



Lung In Vitro event for innovative & predictive models
8-9 October 2026 ■ Archamps, France

PROGRAMME BOOKLET

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Words of introduction

It is my honor and pleasure to welcome you to the **5th Lung In Vitro Event** (LIVe2026), organized by Epithelix and Alvertox.

As with previous editions, the objective of LIVe2026 is to provide a unique platform for exchange among scientists engaged in respiratory and lung research, as well as stakeholders from academia, the pharmaceutical, biotechnology, chemical, tobacco, consumer goods, and medical device industries, contract research organizations (CROs), NGOs, and regulatory agencies.

Over the past decade, lung modeling has experienced remarkable growth across numerous fields of application, and *in vitro* models have become invaluable tools for research and decision-making.

LIVe2026 is uniquely dedicated to innovative and predictive *in vitro* lung models, making it a distinctive event in this rapidly evolving field. Through this congress, we aim to foster **collaborations**, stimulate the **exchange** of ideas, share **scientific advances**, and contribute to the validation of **emerging methods and technologies**. The participation of speakers, sponsors, and attendees from many countries and continents gives this event a truly global dimension.

I would like to personally thank each of you for joining us, and for sharing your expertise, results, experiences, and the challenges you encounter in your work.

LIVe2026 features oral presentations, poster sessions, and dedicated networking opportunities. In addition, for the first time, we have organized a pre-congress hands-on workshop as well as a post-congress social hiking experience in the Chamonix Valley.

We have chosen Archamps, in the Greater Geneva area, as the venue for this event—a region of particular significance as the birthplace of Epithelix. LIVe2026 also provides a special opportunity to **celebrate the 20th anniversary of Epithelix**.

On behalf of the Epithelix and Alvertox teams, I extend a warm welcome to all of you. We hope that LIVe2026 will be both scientifically enriching and personally rewarding, and that together we will make this congress a great success and continue to establish it as a leading international event for the *in vitro* lung research community.

We wish you an inspiring and enjoyable congress.

Dr. Samuel Constant,
co-founder and CEO of Epithelix





Throughout the congress,
share your pictures and
your thoughts with the
hashtag #LIVe2026

SHARE
YOUR
SCIENCE!

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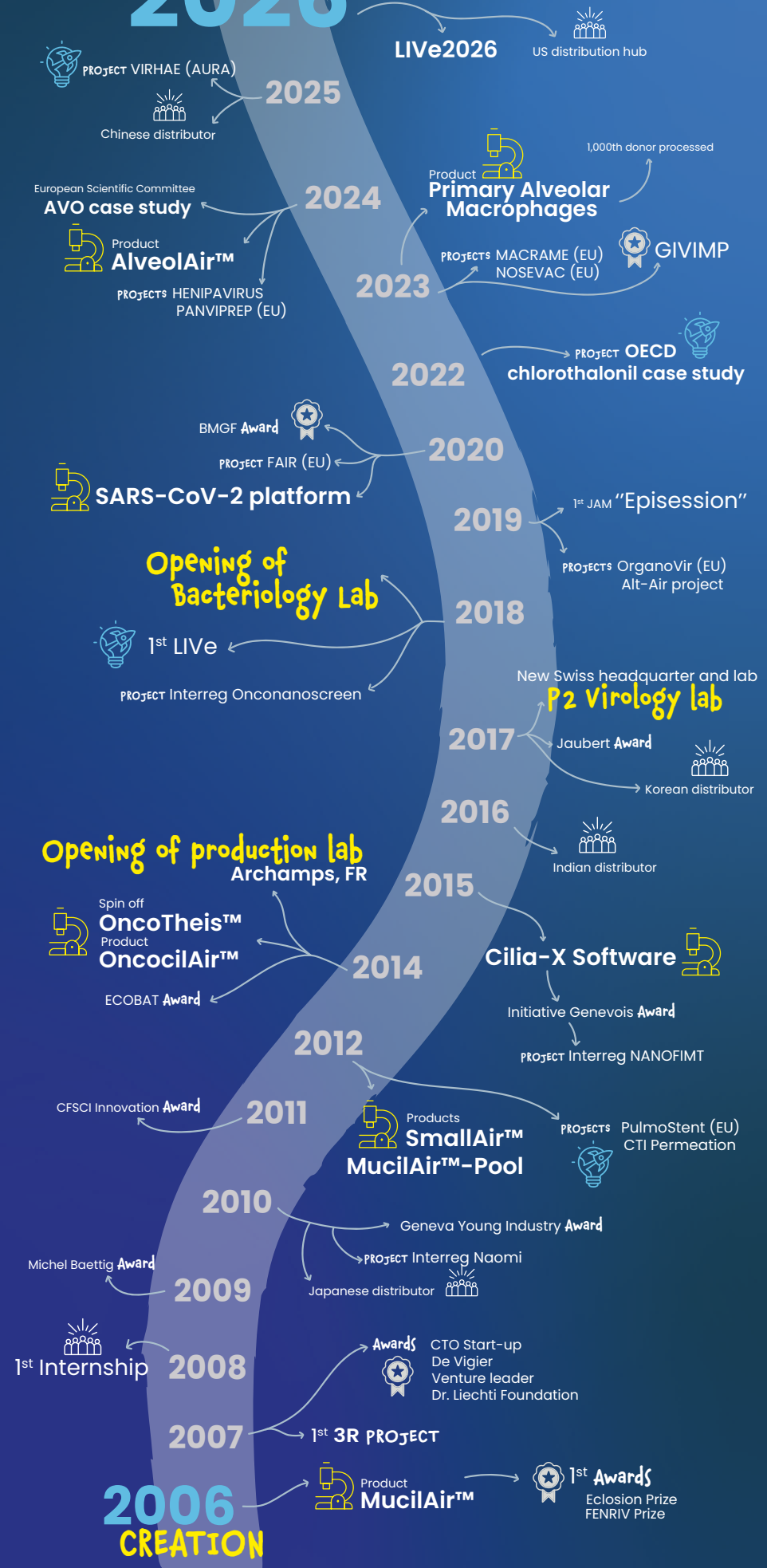
20 YEARS JOURNEY

-  #LEADERSHIP
-  #QUALITY
-  #INNOVATION
-  #COMMUNITY

20 YEARS ANNIVERSARY

2026

5 LIVE congresses
14 international granted projects
>1'200 publications
18 prizes
...and still on fire !



Session 1

Inhalation Toxicity Testing



Human-relevant in vitro lung models deliver predictive, mechanistic data for inhaled substances, supporting safety assessment and regulatory decision-making.

Keynote speaker:

Dr. Shaun D. McCullough

- Team Lead and Senior Principal Scientist, Predictive Toxicology and Exploratory Biology - **RTI International**

Session 2

Viral Infections



Innovative lung in vitro platforms provide controlled, human-relevant environments to study virus-host interactions and accelerate antiviral therapeutic development for both emerging and established respiratory pathogens.

Keynote speaker:

Prof. Caroline Tapparel

- Medical faculty - **University of Geneva**

Session 3

Bacterial and Fungal Infections



Cutting-edge in vitro lung models incorporating immune complexity capture the complex dynamics of host-pathogen interactions to advance antimicrobial and antifungal therapy development.

Keynote speaker:

Dr. Jakson Luk

- Laboratory of Host-Pathogen Interactions in Tuberculosis
- **The Francis Crick Institute**

Session 4

Modeling COPD/Asthma/IPF/CF/PCD *in vitro*



Advanced human in vitro lung models enable mechanistic insights into chronic respiratory diseases while reducing reliance on animal studies through disease-specific phenotypes and functional readouts.

Keynote speaker:

PD. Dr. Katherina Sewald

- Department of Preclinical Pharmacology, Head of Department - **Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM)**

Biological Barriers, MPS & Drug Delivery



Advanced microphysiological and organ-on-chip models replicate lung barrier integrity, permeability, and disease-related dysfunction to drive more translatable respiratory research.

Keynote speaker:

Prof. Olivier Guenat

- Professor of Biomedical Engineering - **Bern University**

Regulatory considerations, Exposure devices and Deposition Modelling



Integrated exposure systems and deposition modeling approaches enable physiologically relevant and reproducible aerosol delivery to in vitro lung models.

Keynote speaker:

Dr. Clive Roper

- Director - **Roper Toxicology Consulting Limited**

Mucus, Surfactant, Cilia Functions and Modulations



Incorporating mucus, surfactant, and ciliary function into in vitro lung models enhances physiological relevance for airway protection, disease, and drug delivery studies.

Keynote speaker:

Dr. Lydia Esteban Enjuto

- Chief Technology Officer - **MucFlow**

Think Different



Disruptive technologies, novel methodologies, and cross-sector collaboration challenge traditional paradigms to accelerate discovery and shape the future of human-relevant respiratory science.

Keynote speaker:

Dr. Lauriane Cabon

- Group lead - Applied Organoid Technologies, Institute of Human Biology (IHB) - **Roche**

DAY 1 – Thursday Oct 8 2026

08:30	Registration	
	Arrival of participants in the venue and poster hanging	
09:00	Welcome	Dr. Samuel Constant CEO, Epithelix
Session 1. Inhalation Toxicity Testing		
09:15	Keynote Beyond “Human-Relevant” : Decision-Relevant Inhalation Toxicology by Design	Dr. Shaun D. McCullough Team Lead and Senior Principal Scientist, Predictive Toxicology and Exploratory Biology RTI International
09:45	Long-term <i>in vitro</i> exposure of an air- liquid interface immunocompetent tri-culture human bronchial cell model to traffic-related ambient air	Dr. Mathilde Delaval Research team leader, Helmholtz Munich / Lung Health and Immunity institute
10:10	A Hypothesis-driven Framework for Screening Potential Respiratory Sensitizers	Dr. Nikaeta Sadekar, DABT Principal Scientist & Supervisor Dr. Jake Muldoon Chemist/Scientist Research Institute for Fragrance Materials (RIFM)
10:35	Coffee Break, Visiting Exhibition Area, Poster Viewing	
Session 2. Viral infections		
11:05	Keynote From Mechanistic Insights to Antiviral Discovery: over a Decade of Respiratory Virus Research in Human Airway Epithelia	Prof. Caroline Tapparel Professor Medical faculty, University of Geneva
11:35	Long-term inflammatory consequences of SARS-CoV-2 infection	Sang Hyun (Ryan) Chun (M.S) Ph.D. Student, Graduate School of Biomedical Sciences, University of Texas Health Science Center, San Antonio
12:00	Development of a PBMC- bronchial MucilAir™ co-culture model to decipher asthma exacerbation mechanisms induced by Respiratory Syncytial Virus infection	Dr. Remi Pereira De Oliveira Researcher, International Research Laboratory RespiVir France-Canada, CIRI - Centre International de Recherche en Infectiologie, Faculté de Médecine RTH Laennec
12:25	Lunch Break, Visiting Exhibition Area, Poster Viewing	



Session 3. Bacterial and fungal infections		
13:55	<p>Keynote</p> <p>An autologous human iPSC-derived multicellular Alveolus-on-Chip reveals early pathological events of M. tuberculosis infection</p>	<p>Dr.Jakson Luk <i>Researcher Laboratory of Host-Pathogen Interactions in Tuberculosis The Francis Crick Institute</i></p>
14:25	<p>Mechanistic insights into the early stage of Bordetella pertussis respiratory infection using fully-differentiated human nasal epithelial cells</p>	<p>Dr. Nathalie Mielcarek <i>Deputy Director, Center for Infection and Immunity of Lille</i></p>
14:50	<p>A Primary Human ALI Infection Model with Bioluminescent MDR Pathogens for Real-Time Antimicrobial Efficacy Assessment</p>	<p>Dr. Marc Vandamme <i>R&D program manager, ERBC Group</i></p>
15:15	Coffee Break, Visiting Exhibition Area, Poster Viewing	
Session 4. Modeling COPD / Asthma / IPF / CF / PCD in vitro		
15:45	<p>Keynote</p> <p>Advanced in vitro and ex vivo models for the development of respiratory therapeutics</p>	<p>PD Dr. Katherina Sewald <i>Division Co-Director of Preclinical Pharmacology and Toxicology and Head of Department of Preclinical Pharmacology & Infection and Immunology, Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM)</i></p>
16:15	<p>Patient-Derived Airway Organoids for Modelling COPD</p>	<p>Miss Serena Bateman (BSc) <i>Scientist, Complex Models, Human Biology, GlaxoSmithKline (GSK)</i></p>
16:40	<p>Adopting and deploying the precision-cut lung slice model for airway constriction studies – challenges, pitfalls, and lessons learned</p>	<p>Prof. Ynuk Bossé <i>Professor, Faculty of Medicine, Université Laval</i></p>
18:00	Departure to Sunset Dinner Experience at Panoramiques du Salève from the Conference Center	

DAY 2 – Friday Oct 9 2026

Session 5. Biological barriers, MPS & Drug Delivery

09:00	Keynote The Alveolar Barrier on Chip: Are We There Yet?	Prof. Dr. Olivier Guenat <i>Professor of Biomedical Engineering Bern University</i>
09:30	Membrane-free human airway-on-a-chip integrating resident memory T cells to model respiratory viral infection	Dr. Yann Tinguely <i>Post Doctorate Scientist, Institute of Human Biology (IHB) at Roche</i>
09:55	Beyond nominal concentration: A framework for standardizing <i>in vitro</i> lung epithelial permeability assessment	PharmD. Rayane Boufalaas <i>Researcher, INERIS- NAHE unit, University of Technology of Compiègne (UTC) – BioMechanics and BioEngineering (BMBI) laboratory</i>

10:20 Coffee Break, Visiting Exhibition Area, Poster Viewing

Session 6. Regulatory considerations, Exposure Devices and Deposition Modelling

10:50	Keynote Exposure scenarios and regulatory considerations for <i>in vitro</i> lung models	Dr. Clive Roper <i>Director, Roper Toxicology Consulting Limited</i>
11:20	Relevance of Characteristic Aspects of <i>in vitro</i> Lung Models: Mechanisms of Pulmonary Aerosol Deposition and Mechanobiology	Dr. Otmar Schmid <i>Head of Research Group, Helmholtz Munich, Institute of Lung Health and Immunity</i>
11:45	Advancing Human-Relevant <i>In Vitro</i> Methods to Assess Respiratory Tissue Irritation	Dr. Andreas Stucki <i>Advisor, PETA Science Consortium International e.V.</i>

12:10 Lunch Break, Visiting Exhibition Area, Poster Viewing

Session 7. Mucus, Surfactant, Cilia Functions and Modulations

13:40	Keynote Physiologically Relevant Airway Mucus in ALI Models	Dr. Lydia Esteban Enjuto <i>Chief Technology Officer, MucFlow</i>
14:10	High throughput screening for compounds allowing readthrough of premature termination codons based on genetically engineered PCD patient specific hiPSCs	David Puertollano Martín (M.Sc.) <i>Hannover Medical School (MHH) Leibniz Forschungslaboratorien für Biotechnologie und künstliche Organe (LEBAO)</i>



14:35	Nanoscale structural insights into human pulmonary mucus and its snail slime-based surrogate	Dr. Marine Le Goas <i>CNRS researcher, Institut Galien Paris-Saclay, Université Paris-Saclay</i>
15:00	Coffee Break, Visiting Exhibition Area, Poster Viewing	
Session 8. Think Different		
15:30	Human-relevant 3D ALI models of NSCLC for diagnostic biomarker discovery	Dr. Dania Movia <i>Assistant Professor in Biomedical and Health Sciences, Department of Biology, Maynooth University, Ireland</i>
16:00	Next-Generation Lung <i>In Vitro</i> Models with Integrated Vasculature	PD Dr. Anja Lena Thiebes <i>Group Leader, RWTH Aachen University</i>
16:25	Keynote Innovative Human Lung Models: Immune Microenvironments and Scaled Discovery	Dr. Lauriane Cabon <i>Group lead, Applied Organoid Technologies, Institute of Human Biology (IHB) Roche</i>
16:50	Closure of LIVE2026	

DAY 1 – Thursday Oct 8 2026

Session 1. Inhalation Toxicity Testing

09:15	<p>Keynote</p> <p>Beyond “Human-Relevant” : Decision-Relevant Inhalation Toxicology by Design</p>	<p>Dr. Shaun D. McCullough Team Lead and Senior Principal Scientist, Predictive Toxicology and Exploratory Biology RTI International</p>
<p>Innovation in the field of <i>in vitro</i> toxicology is often associated with the development of increasingly sophisticated model systems that more closely replicate human biology. Advances such as organoids, organs-on-chips, and complex multicellular culture systems have transformed <i>in vitro</i> toxicology research and expanded opportunities to leverage <i>in vitro</i> toxicity testing paradigms instead of traditional animal testing. Increasing biological complexity can make <i>in vitro</i> models more similar to their respective tissues <i>in vivo</i>; however, a human-relevant <i>in vitro</i> model alone does not ensure the decision relevance of study data. Progressing from a traditionally innovative human-relevant model to decision-relevant data requires a more practical form of innovation that leverages the consideration of biological and technical factors, thorough characterization, and deliberate study design and execution. Focusing on practical innovation will fundamentally revolutionize inhalation toxicology through the generation of decision-relevant data with reliable, well-characterized, and reproducible <i>in vitro</i> methods.</p> <p>This lecture will explore how innovation in <i>in vitro</i> toxicology extends beyond progressively complex model development and increasingly depends on how experimental systems are characterized and used for inhalation toxicology research and testing. Using examples from <i>in vitro</i> respiratory toxicology studies, this presentation will highlight how practical innovation can be applied to advance human-relevant <i>in vitro</i> respiratory models to impactful decision-relevant tools for inhalation toxicology. Examples will highlight how: 1.) defining the role of inter-individual variability in primary cell-based <i>in vitro</i> models; 2.) endpoint selection and characterization; and 3.) understanding the benefits and limitations of exposure paradigms advance human-relevant <i>in vitro</i> models to decision-relevant tools for the reliable identification and interpretation of inhalation exposure outcomes.</p>		
09:45	<p>Long-term <i>in vitro</i> exposure of an air-liquid interface immunocompetent tri-culture human bronchial cell model to traffic-related ambient air</p>	<p>Dr. Mathilde Delaval Research team leader, Helmholtz Munich / Lung Health and Immunity institute</p>
<p>Air pollution is a major factor contributing to the global burden of respiratory and cardiovascular diseases. Little research, however, has addressed the pulmonary effects of complete ambient air exposure using <i>vitro</i> lung models to decipher the underlying toxicological mechanisms, largely due to technical limitations.</p> <p>A tri-culture cell model combining Calu-3 bronchial epithelial cells, differentiated THP-1 macrophage-like cells and MRC5 fibroblasts was cultured at the ALI and exposed to traffic-related ambient air sampled in Munich for 24 h or 72 h, using the optimized Vitrocell® automated exposure station. To capture the airway immune response to aeroallergens or chemical respiratory sensitizers, the triculture bronchial cell models were post-incubated with or without MUTZ-3-derived dendritic cells for 24 h following each exposure period. Comprehensive physicochemical characterization of the ambient aerosol (particle size distribution, particle mass, black carbon, VOCs, NOx, ozone and PAHs) was performed in parallel with biological endpoints including cytotoxicity (LDH release and metabolic activity), epithelial barrier integrity (TEER), xenobiotic metabolism (CYP1A1/1B1 activity) and pro-inflammatory cytokine release (IL-6, IL-8, TNF-α).</p> <p>Physicochemical characterization confirmed a traffic-related profile of the sampled ambient air, with low PAH concentrations over the 24 h exposure period. A decrease in TEER was observed following 72 h of ambient air exposure compared to ambient air, irrespective of post-incubation with dendritic cells, indicating an impairment of epithelial barrier integrity. IL-8 secretion was not significantly altered, although a slight decrease was observed in the dendritic-cell co-incubated condition. Transcriptomic and genotoxicity analyses are currently ongoing.</p> <p>These results represent, to our knowledge, the first long-term ALI exposure campaign performed with an immunocompetent tri-culture model under real ambient air conditions, and pave the way for a mechanistic, multi-endpoint approach for characterizing the innate and adaptive airway epithelial response to traffic-related air pollution.</p>		

10:10	A Hypothesis-driven Framework for Screening Potential Respiratory Sensitizers	Dr. Nikaeta Sadekar, DABT <i>Principal Scientist & Supervisor</i> Dr. Jake Muldoon <i>Chemist/Scientist</i> <i>Research Institute for Fragrance Materials (RIFM)</i>
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Respiratory sensitization triggered by inhalation exposure to low-molecular-weight (LMW) chemicals can lead to chronic and severe diseases such as occupational asthma. Despite its importance for human health and regulatory decision-making, respiratory sensitization remains poorly understood, with a narrow set of recognized causative chemicals and no validated methods for their systematic identification. Current risk assessment frameworks often extrapolate from skin sensitization paradigms; however, emerging evidence suggests that respiratory sensitization follows a distinct biology shaped by airway-specific chemistry, metabolism, and immune responses.

In this presentation, we explore a mechanistic basis for respiratory sensitization that moves beyond analogy to skin. We propose that respiratory sensitizers are characterized by bifunctional electrophilic structures capable of protein crosslinking within airway tissues, promoting neo-antigen formation and immune activation. By integrating clinical evidence with chemical structure and metabolism-informed insights, we examine how respiratory sensitizers differ fundamentally from skin sensitizers and from non-sensitizing irritants.

Together with clinical case evidence, these findings support specific protein-chemical interactions as one of the key initiating events in the respiratory sensitization adverse outcome pathway, grounded in lung-relevant chemistry, metabolism, and immune biology. This work highlights the limitations of transposing dermal paradigms to inhalation endpoints and underscores the importance of developing new approach methodologies (NAMs) tailored to respiratory sensitization. By defining key chemical and mechanistic drivers, this research lays critical groundwork for future screening and testing strategies and regulatory frameworks aimed at identifying respiratory sensitizers without reliance on animal testing.

Session 2. Viral infections

11:05	Keynote From Mechanistic Insights to Antiviral Discovery: over a Decade of Respiratory Virus Research in Human Airway Epithelia	Prof. Caroline Tapparel <i>Professor</i> <i>Medical faculty, University of Geneva</i>
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Respiratory viruses are a leading cause of acute respiratory infections worldwide, yet effective antiviral therapies are available for only a limited number of these pathogens. Over the past decade, we have used reconstituted human airway epithelia to study the pathogenesis of respiratory viruses in physiologically relevant models. These differentiated tissues reproduce key features of the respiratory mucosa, including mucociliary clearance, epithelial regeneration, and innate immune responses.

These models have enabled us to compare the pathogenesis of various respiratory viruses, study viral interference during co-infections, and identify epithelial factors that determine susceptibility to infection. Recently, we have investigated the impact of chronic respiratory diseases and transplantation-associated treatments on epithelial function and viral replication. Additionally, we have expanded our efforts to develop advanced airway organoid models that support antiviral discovery in a format compatible with high-throughput screening.

Together, these studies demonstrate how human airway models can bridge the gap between fundamental virology and antiviral discovery, offering new insights into virus-host interactions and respiratory disease mechanisms.

11:35	Long-term inflammatory consequences of SARS-CoV-2 infection	Sang Hyun (Ryan) Chun (M.S) <i>Ph.D. Student,</i> <i>Graduate School of Biomedical Sciences, University of Texas Health Science Center, San Antonio</i>
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SARS-CoV-2 has become endemic in the human population. Our objective is to understand the downstream impact of long COVID – defined as persistent complications and new symptoms following initial infection – particularly inflammation, as it drives COVID-19 symptoms and fatalities. We employ *in silico*, *in vitro* and *ex vivo* approaches to address this overarching question. First, we screen for differential gene expression related to proinflammatory pathways via bioinformatic analyses. Second, we utilize a human airway epithelium (HAE) organoid model cultured at an air-liquid interface – a 3D lung organoid model suitable for respiratory infection – to infect with multiple SARS-CoV-2 strains and recapitulate SARS-CoV-2-mediated pathophysiology, enabling us to examine inflammatory responses. Lastly, we assess clinical biospecimens from individuals with a history of SARS-CoV-2 infection using a panel of inflammatory-associated biomarkers. Together, these approaches will pave the way for a deeper dive into uncovering the long-term consequences of COVID-19.



