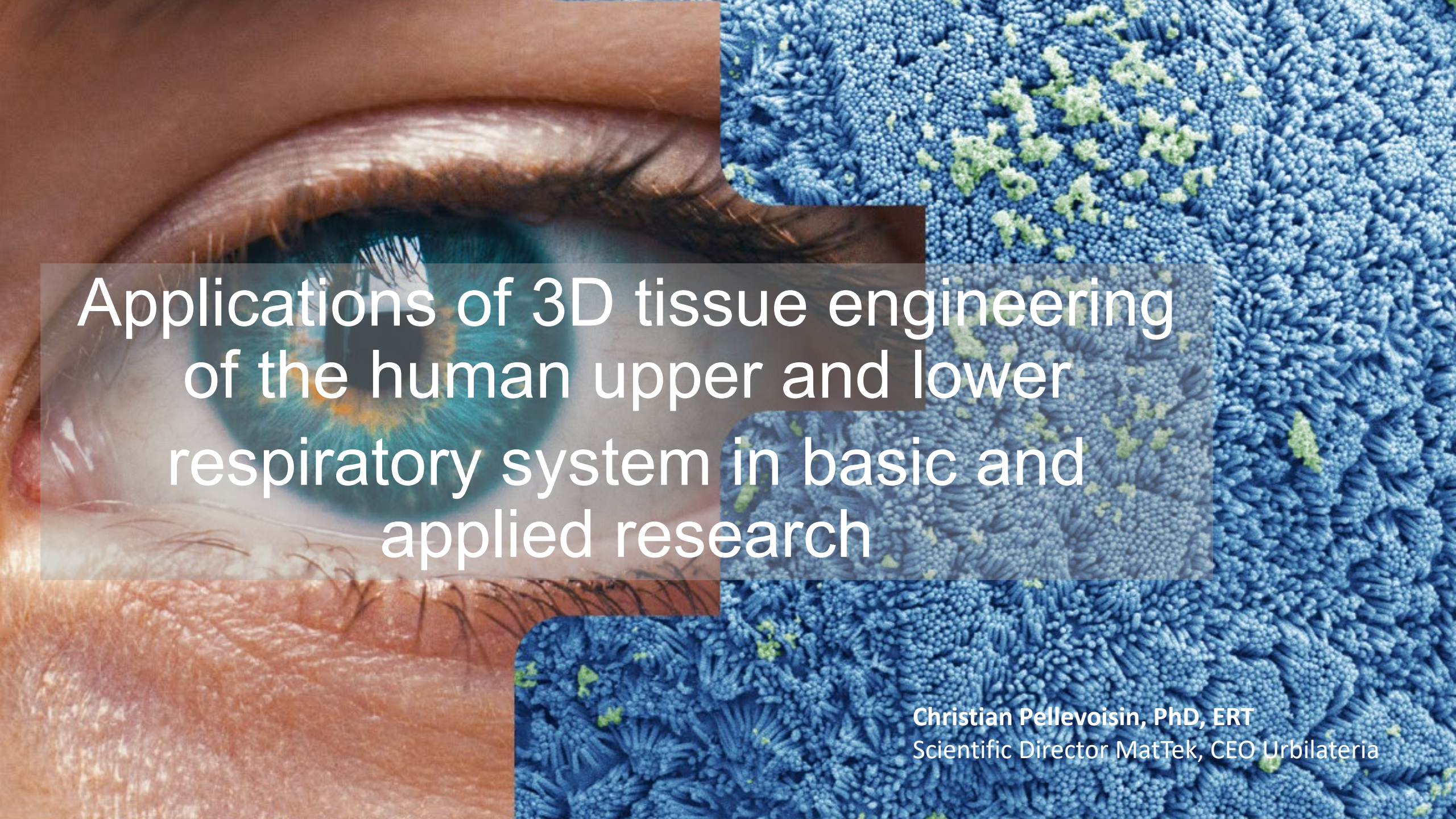
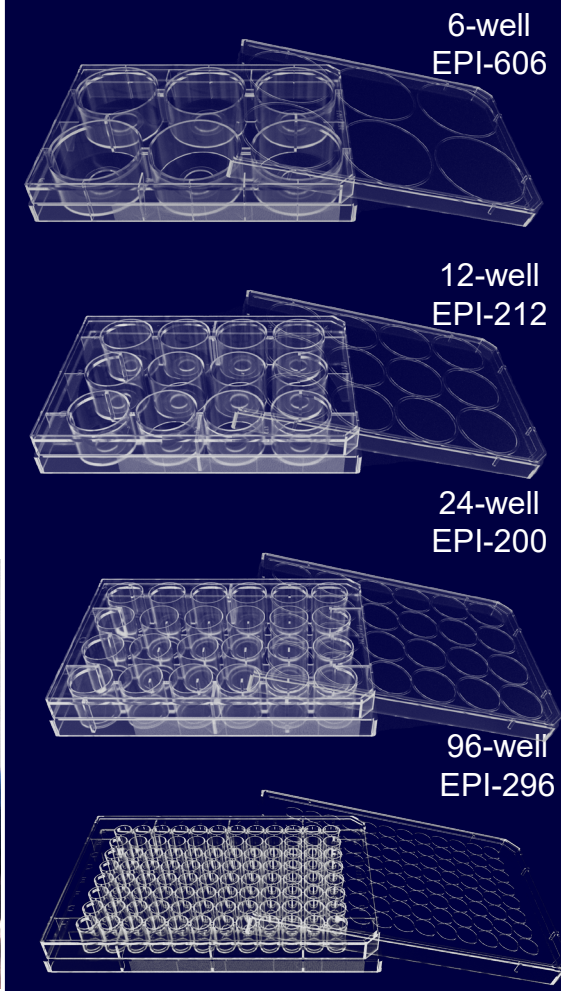


