An integrated Lung-on-Chip platform investigating molecular responses to whole cigarette smoke exposure:

ALEXIS

IN COLLABORATION WITH

AlveoliX — In-vitro models inspired by nature

VITROCELL® SYSTEMS

RATIONALE

The inhalation of tobacco products, including traditional cigarettes, e-cigarettes, and emerging alternatives, significantly contributes to the progression of chronic lung diseases such as Chronic Obstructive Pulmonary Disease (COPD) and lung cancer. These products release around 4500 reactive chemicals that cause inflammation in lung tissue, particularly in the alveoli. Current animal models struggle to replicate the complexities of human lung exposure to these products, underscoring the need for advanced in vitro models. The Alexis smoke inhalation system offers a novel solution by simulating real-world inhalation on breathing cells, enabling more accurate studies of tobacco-related health impacts.

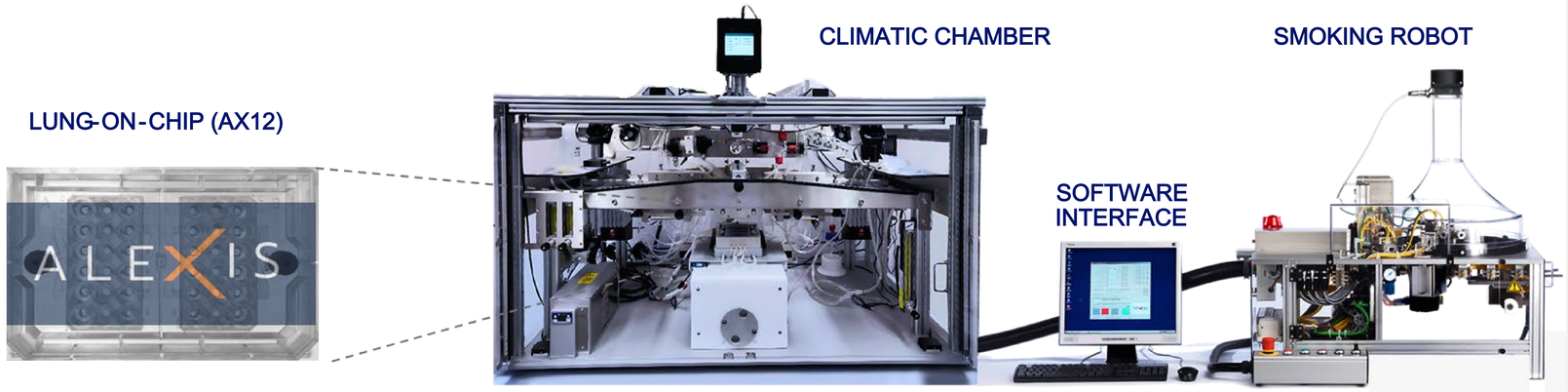
OBJECTIVE

The goal of this study is to evaluate the health risks of combustion tobacco products using our invitro CFAX12 smoke inhalation platform. By simulating both acute and chronic exposure, we aim to assess how different product formulations affect lung cells in mono- and co-culture with immune cells. Specifically, we seek to observe cytotoxicity and inflammatory responses, providing valuable data to support safer product development and regulatory decisions.

TECHNOLOGY

In this study, the Alexis Smoke inhalation platform was utilized to carry out comprehensive hazard assessments of a range of tobacco and alternative products. The system was integrated with complex co-culture bio-models of human lung cells, seeded in Lung-on-chip (AX12) developed by AlveoliX. Exposure regimens were carefully designed to simulate various smoking behaviors and inhalation patterns, representing different user experiences across tobacco products. Controlled doses of smoke and aerosolized vapors were applied, while real-time monitoring of cellular responses—including barrier integrity, oxidative stress marker generation, and inflammatory signaling—was conducted.

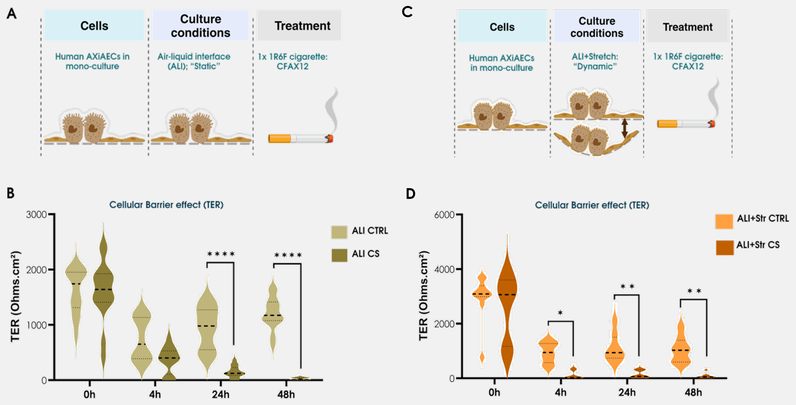
CFAX12: SMOKE ON-CHIP INHALATION PLATFORM