12:00	Development of a PBMC- bronchial MucilAir™ co-culture model to decipher asthma exacerbation mechanisms induced by Respiratory Syncytial Virus infection	Dr. Remi Pereira De Oliveira <i>Researcher, International Research Laboratory RespiVir France-Canada, CIRI - Centre International de Recherche en Infectiologie, Faculté de Médecine RTH Laennec</i>
<p>Asthma is a chronic inflammatory lung disease related to a skewed T2 immune response, facilitating mucus hypersecretion, smooth muscles contraction, bronchial inflammation and remodeling, contributing to lung function deterioration and asthma exacerbation. Respiratory Syncytial Virus (RSV) is a well-known trigger of bronchial inflammation and frequently implicated in asthma exacerbation. Furthermore, infection can reduce the effectiveness of corticosteroids and β2-agonists treatments. However, mechanisms of RSV-induced asthma exacerbation and related immune responses need to be better deciphered by using relevant model that could lead to define new molecular targets for innovative therapeutic approaches.</p> <p>We developed a relevant <i>in vitro</i> human airway model to evaluate the impact of RSV infection. Human bronchial epithelium (bronchial Mucil Air™, Epithelix) from healthy or asthmatic donors were cultured at air/liquid interphase in coculture with peripheral blood mononuclear cells (PBMC), and infected or not with RSV. Viral kinetics were measured through RTqPCR and viral titration. Expression of genes of interests were quantified by RTqPCR. Lymphocytes orientation were analyzed by flow cytometry to differentiate lymphocyte helper 1 (Th1) and 2 (Th2).</p> <p>RSV replication kinetic is comparable in both healthy and asthmatic. RSV infection induced upregulation of TNF-α and IL-6 genes in asthmatic, whose baselines were initially upregulated compared to healthy. Similarly, thymic stromal lymphopoietin, an important alarmin for asthma, is more expressed in asthmatic than healthy. Furthermore, RSV led to a significant imbalanced Th2/Th1 ratio, to the profit of Th2 lymphocytes, in line with an asthma exacerbation profile.</p> <p>We developed and validated a new <i>in vitro</i> bronchial MucilAir™ model cocultured with immune cells. Our results highlight overexpression of pro-inflammatory and exacerbation markers with the induction of a T2 immune response profile in the context of RSV infection. Further experiments using specific antibodies to characterize mechanisms of RSV-induced exacerbation of asthma and explore their potential therapeutic efficacy are currently ongoing.</p>		
Session 3. Bacterial and fungal infections		
13:55	<p>Keynote</p> <p>An autologous human iPSC-derived multicellular Alveolus-on-Chip reveals early pathological events of M. tuberculosis infection</p>	Dr. Jakson Luk <i>Researcher Laboratory of Host-Pathogen Interactions in Tuberculosis The Francis Crick Institute</i>
<p>Immunocompetent and experimentally accessible alveolar systems to study human respiratory diseases are lacking. Here, we developed a single-donor human induced pluripotent stem cell-derived lung-on-chip (iLoC) containing type II and I alveolar epithelial cells, vascular endothelial cells, and macrophages in a microfluidic device that mimic lung three-dimensional mechanical stretching and air-liquid interface. Imaging and single-cell RNA sequencing analysis revealed that the iLoC recapitulated cellular profiles present in the human distal lung. Infection of the iLoC with the human pathogen Mycobacterium tuberculosis (Mtb) showed that both macrophages and epithelial cells were infected but not permissive to bacterial replication. Stochastically, large macrophage clusters containing necrotic macrophages supporting Mtb replication were observed. A genetically engineered autophagy-deficient iLoC revealed that after Mtb infection, macrophage necrosis was higher upon ATG14 deficiency without bacterial replication. Together, we report an autologous, genetically tractable human alveolar model to study lung diseases and therapies.</p>		
14:25	Mechanistic insights into the early stage of Bordetella pertussis respiratory infection using fully-differentiated human nasal epithelial cells	Dr. Nathalie Mielcarek <i>Deputy Director, Center for Infection and Immunity of Lille</i>
<p>Bordetella pertussis is a Gram-negative bacterium responsible for whooping-cough. Despite a large vaccine coverage, this respiratory disease still represents a major health issue worldwide, as it was recently highlighted with the 2024 epidemic post-COVID19 in Europe. The ability of B. pertussis to colonize and persist in the nasal cavity of asymptomatic individuals might represent a key mechanism underlying the continuous circulation of the bacterium, even among vaccinated individuals. In this study, we used fully-differentiated human airway epithelial cells cultured at the Air-Liquid Interface (ALI) and reconstituted from a pool of 14 nasal health donors (MucilAir™ nasal pool) with the objective of increasing our understanding of the host-pathogen interaction in the human nasal cavity.</p>		

We evaluated a panel of endpoints induced by *B. pertussis* infection such as cytotoxicity, mucus production, mucociliary clearance and inflammation. We showed that *B. pertussis* interaction with nasal epithelial cells induced a dose-dependent cytotoxic effect associated with a loss of tissue integrity and a severe impairment of mucociliary clearance. These alterations occurred without any bacteria being detected inside nasal epithelial cells, the bacteria remaining confined to the surface of the ciliated epithelium. All together, these results suggest that *B. pertussis* induces profound functional defects in human nasal epithelium which provide mechanistic insight into the early stage of *B. pertussis* infection in the nasal cavity.

This work was supported by the European NOSEVAC Consortium, funded by the European Union's Horizon Europe research and innovation programme (Grant N°101080528).

14:50

A Primary Human ALI Infection Model with Bioluminescent MDR Pathogens for Real-Time Antimicrobial Efficacy Assessment

Dr. Marc Vandamme
*R&D program manager,
ERBC Group*

The increasing prevalence of multidrug-resistant (MDR) respiratory pathogens demands predictive human-relevant models capable of supporting translational antimicrobial research while reducing animal use. Conventional *in vitro* assays fail to recapitulate airway complexity, and animal models remain costly and ethically challenging. We developed an infection platform combining human primary airway epithelia cultured at the air-liquid interface (ALI) with bioluminescent MDR bacterial strains for real-time, non-destructive infection monitoring. ALI cultures were generated from basal cells isolated from human lobectomy healthy margins. Fully differentiated epithelia exhibited pseudostratified architecture, mucociliary activity, epithelial polarity, and tight-junction integrity, as characterized by confocal microscopy, histology, and flow cytometry. We applied this system to *Acinetobacter baumannii* (ABA) and *Klebsiella pneumoniae* (KP), two WHO critical-priority pathogens associated with nosocomial pneumonia. Recently isolated MDR clinical strains were engineered to stably express the lux operon, enabling longitudinal quantification of bacterial proliferation through bioluminescence. Following apical infection of fully differentiated ALI epithelia, progressive tissue colonization was monitored by bioluminescence imaging and colony-forming unit (CFU) enumeration. Epithelial barrier disruption occurred 18–21 hours post-infection, evidenced by transepithelial electrical resistance (TEER) decline and detection of viable bacteria in the basolateral compartment, confirming bacterial translocation. Bioluminescence closely paralleled CFU counts, validating it as a reliable, non-destructive readout. To demonstrate drug screening capability, tigecycline efficacy was evaluated at four concentrations (0.5–16× MIC) via delayed basolateral administration mimicking systemic exposure. A dose-dependent reduction in bacterial burden was observed, confirming quantitative therapeutic assessment capacity. The platform is compatible with antibiotics, monoclonal antibodies, and host-directed therapies. This ALI-bioluminescence model provides a predictive, dynamic tool bridging conventional *in vitro* assays and *in vivo* studies, supporting antimicrobial development in line with 3R principles.

Session 4. Modeling COPD / Asthma / IPF / CF / PCD *in vitro*

15:45

Keynote

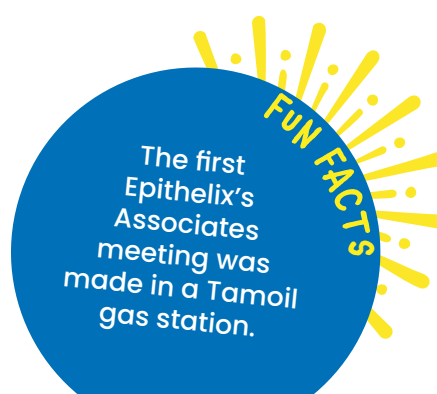
Advanced *in vitro* and *ex vivo* models for the development of respiratory therapeutics

PD Dr. Katherina Sewald
Division Co-Director of Preclinical Pharmacology and Toxicology and Head of Department of Preclinical Pharmacology & Infection and Immunology, Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM)

Respiratory drug development is shifting from broad anti-inflammatory and bronchodilatory approaches toward increasingly targeted interventions, including biologics, RNA-based medicines, pathway inhibitors and immune-modulating therapies. This transition places new demands on preclinical models. It is no longer sufficient to demonstrate target engagement; models must reproduce the biological context that converts target modulation into a clinically relevant response.

This presentation will discuss how advanced human *in vitro* and *ex vivo* models can support the development of respiratory therapeutics by linking mechanism, efficacy, responder biology and safety. Particular emphasis will be placed on matching model complexity to the specific development question.

Acute induced systems, air/liquid interface cultures, organoids and immune co-cultures are valuable for mechanistic proof-of-concept, although they may only partially reflect chronic remodelling and established disease history. ...



Patient-derived cells and precision-cut lung slices preserve tissue architecture, extracellular matrix, resident immune populations and donor-specific disease characteristics, providing a translational bridge to the clinical situation. Representative examples will illustrate the functional assessment of virus-triggered exacerbation biology, IL-4/IL-13 pathway modulation, anti-IgE strategies, inflammation and fibrotic tissue responses. A particular focus will be placed on precision-cut lung slices (PCLS), which preserve native tissue architecture, multicellular interactions and functional responses in a human-relevant *ex vivo* setting. These models can reveal endotype-dependent activity, distinguish antiviral effects from host-response modulation, and connect molecular changes with functional endpoints such as airway hyperresponsiveness, histamine release or tissue stiffness. Immune-competent systems further enable the assessment of adverse pharmacology, including impaired host defence, barrier injury and immune-mediated lung toxicity.

The central message is that no single model is sufficient. Successful translation requires a question-driven portfolio of complementary human models that preserves the biological complexity relevant to patients and identifies a meaningful therapeutic window.

16:15

Patient-Derived Airway Organoids for Modelling COPD

Miss Serena Bateman (BSc)
*Scientist,
Complex Models, Human Biology, GlaxoSmithKline (GSK)*

Background: Patient-derived airway organoids offer a long-term, passagable, scalable 3D model of differentiated respiratory epithelium. Conventional “apical in” organoids entrap motile cilia and secrete mucins within the lumen, limiting apical access, functional readouts and addition of stimuli. We developed extracellular matrix (ECM) free “apical out” organoids to expose the apical surface, enhance relevant cell populations and enable physiological stimulation for COPD disease modelling.

Methods and results: Organoids from healthy and COPD donors were cultured as ECM-embedded apical in controls and re polarised to an ECM free apical out orientation. Samples across multiple timepoints and media compositions were profiled by qPCR, immunofluorescence and effluent analysis (cytokines, mucus). Apical in organoids showed goblet cell markers but limited ciliation. In contrast, apical out organoids exhibited both goblet cells and motile cilia, with expression patterns dependent on time and media composition. The exposed apical surface can facilitate direct physiological stimulation and straightforward effluent sampling, improving suitability for target identification, compound screening and modelling COPD exacerbations.

Conclusion: Apical out airway organoids increase apical accessibility and cellular functionality versus apical in cultures, supporting their use for physiological assays and screening. Ongoing work focuses on assay automation and expanded phenotypic profiling to define how apical orientation affects cellular composition and disease-relevant responses.

16:40

Adopting and deploying the precision-cut lung slice model for airway constriction studies – challenges, pitfalls, and lessons learned

Prof. Ynuk Bossé
*Professor,
Faculty of Medicine, Université Laval*

Introduction: The technique of precision-cut lung slices (PCLS) is widely used for studying airway constriction in a setting that closely mimics a native lung environment. However, many technical and biological factors influence the extent of airway constriction in mouse PCLS. The optimization of these factors and the extent to which they can be adjusted to accommodate different experimental conditions remain to be determined.

Objectives: We thus far focused on three objectives: 1) Optimizing the duration of short-term incubations in mouse PCLS; 2) determining the number of airways to be analyzed in order to allocate a reliable value of constriction in one mouse; and 3) determining the effect of experimental asthma on airway constriction.

Methods: PCLS were isolated from male BALB/c or C57BL/6 mice with or without experimental asthma induced by repeated exposures to house dust mite. PCLS were stimulated once at 24 hours post-incubation or every 24 hours for three days with increasing concentrations of methacholine.

Results: Airway constriction in a single mouse was extremely variable, ranging from 0 to 100%, but it settled to a consistent average when about 35 airways were analyzed. The level of constriction did not differ between 24, 48, and 72 hours of incubation. Experimental asthma did not influence airway constriction.

Discussion: Airway constriction in PCLS is reproducible, it can be maintained to an optimal level for at least 72 hours, and is not different in PCLS from mice with or without experimental asthma. It is recommended that at least 35 airways per mouse be studied to confidently attribute a level of airway constriction in one mouse.

DAY 2 – Friday Oct 9 2026

Session 5. Biological barriers, MPS & Drug Delivery

09:00	<p>Keynote</p> <p>The Alveolar Barrier on Chip: Are We There Yet?</p>	<p>Prof. Dr. Olivier Guenat Professor of Biomedical Engineering Bern University</p>
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The human lung is an extraordinarily complex organ whose function emerges from a fragile yet resilient 3D architecture governed by ECM tension, surfactant balance, and air-liquid interface mechanics. Although lung on chip systems have advanced considerably, current models still capture only selected aspects of this *in vivo* complexity. In this keynote, we present a platform built around a thin, suspended collagen-elastin membrane that mimics the mechanical fragility and dimensions of the native alveolar basement membrane while supporting a geometrically defined array of alveolar units.

Precision injection molded microfluidics provide the rigid support required for reproducible micron scale deflections, enabling physiologically relevant cyclic actuation within an SBS compatible format. Co culture of human alveolar epithelial cells and fibroblasts under these subtle mechanical cues reveals amplified TGF β 1-driven fibrotic signaling and a measurable anti fibrotic response to nintedanib. Together, this architecture demonstrates how extremely small mechanical stresses can shape cellular behavior and supports an emerging *in vitro* IPF model, offering a robust and mechanistically faithful platform for probing early disease pathways and evaluating therapeutic interventions.

09:30	<p>Membrane-free human airway-on-a-chip integrating resident memory T cells to model respiratory viral infection</p>	<p>Dr. Yann Tinguely Post Doctorate Scientist, Institute of Human Biology (IHB) at Roche</p>
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Respiratory viral infections remain a leading cause of mortality, yet effective treatments against many pathogens are still lacking. Furthermore, animal models present ethical and experimental constraints and frequently result in poor clinical translation due to interspecies differences. Consequently, there is a critical need for physiologically relevant human *in vitro* models of the respiratory system to advance our understanding of viral pathogenesis and facilitate targeted therapeutic strategies. Most existing *in vitro* models rely on epithelial cells cultured on synthetic membranes, which fail to mimic the extracellular matrix in terms of mechanical and biochemical properties and create an artificial barrier impeding co-culture with relevant immune cells. Moreover, *in vitro* infection studies have largely focused on innate immune cell recruitment of neutrophils, while the contribution of adaptive components remains less explored. Notably, tissue-resident memory T cells (TRMs) have been shown to play a crucial role in the frontline defense against invading pathogens *in vivo* but are currently neglected in most other *in vitro* models. To address these limitations, we developed a hydrogel-based lung-on-a-chip derived from human airway organoids, designed to recreate an *in-vivo* like microenvironment. The hydrogel-based system supported long-term culture of the airway epithelium at an air-liquid interface and recapitulated key functional features including cell type diversity, barrier integrity, mucus secretion, and ciliary beating. Importantly, the model enabled barrier-free co-culture with autologous lung TRMs, which integrated into the epithelial layer, recapitulating the intraepithelial localization observed *in vivo*. Upon rhinovirus infection, the co-culture model responded with rapid interferon gamma release, enhanced TRM patrolling behavior and a donor-specific attenuation of viral induced loss of ciliated cells, demonstrating the platform's capacity to model complex immune responses. This advanced hydrogel-based platform offers a powerful tool for dissecting human respiratory responses and holds significant potential to study respiratory diseases and evaluate potential treatments.

09:55	<p>Beyond nominal concentration: A framework for standardizing <i>in vitro</i> lung epithelial permeability assessment</p>	<p>PharmD. Rayane Boufalaas Researcher, INERIS- NAHE unit, University of Technology of Compiègne (UTC) - BioMechanics and BioEngineering (BMBI) laboratory</p>
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Inhalation is a major route of human exposure to environmental and industrial chemicals. Yet, the fate of inhaled compounds across the pulmonary epithelium remains insufficiently characterized due to accessibility challenges and animal model limitations. *In vitro* lung epithelial models offer valuable insights into absorption. However, reliance on nominal concentrations and lack of a standardized model led to inaccurate permeability assessment. This study aimed to harmonize *in vitro* pulmonary permeability assays by comparing bronchial and alveolar epithelial models cultured under air-liquid interface (ALI) conditions and identifying key factors influencing permeability measurements.

Barrier integrity of the bronchial Calu-3 and h-AELVi models were monitored over a two-week at ALI culture using transepithelial electrical resistance (TEER), Lucifer Yellow permeability, occludin expression. Apparent permeability (Papp) was measured under identical experimental conditions in both models for five xenobiotics with a wide physicochemical range (propranolol, sumatriptan succinate, mono-(2-ethylhexyl) phthalate, thymol, and hippuric acid). Then, the permeability of mixture of ten per- and polyfluoroalkyl substances (PFAS) was investigated in Calu-3 cells under the harmonized experimental conditions.



Both models developed stable and tight epithelial barriers. Despite different cell origins and growth kinetics, both models provided nearly identical Papp values. Mass balance recovery was high for 9/10 PFAS. N-methyl-perfluorooctane sulfonamide (MeFOSA, logP > 5), showed a markedly lower recovery, indicating substantial compound loss during the assay. PFAS permeability was influenced by functional group and chain length, with short-chain perfluoroalkyl carboxylic acids (PFCA) more permeable than long-chain PFCA and perfluoroalkyl sulfonic acids (PFSA), while perfluorooctane sulfonamide (FOSA) showed substantial cellular retention. In conclusion, effective concentrations ensure consistent Papp values across both models. Unaccounted chemical losses through plastic binding, volatilization and metabolic degradation lead to an underestimation of Papp values. A framework based on experimental results was proposed to standardize *in vitro* lung epithelial permeability assays for drug delivery and safety assessment.

Session 6. Regulatory considerations, Exposure Devices and Deposition Modelling

10:50	<p>Keynote</p> <p>Exposure scenarios and regulatory considerations for <i>in vitro</i> lung models</p>	<p>Dr. Clive Roper Director, Roper Toxicology Consulting Limited</p>
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The inhalation route of exposure in the human can be considerable for many chemicals and formulations in liquid, vapour, powder, and nebulised formats. The upper respiratory epithelium is the first tissue that these inhaled materials directly interact with, and many chemicals can reach past the conducting airways into the lower, alveolar, tissues. Further absorption into the systemic circulation can occur at different locations within the respiratory tract and may result in systemic toxicity. Acute inhalation toxicity testing for regulatory purposes is currently performed in rodents following OECD Test Guideline Nos. 403, 433, and 436. Due to the differences in structural architecture and physiology between the human and rodent lung and the differences in cell sensitivity to toxicant between species, considerable effort has been placed into developing human-relevant *in vitro* models to replace animals. Many human-derived 2D cell and complex 3D co-culture test systems and test methods have been developed. Although no single *in vitro* test method has been accepted by regulatory agencies as a stand-alone replacement for inhalation toxicity testing in animals, new approach methods incorporating chemical, *in vitro*, and computational toxicology models have been more successful. Since no single test will cover all respiratory toxicities, validation of an individual test will be unlikely completed. Publishing case studies demonstrating successful qualifications of a single context of use are recommended to build confidence in the new test methods. A couple of programmes are moving through or towards the OECD test guidelines programme.

11:20	<p>Relevance of Characteristic Aspects of <i>in vitro</i> Lung Models: Mechanisms of Pulmonary Aerosol Deposition and Mechanobiology</p>	<p>Dr. Otmar Schmid Head of Research Group, Helmholtz Munich, Institute of Lung Health and Immunity</p>
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Air-liquid interface (ALI) pulmonary models are increasingly favored over submerged cultures for their enhanced physiological and clinical relevance in inhalation therapy and toxicology. To increase accuracy, these models now incorporate complex features such as multicellular 3D arrangements, biomimetic basement membranes, perfusion, varied aerosol deposition mechanisms, and cyclic stretch. However, the resulting increase in cost and complexity necessitate a critical evaluation of their necessity.

This analysis examines two specific features: aerosol-cell deposition mechanisms and cyclic tissue stretch. Pulmonary aerosol deposition is mainly governed by diffusion, impaction, and gravitational settling depending on aerosol characteristics and lung region. While Brownian diffusion dominates for particles smaller than 500 nm, larger particles (2-10 µm) exhibit more complex behavior: inertial impaction prevails in the upper bronchial region, whereas gravitational settling and breathing profiles govern deposition in the distal bronchial and alveolar regions. Given these regional and size-dependent mechanisms, should one work with region-specific aerosol-cell exposure systems that mimic the most relevant deposition mechanisms according to particle size?

Mechanobiology is an emerging field of research with high relevance for the lung. Breathing-induced cyclic tissue stretch is a key stimulus for activation of mechanobiological mechanisms. Moreover, blood flow induces shear stress in the vascular system triggering mechanobiological pathways. We provide a brief introduction to mechanobiology in the context of the lung, discuss some examples of micro-/millifluidic lung systems capturing mechanobiological aspects and discuss the justification of the resulting enhanced model complexity in light of its clinical relevance.



