Development of Organotypic Air-Liquid Interface Cultures as Models for Smoking-Related Lung Disease Endpoints

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Introduction

In vitro models that replicate the structural integrity and functional responses of the lung inform of the toxicological and biological effects of inhaled toxicants and mechanisms of multi-dimensional diseases such as lung cancer and COPD. Such mechanistic models are critical for the development of novel alternative methods to replace animal testing for regulatory purposes. Here, we describe an application of a novel organotypic air-liquid interface (ALI) cellular model of lung airway cells for the evaluation of perturbed lung physiology and the potential risk of lung diseases from the usage of tobacco products [1-5].

Methods

Cell Culture: Primary normal human bronchial epithelial (NHBE) cells (without identifiers; exempt status from the Institutional Review Board) were provided by Nationwide Children's Hospital Epithelial Cell Core (Columbus, OH). Passage 1 primary NHBE cells were seeded on collagen type IV coated Transwell and grown at the air-liquid interface (ALI). These differentiated cultures display pseudostratified epithelium of basal, ciliated, and goblet cells and phenotypic endpoints of ion channel function (CFTR protein and ENAC), which are key for the fluid homeostasis of ion and fluid balance, and mucociliary clearance. Thus, the ALI cultures replicate select structural and functional features of the human lung [1].

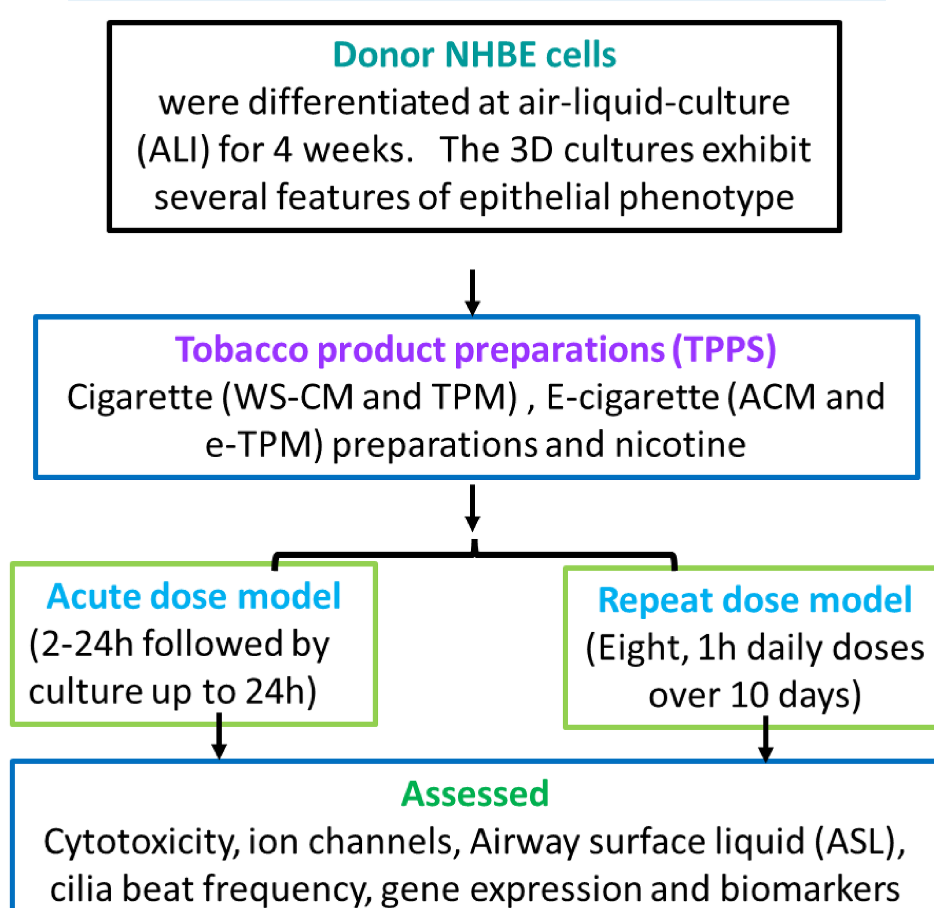
Tobacco Product Preparations (TPPs): Whole-smoke conditioned media (WS-CM) and total particulate matter (TPM) from 3R4F cigarettes were prepared by bubbling mainstream smoke through RPMI 1640 media and stored at -80°C. ENDS preparations (Aerosol Conditioned Media (ACM) and TPM from ENDS (e-TPM)) were prepared by bubbling aerosol generated from commercially available e-liquid into RPMI 1640 medium. The final nicotine content of the WS-CM, TPM, and ACM was used to normalize exposure of cells and is expressed as μg/mL Equi-Nicotine (Eq-Nic.) units.