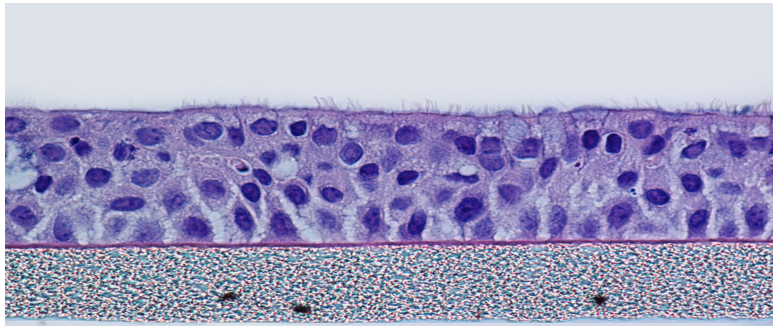
# Applications of 3D tissue engineering of the human upper and lower respiratory system in basic and applied research

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

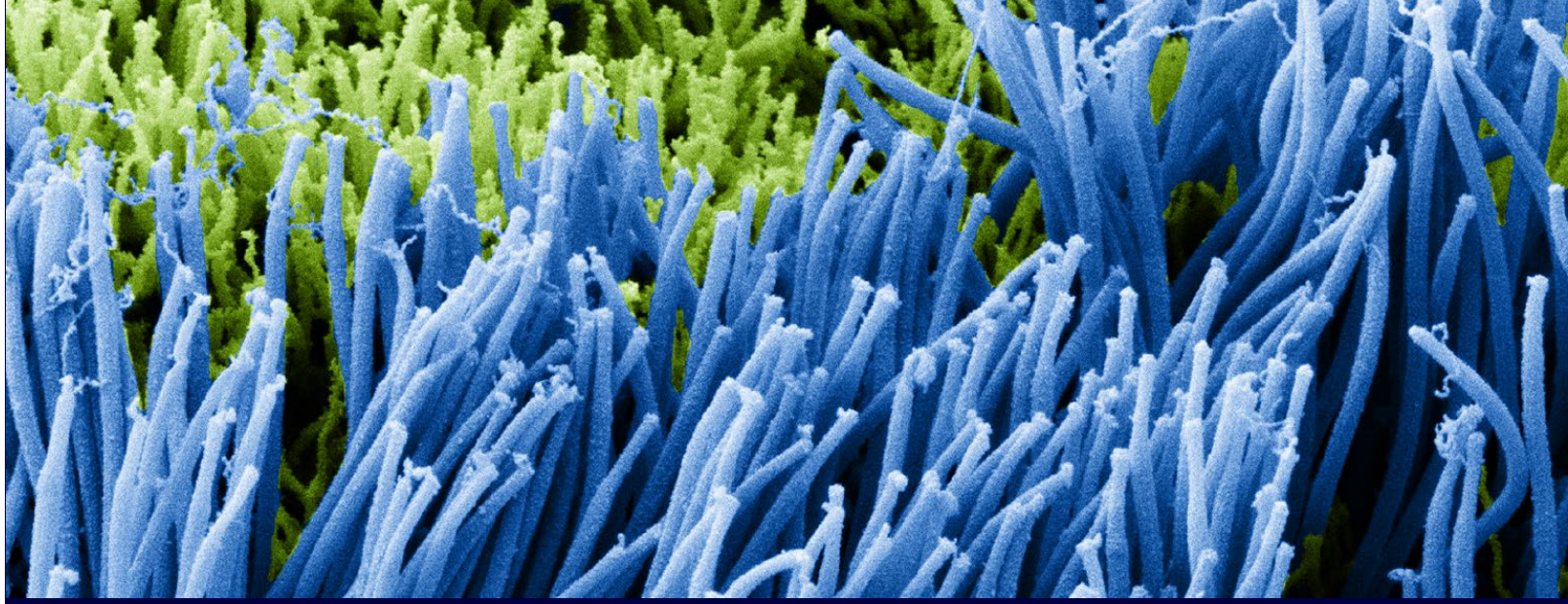
# Principle of Reconstruction of Organotypic Models