11:45	Advancing Human-Relevant <i>In Vitro</i> Methods to Assess Respiratory Tissue Irritation	Dr. Andreas Stucki Advisor, PETA Science Consortium International e.V.
<p>Inhalation toxicity is a complex area for toxicological assessment due to the anatomical and functional diversity of the respiratory tract and the range of potential adverse outcomes. Regulatory decisions have historically relied heavily on animal tests; however, differences in rat and human respiratory anatomy and biology have led to an increase in the use of <i>in vitro</i> human cell-based testing approaches. This work presents collaborative efforts—from workshop to multi-laboratory study—aimed at building scientific confidence and regulatory acceptance for an <i>in vitro</i> method to assess respiratory tissue irritation.</p> <p>After an initial international workshop, two case studies built experience across respiratory models: two silane vapors were tested in a bronchial cell line and bronchial reconstructed human respiratory epithelium (RHRE), showing that highly hydrolytic substances can be tested <i>in vitro</i>; and two surfactants were tested following single, liquid exposure in a bronchial cell line, a bronchial RHRE, and an alveolar RHRE, with repeat exposures additionally assessed in the bronchial RHRE. In parallel, literature reviews summarized differences between the human and rat respiratory tracts and their implications for toxicological assessments, and evaluated the human relevance of RHRE.</p> <p>The aforementioned work and a review compiling existing <i>in vitro</i> protocols and chemicals, informed the design of a three-laboratory study. A commercially available nasal RHRE (MucilAir, Epithelix) was exposed to ~20 chemicals, applied apically as liquids (30 µL; ~100 µL/cm²) for 24 h. Barrier integrity and cell health were assessed using transepithelial electrical resistance (TEER), cytotoxicity (lactate dehydrogenase), and viability (PrestoBlue). Most chemicals showed concentration-dependent responses; analyses are ongoing to quantify interlaboratory reproducibility and predictivity for identifying respiratory tissue irritants. Overall, this multipronged approach provides a regulatory-ready dataset with the ultimate aim to support the development of a fit-for-purpose Organisation for Economic Co-operation and Development (OECD) test guideline for local, point-of-contact respiratory tissue irritation using RHRE.</p>		
Session 7. Mucus, Surfactant, Cilia Functions and Modulations		
13:40	Keynote Physiologically Relevant Airway Mucus in ALI Models	Dr. Lydia Esteban Enjuto Chief Technology Officer, MucFlow
<p>Airway mucus is a key component of the respiratory defence system, yet its physicochemical properties are often poorly reproduced in <i>in vitro</i> Air-Liquid Interface (ALI) models. This study will discuss strategies to improve the physiological relevance of ALI-derived mucus by comparing its rheological properties with those of native human airway mucus. We will present approaches to tune mucus produced by young ALI cultures to generate a model mucus with rheological properties that more closely resemble those observed <i>in vivo</i>.</p> <p>The study will also describe ongoing efforts to investigate the rheological properties of mucus across different respiratory diseases and airway regions, including nasal and bronchial mucus. In addition, it will provide an overview of how physiologically relevant mucus models and mucus rheology can support preclinical research, including applications in the evaluation of different therapeutic molecules and the assessment of interactions with environmental pollutants. Overall, this work contributes to the development of more physiologically relevant <i>in vitro</i> airway models for studying respiratory biology, advancing preclinical drug development, and improving the predictive value of respiratory <i>in vitro</i> models.</p>		
14:10	High throughput screening for compounds allowing readthrough of premature termination codons based on genetically engineered PCD patient specific hiPSCs	David Puertollano Martín (M.Sc.) Hannover Medical School (MHH) Leibniz Forschungslaboratorien für Biotechnologie und künstliche Organe (LEBAO)
<p>Primary Ciliary Dyskinesia (PCD) is a rare genetic disease characterized by impaired mucociliary clearance in the airways, leading to recurrent respiratory infections, bronchiectasis and ultimately lung failure. The disease is caused by mutations in genes essential for ciliary function, resulting in defective ciliated cells. More than 50 genes have been implicated in PCD, and mutations across these loci give rise to a broad spectrum of clinical phenotypes, none of which currently have a curative treatment. Notably, approximately 25% of all PCD-causing mutations are premature termination codons (PTCs), which represent a potentially targetable class through PTC suppression therapies.</p> <p>In this study, DNAIL-dTomato reporter human induced pluripotent stem cells (hiPSCs) were generated using Cas9-mediated gene editing and subsequently differentiated into proximal airway epithelia in air-liquid interface (ALI) cultures, where dTomato-expressing motile ciliated cells developed. A second reporter line carrying a patient-specific PTC in exon 9 was generated in homozygosity. Upon ALI differentiation, these cells formed immotile ciliated cells with a markedly reduced dTomato mean fluorescence intensity (MFI). Western blot and PCR analyses revealed, in addition to the canonical transcript variant, an unexpected exon-skipped isoform lacking the PTC, which accounts for the observed dTomato expression.</p> <p>To assess the system's utility for drug screening, PTC suppressors were applied intermittently to 96-well ALI cultures, and dTomato MFI was quantified over time. Gentamicin and Escin sustained higher MFI levels compared with the DMSO control, whereas other compounds produced an initial increase followed by a pronounced decline. Additionally, differentiation into respiratory ciliated cells was achieved in submerged 384-well cultures, establishing a platform suitable for high-throughput screening.</p>		

14:35	Nanoscale structural insights into human pulmonary mucus and its snail slime-based surrogate	Dr. Marine Le Goas <i>CNRS researcher, Institut Galien Paris-Saclay, Université Paris-Saclay</i>
<p>Pulmonary drug delivery provides a local, non-invasive, and accessible method to treat respiratory diseases. However, the mucus layer lining the bronchial airways acts as an effective physical barrier, trapping therapeutic agents and preventing them from reaching underlying target cells.[1] The nanoscale structure of mucus critically determines the penetration ability of pharmaceutical formulations. Yet, this structure remains poorly understood due to technological limitations in preserving and characterizing its native architecture.[1,2]</p> <p>In this study, we present a novel approach combining high-pressure freezing, cryosubstitution, and transmission electron microscopy (TEM) to capture the nanoscale structure of patient-derived pulmonary mucus. By integrating automated image processing, we provide the first quantitative characterization of its architecture, revealing a fine, interconnected fibrous network with pore sizes consistently below 500 nm, delineated by thin fibers. These findings challenge previous reports of micrometer-scale pores, which likely arose from artifacts introduced by conventional sample preparation and observation methods. To address the need for structurally relevant mucus models, we applied the same methodology to cross-linked snail slime, a mucin-containing hydrogel. Beyond its previously documented rheological similarity to human mucus,[3] we demonstrate that cross-linked snail slime faithfully replicates the nanoscale fibrous architecture of pulmonary mucus.</p> <p>This work not only clarifies the true structural organization of human airway mucus but also establishes cross-linked snail slime as a robust biomimetic model for studying drug diffusion and barrier properties. Additionally, the detailed protocol for TEM characterization and automated image analysis presented here offers a reproducible framework for future investigations of mucus and its surrogates, ensuring precision and consistency in structural assessments.</p> <p><i>References</i> 1. Murgia et al. <i>Adv Drug Deliv Rev</i> 2018; 124: 82–97. 2. Meziu et al. <i>Int J Pharm</i> 2021; 597: 120238. 3. Milian et al. <i>Biomacromolecules</i> 2023; 25: 400–412.</p>		
Session 8. Think Different		
15:30	Human-relevant 3D ALI models of NSCLC for diagnostic biomarker discovery	Dr. Dania Movia <i>Assistant Professor in Biomedical and Health Sciences, Department of Biology, Maynooth University, Ireland</i>
<p>Non-small cell lung cancer (NSCLC) remains a leading cause of cancer-related mortality worldwide, largely due to late-stage diagnosis. There is an urgent need for clinically relevant diagnostic biomarkers. Bronchoalveolar lavage fluid (BALF) reflects the lung tumour microenvironment and has strong diagnostic potential in NSCLC; however, limited access to patient-derived BALF samples and the lack of human-relevant BALF animal models hamper biomarker discovery. To address this, our project investigated the use of 3D air-liquid interface (ALI) <i>in vitro</i> models of NSCLC to generate BALF-like samples and identify soluble and extracellular vesicle (EV)-associated biomarkers. Mono- and co-culture ALI cancer models from human NSCLC cells, alongside three healthy primary alveolar ALI models, were used. BALF-like samples were harvested from the models via apical saline washes, mimicking clinical procedure, and then analysed for protein content, oncogenic proteins, and EV-associated surface markers. Cancer ALI models showed significant up-regulation of 11 soluble biomarkers, 8 of which were previously reported in clinical literature as present in NSCLC patient-derived BALF. Key findings were validated by ELISAs and through comparison with biomarker expression in BALF samples from patients with inflammatory respiratory disease. Our results highlight the value of 3D ALI models as human-relevant, non-animal platforms for translational biomarker discovery and open the way to BALF-based diagnostics for NSCLC.</p>		
16:00	Next-Generation Lung <i>In Vitro</i> Models with Integrated Vasculature	PD Dr. Anja Lena Thiebes <i>Group Leader, RWTH Aachen University</i>
<p>Vascularization is essential for tissue homeostasis in most organs. Beyond ensuring oxygen and nutrient delivery, endothelial and perivascular cells actively regulate cell-cell communication and tissue remodeling, particularly in disease contexts. Consequently, advanced <i>in vitro</i> airway disease models must incorporate multiple cell types, including vascular cells, to more accurately recapitulate physiological complexity.</p> <p>We developed a three-dimensional airway model composed of three human primary cell types: tracheal epithelial, endothelial, and mesenchymal cells, embedded within fibrin hydrogels. These constructs are cultured for 28 days at air-liquid interface in a specialized differentiation medium that supports both epithelial maturation and vascular network formation. .../...</p>		

Within the model, endothelial cells organize into hollow, capillary-like structures in cooperation with mesenchymal cells, while a fully differentiated epithelium emerges, consisting of ciliated, secretory, and basal stem cells. This system provides a powerful platform for investigating airway diseases, including viral pathogenesis such as SARS-CoV-2 infection, using the virus as well as inactivated viral particles. Additionally, the application of mechanical cues such as cyclic stretch, compression, and altered matrix stiffness enables simulation of disease-specific conditions and the study of tissue remodeling processes. The model was also adapted for integration into airway-on-chip systems with perfusable vasculature and used to explore interactions between lung tumors and capillarization. Overall, we have established an innovative platform that combines mature respiratory mucosa with a vascularized submucosa, offering broad potential for complex disease modeling and personalized medicine, while supporting the reduction and replacement of animal experimentation.

16:25

Keynote

**Innovative Human Lung Models:
Immune Microenvironments and
Scaled Discovery**

Dr. Lauriane Cabon

*Group lead, Applied Organoid Technologies,
Institute of Human Biology (IHB)
Roche*

Translating complex human pulmonary biology into predictive *in vitro* assays requires capturing the cellular crosstalk of the lung immune microenvironment while establishing reproducible, medium-throughput formats for industry applications. At the Roche Institute of Human Biology, we address this challenge by developing bioengineered human multicellular alveolar platforms that combine high physiological relevance with practical scalability.

In this talk, I will introduce an autologous alveolar co-culture platform derived from primary human lung resections, incorporating AT2 organoids alongside matched alveolar macrophages and tissue-resident memory T cells (TRMs). I will detail how we use this model to investigate the cellular mechanisms behind checkpoint inhibitor-induced pneumonitis through immune cell flow cytometry, cytokine profiling, and epithelial viability assays. By testing matched tumor co-cultures alongside the lung tissue, we can also evaluate whether candidate immunosuppressive drugs reduce toxicity without blunting anti-tumor efficacy.

Beyond safety testing, I will show how we adapt this co-culture system into 96-well formats for target validation in chronic obstructive pulmonary disease (COPD), using high-content imaging to measure organoid growth, morphology, and viability. Lastly, I will describe configuring these alveolar models into transwell systems to study host-pathogen interactions and model viral exacerbations, a critical driver of COPD progression. Together, these autologous models provide practical, human-relevant systems to study disease mechanisms, validate targets, and assess drug safety.

16:50

Closure of LIVE2026

EpiSession

Click to jam!

Scan the code
to enjoy music
by the Epithelix team.



THE UNOFFICIAL EPITHELIX DICTIONARY

20 years. 20 words. Our definitions.

ABSTRACT

250 words, several drafts, one deadline extension.

ALI

Air-liquid interface. Also: the place where everyone's patience is tested.

ANNIVERSARY

Proof that the risk was worth taking.

AOP

'Appellation d'Origine Contrôlée' for the French cheese lovers. 'Adverse Outcome Pathways' for the others.

APERO

Another way to spend time at work.

ASAP

When you definitively know that it will take a lot of time.

BRAINSTORM

A meeting with a lot of coffee and no deadline.

CILIA-X

A CBF analysis software (do not forget the hyphen if you google it).

COFFEE

Critical laboratory reagent.

CONGRESS

See 'APERO'.

DEADLINE

A concept. Rarely a date.

EU PROJECT

A shared adventure with more acronyms than participants.

INCUBATOR

A machine that somehow always needs checking.

IT GUY

Has the superpower to solve your computer issue just by being there when you want to show him.

KICK-OFF MEETING

The one day everyone is optimistic about the timeline.

LINKEDIN

Not just a platform, a mindset for collaborators to high score their network optimization and proactively leverage their cross-functional relationships.

MEETING

A gathering in which someone eventually says, 'Let's take this offline'.

ORGANOID

Small, complicated, occasionally the star of the meeting.

PIPETTE

An extension of the scientist's hand.

QUICK QUESTION

Rarely quick. Rarely one question.

P-001 Fabienne Archer
 P-002 Fresia Arellano
 P-003 Mireille Caul-Futy
 P-004 Marianne Costa
 P-005 Giulia Gorgoni
 P-006 Sebastian Reusch
 P-007 Caroline Weight
 P-008 Fabienne Archer
 P-009 Matthew Sheavyn
 P-010 Shaginth Sivakumar
 P-011 Georgios Stroulis
 P-012 Pauline Zamprogno
 P-013 Takao Ashikaga
 P-014 Guy Barbin
 P-015 Emilia Barreto Duran
 P-016 Marion Blayac
 P-017 Gerrit Bredeck
 P-018 Aline Chary
 P-019 Shaun D. McCullough
 P-020 Shaun D. McCullough
 P-021 Manuel Garcia-Käufer
 P-022 Arno Gutleb
 P-023 Michael Hollings
 P-024 Amanda Ulrey
 P-025 Darleen Koehn
 P-026 Nuria Roldan
 P-027 Clive Roper
 P-028 Sandra Verstraelen
 P-029 Ludovic Vieille-Petit
 P-030 Pauline Zamprogno
 P-031 Pauline Zamprogno
 P-032 Ghislaine Arib
 P-033 Phillip Clapp
 P-034 Laurien Czichon
 P-035 Mathieu Hubert
 P-036 Eloïse Latouille
 P-037 Thamara Rioline
 P-038 Beatrice Spagnoli
 P-039 Yuki Yamamoto
 P-040 Laura von Schledorn
 P-041 Cyrill Roth
 P-042 Jorid Sørli
 P-043 Mathilde Delaval
 P-044 Paola Haemmerli
 P-045 Song Huang
 P-046 Nouha Mannour
 P-047 Lea Nawatzky
 P-048 Maria Steinke
 P-049 Samanta de Matos Silva
 P-050 Ugne Ceplaitė
 P-051 Julia Dubois
 P-052 Axelle Fransen
 P-053 Hulda Run Jonsdottir
 P-054 Hulda Run Jonsdottir
 P-055 Victoria Meliopoulos
 P-056 Joelle Mettier
 P-057 Rémi Pereira de Oliveira
 P-058 Loison Sheryline
 P-059 Jolly Thomas

Information for poster presenters:

POSTER HANGING TIME:

8:30 AM – 9:00 AM – THURSDAY OCT 8 2026

Poster Display

- Posters are displayed in the poster presentation area throughout the duration of the congress.

Poster allocation

- The list of poster presenters is provided here. Please find your name and note your poster number, which corresponds to the position assigned to your poster on the poster grid.
- Your poster should be displayed in the space marked with your assigned poster number.

Poster Take Down

- Presenters are responsible for setup and takedown. Posters must be taken down immediately after the closure of LIVE2026 on Friday October 09.

Poster Viewing

- Poster presenters are encouraged to be available to present their posters during the coffee and lunch breaks to ensure maximum exposure for their research.

BACTERIAL INFECTIONS AND FUNGAL INFECTIONS

P-001

Modeling mycoplasma infections: insights from Air-Liquid Interface cultures of primary bronchial epithelial cells

Fabienne ARCHER

BACTERIAL INFECTIONS AND FUNGAL INFECTIONS

Although *Mycoplasma (M.) bovis* is widely recognized as an emerging bacterial pathogen associated with bovine respiratory disease, the pathophysiological processes underlying *M. bovis* infections remain poorly understood. Another mycoplasma, *Mycoplasma (M.) bovirhinis* is frequently found in association with *M. bovis* and is suspected to contribute to the overall clinical signs, despite being considered a commensal or opportunistic organism. The present study aims to compare the interactions of *M. bovis* and *M. bovirhinis* using different pulmonary bovine *in vitro* models. Both the embryonic bovine lung (EBL) immortalized cell line, which is easy to grow and manipulate, and bovine primary lung epithelial cells cultured at an air-liquid interface (BAE – ALI), which better mimic *in vivo* tissues, were used.

We confirmed the ability of *M. bovis* to invade epithelial cells, including the EBL cell line – as previously demonstrated – as well as type II alveolar cells and ciliated cells in ALI-cultured primary lung cells. In contrast, the commensal *M. bovirhinis* efficiently invaded EBL cells but did not invade alveolar or ciliated cells in ALI-cultured primary lung cells. Both mycoplasmas induced a strong inflammatory response of epithelial cells within our BAE – ALI model. However, differences were observed in the dynamics of expression of genes encoding receptors involved in pathogen detection, chemokines, cytokines and their receptors as well as antimicrobial defense effectors. The distinct patterns of cell tropism and invasion capacity observed between the two species (a pathogen and a commensal) correspond well with their differential pathogenicity *in vivo* and may be attributable to differences in their cell-adhesion properties.

P-002

Air-liquid interface culture as a tool for deciphering of *Bordetella pertussis* interactions with human upper airway mucosa

Fresia Arellano

BACTERIAL INFECTIONS AND FUNGAL INFECTIONS

Bordetella pertussis is a Gram-negative bacterium that causes a severe respiratory illness known as pertussis or whooping cough. Despite high vaccination coverage in developed countries, pertussis outbreaks continue to occur, making it pertussis to the least controlled vaccine-preventable bacterial infectious disease. The bacteria specifically adhere to ciliated airway epithelial cells, a system that is challenging to replicate under laboratory conditions. In this study, we employ a primary tissue culture model of human nasal epithelial cells at the air-liquid interface to investigate *B. pertussis* interactions with the upper respiratory mucosa. Flow cytometry, microscopy and histological analyses revealed that cells obtained from nasal swabs of healthy volunteers differentiated into a polarized airway epithelium, characterized by the presence of ciliated cells with actively beating cilia. Experiments using a comprehensive set of *B. pertussis* mutants revealed that pertussis toxin, adenylate cyclase toxin, the type 3 secretion system and several other known virulence factors are dispensable for bacterial adhesion to ciliated cells. In contrast, mutants lacking the filamentous hemagglutinin (FhaB) or fimbriae (Fim) adhesins failed to bind the ciliated cells. This finding aligns with *in vivo* data showing that the fhaB and fim mutants exhibit significantly reduced colonization of the mouse nasal cavity compared to the parental strain (Holubova et al, 2022, PLoS Pathogens 18:e1010402). These results demonstrate that the air-liquid interface culture of human nasal epithelial cells provides a physiologically relevant model system for studying *Bordetella* adhesion strategies in the upper respiratory mucosa.

P-003

Development of fully primary human alveolar-resident macrophages to study streptococcus pneumoniae infections

Mireille CAUL-FUTY

BACTERIAL INFECTIONS AND FUNGAL INFECTIONS

Antibiotic resistance is a growing global health threat, yet development is hindered by the lack of physiologically relevant lung infection models. *Streptococcus pneumoniae* pneumonia depends on early interactions between alveolar epithelial cells and macrophages, therefore *in vitro* models incorporating immune components may improve studies of pathogenesis and antimicrobial efficacy.

An immunocompetent human alveolar co-culture was developed by co-culturing primary resident alveolar epithelial cells and resident lung macrophages. The AlveolAir™ model forms a tight epithelial barrier of type I and II pneumocytes at the air-liquid interface, supported by endothelial cells, with macrophages added apically to mimic the alveolar immune compartment.

Macrophages were introduced 24 h prior to infection with *S. pneumoniae* serotype 19F. Bacterial growth, transepithelial resistance (TEER), lactate dehydrogenase (LDH) release, and protein expression were assessed up to 72 h, comparing cultures with and without macrophages and with meropenem-treated controls. Experiments included macrophages from three donors to evaluate donor variability.



The initial inoculum of 100 CFU expanded to 10^7 CFU at 24–72 h in epithelial cultures alone, whereas no CFU were detected in cultures containing macrophages or treated with meropenem. Infection progressively reduced TEER and caused >50% cytotoxicity at 72 h, while other conditions remained unaffected. Macrophages also increased surfactant protein A expression, not seen with infection alone.

This model demonstrates the essential role of resident macrophages in controlling *S. pneumoniae* and maintaining alveolar function, aiming to provide a more predictive platform for preclinical antibiotic screening.

P-004

Developing a persister *Pseudomonas aeruginosa* model strain to mimic chronic and recalcitrant infections in CF and COPD.

Marianne Costa

BACTERIAL INFECTIONS AND FUNGAL INFECTIONS

Chronic *Pseudomonas aeruginosa* (PA) lung infection is considered a hallmark of cystic fibrosis (CF), and despite the introduction of CFTR modulators transforming CF treatment these lung infections generally persist. Similar PA infections are also increasingly recognised in patients with chronic obstructive pulmonary disease (COPD). For patients, the presence of chronic PA infection is associated with sustained airway inflammation, progressive decline in lung function and increased mortality. Isolates of such infections are commonly found in the laboratory to be sensitive to the antibiotics administered, yet they fail to eradicate the infection. Therefore, once the infection becomes chronic the focus shifts to management of the infection rather than treatment. Persisters are gaining attention as the primary reason for this clinical failure.

Studies have shown that CF patients often develop variants of PA that produce a higher percentage of persisters over the course of long-term infection. These persisters are able to survive lethal antibiotic exposure via metabolic shutdown, which disables the pathways typically targeted by these molecules. The observed tolerance is phenotypic, and once the stressor is removed the bacteria are able to resume growth and consequently cause a relapse. As conventional antibiotics are ineffective, new treatments are needed that specifically target and eradicate persisters. To achieve this we need to be able to screen against persisters- which is not straightforward owing to their low abundance, phenotypic heterogeneity and transient nature. Thus, Olgram has developed and characterised a genetically inducible PA persister strain for use in an antipersister molecule screening platform or *in vitro* lung tissue models. Our PA persister strain is able to survive treatment with multiple classes of bactericidal antibiotics and has been found to be resistant to phagocytosis. The persister strain has been used to identify antipersister hits that target bacterial membranes.

P-005

Modelling pneumococcal colonisation and acute infection in a human Air-Liquid Interface respiratory epithelium

Giulia Gorgoni

BACTERIAL INFECTIONS AND FUNGAL INFECTIONS

Introduction. *Streptococcus pneumoniae* is a major human pathogen causing local and invasive diseases, including sinusitis, otitis media, pneumonia, sepsis, and meningitis. Nasopharyngeal carriage is a reservoir for transmission and a precursor of disease. Air-Liquid Interface (ALI) models may support the study of pneumococcal colonisation and infection of the human respiratory epithelium.

Materials and Methods. ALI cultures were established from human primary nasal epithelial cells (HNEC, Epithelix), seeded onto collagen-coated transwell inserts, maintained in liquid-liquid conditions for 2–4 days, then air-lifted and differentiated for ~21 days into a pseudostratified epithelium (assessed by TEER, immunofluorescence, SEM). Using a fluorescent *S. pneumoniae* D39 strain, two models were developed: (i) acute infection model, with continuous or transient bacterial exposure (MOI 50:1, 10:1) and, (ii) colonisation model for up to 72 hpi, based on a lower MOI (0.1:1) and a modified transient bacterial exposure protocol with apical medium replenishment every 12 h. In both cases, epithelial integrity (TEER) and cell damage as well as bacterial replication/localisation were assessed. **Results.** Acute infection model: MOI 50:1 caused rapid epithelial damage, while MOI 10:1 preserved barrier integrity. Under continuous exposure, bacteria remained predominantly extracellular, whereas transient exposure led to a modest increase of intracellular bacteria over time. Colonisation model: the modified challenge protocol maintained the host-pathogen equilibrium for at least 72 hpi. Viable bacteria were recovered in both extracellular and intracellular compartments, confirming prolonged colonisation of the nasal epithelium.