Exposure Design: Two different exposure conditions (acute and repeated dose) were employed.

Acute dose: Differentiated cultures were treated with different TPPs for 2-24h [2].

Repeat Dose: Differentiated cultures were treated with TPPs for 1h /day, eight times over 10 days [3].

Organotypic lung ALI model



Results

Differentiated NHBE cells exhibit multilayered epithelium

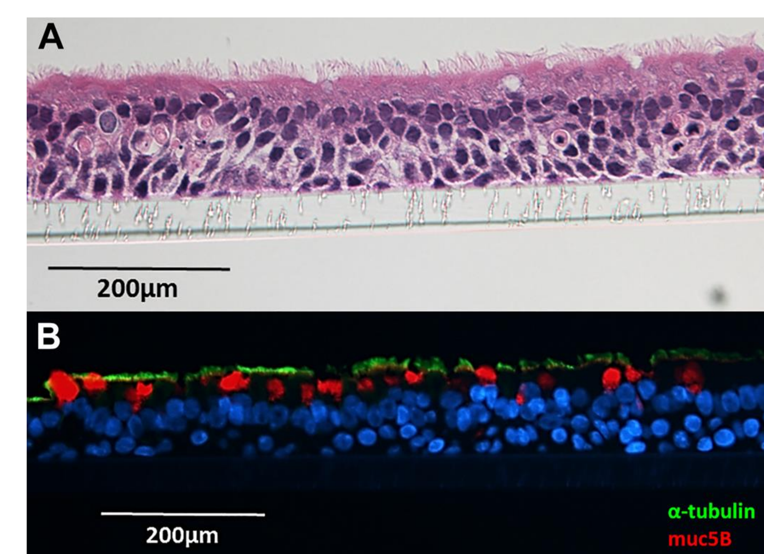


Figure 1. Differentiated NHBE cells exhibit multilayered epithelium with the presence of columnar ciliated cells with goblet cells interspersed (top panel, A). Immunofluorescent staining reveals the presence of ciliated cells identified with tubulin staining (green) and goblet cells stained for MUC5B (red) (bottom panel, B). Nuclei were stained with DAPI (blue) [1].

Acute effects. Cigarette smoke, not ENDS preparations, inhibits ion channel functions