# 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)**

# 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

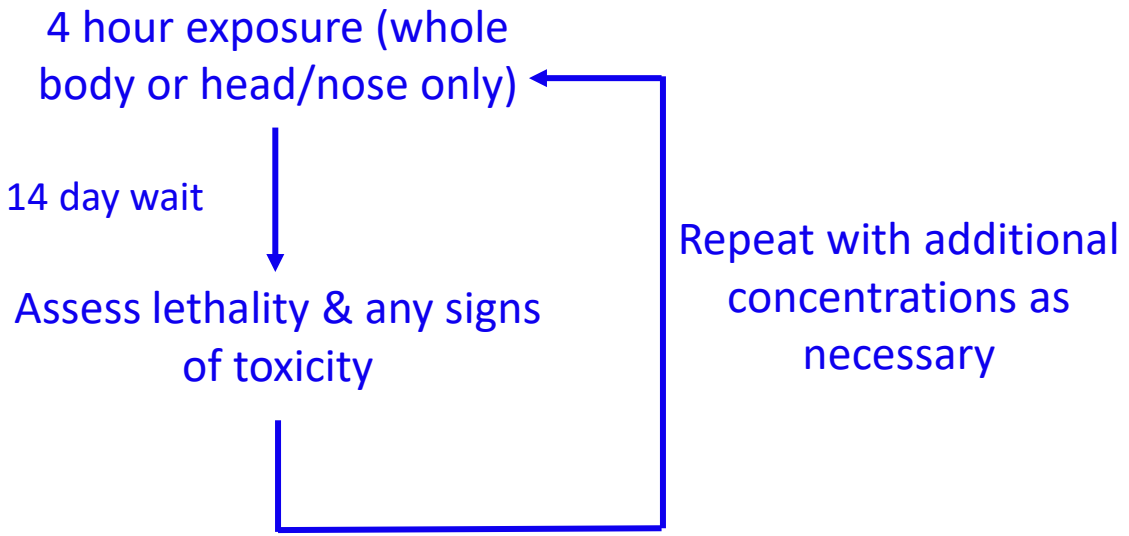
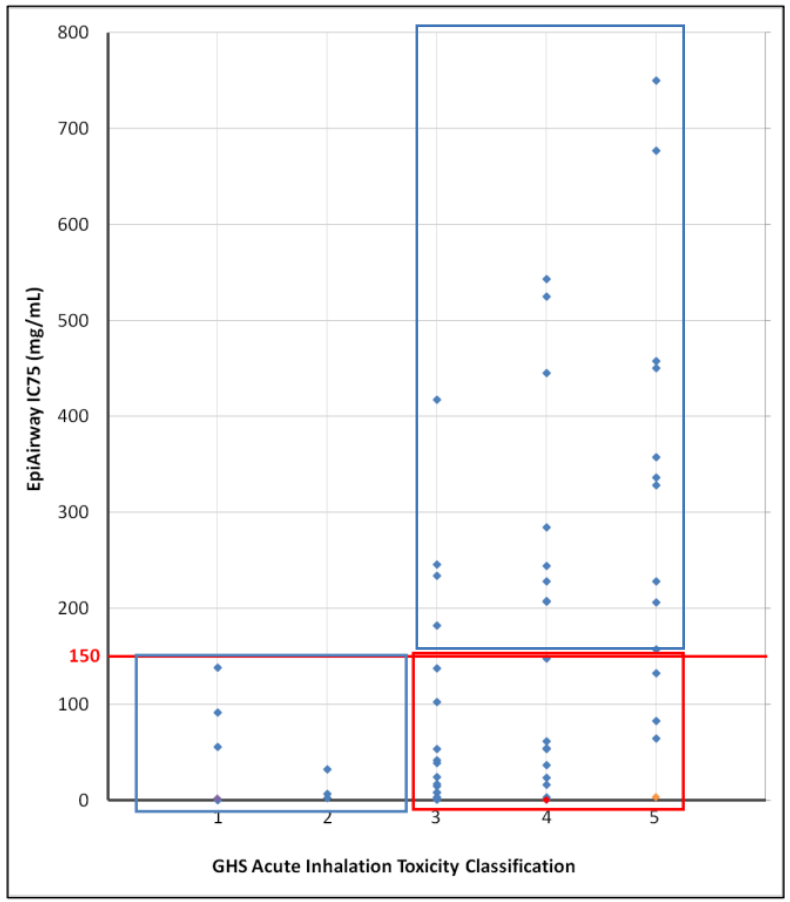