Conclusions. The ALI respiratory model is a flexible platform for studying pneumococcal infection and colonisation. The acute infection model highlighted the importance of MOI and exposure conditions for preserving epithelial integrity. The colonisation model showed that controlled apical exposure allowed stable host-pathogen homeostasis over time, possibly mimicking asymptomatic nasopharyngeal carriage. These two approaches provide two distinct robust systems for studying both pneumococcal colonisation and infection of the human nasal epithelium;



P-006

Using human airway and alveolar organoid-derived monolayers for analysis of host-fungal interactions

Sebastian Reusch

BACTERIAL INFECTIONS AND FUNGAL INFECTIONS

Background and aims:

The WHO Fungal Priority Pathogens List classifies *Cryptococcus neoformans* as a critical-priority fungal pathogen and *Cryptococcus gattii* as a medium-priority pathogen, underscoring their clinical relevance for global fungal disease research and public health. Following inhalation, cryptococci can cause localized pulmonary disease or disseminated infection. Current pathogenicity studies rely mainly on animal models, tumor-derived cell lines, or isolated immune cells, which incompletely reproduce the complexity of the human respiratory tract. More physiologically relevant human *in vitro* models are therefore needed to investigate early host-fungal interactions and cryptococcal virulence in a human context.

Methods:

We co-cultured cryptococcal reference strains (*C. gattii* VGII/R265, a primary lung pathogen, and *C. neoformans* VNI/H99, the main genotype associated with disseminated CNS infection) with organoid-derived monolayers (ODMs) generated from adult stem cells of proximal airway and distal alveolar human respiratory tissue. Barrier function of ODMs was assessed by continuous transepithelial electrical resistance measurements for up to ten days. Fungal adhesion, internalization, and translocation were examined by immunofluorescence stainings, while cryptococcal morphology was assessed by ink stainings. Finally, fungal viability was quantified by cell counting and cultivation.

Results:

Airway ODMs maintained an epithelial barrier integrity following cryptococcal exposure, whereas alveolar ODMs showed a pronounced, strain-specific barrier disruption. Cryptococcal adhesion to and internalization into airway ODMs was rare, while alveolar ODMs were more susceptible to fungal internalization and translocation. Both cryptococcal strains could proliferate on ODMs, and the respiratory cell microenvironment was shown to modulate species-specific cryptococcal morphologies.

Conclusions:

Interactions between *Cryptococcus* species and primary human respiratory cells can be studied using airway and alveolar organoid-derived *in vitro* models and revealed strain- and tissue-specific host responses. Together, these complementary models provide a valuable platform for investigating tissue-specific host responses, early host-fungal interactions, cryptococcal virulence, and disease mechanisms.

P-007

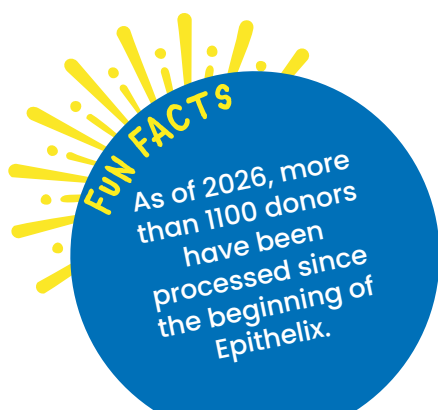
Factors affecting *Streptococcus pneumoniae* infection in Nasal Epithelium

Caroline Weight

BACTERIAL INFECTIONS AND FUNGAL INFECTIONS

The bacterium *Streptococcus pneumoniae* (pneumococcus) commonly lives at the back of the nose without causing problems. However, if it crosses the respiratory epithelium, pneumococcus can cause invasive diseases which kill over one million individuals every year, primarily those under 5 years and over 65 years. In healthy adults, we have previously shown that nasal epithelial cells help to control pneumococcal clearance, so interactions with pneumococcus are pivotal to understanding disease onset. However, disease burden is particularly enhanced in highly polluted and populated areas, like Southeast Asia. Furthermore, 4.2 million deaths/year are attributable to outdoor (particulate matter), and indoor (nanoplastics/microplastics) pollution and plastic absorption is predicted as a leading cause of health complications by 2050, especially for the 250 million vulnerable individuals suffering with respiratory disorders like Asthma.

Here, we used healthy nasal epithelium exposed to air pollutants to determine the molecular processes involved in understanding increased susceptibility to pneumococcal infection. Pneumococci adhered and internalised healthy epithelium at a higher frequency in cells exposed to particulate matter. Particulate matter increased baseline IL-6 and IL-8 secretion, which were exacerbated following pneumococcal infection. These data demonstrate that air pollution stimulates inflammation profiles, which increases epithelial susceptibility to respiratory pathogens. Furthermore, we demonstrate by Electron Microscopy that particulate matter is absorbed into the epithelium and the implications of this are currently being investigated. These data provide scientific evidence for policy makers to develop safer manufacturing alternatives to improve environmental sustainability, to breathe cleaner air and improve human health, especially within vulnerable individuals.



BIOLOGICAL BARRIERS, MPS & DRUG DELIVERY

P-008

Mucus-penetrating lipoparticles enable efficient mRNA delivery to differentiated human airway epithelium

Fabienne Archer

BIOLOGICAL BARRIERS, MPS & DRUG DELIVERY

The induction of protective immunity in the respiratory tract remains one of the main challenges in vaccinology. The emergence and success of messenger RNA (mRNA) vaccines, as demonstrated by their role in controlling the COVID-19 pandemic, has opened perspectives for enhancing vaccine efficacy at the primary sites of respiratory virus entry through new strategies using nasal sprays or aerosols for mRNA delivery. To address this objective, we propose to develop a new generation of LipoParticles, recently described, consisting of a biodegradable poly(lactic acid) core surrounded by a lipid corona capable of protecting and condensing mRNA. Different formulations of mucus-penetrating LipoParticles (mucLPs) were generated, and their capacity to deliver EGFP-encoding mRNA was evaluated in a mucus-secreting lung epithelial cell line (Calu-3 grown at an air-liquid interface, ALI) and a non-mucus-secreting lung epithelial cell line (A549 grown at ALI). The formulation showing the best mucus-penetrating and delivery properties was then tested in a human primary airway epithelium (HAE) model grown at ALI. This model reproduces the pseudostratified organization, barrier properties, and cellular diversity of the respiratory epithelium, including ciliated, goblet, club, and basal cells. In addition to this structural organization, it also recapitulates key lung functions such as ciliary beating and mucus secretion. The selected mucLP formulation successfully transfected HAE cells, demonstrating efficient mRNA delivery and expression in this fully differentiated airway model. These results support the application of this mucus-penetrating mRNA delivery system for the administration of vaccine mRNAs (e.g., SARS-CoV-2, influenza, RSV) and, more broadly, for therapeutic mRNAs (e.g., targeting lung cancer, COPD).

P-009

Using a lung transwell model for the *in vitro* assessment of inhaled defence- and national security-relevant compounds, using ricin as an example.

Matthew Sheavyn

BIOLOGICAL BARRIERS, MPS & DRUG DELIVERY

As scientific research seeks to reduce the reliance on animal models, alternative, validated approaches to accurately model respiratory human biology are necessary to study the effects of compounds *in vitro*. As an example, inhalation of ricin causes severe lung injury and respiratory failure and is often studied *in vivo*. Pathological changes in the lung epithelia include necrosis and the apoptotic deletion of epithelial cells at all levels of the respiratory tract, and marked, neutrophilic inflammation. This study examined ricin's cytotoxic interactions with transwell models of the human lower respiratory tract to identify if *in vitro* results correlate with these *in vivo* observations.

SmallAir™ transwell models (Epithelix), representing the human lower respiratory tract, were exposed to aerosolised ricin, delivered by the VITROCELL® Cloud Alpha MOVE, at deposition densities of ~0.004, 0.4, and 40 ng/cm². At various timepoints after exposure, assays were conducted to assess cytotoxicity, cytokine release, histological profiles, and ricin permeation. Cytotoxicity was determined at 0-, 4-, 24-, and 48-hours with a lactate dehydrogenase (LDH) assay. The highest deposition density of ricin (40 ng/cm²) increased LDH release in a time-dependent manner, indicating increased cytotoxicity. At the lower densities LDH release did not increase, indicating a minimal cytotoxic effect. This observation was consistent with measures of the barrier integrity of the SmallAir™ models, quantified using lucifer yellow permeability assays and transepithelial resistance measurements. Here, the highest deposition density of ricin caused the most disruption to barrier integrity whilst little effect was observed at the lower densities.

These preliminary results are consistent with observations reported *in vivo*, supporting the use of the SmallAir™ lung transwell model as an *in vitro* platform for assessing the respiratory effects of ricin.

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P-010

Novel TEER and bioimpedance spectroscopy measuring device compatible with specific Lung-on-Chip and standard cell culture inserts

Shaginth Sivakumar

BIOLOGICAL BARRIERS, MPS & DRUG DELIVERY

Advanced *in vitro* lung epithelial models, including cell culture inserts and organ-on-chip systems, are increasingly used to investigate respiratory diseases, inhalation toxicity, and drug delivery, while reducing reliance on animal models. In these platforms, reliable functional readouts are required to monitor epithelial barrier formation and disruption over time. Trans-epithelial electrical resistance (TEER) is widely used as a non-invasive indicator of barrier integrity in epithelial models and has been proposed as a functional readout for *in vitro* lung barriers (Srinivasan et al., 2015; Doryab & Schmid, 2022).

As part of the European UNLOOC project, we present a compact, multiplexed electrical impedance spectroscopy platform designed for real-time monitoring of lung epithelial barriers in both the dynamic AXBarrier-on-Chip system (AlveoliX) and standard cell culture inserts in well plates. The platform performs two-electrode



impedance measurements under in-liquid interface conditions over a broad frequency range from 0.1 Hz to 190 kHz, enabling acquisition of both impedance magnitude and phase. Compared to conventional single-frequency TEER measurements (typically at 12 Hz), broadband impedance spectroscopy provides improved insight into the electrical behaviour of epithelial barriers by capturing both resistive and capacitive contributions of the cell layer and surrounding environment (Fernandes et al., 2022).

The system integrates a miniaturized measurement head compatible with different electrode layouts for standard cell culture inserts (12- or 24-well plates) as also the AXBarrier-on-Chip platform, validated using alveolar epithelial cells (AXiAECs). Up to twelve electrode pairs can be monitored with automated acquisition and real-time measurements directly inside the incubator, enabling continuous monitoring during long-term experiments while minimizing environmental perturbations. This platform provides a scalable and non-invasive tool for the functional characterization of *in vitro* lung epithelium, supporting applications in respiratory toxicology, drug testing, and disease modelling using next-generation lung models.

P-011

Enhanced Expansion and Differentiation of Primary Human Airway Epithelial Cells with Optimised Media Conditions

Georgios Stroulis

BIOLOGICAL BARRIERS, MPS & DRUG DELIVERY

Primary airway basal cells cultured at the air-liquid interface (ALI) or as organoids provide invaluable models for studying respiratory pathophysiology and test drug efficacy *in vitro*, yet standard expansion media limit their long-term proliferative and differentiation potential. To address these limitations, we developed PneumaCult™-NGEx (NGEx), an optimised expansion medium for human airway epithelial cells designed to maximize cell yields and differentiation potential. Human bronchial epithelial cells (HBECs) maintained in NGEx exhibited characteristic cobblestone morphology and attained > 60 population doublings (PDs; $n \geq 4$ donors). In contrast, HBECs maintained in five commercial expansion media displayed a notable reduction in expansion, yielding ≥ 20 fewer PDs. Crucially, HBECs expanded in NGEx efficiently differentiated at late passages (i.e. after > 45 PDs) into pseudostratified epithelia. Immunocytochemical analysis verified the presence of ciliated and goblet cells, and transepithelial electrical resistance measurements confirmed the establishment of a robust epithelial barrier ($300 \leq \Omega \times \text{cm}^2$; $n \geq 3$ donors). HBECs expanded in NGEx showed similar early-passage performance compared to HBECs expanded in the next best performing medium, PneumaCult™-Ex Plus. However, NGEx-expanded HBECs exhibited superior differentiation efficiency into ciliated and goblet cells at late passages. Furthermore, human nasal epithelial cells cultured in PneumaCult™-NGEx displayed comparable performance gains, achieving > 35 PDs compared to < 20 PDs in PneumaCult™-Ex Plus and PneumaCult™-Ex, while fully maintaining their ALI and organoid differentiation capacity ($n = 3$ donors). Ultimately, PneumaCult™-NGEx increases the long-term proliferation and differentiation potential of primary human bronchial and nasal epithelial cells. This may allow for more effective modelling of respiratory diseases, including cystic fibrosis, asthma, and viral infections.

P-012

Enabling first-in-human studies with patient-derived lung-on-chip models for immunotherapy safety

Giulia Raggi

BIOLOGICAL BARRIERS, MPS & DRUG DELIVERY

T-cell bispecific antibodies (TCBs) targeting peptide-HLA complexes can engage intracellular tumor antigens but may cause immune-mediated, on-target/off-tumour toxicity. For HLA-restricted TCBs, species differences limit animal-model relevance, increasing the need for predictive human non-animal methods (NAMs). We developed an immunocompetent human lung-on-chip platform to assess HLA-A*02:01-targeting TCB safety, including a MAGE-A4 TCB subsequently advanced to first-in-human studies.

Safety testing was performed on the AXBarrier-on-Chip lung model with patient-derived alveolar epithelial cells on a porous, flexible membrane. Co-culture with peripheral blood mononuclear cells enabled immune-competent testing. We quantified TEER, cytotoxicity (LDH/apoptosis), immune-cell recruitment/activation and cytokines after exposure to HLA-A*02:01 TCBs, including MAGE-A4-TCB and comparators.

In the AXLung-on-Chip model, MAGE-A4-TCB preserved barrier function without cytotoxicity or increased epithelial apoptosis versus non-functional controls. A broader-reactivity control TCB induced cytokine release, immune activation, apoptosis and barrier loss.

The immunocompetent AXLung-on-Chip model provides mechanistic, human-relevant safety readouts for HLA-restricted TCBs. AlveoliX data supported an integrated NAM strategy for regulatory submissions, including IND-enabling activities, and clinical progression of a MAGE-A4-targeting TCB (1). These results underscore the value of organ-on-chip platforms for preclinical decisions and regulatory safety assessment of next-generation immunotherapies.

(1) Translational strategy to support the first-in-human study of a TCR-like T cell bispecific with an *in vitro*-based safety approach. Front. Immunol., 17 April 2026. Volume 17 - 2026 | <https://doi.org/10.3389/fimmu.2026.1736584>



INHALATION TOXICITY TESTING

P-013

An attempt to evaluate the immunotoxicity of inhaled nanomaterials using human iPSC-derived alveolar macrophages

Takao Ashikaga

INHALATION TOXICITY TESTING

With the increasing use of NMs (Nanomaterials), their toxicity concerns are growing and NMs can be absorbed into the body through the respiratory system. Therefore, development of a high-throughput, respiratory-relevant, and certified method to evaluate the immunotoxicity of inhaled NMs is required. This study aimed to develop an *in vitro* immunotoxicity testing method for NMs using human iPSC-derived tissue resident alveolar macrophages (HiTRAM). The test substances used were DNCB (a positive control) and NM-204 (a silicon dioxide nanomaterial). After exposing HiTRAM to these substances at multiple concentrations, changes in marker expression (CD54, CD86, CCL3, CCL5, IL1 β , MMP12, IL1 α) were evaluated with RT-qPCR. Exposure to the positive substance, DNCB, particularly at 2 μ g/mL, strongly induced the expression of each marker, confirming that HiTRAM exhibits relevant responsiveness to substance exposure. Regarding exposure to NM-204, characteristic changes in marker expression, including MMP12 and IL1 α , were consistently observed in a concentration-dependent manner. Furthermore, compared to the control cell group THP-1, differences were observed in the baseline marker expression profile and the responsiveness of some markers in HiTRAM cells. Interestingly, some biomarkers showed higher reactivity in HiTRAM cells than in THP-1 cells, suggesting that the desirability of developing *in vitro* testing methods using HiTRAM cells.

P-014

Coculture of peripheral blood mononucleated cells (PBMC) with primary nasal epithelium (MucilAir™-Pool) responds to airborne or systemic challenges

Guy Barbin

INHALATION TOXICITY TESTING

Nasal mucosa acts as both a physical barrier and an immunological sensor, recruiting immune cells to contain inhaled xenobiotics. Current air-liquid interface (ALI) epithelial models faithfully reproduce epithelial barrier function and cytokine secretion but fail to capture immune responses due to the absence of immune cells. To address this limitation, we cocultured peripheral blood mononuclear cells (PBMCs) in the basal compartment of MucilAir™, a well-established *in vitro* model of the human nasal epithelium.

Nasal epithelia were reconstituted using primary nasal cells from fourteen healthy donors. Heterologous cryopreserved PBMCs were added to the standard epithelial culture medium for three days. The presence of immune cells had no deleterious effect on epithelial viability or ciliary motion. Tissue integrity was maintained, with only a transient, reversible reduction in transepithelial electrical resistance. In contrast, PBMC viability decreased by approximately 15% per day, resulting in an overall survival rate of 55–60% after three days, likely reflecting suboptimal culture conditions.

To model distinct immune challenges, mono- or cocultures were exposed to Poly(I:C) or LPS on the apical side or to a strong pro-inflammatory cocktail (Cytomix: TNF- α , LPS and FCS) in the basal compartment. PBMCs and co-culture showed a strong secretion of cytokines during the first 24 hours. The presence of PBMCs in coculture enhanced the basal secretion of several cytokines compared to epithelial monoculture. Importantly, cocultures exhibited enhanced IP-10 and TNF- α secretion following Poly(I:C) stimulation compared with epithelia or PBMC monoculture, while LPS selectively increased TNF- α secretion in cocultures. Furthermore, basal Cytomix exposure triggered increased secretion of IL-6, IL-8, MIP-1 α , IL-1 β , and GM-CSF.

In conclusion, these preliminary data demonstrate that the nasal epithelium–PBMC coculture responds distinctly to challenges representing viral/bacterial exposure by a basal cytokine secretion profile that differs from monocultures. This model therefore offers potential for studying epithelial–immune cell communication.

P-015

Carcinogen-Induced DNA Mutagenesis in Human Airway Epithelial Organoids

Emilia Barreto Duran

INHALATION TOXICITY TESTING

The airway epithelium is continuously exposed to environmental carcinogens, yet physiologically relevant models to study early mutagenic events remain limited. Here, we use human airway epithelial organoids (HAE) differentiated at the air–liquid interface to investigate carcinogen-induced DNA damage and mutagenesis. HAE cultures were treated with the tobacco-associated carcinogen 4-nitroquinoline 1-oxide (4NQO) in the basolateral media once weekly for one month to model chronic exposure. Using duplex sequencing, we evaluated mutational burden and signatures in host genomic DNA. We observed that 4NQO induces detectable genomic alterations in HAE cultures, demonstrating the ability of this model to capture early mutational events in a physiologically relevant context. These findings establish HAE cultures as a robust platform to study mutation accumulation and early steps in lung carcinogenesis.



P-016

Toxicity ranking of nanomaterials obtained from air-liquid interface cultured lung epithelial cells: A critical meta-analysis

Marion Blayac

INHALATION TOXICITY TESTING

There is a substantial body of *in vitro* nanomaterial toxicity data for air-liquid interface (ALI) cultured lung epithelial cells, which might overcome the limitations of the traditionally used submerged cell culture models. Yet there is currently no standardized guidelines for conducting *in vitro* ALI exposures. We conducted a meta-analysis of ALI nanotoxicological data in lung epithelial cells for the most common endpoints and assessed the *in vitro* predictive power for *in vivo* acute lung toxicity. Out of the 6,629 potentially relevant PubMed and OpenAlex publications, identified using manual and semi-automated, AI-assisted workflows, 76 studies met all our inclusion criteria. Generating particle surface area-based dose-response curves for cell viability, cytotoxicity and pro-inflammatory cytokines, we investigated potential effects due to material type, rate of dose delivery, duration of exposure, and post-exposure incubation time. The BMD20-based toxicity ranking from metabolic activity assays in alveolar epithelial cells (A549) providing the largest body of data revealed copper oxide and zinc oxide as the most toxic materials. Cerium oxide, silicon dioxide and titanium dioxide were identified as medium-level toxic materials while carbon-based materials clustered in a lower toxicity regime. Comparative potency ranking of metabolic activity, LDH release and IL-8 (low amount of data) secretion indicated good inter-assay consistency. Moreover, culturing A549 cells with d-THP-1 macrophages did not affect the dose response pattern. Similarly, dose-response profiles were relatively independent of duration of exposure, i.e. dose delivery rate, post-exposure incubation, and type of aerosol cell-exposure device. Finally, comparison with selected *in vivo* data on acute pulmonary toxicity showed good correlation of *in vitro* and *in vivo* BMD20 values for cerium oxide, copper oxide, zinc oxide, titanium dioxide, carbon black materials and short MWCNT. Overall, relatively simple ALI cell culture models could be suitable for predicting acute *in vivo* pulmonary toxicity of at least some types of nanomaterials.

P-017

Diesel Exhaust Particle-Induced Alveolar Stress Responses and Pulmonary Arterial Endothelial Effects in Human *In Vitro* Models

Gerrit Bredeck

INHALATION TOXICITY TESTING

Traffic-related air pollution contributes to both respiratory and cardiovascular disease, but the molecular mechanisms linking alveolar exposure to pulmonary vascular responses remain unclear. We combined an alveolar and a pulmonary arterial endothelial model to investigate whether diesel exhaust particle (DEP) exposure triggers oxidative, inflammatory, and mitochondrial responses in the alveolar compartment, and assessed indirect effects on remodelling markers in human pulmonary arterial endothelial cells (HPAECs).

We repeatedly exposed the human quasi-air-liquid interface EpiAlveolar™ model with apical (primary alveolar epithelial cells and fibroblasts with THP-1 macrophages) and basolateral compartments (primary microvascular endothelial cells) to non-cytotoxic concentrations of standard DEP (SRM2975) for 96 h. Diluted conditioned medium was applied to primary HPAECs.

In the alveolar model, DEP caused a concentration-dependent increase in the expression of the oxidative stress markers NFE2L2, NQO1, and mitochondrial SOD2, but not the cytosolic SOD1, aligning with altered mitochondrial biogenesis markers (PGC1A↓, PGC1B↑, NRF1↑, TOMM20↑, NDUFB8↑). Apical gene expression of IL1B, IL8, and TNFA increased in a concentration- and time-dependent manner, accompanied by elevated IL-8 secretion. In basolateral microvascular endothelial cells, we observed a small increase in SOD2 expression.

In HPAECs, conditioned medium from DEP-exposed alveolar cultures did not elicit detectable changes in endothelial activation or remodelling markers. By contrast, recombinant TNFA confirmed endothelial responsiveness by upregulating SOD2, IL8, and TNFA expression, inducing gene and surface expression of the adhesion molecules ICAM-1 and VCAM-1, and reducing the remodelling-protective receptor BMPR2.

Altogether, DEP triggered robust oxidative, inflammatory and mitochondrial responses in an advanced human alveolar *in vitro* model, which we will further characterise using transcriptomics and proteomics. The limited changes in healthy-donor HPAECs suggest that vascular effects may require additional susceptibility or a more complex vascular architecture. We are therefore exploring genetically susceptible BMPR2-silenced HPAECs and an endothelial-smooth muscle pulmonary artery-on-a-chip model under flow.

P-018

Exposure of alveolar models to advanced materials under dry powder conditions using the Vitrocell PowderX

Aline Chary

INHALATION TOXICITY TESTING

Background:

Inhalation represents a major exposure route for advanced materials (AdMas), yet current toxicological assessment remains limited by the lack of standardized and physiologically relevant methods for dry aerosol exposure. Advanced lung models combined with dry powder exposure technologies offer a promising alternative to realistically reproduce particle deposition and air-liquid interface (ALI) conditions.