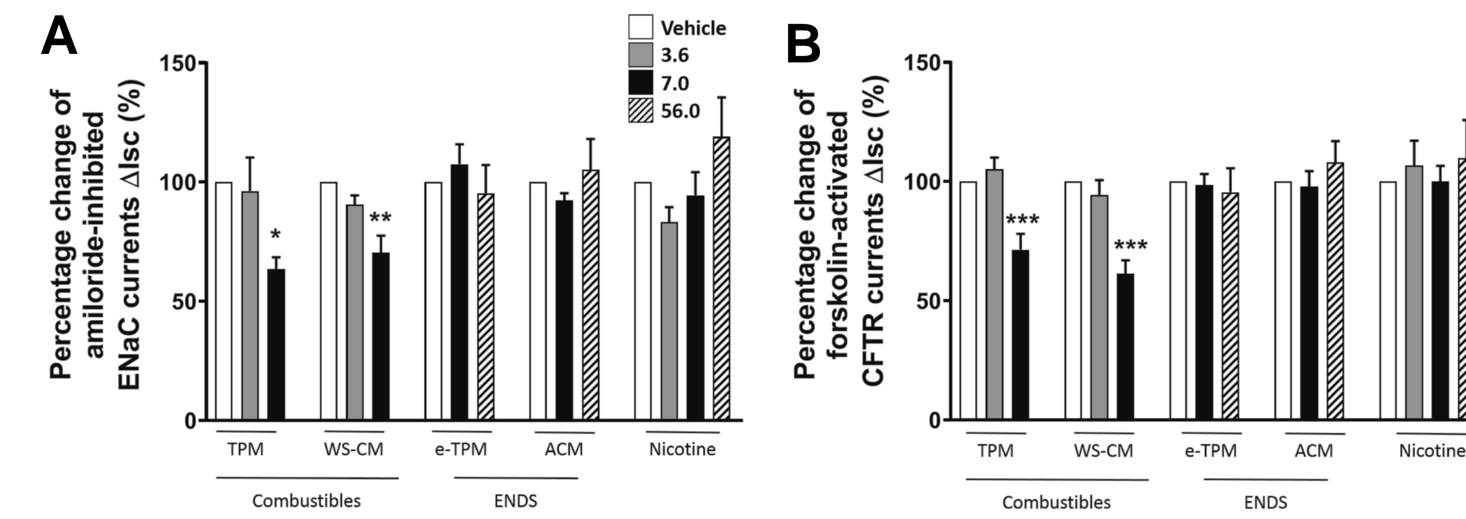


Figure 2. Epithelial sodium channel (ENaC) and CFTR function in the NHBE cultures were measured using Ussing chambers. Differentiated NHBE cultures were exposed to cigarette smoke (TPM) and (WS-CM), ENDS (eTPM) and (ACM)] and nicotine for 24 h at the indicated concentrations [equi-nicotine units (Eq-Nic) for TPM, WS-CM, e-TPM, and ACM; or nicotine units are μg/mL]. (A): ENaC activity was measured after the addition of amiloride. (B): CFTR function was measured after addition of forskolin and then inhibited with CFTR. Statistical significance is compared with vehicle control; ≤ *P 0.05, **P 0.01, ***P 0.001 (three donors; n = 3 for each donor) [2].

Acute exposures to cigarette smoke, not ENDS preparations, markedly alter gene expression in lung cell cultures

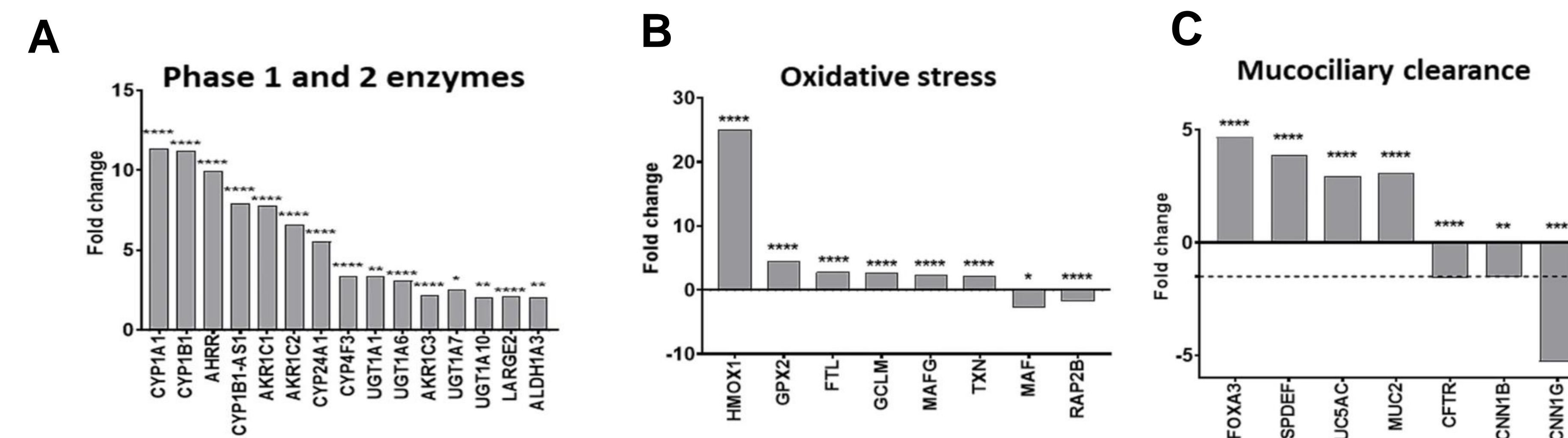


Figure 3: Next Generation Sequence (NGS) analyses show acute exposure to WS-CM induces marked changes in gene expression in the lung 3D cultures (4 donors) in a dose- (3.6-10μg/ml Eq-Nic units/ml) and time (0h, 4h and 24h)-dependent fashion. Treatment with ACM resulted in minimal changes (7-28 μg/ml Eq-Nic Units) (data not shown). Shown here are differentially expressed genes with 10 μg/ml WS-CM treatment for 24h related to phase 1 and phase 2 metabolism (A), oxidative stress (B) and mucociliary clearance (C). Additional details on the methodology, gene expression and pathway analyses are published [4].