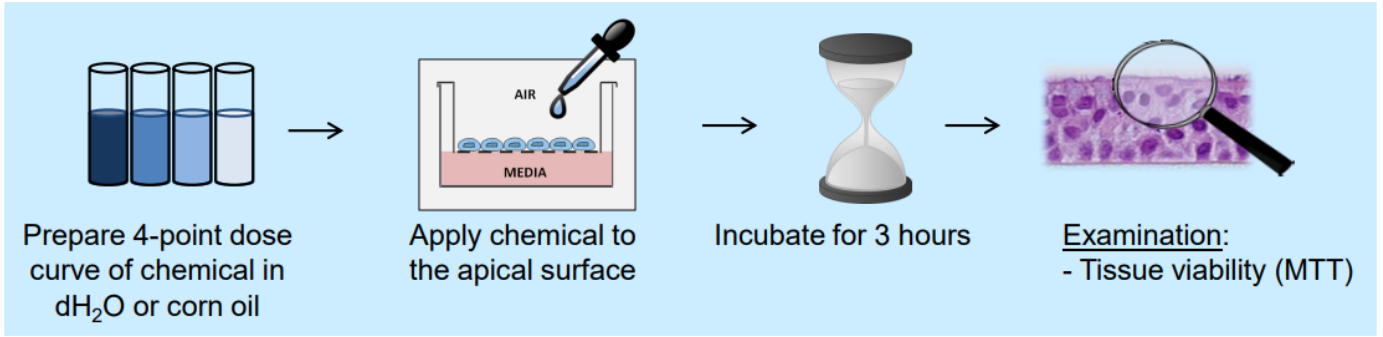


**Figure 1A. Globally Harmonized System (GHS): Acute Toxicity**

| 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.