Methods: Toxicity of AdMas (silica, quartz, multi-walled carbon nanotubes, graphene-based materials, and corundum) was assessed using a tiered *in vitro* approach that combines monocultures (A549 alveolar epithelial



cells) and a complex alveolar 3D coculture grown at the ALI (ALIsens®; A549, EAhy926 endothelial cells, THP-1-derived-dendritic-like cells and THP-1 differentiated-macrophages). ALI exposures were conducted using the Vitrocell® PowderX for dry aerosol deposition. Deposition was estimated by gravimetry, quartz crystal microbalance and SEM. Evaluation of biological endpoints included metabolic activity, ROS production, cell surface markers expression, cytokine secretion, and high-resolution imaging.

Results: Theoretical dose modelling overestimated the amount of material deposited, while gravimetry provided reliable quantification of the effective dose. Material-dependent cytotoxic and inflammatory responses were observed already at the level of monoculture. The ALIsens® model provided additional information in terms of epithelial stress responses and pro-inflammatory signalling. Detailed analysis revealed particle-cell interactions, including internalization and morphological changes.

Conclusions: The integration of ALI models with dry powder aerosolization systems such as the PowderX provides a more physiologically relevant framework for inhalation toxicity testing, especially when the materials to be tested are incompatible with liquid dispersion.

P-019

First Use of *In Vitro* Respiratory Tract Models for a TSCA Test Order: Evaluating Hexafluoropropylene Oxide (HFPO) Effects Across Three Regions of the Respiratory Tract

Shaun D. McCullough

INHALATION TOXICITY TESTING

In 2023 the EPA issued a Test Order for hexafluoropropylene oxide (HFPO), under Section 4 of the Toxic Substances Control Act (TSCA), which was the first Test Order to specifically require the use of *in vitro* respiratory models for the collection of data to evaluate human exposure effects. We conducted single and 14-day repeated exposures to HFPO (100–2,500 ppm) on primary human cell-based air-liquid interface (ALI) co-culture models of the nasal, bronchial, and alveolar regions of the respiratory tract in multiple donors. Acute exposures caused significant increases in cytotoxicity in the nasal ($\geq 1,250$ ppm) and bronchial ($\geq 1,770$ ppm) models. Acute alveolar co-culture model exposures resulted in increased secretion of VEGFA, a key event in the adverse outcome pathway for pulmonary edema and a consistent finding animals exposed to higher HFPO concentrations from extant data. The effects of 14-day exposures were evaluated in the nasal and bronchial models using two concentrations (880 and 1,770 ppm) determined based on the acute exposure outcomes. Repeated exposures increased epithelial barrier integrity and epithelial remodeling, but not cytotoxicity, in both models. In addition to the effects of HFPO exposures, the use of both vehicle and incubator control conditions in this study enabled a detailed characterization of the effects of repeated clean air exposures on ALI models of the nasal and bronchial epithelium. While no effect was observed on cytotoxicity in either the nasal or bronchial model, repeated clean air exposures caused an increase in trans-epithelial electrical resistance (TEER) that was accompanied by a decrease in goblet cell density in nasal co-cultures. No significant changes in TEER or cell type distribution were observed in bronchial co-cultures. While simple, these data provide valuable characterization of repeated clean air exposure effects and support the suitability of the MucilAir™-HF Nasal and Bronchial co-culture models for 14-day repeated ALI exposures under the conditions used for this study. The outcomes of HFPO exposures observed *in vitro* reflect the outcomes of extant *in vivo* studies where applicable comparable data are available. Observations from this study also demonstrate significant inter-individual variability across donors in some endpoints that supports the value of including more than a limited number of (e.g., 1–3) donors in future *in vitro* studies. Overall, the data presented here provide a range of valuable insights into the use of ALI co-culture models for future *in vitro* respiratory toxicology studies.

P-020

Repeated Exposure Duration and Endpoint Selection Influence the Reliability of Hazard Identification in Air-Liquid Interface Respiratory Models

Shaun D. McCullough

INHALATION TOXICITY TESTING

Acetic acid is widely used in personal care and fragranced products, creating the potential for repeated daily inhalation exposures during fragrance manufacturing and product use. Here, we defined the effects of repeated daily exposures to a range of acetic acid concentrations across a battery of *in vivo*-relevant *in vitro* endpoints using donor-matched differentiated primary human nasal epithelial cell/primary human nasal fibroblast co-cultures. Using co-cultures from five individual donors, we conducted daily two-hour exposures to acetic acid for either three or five days. Of the five concentrations tested, effects in quantitative endpoints were only observed at the highest concentration (120 $\mu\text{g}/\text{cm}^2$). Mild cytotoxicity was observed with a peak after the Day 1 exposure that was progressively attenuated over repeated exposures. Trans-epithelial electrical resistance progressively increased from Day 3 to Day 5 exposures. Percent active ciliated surface area and mucin 5AC secretion were significantly reduced following both Day 3 and Day 5 exposures. Secretion of interleukin (IL)-8, IL-6, and TNF α increased following Day 3 exposures but induction was attenuated following Day 5 exposures. Epithelial layers were disorganized and had lost normal pseudostratified architecture with a notable reduction of ciliated and goblet cells after Day 3 exposures and exhibited stratified squamous morphology after Day 5. Collectively, the observations reported here indicate that repeated exposures cause progressive effects between Day 3 and Day 5 that can be characterized by either potentiation or attenuation of exposure-induced changes in typical endpoints. Further, significant remodeling of the epithelial cell layer can occur in as few as three days of exposures despite mild ($\leq 2\%$) cytotoxicity that would typically not be considered biologically relevant. More broadly, the results reported here also demonstrate the risk to human health protection posed by relying on a limited scope of traditional simple endpoints (e.g., cytotoxicity) for hazard identification.



P-021

Next-Generation High-Throughput *In Vitro* Hazard Assessment: A Comparison of the Mechanistic Effects of Tobacco and Cannabis Aerosols

Manuel Garcia-Käuffer

INHALATION TOXICITY TESTING

Inhalation toxicology is undergoing a major transition toward human-relevant, mechanism-informed New Approach Methodologies (NAMs) that can support both scientific assessment and future regulatory decision-making. Current inhalation models are limited by interspecies differences in airway anatomy, physiology, and particle deposition, which can reduce their translational value for aerosol hazard characterization. To address these limitations, we developed and applied a semi-automated high-throughput exposure platform in a 96-well format combined with air-liquid interface (ALI) continuous-flow technology to generate reproducible concentration-effect data from human lung epithelial cells exposed to combustion aerosols. The generation of test item-specific data enables meaningful comparison and ranking at the effect level and supports the derivation of point-of-departure estimates for comparative aerosol assessment. This technology supports the integrated assessment of cytotoxic, genotoxic, metabolic, and inflammatory endpoints within a single experimental workflow while substantially improving throughput, standardization, and comparability. In light of changing legal frameworks and increasing cannabis use, there is a growing need for robust toxicological data to support evidence-based hazard assessment. In this pilot framework, standardized reference cigarette smoke was used as a benchmark for mechanistic comparison with cannabis smoke, supporting interpretation within an established toxicological context. Beyond providing insight into the biological effects of cannabis smoke on lung epithelial cells, this study illustrates how advanced *in vitro* aerosol exposure systems can contribute to hazard identification, comparative risk assessment, product ranking, and the implementation of the 3Rs principles (Replacement, Reduction, and Refinement), thereby supporting both ethical progress and future regulatory relevance.

P-022

Methyl methacrylate (MMA) is a respiratory irritant, but not a respiratory sensitiser

Arno Gutleb

INHALATION TOXICITY TESTING

Methyl methacrylate (MMA), a widely used low-molecular-weight monomer classified as a weak skin sensitiser and respiratory irritant under the Globally Harmonized System (GHS), has recently been proposed for additional classification as a respiratory sensitiser. This proposal was based on a limited number of poorly documented case reports. To provide a robust, mechanistic assessment, we investigated the respiratory sensitisation potential of MMA using two complementary human-relevant *in vitro* systems.

In the ALIsens® model — a three-dimensional alveolar co-culture of A549, EA.hy926, and THP-1 cells at the air-liquid interface — MMA was tested at 250 and 500 µg/cm². Flow cytometry analysis of CD54, CD86, and TSLPr surface markers on dendritic-like THP-1 cells showed TSLPr expression well below the 150% threshold, indicating no respiratory sensitisation potential. Gene expression analysis of a targeted 20-gene panel in DC-THP-1 cells revealed no genes meeting the predefined criteria for statistical significance and biological relevance (>2-fold change); only a minor, sub-threshold decrease in MAP2K2 was observed.

In human precision-cut lung slices (hPCLS), MMA (30 µg/mL) induced only a modest, isolated upregulation of GCSFR (1.7-fold) without broader immune activation, in contrast to the pronounced transcriptional response elicited by the reference respiratory sensitiser chloramine T trihydrate. Functional endpoints — metabolic activity, cytotoxicity, and CXCL8/IL-8 release — showed no biologically relevant alterations upon MMA exposure.

Taken together, these convergent findings from flow cytometry, targeted transcriptomics, and functional readouts provide a strong weight-of-evidence conclusion that MMA does not possess respiratory sensitisation potential, and that its proposed classification as a respiratory sensitiser is not justified.

P-023

In Vitro Assessment of Acute and Repeated Dose Toxicity of a Refillable ENDS Aerosol Using the MucilAir™ Human Airway Model

Michael Hollings

INHALATION TOXICITY TESTING

The United Kingdom's ENDS (Electronic Nicotine Delivery Systems) retail market is currently dominated by disposable devices, with SKE Crystal Bar, Lost Mary, and Elf Bar. As the prevalence of these products increases, so does the need for robust, human relevant approaches to evaluate their potential health impacts. New Approach Methodologies (NAMs) are increasingly being adopted for inhalation toxicity testing, offering improved safety assessment and hazard identification while reducing reliance on traditional animal models.

This study employed MucilAir™ (Epithelix Sàrl, Switzerland) to characterise biological responses to whole aerosol generated from the Lost Mary BM6000 Pineapple Ice (20 mg/mL nicotine) disposable ENDS device. Both acute and repeated dose (chronic) exposure paradigms were implemented to capture short term and cumulative effects under conditions designed to reflect real world use. This work contributes to the growing evidence base supporting NAMs for ENDS aerosol evaluation and provides insight into potential respiratory impacts associated with high use disposable products.

MucilAir tissues were exposed using a Vitrocell® VC10® smoking robot with puffing conducted according to ISO 20768:2018. Whole aerosol was diluted at defined flow rates, and nicotine collected in liquid traps at the air-liquid interface was quantified as a marker of delivered dose. Chronic Exposures were conducted on Monday, Wednesday



and Friday, with acute exposures performed as a single event. Cytotoxicity was assessed using LDH release and WST 8. TEER, cilia beat frequency, active ciliated area, and pro inflammatory cytokines provided mechanistic insight. Acute exposures produced marked cytotoxicity across all airflow dilutions. In chronic studies, cytotoxicity emerged at 1 L/min after the second exposure, accompanied by exposure dependent increases in oxidative stress and inflammatory markers. These findings demonstrate that repeated exposure to whole ENDS aerosol can elicit measurable adverse biological responses in a human relevant airway model, supporting continued evaluation of commercially available ENDS products.

P-024

Establishing a U.S. distribution and quality framework for 3D *in vitro* airway models

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INHALATION TOXICITY TESTING

Increasing regulatory and commercial emphasis on non-animal approaches for assessing respiratory irritation and sensitization has driven demand for advanced *in vitro* models. New Approach Methodologies (NAMs) are increasingly relied upon to generate human-relevant data while addressing ethical considerations and evolving regulatory expectations to reduce animal testing. Central to the successful implementation of these methods is access to robust, well-characterized *in vitro* systems, such as the Epithelix 3D airway platforms. To support regulatory and research applications, these models must demonstrate consistent performance across multiple biological endpoints. Maintaining this functionality requires controlled culture conditions and use within a defined window of optimal performance, making reliable handling and transport critical. However, broader adoption in the United States has been limited by logistical challenges related to international shipping, customs clearance, and maintaining tissue viability during transit.

To address these barriers, the Institute for *In Vitro* Sciences (IIVS) and Epithelix established a collaborative framework to enable reliable U.S. distribution while ensuring tissue quality and functional performance. This poster presents an overview of the systems and processes developed to ensure consistent availability of high-quality tissues despite transportation challenges. Key elements include post-production quality control (QC) testing prior to shipment and a tiered QC approach implemented at IIVS to verify tissue integrity and performance upon receipt.

This effort culminated in a pilot shipment to a U.S.-based laboratory, where tissues underwent additional QC assessments to confirm model performance. Comprehensive documentation practices were also established to ensure full traceability across the supply chain, from production through end use, enhancing confidence in model consistency and reliability.

Operational challenges, including shipment timing, temperature control, and customs delays, were systematically mitigated through optimized logistics strategies. The resulting framework improves reliability, reduces risk to tissue quality, and expands access to advanced respiratory models in the United States.

P-025

Pulmonary Toxicity of Environmentally Relevant Nanoplastics: Comparative *In Vitro* Assessment of PET and PS

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INHALATION TOXICITY TESTING

Humans are chronically exposed to airborne Micro- and Nanoplastic particles (MNP), yet their effects on the lung barrier with respect to uptake, clearance and translocation remain poorly understood. Existing studies predominantly focus on polystyrene (PS) at high, non-environmental concentrations, while other abundant polymers such as polyethylene terephthalate (PET), commonly released into the air from textile fibers and packaging, and realistic exposure concentrations remain largely unexplored.

We therefore systematically investigated the effects of PS and PET nanoplastics across a concentration range spanning commonly used experimental doses to environmentally relevant levels, i.e., 100 µg/mL – 0.1 µg/mL, using two complementary cell types of the alveolar region: monocyte-derived macrophages (MDMs) and A549 alveolar type II epithelial cells. Initial screening across different post-exposure time points revealed that neither PS nor PET induced acute cytotoxicity at any concentration. However, MDMs exhibited a time-dependent increase in metabolic activity at the highest concentrations, suggesting a concentration-dependent sub-cytotoxic cellular stress response.

These findings identify alveolar macrophages as potential sensitive responders to environmentally relevant MNP.

Ongoing work aims to resolve underlying molecular mechanisms using multi-omics approaches and to extend these observations to more physiologically relevant *in vitro* models of the alveolar epithelial tissue barrier.

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P-026

Transcriptomic characterization of metabolic competency in reconstructed human respiratory epithelium (RHRE)

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INHALATION TOXICITY TESTING

Characterizing the metabolic competency of reconstructed human respiratory epithelium (RHRE) is key to their use in toxicological assessments of inhaled xenobiotics. Here, we analyzed the expression of xenobiotic metabolism-related genes across RHRE from different airway regions and compared them with human reference samples.

RHRE representing the nasal, bronchial, small airway, and alveolar regions were obtained from Epithelix and



analyzed using bulk RNA sequencing. These data were compared with human reference samples generated from single-cell RNA sequencing data from the Human Lung Cell Atlas (HLCA). Analyses included principal component analysis, hierarchical clustering, and linear regression of log₂-transformed expression data. Percentile thresholds were calculated separately for RHRE and HLCA datasets to compare relative gene expression.

Tissue type explained most of the variance in the full RHRE expression profiles. Nasal, bronchial, and small airway tissues clustered closely, while alveolar samples were more distinct. Similar patterns were observed for metabolic genes. Nasal and bronchial RHRE shared comparable metabolic profiles, followed by small airway RHRE. Comparisons with HLCA showed linear correlation trends for nasal, bronchial, and small airway RHRE ($R^2 > 0.6$). Nasal RHRE aligned well with the human reference sample for a subset of lung-specific, CYP enzymes (e.g., CYP2A6 or CYP2A13). Nasal, bronchial, and small airway RHRE also showed good agreement with the human expression levels of highly expressed, non-CYP, phase I enzymes (e.g., NQO1, CES, and aldo-keto reductases). The lower correlation observed for alveolar RHRE ($R^2 = 0.365$) was explained by differences in cell composition compared with the HLCA reference.

Overall, RHRE with comparable human data (nasal, bronchial, and small airway) largely aligned with their reference in the metabolic gene expression profiles. The data generated here can assist in confirming whether a RHRE expresses the human-relevant set of metabolism-associated genes for the region of interest. Similar approaches can be applied to other RHRE and *in vitro* systems.

P-027

In Vitro Respiratory Toxicity Assay of Lambda-cyhalothrin 5% EC (LC), Azoxystrobin 23% SC (AS) and Spinetoram 11.7% SC (SC) Using EpiAirway™

Clive Roper

INHALATION TOXICITY TESTING

Crop protection products may cause respiratory tract damage in exposed spray operators or bystanders.

Historically, regulatory inhalation toxicity was evaluated using rats. As part of JRFs 3Rs program, MatTek EpiAirway™ AIR-100 was used to test the respiratory toxicity of LC, AS, and SC.

TEER was measured before dosing and at 24 h post dose. LC, AS, and SC were applied at 0.1–5.0, 0.5–500, and 0.25–100 mg/mL in DPBS to tissues (100 µL). The negative, vehicle and positive controls were untreated, DPBS and formaldehyde (10 mg/mL) treated tissues. Tissues were incubated for 24 h at 37°C, humidified and 5% CO₂. At 24 h, LDH was measured in the medium and tissue viability assessed using MTT.

Vehicle and positive control MTT, TEER and LDH responses met acceptance criteria confirming assay reliability. Dose-dependent increases in cytotoxicity (MTT) were observed at concentrations greater than 1 mg/mL (LC), 250 mg/mL (AS) and 50 mg/mL (SC). EC₅₀ values were between 1.0–2.5 mg/mL (LC), 10–50 mg/mL (AS) and 100–250 mg/mL (SC). Epithelial barrier disruption (TEER) begun at 0.5 mg/mL (LC) and 50 mg/mL (SC) with no effects observed until 500 mg/mL (AS). Cytotoxicity (LDH) was observed at concentrations >1.0 mg/mL (LC), >50 mg/mL (SC) and 500 mg/mL (AS). Histopathological examination revealed changes consistent with biomarker findings.

In conclusion, based on effect severity observed, the LOAELs were 0.1 mg/mL (LC), 0.5 mg/mL (AS) and 0.25 mg/mL (SC). The results support the utility of this test to replace *in vivo* tests or screen chemicals prior to regulatory *in vivo* testing.

P-028

High-Content Imaging-Based Characterization of an Inverted 3D Human Bronchial Model for Airborne Exposure Testing Using the NAVETTA System

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INHALATION TOXICITY TESTING

Three-dimensional (3D) human bronchial epithelial cultures grown at the air-liquid interface (ALI) are among the most biologically relevant epithelial-only platforms supporting New Approach Methodology (NAM)-based inhalation research, as they restore key airway features including epithelial polarity, barrier function, mucus production, ciliation, mucociliary activity, and an air-facing exposure surface. Here, we describe the establishment of inverted 3D bronchial epithelial tissues derived from primary Normal Human Bronchial Epithelial (NHBE) cells tailored for NAVETTA system-based airborne exposure testing. Comparative histological analysis of our inverted primary 3D bronchial epithelial model and the established MucilAir™ tissue model confirmed similar epithelial organization and maturation features. We then implemented a high-content imaging (HCI)-based workflow for longitudinal, multiplex fluorescence-based characterization of the model over a 28-day culture period, with the aim of assessing its suitability for future culture quality control. This single multiplex HCI assay enabled simultaneous visualization of key epithelial maturation parameters, including tight-junction formation, ciliation, mucus-secreting cell development, and pseudostratified architecture, and showed strong concordance with the histological findings. These imaging-based data were further complemented by two functional assays: transepithelial electrical resistance (TEER) measurements, used as a quantitative indicator of barrier integrity, and ciliary activity analysis, used as a quantitative measure of ciliary function. Together, our findings establish an inverted primary 3D bronchial epithelial model, supported by an efficient imaging-driven characterization workflow that can serve as a quality control tool to verify culture readiness prior to NAVETTA-based airborne exposure studies.



P-029

Integrating the 3R Principle and STOP Strategy in Occupational Toxicology for Feed and Fragrance Intermediates

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INHALATION TOXICITY TESTING

Occupational exposure to the structurally related feed and fragrance intermediates Isopropenyl Methyl Ether (IPM) and Butenyl Methyl Ether (BME) is potentially critical, particularly via inhalation in industrial environments. To establish robust internal occupational exposure limits (IOELs) addressing both systemic toxicity and local respiratory effects, a multidisciplinary approach guided by the 3R principle was used. Systemic effects were assessed through an *in vivo* 28-day oral study in rats with IPM, from which a systemic IOEL was derived. For local toxicity, *in vitro* studies using the human 3D airway epithelial tissue model MucilAir™-Pool Bronchial were prioritized to simulate repeated inhalation exposure.

In this assay, IPM and BME at concentrations equivalent to airborne exposures of 40, 80, and 120 ppm were applied once daily over four days, corresponding to 1-, 2-, and 3-times the systemic IOEL for IPM. Endpoints included tissue integrity (TEER), cytotoxicity (LDH release), pro-inflammatory response (IL-8 secretion), cilia beating frequency (CBF), and mucociliary clearance (MCC). Results showed no significant changes in tissue viability, barrier function, or mucociliary clearance at any concentration for either substance, and no increases in LDH or IL-8 levels, indicating no cytotoxic or inflammatory effects.

These findings confirm that inhalation exposure to IPM at or above the systemic IOEL of 40 ppm does not compromise bronchial epithelial health. Applying a read-across principle for the structurally similar BME, an IOEL of 34 ppm was derived, also covering the absence of local effects from inhalation. This project demonstrates the value of integrating *in vivo* and *in vitro* toxicology with occupational hygiene to derive health-based OELs, highlighting the importance of the new approach methodology (NAM) testing strategies and collaboration between toxicology and industrial hygiene to protect worker health and ensure regulatory compliance.

STOP principle: S – substitution, T – technical controls, O – organizational controls, P – personal protective equipment

P-030

A Multimodal Continuous-Flow Platform for Investigating Smoke-Induced COPD Pathophysiology

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INHALATION TOXICITY TESTING

COPD is a major global health challenge and the third leading cause of death worldwide, imposing a heavy burden on patients and healthcare systems. Tobacco smoke contributes significantly to COPD, EVALI, and lung cancer due to reactive chemicals that damage and inflame alveoli. However, most current *in vitro* studies rely on single-cell models or cigarette smoke extracts in submerged cultures, which fail to replicate the complex, dynamic microenvironment of the distal lung.

We established a novel inhalation model combining a continuous-flow smoke exposure platform (CFAx12) with AX Lung-on-Chip technology (AlveoliX AG) [Sengupta et al., 2023] to mimic realistic whole cigarette smoke (CS) exposure. The co-culture system comprised human alveolar epithelial cells (AXiAECs) [Sengupta et al., 2022], lung microvascular endothelial cells, and macrophages, recreating the air-blood barrier under air-liquid interface (ALI) conditions with or without dynamic stretch. Native porcine surfactant (Curosurf) was added to assess its protective role during smoke exposure.

Cigarette smoke exposure induced pronounced disruption of the air-blood barrier and cytotoxicity, reflected by a ~50–80% reduction in transepithelial/transendothelial electrical resistance (TEER), structural barrier damage, and earlier onset of effects in triple co-cultures compared to monocultures. At 48 hours post-exposure, LDH levels increased approximately threefold relative to controls, indicating acute cytotoxicity. Gene expression analysis demonstrated upregulation of inflammatory markers (IL6, IL8, TNF- α) and oxidative stress-associated genes (SOD1, Catalase), accompanied by a comparable (~threefold) elevation in IL8 cytokine secretion. Remarkably, surfactant inclusion on-chip substantially attenuated these smoke-induced effects compared with untreated controls [Sengupta et al., 2025].

The integrated CFAx12 Lung-on-Chip model advances the understanding of molecular mechanisms linked to tobacco exposure and offers a physiologically relevant platform for toxicological testing of conventional and novel tobacco products. This approach supports the discovery of biomarkers and therapeutic targets for tobacco-induced lung diseases while improving preclinical tools for risk assessment and regulation.