Repeated exposures of Cigarette smoke, not ENDS, preparations decrease ASL

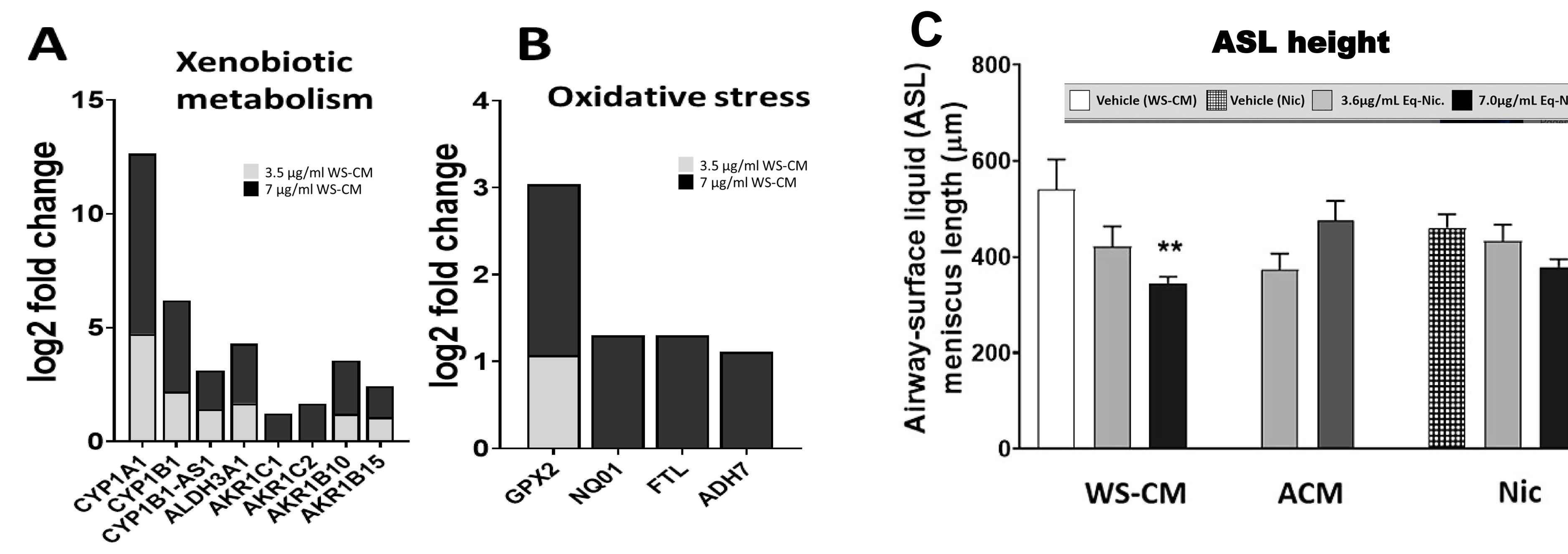


Figure 4: Treatment of differentiated ALI cultures with WS-CM and ACM for 10 days induced several molecular, morphological, and functional changes, but not cytotoxicity (data from 3 donors). Repeated exposures of WS-CM (3.5 μg/ml, gray bars and 7 μg/ml; black bars) sustain upregulation of genes to xenobiotic metabolism (A) and oxidative stress (B). Further, WS-CM, not ACM, exposure for 10 days decreased ASL height (C). Additional effects of WS-CM and ACM on ion channel function, NGS gene expression, and pathway analyses are published [3,5].