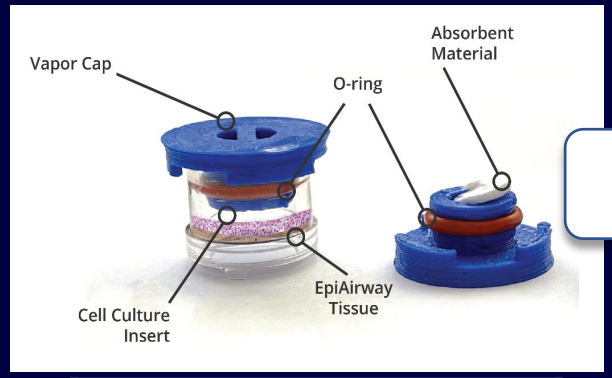
P16-46

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

M. Spacir[1], **S. Valasikova**[1], Y. Kaluzhny[2], J. Markus[1], C. Pellevoisin[2], G. R. Jackson[2], P. Kearney[2], M. Klausner[2], A. Armento[2]

[1] MatTek In Vitro Life Science Laboratories, Bratislava, Slovakia

[2] MatTek Life Sciences, Ashland, Massachusetts, United States of America




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

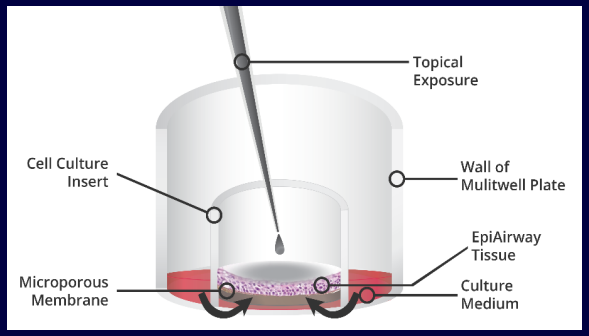
Exposure 4h
Covered with Vapor Cap
Wash with PBS

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

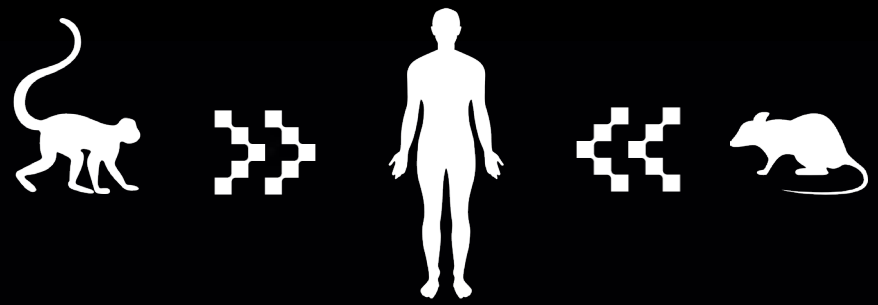
| Vapor Cap | GHS hazard classification | | | |
|---|---|---|---|---|
| | MTT | | TEER | |
| | MatTek | IVLSL | MatTek | IVLSL |
| Sensitivity (%) | 70,8 | 71,9 | 64,4 | 67,1 |
| Specificity (%) | 83,2 | 83,2 | 78,5 | 80,1 |
| Accuracy (%) | 77 | 77,5 | 71,5 | 73,6 |

## 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

R. Jackson, S. Durand, K. Coen, T. Landry, M. Klausner, Y. Kaluzhny, A. Armento, and S. Ayehunie – MatTek Life Sciences, Ashland, MA

MATTEK A BICO COMPANY

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**).

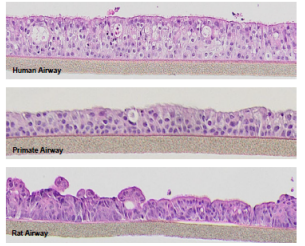
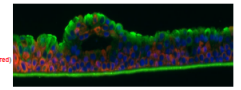
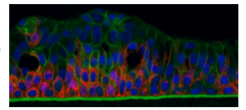



**Figure 1:** H&E-stained histological cross-sections of in vitro reconstructed airway tissue models reconstructed using cells from multiple species (human, rhesus monkey, and rat). Tissues show well developed cilia on the apical tissue surface.



