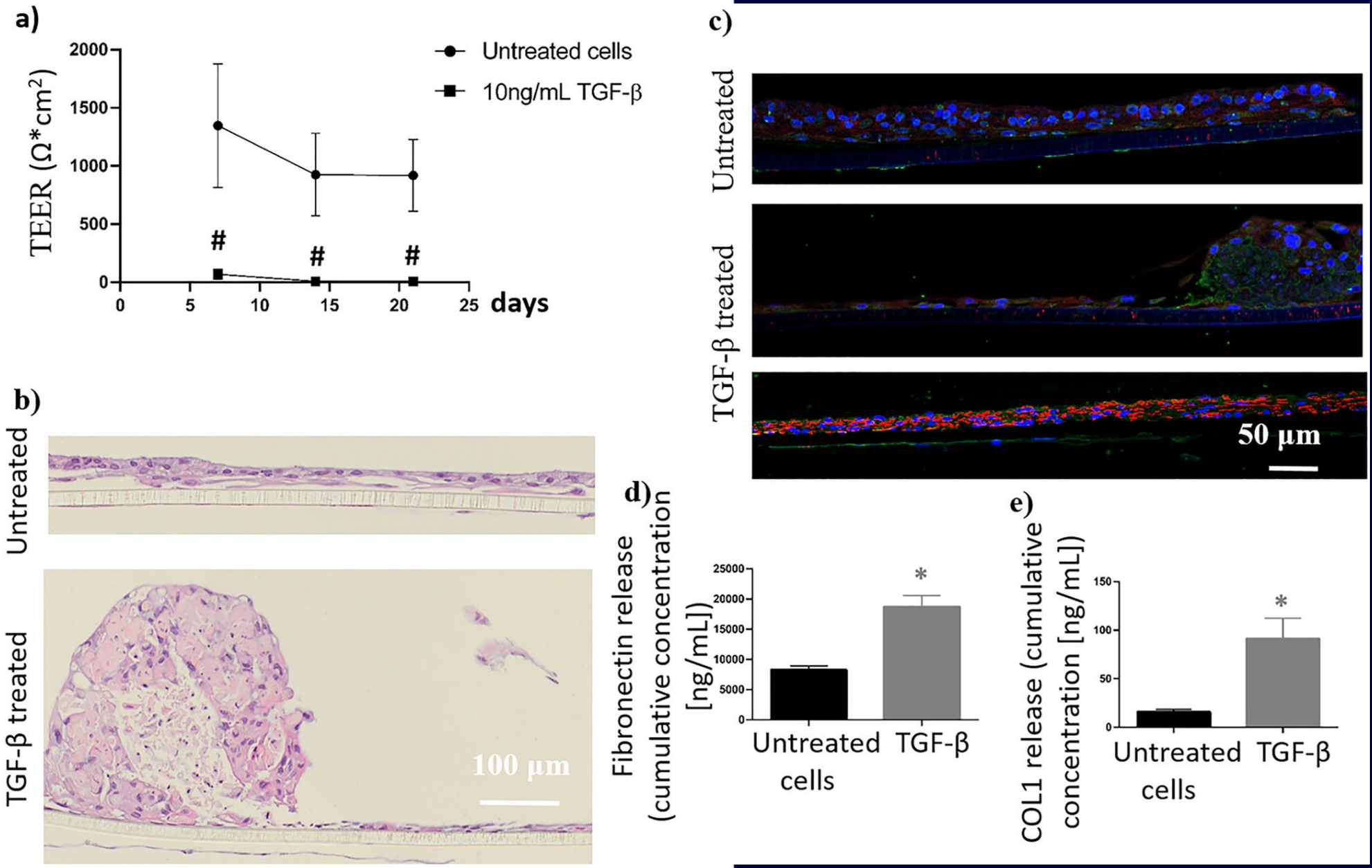


Figure 2. Characterization of EpiAlveolar model response.



RESEARCH ARTICLE
Therapeutics and Prevention

Inhibition of Coronavirus Entry *In Vitro* and *Ex Vivo* by a Lipid-Conjugated Peptide Derived from the SARS-CoV-2 Spike Glycoprotein HRC Domain

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ABSTRACT The emergence of severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), the etiological agent of the 2019 coronavirus disease (COVID-19), has erupted into a global pandemic that has led to tens of millions of infections and hundreds of thousands of deaths worldwide. The development of therapeutics to treat infection or as prophylactics to halt viral transmission and spread is urgently needed. SARS-CoV-2 relies on structural rearrangements within a spike (S) glycoprotein to mediate fusion of the viral and host cell membranes. Here, we describe the development of a lipopeptide that is derived from the C-terminal heptad repeat (HRC) domain of SARS-CoV-2 S that potently inhibits infection by SARS-CoV-2. The lipopeptide inhibits cell-cell fusion mediated by SARS-CoV-2 S and blocks infection by live SARS-CoV-2 in Vero E6 cell monolayers more effectively than previously described lipopeptides. The SARS-CoV-2 lipopeptide exhibits broad-spectrum activity by inhibiting cell-cell fusion mediated by SARS-CoV-1 and Middle East respiratory syndrome coronavirus (MERS-CoV) and blocking infection by live MERS-CoV in cell monolayers. We also show that the SARS-CoV-2 HRC-derived lipopeptide potently blocks the spread of SARS-CoV-2 in human airway epithelial (HAE) cultures, an *ex vivo* model designed to mimic respiratory viral propagation in humans. While viral spread of SARS-CoV-2 infection was widespread in untreated airways, those treated with SARS-CoV-2 HRC lipopeptide showed no detectable evidence of viral spread. These data provide a framework for the development of peptide therapeutics for the treatment of or prophylaxis against SARS-CoV-2 as well as other coronaviruses.

IMPORTANCE SARS-CoV-2, the causative agent of COVID-19, continues to spread globally, placing strain on health care systems and resulting in rapidly increasing numbers of cases and mortalities. Despite the growing need for medical interven-

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FIG 4 SARS-CoV-2-derived cholesterol-conjugated peptides block **SARS-CoV-2-mNeonGreen viral** spread in human airway epithelial cells (HAE). (A) HAE cells were infected with SARS-CoV-2 (2,000 PFU/well for a multiplicity of infection of ~0.02) for 90 min before adding SARS-CoV-2 peptide. Fluid was collected from the apical or basolateral surfaces daily for 7 days (as shown in the schematic in panel A). (B) Spread of fluorescent virus is shown at the indicated days with or without peptide treatment. (C) Viral genome copies in apical or basolateral fluids were determined by RT-qPCR at the indicated time points (days postinfection [DPI]). (D) Infectious viruses released were quantified by titration from the apical or basolateral spaces. The median values are represented by horizontal bars, and the detection limits are indicated by the dotted lines. RT-qPCR and viral titration were performed on the same fluids collected from the same HAE wells the pictures were taken. Data were from three separate wells for infection treated and two separate wells for infection untreated.

https://pmc.ncbi.nlm.nih.gov/articles/PMC7587434/

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