Results

Repeated exposure of lung 3D cultures to WS-CM alters epithelial morphology and promotes cell proliferation but not in ACM or nicotine

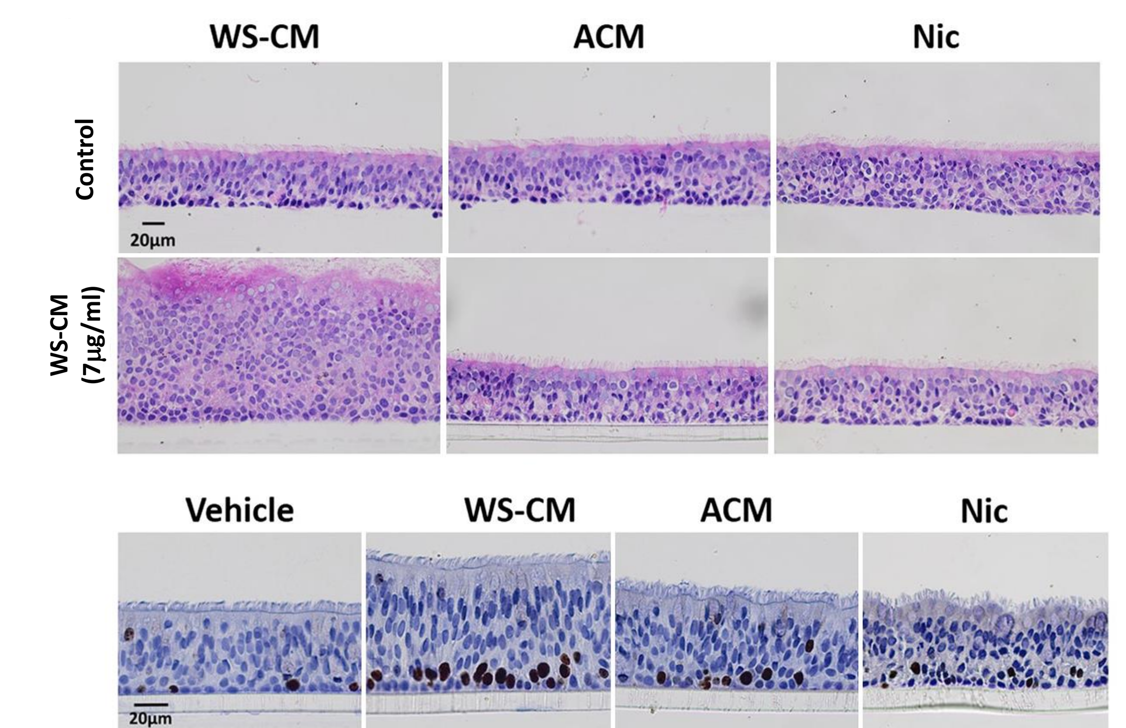


Figure 5. Fully differentiated primary NHBE cells (3 donors) were exposed to WS-CM and ACM (7 μg/ml Eq-Nic units), or nicotine (7 μg/ml). Top panels depict H&E representative images for each exposure shown for a donor. Bottom panels show representative IHC staining of ki67 as a marker of cell proliferation [3].

Summary

- The ALI- cultured NHBE cells display morphological features and retain several functional features of lung epithelium. This model is useful for toxicological investigations.
- Here we utilized this model to evaluate the effects of exposure to cigarette and ENDS preparations on molecular and morphological endpoints that are associated with lung diseases.
- Acute exposure to cigarette smoke preparations inhibits ENAC and CFTR function and upregulates genes involved in xenobiotic metabolism, oxidative stress, and mucociliary clearance, whereas ENDS preparations elicited minimal responses.
- Repeated exposure to cigarette smoke preparations inhibited the ion channel function and ASL height, and differentially regulated genes involved in xenobiotic, oxidative stress, and other key biological pathways.
- Repeated exposures to cigarette smoke preparations induced hyperplasia and promoted the proliferation of lung epithelium.
- Overall, nicotine and ENDS preparations exerted minimal effects, compared to cigarette smoke preparations.
- Thus, the ALI model described herein replicates some of the well-established effects observed in cigarette smokers that are relevant to lung disease.

References

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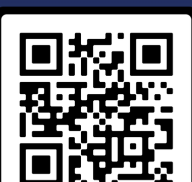

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