P-031

Predicting inhaled drug safety and assessing *in vivo*–*in vitro* translatability with a breathing lung-on-chip

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INHALATION TOXICITY TESTING

Delivering therapeutics via inhalation is a crucial strategy for treating respiratory conditions, as it allows for direct targeting of the lungs. However, the progression of drug candidates in clinical development is frequently hindered by dosage restrictions, which are based on histopathological changes or functional impairments detected during *in vivo* studies. Addressing these safety challenges is vital for advancing compounds that demonstrate appropriate safety profiles. As a result, there is a growing demand for robust *in vitro* models that can reliably predict lung toxicity



and inflammatory reactions across a variety of therapeutic approaches.

This research aimed to determine how well the AlveoliX Lung-on-Chip (AXLung-on-Chip) model predicts respiratory toxicity for eight inhaled agents, each representing different modalities: small molecules (AZ1, AZ2, Tiotropium), antisense oligonucleotides (ASO1, ASO2), a lipid nanoparticle containing mRNA (LNP-eGFP), cadmium chloride (CdCl₂), and lipopolysaccharide (LPS). Initial dose assessments were conducted in a basic 2D co-culture system of alveolar and immune cells, followed by evaluation of single and repeated dosing in the advanced tri-culture AXLung-on-Chip platform under both static and dynamic conditions. Key measurements included cytokine release, cell toxicity, barrier function, gene expression, GFP detection, and comparison to established *in vivo* responses.

While the 2D cell model failed to adequately differentiate responses, treatment using the AXLung-on-Chip system produced clearer outcomes, with multiple endpoints enabling the identification of compounds based on their inflammatory profiles. Incorporating mechanical stretch into the AXLung-on-Chip further heightened cytokine sensitivity after exposure. Notably, direct comparisons with *in vivo* toxicology data from cynomolgus monkeys and rats exposed to AZ2 showed that the AXLung-on-Chip reliably anticipated drug-induced lung inflammation, capturing a spectrum of pulmonary pathologies.

These findings highlight the value of the AXLung-on-Chip for evaluating the toxicity of inhaled compounds across multiple modalities, and underscore its role in bridging *in vivo* animal studies with human-relevant preclinical data.

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P-032

Beyond Slicing: Automated 3D Imaging of MUC5AC in Engineered COPD Airway Models

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MODELING COPD/ASTHMA/IPF/CF/PCD IN VITRO

Chronic obstructive pulmonary disease (COPD) is a major respiratory disorder driven by a chronic inflammatory response to noxious particles, notably cigarette smoke. Over time, persistent inflammation causes irreversible airflow limitation, characterized by goblet cell hyperplasia and MUC5AC mucin overproduction. Traditionally, MUC5AC quantification relies on immunohistochemistry (IHC) on thin tissue sections. While powerful, IHC is a tedious, time-consuming, and destructive method requiring high technical expertise.

To overcome these limitations, we developed a non-destructive alternative combining 3D confocal microscopy of immunofluorescent-labeled whole tissues with a proprietary image-analysis algorithm. Using Epithelix IL-13-induced COPD models, our method successfully quantified volumetric MUC5AC overproduction and confirmed that treatment with Lebrikizumab, an IL-13 antagonist, effectively blocks this phenotype demonstrating the platform's capacity to detect therapeutic efficacy. Interestingly, tissue models derived from the lower airways (MucilAir™ Bronchial and SmallAir™) replicated the hyperplasia phenotype more accurately than nasal tissue (MucilAir™ Nasal). This automated 3D immunofluorescence platform offers a robust, rapid, high-throughput alternative to traditional IHC for evaluating goblet cell metaplasia and serves as a powerful tool for accelerating preclinical drug screening in fully representative human airway models.

P-033

Differentiation conditions shape baseline phenotype and function of primary human airway epithelial ALI models

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MODELING COPD/ASTHMA/IPF/CF/PCD IN VITRO

Primary human bronchial epithelial cells differentiated at an air-liquid interface (ALI) are widely used to investigate airway biology, model respiratory health and disease, and evaluate inhaled toxicants and therapeutics. Yet the extent to which differentiation medium and porous support material influence baseline epithelial phenotype and function remains poorly defined. Because baseline epithelial characteristics influence experimental outcomes, we systematically evaluated how commonly used differentiation conditions shape the morphology, cellular composition, and electrophysiological function of well-differentiated primary human airway epithelial ALI cultures. Primary bronchial epithelial cells isolated from three donors with cystic fibrosis (CF) were expanded by conditional reprogramed cell (CRC) methods and differentiated at ALI on polytetrafluoroethylene (PTFE) or polyester (PET) porous supports using four commonly used differentiation media (UNC-ALI, PneumaCult ALI, Vertex ALI, and modified Lonza ALI). Epithelial morphology, barrier integrity, cell composition, and ion transport were assessed by quantitative histology, transepithelial electrical resistance (TEER), and electrophysiological measurements.

All differentiation conditions supported successful mucociliary differentiation but produced distinct baseline phenotypes. PTFE supports generated thicker epithelia with greater goblet cell abundance than PET supports. PneumaCult ALI promoted epithelial hyperplasia characterized by increased epithelial thickness and ciliated cell numbers, whereas Vertex ALI on PTFE produced the greatest goblet cell differentiation. Barrier integrity and ion transport also varied by culture condition, with UNC-ALI producing the highest TEER and PneumaCult ALI and Vertex ALI exhibiting greater ENaC activity.

These findings demonstrate that differentiation conditions are major determinants of baseline epithelial phenotype and function rather than passive components of ALI culture systems. Differentiation medium and substrate should therefore be selected according to the biological question and experimental endpoint rather than viewed as interchangeable methodologies. Careful selection and transparent reporting of culture conditions will improve reproducibility and support the rational selection of fit-for-purpose ALI models for respiratory disease research, inhalation toxicology, and pharmacological testing.



P-034

Immunocompetent Airway Model for Studying the Effects of CFTR Modulator Therapy on *Pseudomonas Aeruginosa* Infections in Cystic Fibrosis using hiPSCs

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MODELING COPD/ASTHMA/IPF/CF/PCD *IN VITRO*

Model systems for the respiratory tract and the lung microenvironment are essential for robust research on Cystic Fibrosis (CF). To date, these systems largely rely on animal models, as well as primary or immortalized cells. However, such approaches fail to fully recapitulate the complexity of lung physiology while maintaining scalability, disease relevance and translational applicability to the human biological context. This limitation is particularly critical for inflammation-driven diseases with immune dysregulation such as CF, where the immune system – and especially macrophages as key first responders to pathogens – plays a central role. To overcome these challenges, this study aims to establish and characterize an immunocompetent airway model based on human induced pluripotent stem cell (hiPSC)-derived airway epithelial cells (iAECs) in combination with hiPSC-derived macrophages (iMacs), both carrying the CF genotype and its isogenic control. The two cell systems closely mimic their primary counterparts in both molecular and functional terms. Co-culture of iAECs and iMacs supports the long-term viability of both cell types for at least 14 days. Within this environment, iMacs display lateral motility and form clusters on the epithelial surface – behaviors that closely resemble their *in vivo* dynamics. Infection with *Pseudomonas aeruginosa* revealed pronounced defects in the phagocytic capacity of CFTR-mutant macrophages when co-cultured with CFTR-deficient epithelium, pointing to a synergistic disease mechanism. Ongoing studies focus on dissecting the effects of CFTR modulator therapy on intrinsically versus extrinsically driven CF macrophage phenotypes during infections with *Pseudomonas aeruginosa*. These analyses include transcriptional profiling on single cell resolution, secretome characterization, and assessment of pathogen uptake.

P-035

Development and use of pathological apical-out airway organoids to study respiratory viral infections

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MODELING COPD/ASTHMA/IPF/CF/PCD *IN VITRO*

Airborne viruses represent a major global health burden and are key drivers of morbidity and mortality. Beyond their intrinsic pathogenicity and their capacity to promote bacterial superinfections, several respiratory viruses are major triggers of exacerbations in chronic inflammatory airway diseases such as asthma and chronic obstructive pulmonary disease (COPD). Conversely, patients with pre-existing airway conditions are at increased risk of severe viral infections. The epithelial mechanisms underlying this bidirectional relationship remain poorly understood, largely due to the limited availability of scalable relevant *in vitro* models of such chronic lung diseases. Here, we developed apical-out airway organoids (AOAOs) derived from primary human airway basal cells obtained from healthy donors and patients with asthma, COPD, and cystic fibrosis (CF), to investigate how intrinsic epithelial alterations influence susceptibility to respiratory viral infections and shape host responses. Structural characterization revealed disease-specific features, including goblet cell hyperplasia in asthma- and COPD-derived AOAOs. Functional analyses confirmed that CF-AOAOs failed to respond to forskolin treatment in contrast to healthy controls. Moreover, asthma-derived AOAOs exhibit increased susceptibility to respiratory syncytial virus (RSV) infection. Ongoing work aims to evaluate the antiviral efficacy of selected compounds in both healthy and pathological organoid models. Altogether, this study provides novel insights into the role of the airway epithelium in modulating viral susceptibility in chronic respiratory diseases and establishes a versatile platform for translational and therapeutic applications.

P-036

Advancing COPD research: improved freeze-thaw methods for patient-derived lung organoids

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MODELING COPD/ASTHMA/IPF/CF/PCD *IN VITRO*

COPD is a heterogeneous disease, which complicates the development of effective therapeutic strategies. Patient-derived organoid biobanks offer a powerful platform for drug discovery and personalized medicine. While successful cryopreservation of lung organoids has been reported, primarily in non-diseased condition, the effects of cryoconservation on organoid integrity and function remain poorly understood, especially in disease-derived models such as COPD organoids. Here, we aimed to evaluate methods for biobanking COPD organoids. Airway organoids were generated from basal cells purified from bronchial tissue. We employed the conventional slow-freezing method to cryopreserve both dissociated cells and whole organoids after the first passage, and developed an optimized rapid-thawing protocol using a series of warming solutions with gradually decreasing sucrose concentrations. Cryoconservation-associated cell death was assessed via annexin-PI staining and flow cytometry, while proliferation capacity was evaluated by monitoring organoid growth. Preliminary results indicate that our optimized freeze-and-thaw approach improves cell recovery and preserves growth potential compared with conventional rapid-thawing methods, providing a practical strategy for both basic and translational COPD research.



P-037

Development of an *in vitro* bleomycin model for the screening of antifibrotic compounds based on primary human alveolar epithelium

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MODELING COPD/ASTHMA/IPF/CF/PCD *IN VITRO*

Idiopathic pulmonary fibrosis (IPF) is a fatal interstitial lung disease characterised by recurrent alveolar injuries, dysregulated fibrotic and inflammatory responses, leading to progressive scarring of lung tissues and excessive extracellular matrix (ECM) deposition.

This study aimed to develop a new *in vitro* platform for the screening of antifibrotic compounds based on specific IPF biomarkers and an *in vitro* alveolus epithelium model, AlveolAir™, composed of human types I and II pneumocytes co-cultured with endothelial cells at the air-liquid interface.

AlveolAir™ cultures were exposed during 10 days to a mix of TGF-β and TNF-α recapitulating the pro-fibrotic and pro-inflammatory microenvironment observed in IPF. To strengthen translational relevance and bridge the gap between classical animal models and human systems, the fibrogenic activation was induced in parallel with bleomycin, since the most widely used model for the study of IPF is the bleomycin mouse model. The different key steps of the establishment of IPF, inflammation, fibrotic mediators' response, epithelial-to-mesenchymal transition (EMT) and extracellular matrix (ECM) deposition, were assessed through basal secretion of cytokines and gene expression analysis. The model was validated across multiple human cells donors, demonstrating strong reproducibility despite inter-individual variability.

The applicability of this system for antifibrotic compounds screening was then evaluated. Reference antifibrotic compounds, Nintedanib and Pirfenidone, used for the treatment of IPF patients for about 10 years, were tested on the model concurrently to TGF-β and TNF-α or bleomycin. A decrease in fibrotic markers was observed, demonstrating the suitability of the model for antifibrotic compounds screening.

P-038

Targeting Transglutaminase 2 in Idiopathic Pulmonary Fibrosis: Insights from 2D Cell Cultures and Advanced 3D Lung Models

Beatrice Spagnoli

MODELING COPD/ASTHMA/IPF/CF/PCD *IN VITRO*

Idiopathic pulmonary fibrosis (IPF) is a chronic interstitial lung disease marked by progressive respiratory decline due to replacement of normal lung tissue with fibrotic matrix.

It is now considered an aberrant wound-healing response triggered by repeated alveolar epithelial injury.

The damaged epithelium releases pro-fibrotic mediators, including TGF-β, which activate resident fibroblasts and drive their differentiation into matrix-producing myofibroblasts.

Transglutaminase 2 (TG2) is emerging as a potential therapeutic target. This calcium-dependent enzyme cross-links extracellular matrix proteins such as fibronectin and collagen, increasing matrix stability.

TG2 also enhances pro-fibrotic signaling by promoting TGF-β deposition and amplifying integrin- and SMAD2/3-mediated pathways. Non-canonical pathways, including MAPK, NF-κB, and YAP/TAZ, further sustain fibroblast proliferation, myofibroblast differentiation, and epithelial-mesenchymal transition.

A pathogenic feedback loop occurs as TGF-β induces TG2, which in turn reinforces fibrotic signaling. In bleomycin-induced fibrosis models, TG2 inhibition reduces matrix

deposition and improves lung function, supporting its therapeutic relevance.

To investigate these mechanisms, primary lung fibroblasts from healthy donors (NHLF) and IPF patients (DHLF) were studied in 2D cultures and advanced systems. The Air-Liquid Interface (ALI) model supports epithelial differentiation and allows analysis of epithelial-stromal interactions in a multicellular context, while human Precision-Cut Lung Slices (PCLS) preserve native lung architecture, enabling study of TG2 function in an *ex vivo* tissue environment.

The integration of 2D cultures, ALI systems, and human PCLS provides a comprehensive platform to assess TG2 at cellular and tissue levels, advancing understanding of IPF pathophysiology and supporting the identification of new therapeutic targets.

P-039

Modeling chronic lung diseases using engineered organoids

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MODELING COPD/ASTHMA/IPF/CF/PCD *IN VITRO*

Organoid technology is a promising approach to recapitulate the lung *in vitro*, as it enables the reconstruction of multiple constituent cell types and complex three-dimensional structures. However, its physiological relevance and broader applicability have been limited by several challenges: (i) strong dependence on stem cell self-organization, resulting in poor controllability of organoid size and differentiation and batch-to-batch variability; (ii) the inability to reproduce respiratory dynamics and mechanical cues associated with breathing; and (iii) the closed luminal structure of conventional organoids, which hampers the modeling of inhalation exposure.

To overcome these limitations, we advanced our iPS cell-derived alveolar organoid system by integrating tissue engineering approaches and developed pulmonary aciniform organoids (PAcInOs) that recapitulate the pulmonary lobular architecture, a key anatomical unit of the lung. Incorporation of automated manufacturing processes enabled tight control over organoid size and number, significantly reducing variability while allowing efficient large-scale production. Furthermore, by combining PAcInOs with microneedles and a custom-designed device, we achieved luminal perfusion and successfully established dynamic organoids capable of mimicking respiratory

motions such as contraction and expansion.

Induction of fibrosis in these dynamic organoids resulted in reduced organoid volume and compliance, closely reflecting physiological hallmarks of pulmonary fibrosis. Treatment with antifibrotic drugs restored organoid volume and compliance along with a reduction in fibrotic markers, demonstrating the utility of this system for diseases and drug-evaluation model. In addition, we established a novel method to differentiate functional tissue-resident alveolar macrophages from iPS cells. Co-culture of these iPS-derived tissue-resident alveolar macrophages (iTRAMs) with lung organoids enabled the reconstruction of complex epithelial-immune cell interactions. Together, these systems provide a versatile foundation for modeling chronic respiratory diseases and investigating multicellular pathophysiology in a highly relevant human lung system.

P-040

Exploring the therapeutic potential of hiPSC-derived basal cells in the context of primary ciliary dyskinesia

Laura von Schledorn

MODELING COPD/ASTHMA/IPF/CF/PCD *IN VITRO*

Primary ciliary dyskinesia (PCD) is a genetic disorder affecting motile airway cilia. Mutations in more than 50 genes disrupt cilia assembly or function, leading to impaired mucociliary clearance, recurrent infections, and progressive lung damage. Current treatments are symptomatic and do not restore epithelial function. Regenerative approaches using human induced pluripotent stem cell (hiPSC)-derived airway epithelial cells may address these deficits, but key translational challenges remain, including scalable animal-free cell production and identifying the proportion of functional cells required for therapeutic benefit.

To explore the therapeutic potential of hiPSC-derived airway epithelial cells in PCD context, we are developing a co-culture system composed of healthy hiPSC-derived airway basal cells (iBCs) and PCD patient-derived nasal epithelial cells (PCD NECs). By co-cultivating both cell sources, this system enables the assessment of iBC dose-dependent contributions to MCC restoration in a PCD-diseased epithelium, as well as the implications of the underlying PCD phenotype itself, thereby providing important benchmarks for future clinical translation.

To facilitate this approach, we generated a reporter hiPSC line, which enables tracking and quantification of hiPSC-derived ciliated cells in those co-cultures. In parallel, we established a PCD NEC biobank that includes the most frequently mutated PCD genes and encompasses a diverse range of functional phenotypes, such as cilia agenesis, immotility, and residual ciliary function. To establish baseline functional properties, we thoroughly characterized iBC- and PCD NEC-derived airway epithelia, including ciliary beating frequency, cilia length, cell type composition, and mucociliary transport. Further efforts focus on scaling up the iBC differentiation protocol and replacing animal components to pave the way for clinical translation.

This work will provide important insights into the feasibility and design parameters of future iBC-based cell therapies for PCD and beyond this for other chronic airway diseases, such as cystic fibrosis and COPD.

MUCUS, SURFACTANT, CILIA FUNCTIONS AND MODULATIONS

P-041

Miniaturizing Complexity: A Fully Automated 384-Well Air-Liquid Interface Platform for High-Throughput Airway Modeling

Cyrill Roth

MUCUS, SURFACTANT, CILIA FUNCTIONS AND MODULATIONS

Background: Air-liquid interface (ALI) cultures of primary human bronchial epithelial cells are the gold standard for recapitulating the mucociliary apparatus *in vitro*. However, their application in large-scale drug discovery and functional genomics screens has historically been limited by low throughput, high material costs, and manual labor intensity.

Methods: To overcome these barriers, we developed a high-throughput airway ALI workflow utilizing the Microwells Embedded with Membranes (384MEM) plate, a proprietary platform engineered at the Roche Institute of Human Biology (IHB). The 384MEM plate features bespoke membrane topography and pore characteristics optimized for both long-term cell differentiation and high-content imaging compatibility. We integrated this platform into a fully automated end-to-end pipeline, utilizing liquid handling robotics for precise cell seeding, apical mucus washes, and basal media exchange.

Results: Morphological and functional benchmarking against conventional 24-well Transwell inserts confirmed that the 384MEM format supports the development of a robust, pseudostratified epithelium with comparable ratios of ciliated, goblet, and basal cell populations. To enable deep phenotyping, we validated a modular assay toolbox including: a fluorescent bead assay as a proxy for mucociliary clearance, a cascade blue permeability assay to assess barrier integrity, bulk RNA sequencing to measure changes in gene expression and an immunofluorescent panel to quantify changes in cell subset populations. The platform's sensitivity was verified using pharmacological benchmarks, such as IL-13 to induce goblet cell hyperplasia and DBZ to disrupt Notch-mediated ciliated cell lineage specification, showing high reproducibility across automated runs.

Conclusion: We have successfully bridged the gap between physiological relevance and screening throughput. This automation-ready, miniaturized ALI system enables the interrogation of complex lung biology at an unprecedented scale. We are currently deploying this platform in a medium-scale knock-out screen to validate novel molecular drivers of impaired mucociliary clearance in Chronic Obstructive Pulmonary Disease (COPD).



P-042

The predictiveness of the *in vitro* lung surfactant inhibition assay for impaired lung function in humans and animals

Jorid Sørli

MUCUS, SURFACTANT, CILIA FUNCTIONS AND MODULATIONS

Background

Acute inhalation toxicity testing for regulatory purposes relies on the use of guideline studies in rodents. The results from acute inhalation toxicity testing are systemic (lethality or evident signs of toxicity). Systemic toxicity is difficult to predict using *in vitro* methods that measure effects at the portal-of-entry, i.e. the lungs. However, understanding the effect on the lungs is essential for replacement of animals for acute inhalation testing, thus we use lung function as the *in vivo* endpoint we compare *in vitro* results to. Here the reliability and relevance of a cell-free method based on the monitoring of the biophysical function of lung surfactant during exposure to a test chemical, for predicting acute effects on lung function in exposed humans or experimental animals will be presented.

Method

Measuring lung surfactant function addresses the molecular initiating event of the adverse outcome pathway AOP 302. The main function of lung surfactant is to regulate the surface tension at the respiratory air-liquid interface to avoid alveolar collapse *in vivo*. Inhibition of function starts a cascade that leads to decreased lung function, the adverse outcome in AOP 302. The effect of more than 150 different chemicals and products have been tested, covering consumer products, occupational exposures, inhaled pharmaceuticals and single chemicals.

Results

The current applicability domain of the method will be explored, and how the results of this *in vitro* test relates to outcomes in accidentally exposed humans, guideline studies for acute inhalation toxicity and exposure of mice in whole body plethysmographs.

Discussion and Conclusion

This cell-free method is a promising candidate for prioritization and screening of chemicals, and its inclusion in an integrated approach to testing and assessment will contribute to the reduction of the use of rodents for acute inhalation toxicity testing.

THINK DIFFERENT

P-043

A system to analyse the temperature-dependent release of plant volatile organic compounds and their effect on lung epithelial cells

Mathilde Delaval

THINK DIFFERENT

Climate change, characterized by rising global temperatures, profoundly affects terrestrial ecosystems and human health. As temperatures increase, plants respond by altering their emission of biogenic volatile organic compounds (BVOCs) a diverse group of reactive molecules including terpenes, isoprenoids, and phenolics. Emerging evidence suggests that certain BVOCs possess anti-inflammatory and antioxidant properties that may influence human respiratory health. Specifically, inhalation of some BVOCs has been associated with reduced pulmonary inflammation and improved lung function, potentially counteracting the adverse respiratory outcomes linked to air pollution and heat stress. We established a VOCs exposure system, which allows us to treat lung cells, grown in air liquid interface conditions, with VOCs released from fir trees under moderate (22°C) and high (40°C) temperature. VOCs concentrations were monitored using proton-transfer-reaction mass spectrometry (PTR-MS), which indicated total monoterpene levels in the plant cuvette ranging from approximately 1.4 to 1.8 ppb which is comparable to concentrations typically found in forest environments (0.1-2 ppb). In addition, VOCs were collected on Tenax tubes for GC-MS analysis to identify and quantify the specific composition of monoterpenes, sesquiterpenes, and diterpenes present during the exposure. In comparison to 22°C, the fir trees release higher levels as well as a different set of VOCs at 40°C. The effect of released VOCs on the immune response of a co-culture lung model comprising Calu-3 bronchial epithelial cells and THP-1 macrophages has been analyzed. The cells were exposed to the VOCs for 24 h and subsequently treated with 250 ng/cm² LPS using a cloud system. VOCs showed no cytotoxic effect, as confirmed by Alamar Blue and LDH assays. BVOC pre-exposure attenuated LPS-induced TNF-α release, with a more pronounced reduction at higher temperature.

Conclusion: Our project highlights the nature of plant-emitted volatiles in the context of a warming climate and as potential natural reducers of inflammatory lung disease.

We have implemented a music synth in the Cilia-X software to generate sounds from the acquisition data.