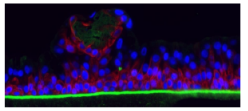
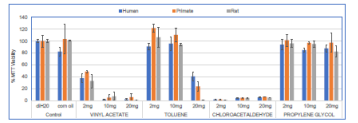




**Figure 2:** Imuno-stained histological cross-sections of in vitro reconstructed rhesus monkey airway tissue model. The tissues model show well developed epithelium (CK5) and cilia (β-tubulin), tight junction (E-cadherin), and mucus producing goblet cells (MUC 5B.



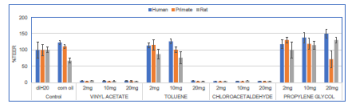




**Figure 3:** Tissue viability (MTT) and barrier integrity (TEER) of in vitro 3D airway tissue models reconstructed using cells from multiple species (human, rhesus monkey, and rat) following exposure to different concentrations of test chemicals.

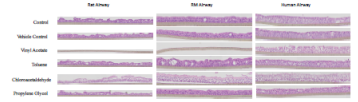



**Figure 4:** H&E-stained histological cross-sections of in vitro reconstructed airway tissue models from multiple species (rat, rhesus monkey, and human) exposed to chemical irritants (10 mg/ml) for 4 hr followed by a 20 hr recovery period.

### 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.

# 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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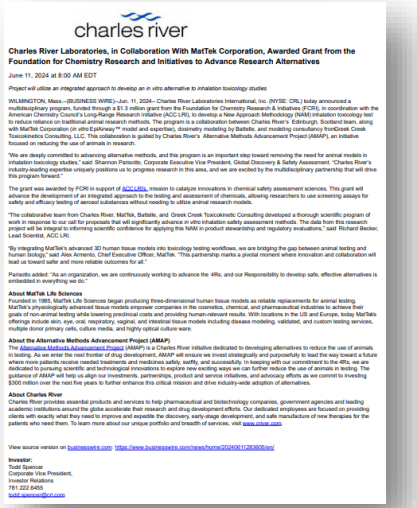
charles river

$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.

View source version on businesswire.com: https://www.businesswire.com/news/home/20240611283605/en/

**Investor:**
Todd Spencer
Corporate Vice President,
Investor Relations
781.222.6455
todd.spencer@crl.com

**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 is to develop an in vitro integrated approach using 3D models as alternative to inhalation toxicology studies

Ecotoxicology and Environmental Safety 273 (2024) 116090

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journal homepage: www.elsevier.com/locate/ecoenv

ELSEVIER

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

Eunsook Park [a], Bu-Yeo Kim [b], Seahyoung Lee [c], Kuk Hui Son [d], Jihye Bang [a], Se Hyang Hong [a], Joong Won Lee [a], Kyung-Ok Uhm [a], Hyun-Jeong Kwak [d], Hyun Joung Lim [a,*]

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[c] Institute for Bio-Medical Convergence, College of Medicine, Catholic Kwandong University, Gangneung-si, Gangwon-do, South Korea
[d] Department of Thoracic and Cardiovascular Surgery, Gachon University Gil Medical Center, College of Medicine, Gachon University, Incheon 21565, South Korea
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ARTICLE INFO

Edited by: Bing Yan

Keywords:
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Normal human bronchial epithelial cell
Air-liquid interface
Ciliary function
Mucociliary clearance

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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https://www.sciencedirect.com/science/article/pii/S0147651324001659?via%3Dihub

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

E.Park et al.

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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.