The CFAX12 Smoke Inhalation platform comprises of three key components: the software-controlled interface, the smoking robot, and the climatic chamber. Cigarettes are automatically loaded and ignited using an electric lighter. The lung-on-chip consumable, AX12, is used for exposure. The smoking robot delivers smoke to the dilution system, where it is mixed with dilution air and directed to the cells in the AX12 through "trumpet" inlets. After exposure, the smoke is exhausted through an exhaust tube, with continuous airflow ensuring no residual smoke remains.

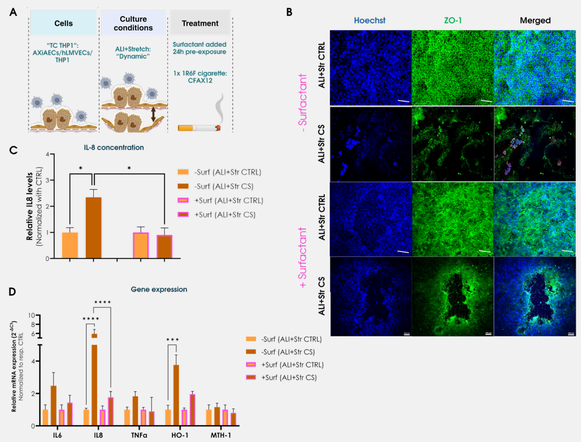
RESULTS

Breathing dynamics with ALI amplifies smoke -induced Alveolar Barrier Dysfunction



(A,B) Cigarette smoke (CS) exposure significantly compromises alveolar epithelial barrier integrity, as measured by trans-barrier electrical resistance (TER). Under static air–liquid interface (ALI) conditions, CS induces a progressive reduction in TER at 24 h and 48 h post-exposure, indicating barrier disruption. (C,D) However, when cells are cultured under physiologically relevant ALI + stretch (ALI + Str) conditions—simulating breathing-like mechanical forces—the response is substantially more pronounced. TER measurements reveal early and dramatic barrier collapse as early as 4 h post-exposure, which persists and worsens through 48 h. These findings underscore the critical role of biomechanical forces in reproducing realistic smoke-induced lung pathology and highlight why static models alone may significantly underestimate cigarette smoke-related barrier dysfunction.

Interfacial pulmonary surfactant layer mitigates smoke -induced inflammatory responses



Pulmonary surfactant is a critical component of the alveolar lining fluid that modulates immune and epithelial responses. (A) Here, we investigated whether a clinical surfactant preparation (Curosurf®) can attenuate cigarette smoke (CS)-induced damage. (B) IF staining shows that surfactant pre-treatment substantially preserves epithelial barrier integrity and cell viability following CS exposure, as evidenced by enhanced ZO-1 tight junctions (in green) and higher nuclei density (Hoechst in blue). (C,D) Surfactant-treated, CS-exposed cells demonstrate significantly reduced IL-8 secretion at both protein and gene expression levels compared to untreated CS-exposed controls. Additionally, surfactant shows a protective trend in reducing cytotoxicity under dynamic smoke exposure. These findings demonstrate that the surfactant layer provides a protective, anti-inflammatory shield against smoke-induced epithelial damage.

CONCLUSION

Bridging Translational Gaps in Respiratory Disease Modeling: The CFAX12 represents a paradigm shift in cigarette smoke toxicity assessment, uniquely integrating whole smoke exposure with breathing-like mechanical stretch in a human-relevant three-dimensional alveolar microenvironment. Our results demonstrate that smoke-induced epithelial barrier dysfunction, oxidative stress amplification, and macrophage-driven inflammatory responses are substantially more pronounced under physiologically relevant dynamic conditions than traditional static models—highlighting the critical role of mechanical forces and cellular complexity in capturing disease pathogenesis. By replacing outdated cigarette smoke extract protocols with controlled, reproducible whole-smoke delivery, the CFAX12 overcomes significant methodological limitations and provides a robust, regulatory-aligned platform for COPD drug discovery, next-generation product safety assessment. This advancement not only accelerates the translation of preclinical findings into clinical benefits but also significantly reduces the need for animal testing—positioning human lung-on-chip technology as a cornerstone of modern respiratory toxicology and precision medicine

REFERENCES

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