FUN FACTS



P-044

Human airway organoids as a high-throughput screening platform for antiviral natural products discovery

Paola Haemmerli

THINK DIFFERENT

Respiratory syncytial virus (RSV) is a leading cause of lower respiratory tract infections in young children and immunocompromised individuals and remains a major global health burden. Despite recent progress in prevention, including vaccines and IgG-based prophylaxis, effective antiviral treatments remain limited, highlighting the need for new therapeutic strategies. As respiratory viruses infect a complex pseudostratified airway epithelium *in vivo*, physiologically relevant models are essential for antiviral discovery. In this context, natural products (NPs) represent a vast and underexplored source of chemical diversity for identifying novel antivirals. Here, we established a scalable screening workflow based on apical-out airway organoids (AOAOs), combined with a parallel screen in A549 cells, a widely used model for drug testing. Using this approach, we comparatively evaluated 764 plant natural extracts (NEs) from the Pierre Fabre Laboratories collection and identified antiviral NEs. This subset covered a broad chemical space, with annotated structures matching over 8.5% of NPs catalogued in LOTUS. Primary screening identified active NEs in both models, with more hits detected in AOAOs (70 NEs). We selected four NEs based on predicted chemical classes and activity profiles, including one NE active exclusively in AOAOs, and generated a chemiodiverse library of purified compounds. This approach yielded 45 NPs. All active NPs in A549 cells retained activity in AOAOs, whereas phylligenin displayed antiviral activity exclusively in AOAOs. Mechanistic studies showed that phylligenin acts at a late stage of the viral cycle and synergizes with ziresovir, an RSV fusion inhibitor. It also retained antiviral activity in human airway epithelia cultured at the air-liquid interface after both apical and basolateral administration. Together, these findings position AOAOs as a scalable and physiologically relevant platform for antiviral discovery and highlight NEs as a promising source of RSV inhibitors.

* PH and MH: co-first authors and SC, JLW, CT: co-last authors

P-045

A Novel Type of Human Lung Cells with a Hybrid Identity between Antigen-Presenting and Epithelial Cells

Song Huang

THINK DIFFERENT

Introduction: Pulmonary dendritic cells (DCs) are vital sentinel cells that bridge innate and adaptive immunity. Following unresolved immunocompetent models using monocyte-derived DCs (moDCs) due to technical and secretory limitations, resident DCs have become a priority. Consequently, resident DCs have emerged as a more relevant target for study. The primary cell population DC1038, isolated from human lung parenchyma, was selected as the focal point of this characterisation study to provide an opening for the investigation of resident immune cells. **Objectives:** The study aimed to: 1) establish a robust protein and transcriptional biomarker profile for DC1038 at steady state and under stimulation, and 2) optimisation of isolation technique.

Materials and Methods: The phenotyping was performed via Immunofluorescence, RT-qPCR, and Western Blot using a panel of over 20 markers, including immune (Langerin, CD11c, CD80/CD86, CD40, OX40L, CD74), mesenchymal and fibroblastic (Vimentin, TE-7) and epithelial (DSG3, KRT13, SERPIN B2).

Results: Biologically, DC1038 cells express key antigen-presentation markers such as CD40, CD80, CD74, and CSF1R. However, the consistent absence of canonical markers (CD103, CD11c, CD11b) challenges their classification as classical resident DCs. Unexpectedly, the cells display a robust signature of Hillock cells (expressing KRT13, DSG3, and Serpin B2), which are rare, injury-resistant basal stem cells of the airway epithelium alongside mesenchymal and fibroblastic profiles (Vimentin, TE-7).

Conclusion: DC1038 exhibits a complex hybrid identity, combining immune sentinel characteristics with the properties of plastic epithelial «Hillock» stem cells. This dual nature suggests a novel cell type for studying the immune competence of the epithelial cells rather than a pure resident DC population. Ongoing functional assays, such as pinocytosis and mixed lymphocyte reactions, are essential to confirm their definitive capacity for antigen presentation.

P-046

Interactions between respiratory epithelial cells and chondrogenic progenitor cells following tracheal replacement with a cryopreserved aortic allograft

Nouha MENNOUR

THINK DIFFERENT

Introduction

Cryopreserved aortic allograft transplantation supported by a stent is an innovative strategy for reconstructing extensive tracheobronchial defects, enabling the formation of a functional neo-trachea through respiratory epithelial regeneration from recipient-derived stem cells and neo-cartilage formation, whose cellular origin remains unknown. However, neochondrogenesis required for graft stiffening and stent removal remains slow, increasing the risk of complications. Understanding the mechanisms underlying this process is essential to accelerate regeneration. Based on the critical role of epithelial-mesenchymal crosstalk during tracheal development, we hypothesize that respiratory epithelium colonizing the aortic graft secretes key factors that modulate recruited chondrogenic progenitors during tracheal regeneration.



Methods Human bronchial epithelial cells (hBECs), isolated from bronchi of organ donors, were cultured and the conditioned media at proliferative and differentiated stages was collected. The impact of these conditioned media was evaluated on the viability, proliferation, migration, invasion and initiation of chondrocyte differentiation of human bone marrow mesenchymal stem cells (hBM-MSCs). The secretomes were analysed by multiplexing ELISA to identify molecules potentially involved in the hBEC-hBM-MSC interaction.

Results hBECs secretomes differentially regulated hBM-MSCs biological properties depending on the epithelial differentiation stage. Proliferating hBECs secretomes enhanced hBM-MSCs viability and migration, whereas differentiated hBECs secretomes promoted MSC proliferation, invasion, and SOX9 expression, suggesting chondrogenic commitment. Secretome analysis revealed high levels of IL-8, SDF-1 α , VEGF-A, and fractalkine during hBECs differentiation.

Conclusions During tracheal replacement using a cryopreserved aortic graft, the respiratory epithelium recolonizing the luminal surface of the graft may be involved in neo-tracheal formation by regulating hBM-MSCs behavior through the secretion of mediators whose profile depends on the epithelium's state of differentiation. These findings suggest that these epithelial signals may contribute to the reactivation of embryonic developmental pathways, thereby potentially replicating the mechanisms involved in tracheal organogenesis.

P-047

Cold atmospheric plasma and alveolar epithelial cells: Safety and efficacy for therapeutic use in bacterial lung infections

Lea Nawatzky

THINK DIFFERENT

Background

Ventilator-associated pneumonia (VAP) is a serious complication in ICUs, affecting up to 26% of critically ill patients and associated with high mortality. Current treatment strategies rely on broad-spectrum antibiotics, which contribute to the emergence of multidrug-resistant (MDR) pathogens. Cold atmospheric plasma (CAP) has potent antimicrobial activity and represents a promising alternative to conventional antibiotic therapy. Its reactive components disrupt bacterial membranes, induce protein degradation and modification, and ultimately lead to microbial cell death.

Objective/ Using a physiologically relevant model, we aim to systematically evaluate the therapeutic potential of CAP in VAP by identifying an effective and safe application strategy for future clinical use.

Methods and Results

We employ Air-Liquid Interface (ALI) cultures of CI-huArlo cells as model system to replicate the alveolar epithelium. These cultures are exposed to CAP in the P.R.I.T. ExpoCube.

This study examined the effects of CAP treatment on barrier integrity, cellular architecture, inflammation and toxicity. The effects of CAP are dose- and time-dependent. Low doses were tolerated, preserving barrier integrity and architecture with no relevant cytotoxicity. In addition, our results show that short CAP exposure does not significantly affect the inflammatory response of the cells. In parallel, the reactive species generated by CAP at low doses are sufficient to eliminate relevant respiratory pathogens such as *S. aureus*, *K. pneumoniae* and *P. aeruginosa*, even when they are multidrug-resistant.

Conclusion/ This study examines the therapeutic potential of CAP for the treatment and prevention of VAP, emphasizing its promise as a novel therapeutic approach for respiratory infections.

P-048

Human respiratory tissue models are suitable to study xenobiotic metabolism

Maria Steinke

THINK DIFFERENT

Introduction: Nasal xenobiotic metabolizing enzymes (XMEs) protect against inhalable toxicants and play a key role in olfactory peri-receptor events by metabolizing odorants. Inflammatory diseases, such as chronic rhinosinusitis, may affect the sense of smell by modulating XME abundance and activity. In the present study, we investigated the contribution of the nasal respiratory mucosa to xenobiotic metabolism and analyzed the impact of the Th2 cytokine interleukin (IL) 13 on the XME protein profile *in vitro*.

Material and Methods: We generated human nasal tissue models at the air-liquid interface (ALI) using donor-matched respiratory epithelial cells and fibroblasts. To study the overall XME profile, we applied single-cell RNA sequencing. XME metabolic activity was assessed by treating tissue models with defined odorants, and metabolites were analyzed by gas chromatography-mass spectrometry. Moreover, XMEs were comparatively quantified in IL13- and non-treated ALI models using Western blot.

Results: We identified >60 phase I and phase II XME genes in the tissue models. From this panel, we selected the phase I XMEs dicarbonyl and L-xylulose reductase (DCXR), aldehyde dehydrogenase (ALDH) 1A1 and ALDH3A1 for further analyses. These XMEs are relevant in food odorant metabolism and we verified their protein abundance in the tissue models. The respiratory cells were capable of metabolizing substrates of these XME families, for instance, 3,4-hexanedione to 4-hydroxy-3-hexanone and benzaldehyde to benzyl alcohol and benzoic acid. Preliminary data indicate that a 14 days treatment with IL13 alters the XME protein amount in the nasal tissue models.

Conclusion: Human nasal tissue models are highly suitable to study xenobiotic metabolism. The *in vitro* models express several functional XMEs and show metabolic activity towards representative aroma compounds occurring in food. IL13-induced inflammation appears to affect the nasal XME profile, which might be linked to an altered sense of smell in chronic inflammatory diseases.



P-049

A 3D human airway tissue platform integrating pooled CRISPR-Cas9 macrophages for high-throughput functional genomics of respiratory diseases

Samanta de Matos Silva

THINK DIFFERENT

Infectious respiratory diseases remain a major global health challenge, highlighting the need for physiologically relevant *in vitro* models to investigate host-pathogen interactions and disease mechanisms. Here, we developed a cost-effective and reproducible 3D human airway tissue platform integrating macrophage-like cells carrying a pooled CRISPR/Cas9 knockout library targeting 18,663 human genes for functional genomics studies. Human type I collagen was selected as the scaffold based on its physiological relevance and tissue-forming capacity. The tissue was assembled using a layer-by-layer air-liquid interface (ALI) approach with MRC-5 fibroblasts, PMA-differentiated THP-1 macrophage-like cells, and either Calu-3 or A549 epithelial cells to generate tracheal or bronchiolar models, respectively. The engineered tissues were maintained for 30 days and validated by glucose consumption, lactate dehydrogenase (LDH) release, histology, digital PCR, scanning electron microscopy (SEM), and multiphoton multiparametric microscopy (MMM). The platform maintained high cell viability (>90%) throughout culture, with increased glucose consumption during the first 14 days followed by stabilization and consistently low LDH release, indicating minimal cytotoxicity. Histological and SEM analyses demonstrated progressive epithelial differentiation, tissue stratification, and the development of ciliated and alveolar-like structures. MMM confirmed preservation of tissue architecture, appropriate spatial organization of fibroblasts, macrophages, and epithelial cells, and sustained cellular proliferation over 21 days. For CRISPR engineering, THP-1 cells were transduced with the Human Activity-Optimized CRISPR Knockout Library. The pooled sublibraries were amplified, sequence-validated, packaged into lentiviral particles, and used to generate edited macrophages, which were selected by puromycin and fluorescence-activated cell sorting before incorporation into the tissue. A tissue dissociation protocol combining type I collagenase and Accutase (1:2, 37°C, 20 min) achieved the highest viability and dissociation efficiency, enabling downstream third-generation sequencing. Collectively, this integrated 3D airway platform provides a robust and scalable model for functional genomics studies of infectious respiratory diseases and the identification of novel therapeutic targets.

VIRAL INFECTIONS

P-050

Development of iPSC-Derived Human Lung Organoids for Microbiome Research

Ugne Ceplaite

VIRAL INFECTIONS

There is extensive research on how the human microbiome affects health. However, its effects on the lung are poorly understood. While many studies have shown a connection between the gut microbiota and lung health, the exact mechanisms behind this relationship remain unknown. Because animal models cannot recapitulate all complexities of human physiology, developing human model systems is necessary for a better understanding of these interactions. Organoids are self-organizing 3D structures that serve as physiologically relevant *in vitro* models for investigating various biological questions. Compared to classical cell culture models, they better reflect tissue physiology, cellular and structural complexity, making them a valuable tool for microbiome research.

Using human induced pluripotent stem cells (hiPSCs), we established two human lung organoid models: airway and alveolar organoids. hiPSCs were differentiated into lung progenitor cells, which were sorted and embedded for organoid formation. The airway and alveolar organoids were then characterized using flow cytometry and microscopy approaches. Airway organoids contained basal, ciliated, and goblet cells, while alveolar type 1 (AT1) and alveolar type 2 (AT2) cells were absent. Conversely, alveolar organoids mainly differentiated into AT1 and AT2 cells, with no airway-specific cells detected.

Human airway and alveolar organoids can now be used as a novel tool to investigate various questions in microbiome research, including how the microbiome affects diseases such as asthma, chronic obstructive pulmonary disease, and pulmonary fibrosis. Furthermore, these models can be used to study the effects of microbial metabolites and to establish co-cultures with immune cells to recapitulate the cellular network present in the human lung.



P-051

A Novel *In Vitro* Primary Human Alveolar Model (AlveolAir™) for H1N1 and SARS-CoV-2 Infection and Antiviral Screening

Julia Dubois

VIRAL INFECTIONS

During respiratory virus infection, antigens and immune mediators are exposed and/or secreted at surface of infected airway cells to recruit cellular mediators of the innate and adaptive immune response. Endothelial cells are at interface between epithelial cells and circulating immune cells and are able to express chemokines to attract immune cells involved in inflammation. In that regard, a robust and reproducible model of reconstructed human lung epithelium co-cultured with endothelial cells could be a relevant tool to overcome existing challenges concerning the use of non-animal models and to study lung physiopathology during respiratory viral infection. In our study, we characterized SARS-CoV-2 and H1N1pdm09 Influenza infections in the AlveolAir™ model (Epithelix), a fully primary *in vitro* 3D human alveolar model comprising primary alveolar epithelial (ATI/ATII) and endothelial cells, cultured at the air-liquid interface. We focused on viral replication kinetics, inflammatory and innate immune responses and impact on AlveolAir™ tissue integrity. Measuring infectious titer, intracellular and secreted viral RNA, our results show a higher permissiveness of the AlveolAir™ model to H1N1pdm09 than to the Delta variant of SARS-CoV-2. Moreover, H1N1pdm09 infection resulted in a strong inflammatory response and impact on tissue integrity whereas Delta SARS-CoV-2 induced mainly an upregulation of interferon genes with no tissue integrity loss. Finally, we confirmed the antiviral efficacy of Baloxavir and Remdesivir treatments on H1N1pdm09 and SARS-CoV-2 infections respectively, and described their subsequent effect on the inflammatory response of the tissues. In conclusion, the AlveolAir™ model represents an interesting tool to study *in vitro* airway physiopathology during respiratory viral infections and a ready-to-use primary alveolar model for antiviral drug evaluation. Article published in Microorganisms 2025, 13, 572. doi: 10.3390/microorganisms13030572

P-052

Exploratory transcriptomic profiling of host responses to RSV clinical isolates in primary bronchial epithelial cells cultured at an air liquid interface

Axelle Fransen

VIRAL INFECTIONS

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Title: Exploratory transcriptomic profiling of host responses to RSV clinical isolates in primary bronchial epithelial cells cultured at an air liquid interface

Abstract: Respiratory syncytial virus (RSV) remains a major cause of acute lower respiratory tract infections (LRTI) in infants and young children. The severity of RSV disease varies widely, ranging from mild upper airway symptoms to severe life-threatening bronchiolitis and pneumonia. The factors driving this variability are not fully understood, and current therapeutic options remain largely supportive, despite recent progress in prophylaxis. Increasing evidence suggests that, besides virus-induced pathology, dysregulated or exaggerated immune responses may also contribute to disease outcome. Although laboratory-adapted RSV strains are widely used in research, their relevance compared to currently circulating viruses is increasingly questioned, underscoring the need to include contemporary clinical isolates in studies investigating RSV biology and pathology.

To address the host responses to RSV infection, we performed a pilot transcriptomic experiment in a controlled *in vitro* system using well-differentiated primary bronchial epithelial cells cultured at an air-liquid interface. Cultures from a single donor were infected with a diverse panel of clinical RSV isolates, representing both subgroups and varying clinical phenotypes, alongside the commonly used reference strains. This approach allowed us to focus specifically on virus-driven differences in epithelial responses, offering a controlled perspective on how viral diversity may shape local virus-host interactions and potentially contribute to disease outcome.

Together, these findings highlight the importance of incorporating representative clinical RSV isolates in transcriptomic studies to advance our understanding of virus-host interactions and support future therapeutic development.

P-053

Establishment of a standardized protocol for antiviral testing against Nipah virus using commercially available reconstituted human airway epithelia

Hulda Run Jonsdottir

VIRAL INFECTIONS

Henipaviruses are highly pathogenic zoonotic viruses within the Paramyxoviridae family, capable of crossing species barriers and causing severe disease in humans. Nipah virus, a member of this genus, originates in fruit bats and can cause infections in humans through direct contact with infected animals, contaminated food, or human-to-human transmission. Since its discovery in the late nineties, Nipah virus has caused multiple outbreaks in South and Southeast Asia, with reported mortality rates ranging from 40% to 75%.

Currently, there are no approved antivirals or vaccines for human use against henipaviruses, highlighting the urgent need for reliable and physiologically relevant platforms to study viral pathogenesis and evaluate antiviral candidates. Nipah virus exhibits dual tropism for the respiratory tract and the central nervous system, posing



significant challenges for therapeutic development.

In this study, we developed a standardized experimental protocol using commercially available reconstituted human airway epithelia (HAE) representing the nasal, bronchial, bronchiolar, and alveolar regions of the respiratory tract. These epithelia were used to investigate Nipah virus replication kinetics and host responses across anatomically distinct epithelial models.

We demonstrate that Nipah virus replicates in all HAE types tested, more efficiently in models from the lower respiratory tract, supporting their suitability as physiologically relevant *in vitro* models of infection. Additionally, the nucleoside analogue Remdesivir exhibits direct antiviral activity against Nipah virus in nasal and bronchiolar epithelia, further supporting their utility for antiviral testing.

Together, these findings establish reconstituted HAE as a robust and reproducible system for studying Nipah virus infection and the development and assessment of antiviral therapeutics *in vitro*.

P-054

Dual antiviral combinations enhance SARS-CoV-2 inhibition in reconstituted human nasal epithelia

Hulda Run Jonsdottir

VIRAL INFECTIONS

The emergence, global spread, and persistence of SARS-CoV-2 resulted in an unprecedented need for effective antiviral drugs. Throughout the pandemic, various drug development and treatment strategies were adopted, including repurposing of antivirals designed for other viruses along with a multitude of other drugs with varying mechanisms of action. Although vaccines show clinical efficacy, they are not equally accessible worldwide, and due to continuous emergence of new variants and short duration of immunity, the development of effective antiviral treatments was of the utmost importance. However, drug-repurposing studies using cellular assays often identify hits that later prove ineffective clinically, highlighting the need for more complex screening models.

We assessed the antiviral activity of 95 single compounds and 30 double drug combinations against SARS-CoV-2 using a standardized *in vitro* model of human nasal epithelium. We evaluated single agents that have either been tested clinically or undergone extensive preclinical profiling. We also assessed multidrug treatment strategies, including combinations of molnupiravir and nirmatrelvir as a baseline, supplemented with suboptimal concentrations of GS-441524 or ivermectin, with the goal of increasing overall antiviral activity while lowering the overall therapeutic dose.

Only a few single agents were highly effective in inhibiting SARS-CoV-2 replication. Selected double combinations containing molnupiravir or nirmatrelvir combined with GS-441524 or ivermectin at suboptimal concentrations showed increased antiviral activity compared to single compound treatment. No triple combinations showed improved inhibition of SARS-CoV-2 replication beyond what was observed for double treatments. While various double combinations exhibit increased antiviral activity over single treatment, the addition of a third compound is not beneficial.

These data indicate that molnupiravir and nirmatrelvir-based dual combinations are worthy of further consideration as potential treatment strategies against COVID-19.

P-055

Susceptibility of bovine respiratory and mammary epithelial cells to avian and mammalian derived clade 2.3.4.4b H5N1 highly pathogenic avian influenza viruses

Victoria Meliopoulos

VIRAL INFECTIONS

Highly pathogenic H5N1 clade 2.3.4.4b influenza viruses recently emerged in cattle, raising concerns of adaptation to mammalian hosts. However, these viruses exhibit a unique tropism for the bovine mammary gland. To better understand species and cellular tropism, we compared replication of avian- and mammalian-derived clade 2.3.4.4b H5N1 viruses in fully differentiated primary mammary and respiratory epithelial cells. Avian-derived isolates could replicate in bovine mammary but not bovine respiratory or human mammary epithelial cells, suggesting this phenotype was unique to cattle. In contrast, mammalian-derived isolates could replicate efficiently in both bovine mammary and respiratory cells. Notably, we observed donor-to-donor variability among receptor expression and abundance, mucin production, and composition of epithelial cell subpopulations, susceptibility to infection, and virus-induced cell death. To identify the host factors underlying these differences, we are performing RNA sequencing on primary bovine epithelial cells from multiple donors infected with avian or mammalian isolates. Finally, we infected a panel of nasal and tracheal epithelial cells from human donors to determine which donor-specific characteristics influence susceptibility to 2.3.4.4b viruses. These studies highlight the importance of considering donor-to-donor variability when determining host susceptibility and zoonotic risk.



P-056

Dissecting the tropism and host cellular response of SARS-CoV-2 Omicron BA.1 in human airways

Joelle Mettier

VIRAL INFECTIONS

The SARS-CoV-2 Omicron BA.1 variant emerged in late 2021 and rapidly displaced Delta. While its antigenic distance contributed to its dominance, BA.1 also exhibited greater intrinsic transmissibility and was associated with less severe disease. Studies in primary human epithelial cells suggest an early replication advantage of BA.1 in the human nasal epithelium, potentially explaining its increased transmissibility. However, the biological mechanisms underlying this phenotype remain unclear. Differences in entry pathways, including enhanced use of matrix metalloproteinases for Spike protein cleavage and differences in the ability to utilise ACE2 when complexed with other proteins, have been reported using drug inhibition tests and pseudovirus entry assays.

Using primary airway cells and iPSC-derived models that recapitulate Delta and BA.1 phenotypes, we aimed to (i) assess the co-expression of ACE2 and host factors involved in viral entry across airway cell types, and (ii) characterise the tropism of Delta and BA.1, as well as the single-cell transcriptomic response to infection.

We found that the early replication advantage of BA.1 in the upper airways is not due to an expanded cell tropism. Both BA.1 and Delta primarily target the ciliated cells, however more cells become virus positive at an earlier time point following BA.1 exposure suggesting more efficient entry. This might be explained by a higher proportion of ciliated cells co-expressing ACE2 with additional entry factors that BA.1 can use but Delta does not. Additionally, the attenuated replication of BA.1 in the lower airway cells led to a reduced host immune response.

These findings improve our understanding of the balance between transmissibility and virulence of SARS-CoV-2 variants and highlight viral adaptations that enhance infection by enabling the virus to utilise host factors.

P-057

Towards a model of bronchiolitis induced by Respiratory Syncytial Virus infection in human lower airway epithelium cultured at air-liquid interphase

Rémi PEREIRA DE OLIVEIRA

VIRAL INFECTIONS

Respiratory Syncytial Virus (RSV) is one of the most predominant cause of lower respiratory tract infection in early childhood worldwide. In infants, RSV infection can lead to frequent and persistent wheezing in early life and can be associated to asthma development in childhood. Although there is a vaccine for pregnant women and therapeutic antibodies to prevent RSV infection in infants, once the infection has taken hold, there is no antiviral treatments available to prevent or limit the development of bronchiolitis and persistent physiopathology.