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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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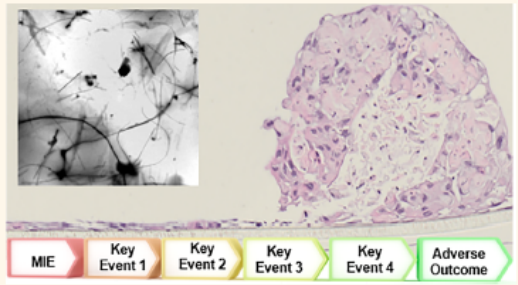
**ABSTRACT:** Expansion in production and commercial use of nanomaterials increases the potential human exposure during the lifecycle of these materials (production, use, and disposal). Inhalation is a primary route of exposure to nanomaterials; therefore it is critical to assess their potential respiratory 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*

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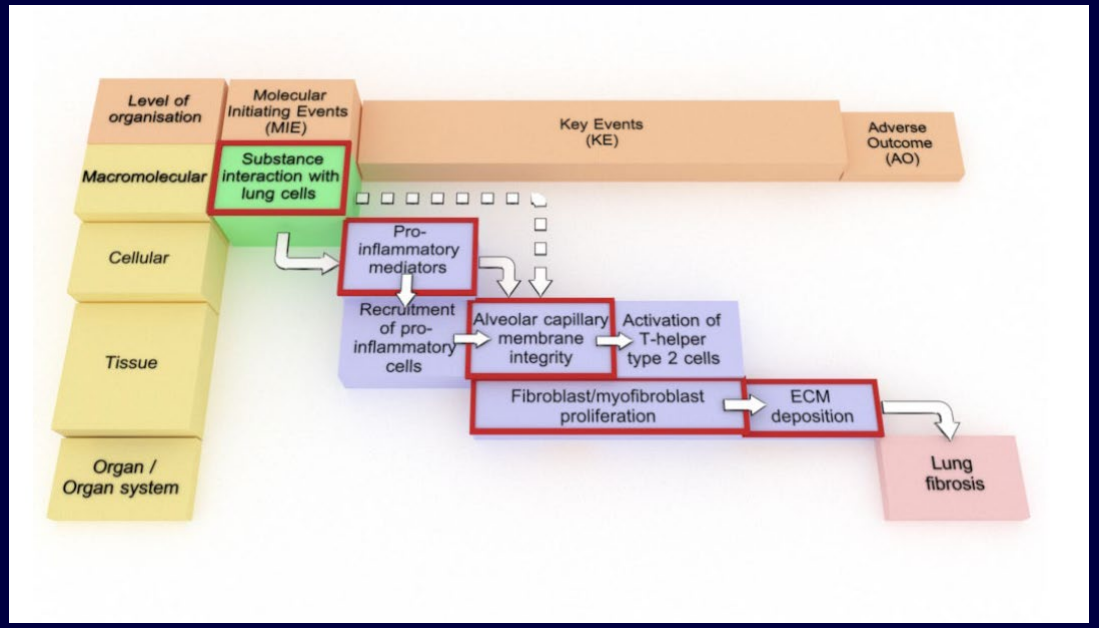
Schematic depicting the adverse outcome pathway (AOP) for pulmonary fibrosis.



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 supernatant fluids sequentially 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.

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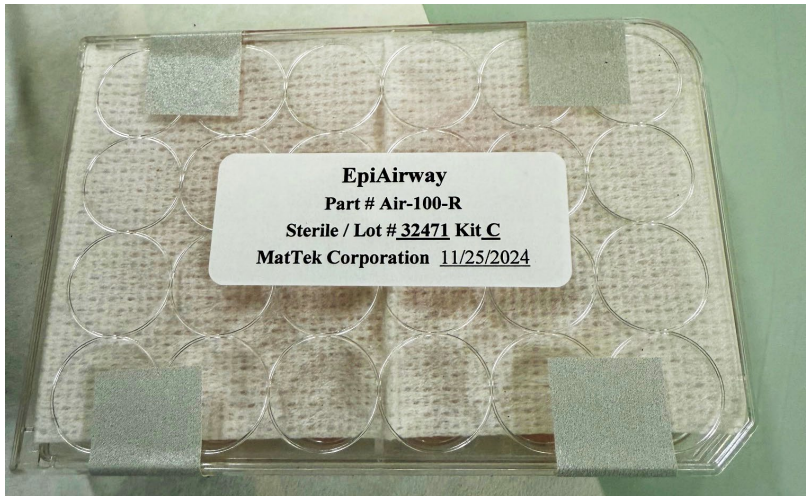



The End