The main objective of the project was to develop and describe a relevant *in vitro* model of bronchiolitis induced by RSV infection. In this study, human lower airway epithelium (SmallAir™, Epithelix) cultured at air-liquid interphase was infected with RSV. Viral replication, impact of the infection on tissue integrity and innate response were assessed over 14 days post-infection (dpi). RSV RNA persistence was observed up to 14 dpi, in term of intracellular and secreted viral genome. In the same way, persistence of infectious particles was detected at the apical side up to 14 dpi. Expression kinetics of host gene (RT-qPCR) showed an upregulation of inflammatory genes, such as CXCL10 and IL-6, an upregulation of MUC5AC (associated Goblet cell gene), and a downregulation of CFAP221 (associated ciliated gene) and CC-10 (associated Club cell gene). Additionally, immuno-histochemistry observations revealed an alteration of the tissue integrity marked by a persistence of infected cells and an important remodeling of tissue and cell morphologies after RSV infection, with a higher number of mucus-secreting cells than un-infected epithelium.

These results suggest that RSV-infected SmallAir™ could replicate part of bronchiolitis physiopathology, making this model suitable for studying the underlying mechanisms and screening antiviral drugs and biologics for this disease.

P-058

Toward Reduction of Animal Use in Antiviral Research : in-depth comparison of murine models and human airway epithelia

Loison Sheryline

VIRAL INFECTIONS

Antiviral development is challenged by the limited predictive value of conventional preclinical models. Cell lines enable rapid compound screening, but they lack the complexity of the human respiratory tract, while animal studies frequently fail to translate to clinical outcome. Ex vivo human airway epithelia (HAE) represent a promising alternative by preserving tissue architecture and epithelial diversity.

We established an experimental framework to evaluate the efficacy of Paxlovid, Molnupiravir and Ivermectin against SARS-CoV-2 across cell lines, HAE and murine models. These compounds were selected based on their contrasting clinical efficacy in humans: Paxlovid and Molnupiravir are clinically used antivirals, whereas Ivermectin has failed to demonstrate clinical benefit. All three compounds showed antiviral activity in cell lines and mice, but their efficacy differed in HAE. Paxlovid and Molnupiravir retained activity, whereas Ivermectin failed to inhibit viral replication in HAE, consistent with its lack of clinical efficacy.

To further assess the biological relevance of these models, we performed single-cell RNA sequencing on infected and non-infected HAE, mouse lungs and nasal turbinates, in the presence or absence of treatment, and integrated these data with publicly available human respiratory datasets. HAE closely resembled the epithelial composition of human nasopharyngeal swabs and recapitulated infection-associated changes in cellular proportions observed



in humans, including ciliated cell loss and increase of secretory cells. In contrast, mouse lungs primarily captured immune responses. Transcriptional signatures revealed that infected ciliated cells from HAE clustered closely with human nasal epithelial cells, indicating conserved epithelial reprogramming across HAE and human upper airway tissues. In Molnupiravir-treated HAE, we identified transcriptional changes in basal cells, suggesting potential off-target effects that were not detected in murine models. Together, these results demonstrate that HAE provide a predictive and physiologically relevant epithelial benchmark for evaluating antiviral therapies while enabling mechanistic insights into both viral infection and drug-induced cellular responses.

P-059

Advancing Antiviral Glycopolymers to Inhalation Therapy Using an Immune-Competent Airway Model

Jolly Thomas

VIRAL INFECTIONS

Inhaled antiviral therapeutics have emerged as a promising strategy to combat influenza virus infections; however, *in vitro* airway models, including air-liquid interface (ALI) cultures, are seldom combined with controlled aerosol delivery and precise dosimetry. Here, we evaluate antiviral glycopolymers in an advanced *in vitro* airway epithelial co-culture model. Human bronchial epithelial cells (Calu-3) were cultured at the air-liquid interface (ALI) and co-cultured with THP-1-derived macrophages in the basolateral compartment to incorporate key epithelial-immune interactions. This setup enables the simultaneous assessment of antiviral efficacy and immune responses, including cytokine modulation. Glycopolymers acting as viral binding inhibitors were first characterized using hemagglutination inhibition assays and microscale thermophoresis to quantify virus-polymer interactions. A lead candidate from a prescreened panel was subsequently aerosolized and compared to the neuraminidase inhibitor, zanamivir. Antiviral activity was assessed by quantifying viral nucleoprotein expression in epithelial cells and determining viral titers (TCID₅₀) in apical and basolateral compartments. Barrier integrity was monitored via transepithelial electrical resistance (TEER). In addition, cytokine responses, including IL-8, IL-1 β , and TNF- α , were analyzed to evaluate both immune activation and infection-associated inflammation. The glycopolymers retained functional antiviral activity following aerosolization and effectively reduced infection in this co-culture model. This platform enables integrated evaluation of antiviral efficacy, epithelial protection, and aerosol delivery under controlled and biologically relevant conditions. Overall, this study establishes a translational *in vitro* system for assessing inhaled influenza therapeutics and supports the further development of glycopolymer-based antiviral strategies for respiratory virus intervention.



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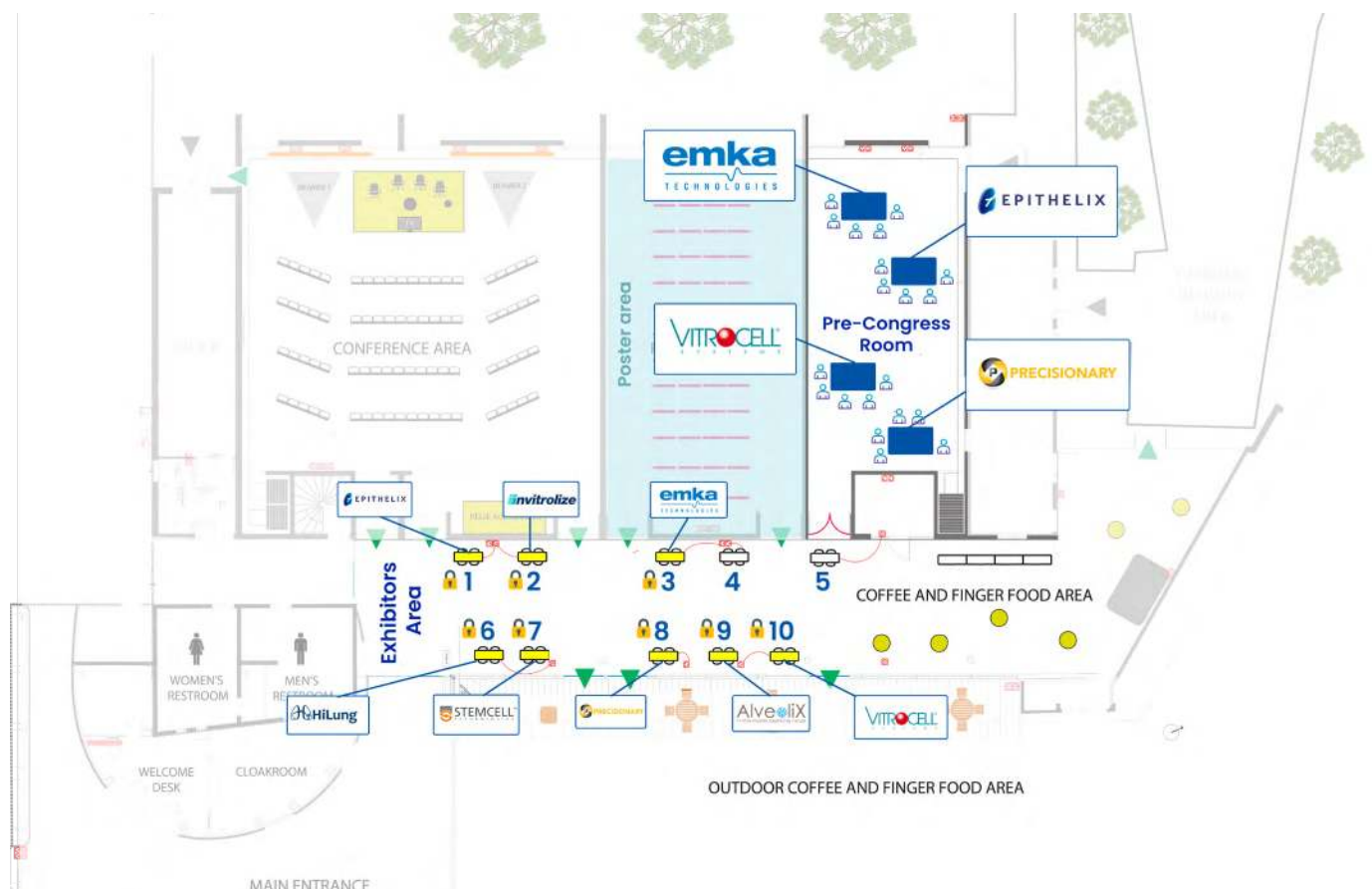


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Floor plan



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Anna	ZERDOUG	<i>Aixial</i>



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EXPOSOME: Building the Case for a Large-Scale Research Cohort



On 28 August, the Exposome Alliance, for which Altertox acts as Secretariat, held a technical meeting at Helmholtz Munich on a potential large-scale European exposome initiative. In discussions chaired by Prof. Annette Peters and Prof. Roel Vermeulen, scientific members worked on the architecture of a shared research cohort and on deliverables aligned with the International Human Exposome Network (IHEN) roadmap.

Civil society and partner initiatives joined the discussions on consent, data use, participant rights and governance, as well as possible models for industry partnership. The Association of European Cancer Leagues, Our Future Health and the Genome of Europe initiative shared their perspectives on how such an initiative could integrate health, exposome and genomic data. The outcomes will feed into the Alliance's technical input to the European Commission and national ministries.

REGULATORY SCIENCE: EMA launches a Voluntary Data Submission pilot

The European Medicines Agency (EMA) has launched a Voluntary Data Submission (VDS) pilot to help regulators gain experience with New Approach Methodologies (NAMs). These include complex *in vitro* systems such as organoids and microphysiological systems, computational models, and combinations of methods.

Companies, method developers, contract research organisations, academic laboratories and other stakeholders can submit NAM data independently of a marketing authorisation application. Participants receive individual, non-binding and non-decisional feedback from an expert group drawn from across the European medicines regulatory network. Submissions are expected until Q3 2027, after which EMA will publish a lessons-learned report.

REGULATORY SCIENCE: EMA explores regulatory sandboxes for innovative medicines

EMA and the European Commission are exploring how to implement regulatory sandboxes under the revised EU pharmaceutical legislation. A sandbox is a time-limited framework in which developers can design, validate and test innovative medicines or methodologies in a controlled, real-world environment under regulatory oversight. It is intended for products that cannot be developed under current rules.

On 21 September, a multi-stakeholder workshop brought together medicines developers, regulators and policymakers to discuss horizon scanning, the development of sandbox plans, and transparency and communication.

Regulatory challenges and opportunities on NAMs at ECHA

This paper examines how the European Chemicals Agency (ECHA) is integrating NAMs into EU chemicals regulation under REACH and CLP. NAMs are already used in read-across, (Q)SAR and grouping approaches, and the authors see scope for wider use in chemical safety assessment and Next-Generation Risk Assessment (NGRA).

For broader regulatory uptake, the paper points to international harmonisation, standardised data and reporting, robust validation, better exposure information, and transparent approaches for integrating evidence and uncertainty. It also outlines ECHA's collaboration with the OECD, the Commission, Member States and other stakeholders.

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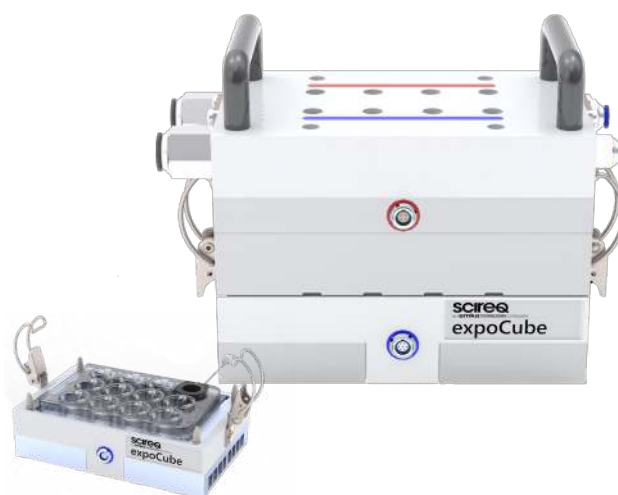
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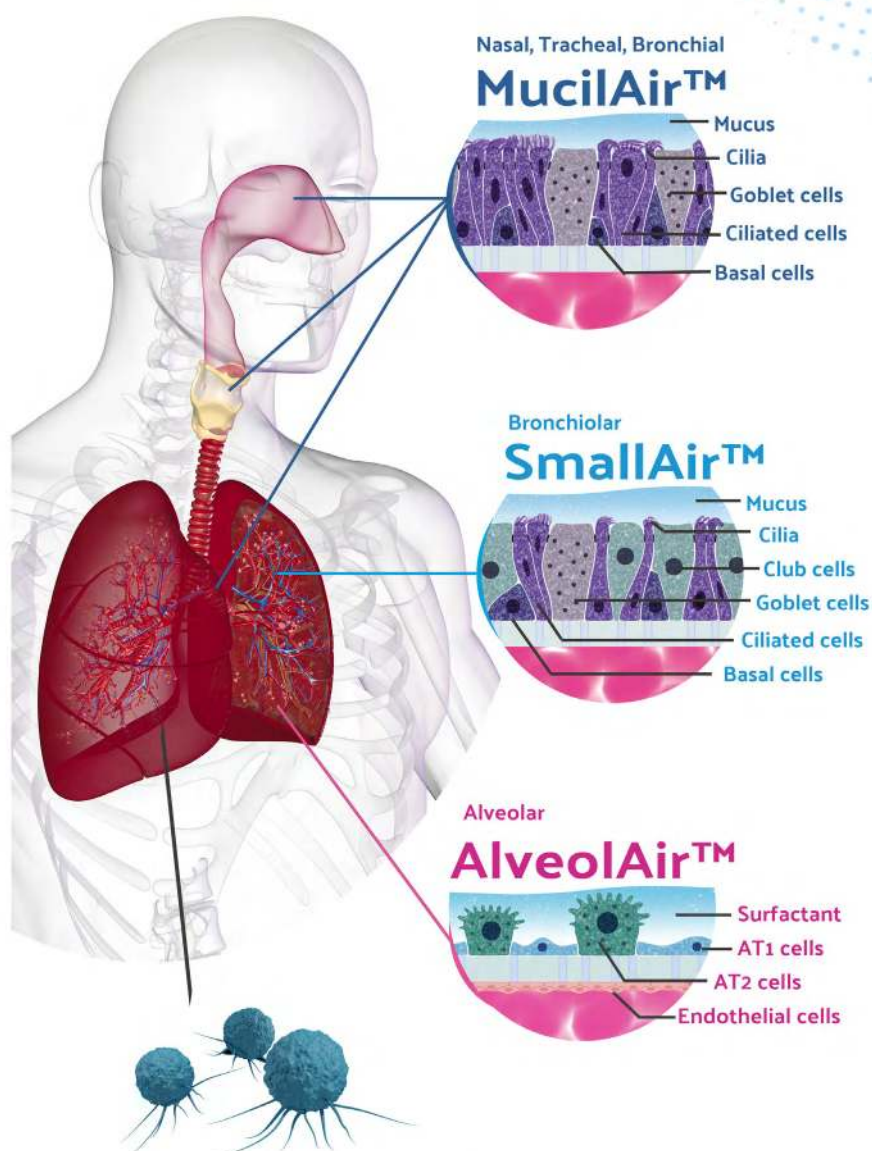




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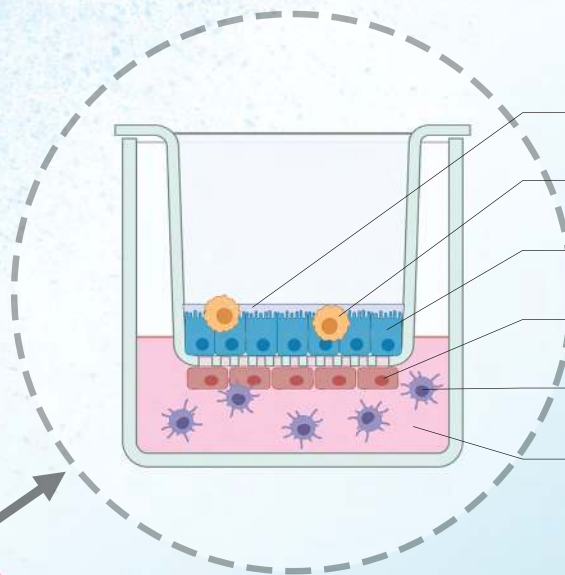
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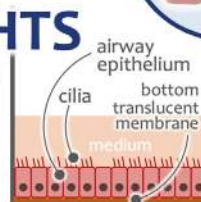
- as conventionally used in respiratory research, but with **unique durability**
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- transmembrane assays; basal 'systemic' access

airway cell plates

Konishi S, et al. *Stem Cell Reports* 2016.
durable lining w/ ciliated & club cells

HiTrach-HTS

- ✓ **months-long studies**, incl. for infection & antimicrobial observations
- ✓ see, e.g., Yin X, et al. *Cell Reports* 2021.

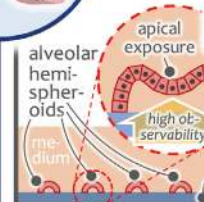


alveolar cell plates

Yamamoto Y, et al. *Nature Methods* 2017.
epithelium w/ both AT1 & AT2 cells

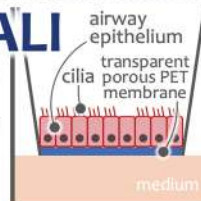
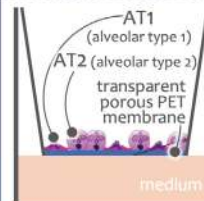
HiAlv-HTS

- ✓ alveolar hemispheroid with polarity of 'outward' apical exposure natural for, e.g., infection



HiTrach-ALI

- ✓ replicating bronchial physiology (e.g., ciliary movements), exposure & infections
- ✓ see, e.g., Kimura I, et al. *Cell* 2022.

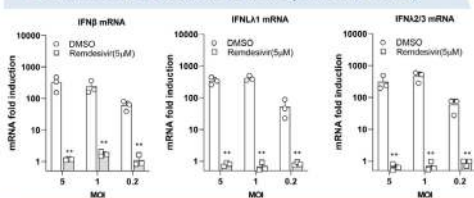


HiAlv-ALI

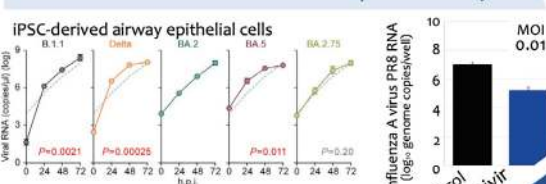
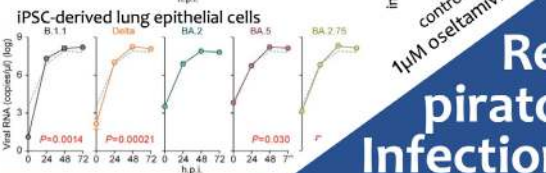
- ✓ uniquely enabling weeks-long alveolar transmembrane ADMET & infection
- ✓ see, e.g., Saito A, et al. *Cell Host Microbe* 2022.

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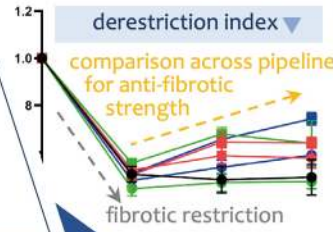
SARS-CoV-2 antiviral therapeutics study



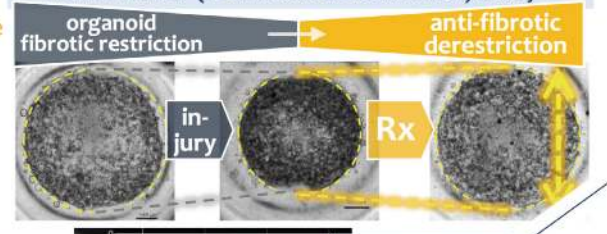
influenza A virus antiviral therapeutics study



▲ Cross-(sub)variant & airway vs alveolar comparison for SARS-CoV-2



HiDEF-ReDer (restriction-derestiction) assay



Fibrosis
(Idiopathic Pulmonary Fibrosis: IPF)

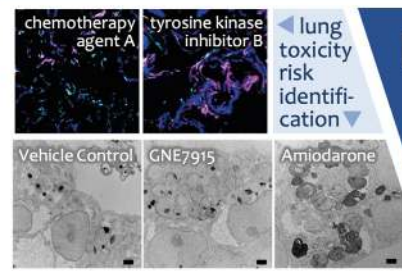
Cystic Fibrosis
(incl. gene variant-dependent CF)

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bd@hilung.com

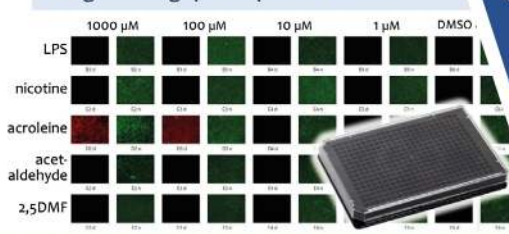
Respiratory Infections & Pandemic Preparedness

Lung Safety & Toxicity
(on-target & off-target)

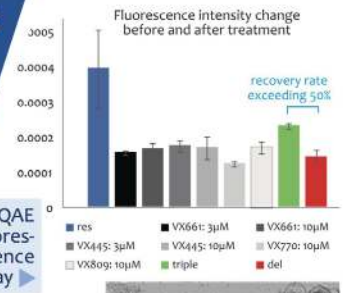
Lung & Airway MPSs
(microphysiological systems)



high-throughput exposure studies

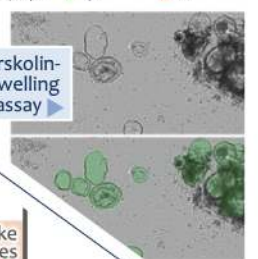
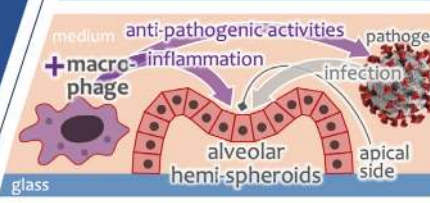
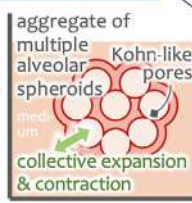


▲ organoid CFTR functional assay: patient iPSC-derived control vs. VX809-treated



MQAE fluorescence assay

label-free forskolin-induced swelling assay



MPS R&D supported in part by AMED's MPS Ph.2 Project, where HiLung is the national PI for the new lung team



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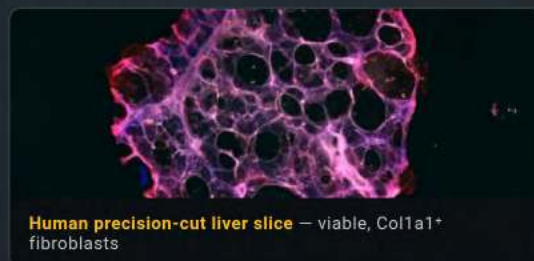
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- Advancing international **harmonization**
- **Collaborating** with international companies and regulatory agencies



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Jiang Lab, BIDMC / Harvard Medical School. eGastroenterology 2026;4(2).

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