Exposure to aerosols from electronic cigarettes using the *MESH*™ technology has a reduced biological impact on bronchial epithelial cell cultures compared with exposure to cigarette smoke: A systems toxicology assessment

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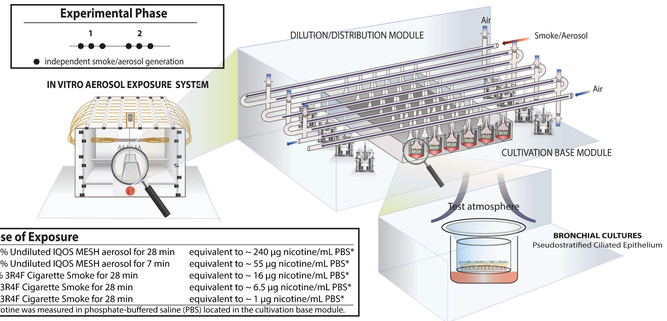
Introduction and objectives

The harmful effects of cigarette smoke (CS) exposure on the respiratory tract are widely known. Electronic cigarette (EC) exposure has been suggested to result in less harm than CS exposure. Many studies have assessed the potential toxicity of ECs *in vitro*. However, most studies have tested the effects of liquid formulations applied directly to cell cultures but not those of formulations applied as vapor/aerosols. In this study, we examined the effects of acute exposure of human bronchial organotypic epithelial cell cultures to whole aerosols generated by a novel EC device that uses MESH™ technology and to diluted CS from the 3R4F reference cigarette (RC). Six independent exposure experiments were conducted in VITROCELL® exposure systems. In each experiment, cell cultures were exposed at the air–liquid interface to undiluted "Classic Tobacco" aerosols generated from the EC for 28 or 112 puffs or to diluted CS for 112 puffs. Deposited nicotine concentrations in the exposure chamber were measured as an exposure marker. Using a systems toxicology approach, we complemented our histological and functional findings with the results of quantitative analysis of molecular changes within a 48-hour recovery period following exposure (global mRNA expression profiles and targeted protein profiles, including those of secretory proteins).

Methods



Dose of Exposure	
100% Undiluted IQOS MESH aerosol for 28 min	equivalent to ~ 240 µg nicotine/mL PBS*
100% Undiluted IQOS MESH aerosol for 7 min	equivalent to ~ 55 µg nicotine/mL PBS*
13% 3R4F Cigarette Smoke for 28 min	equivalent to ~ 16 µg nicotine/mL PBS*
7% 3R4F Cigarette Smoke for 28 min	equivalent to ~ 6.5 µg nicotine/mL PBS*
3% 3R4F Cigarette Smoke for 28 min	equivalent to ~ 1 µg nicotine/mL PBS*

*Nicotine was measured in phosphate-buffered saline (PBS) located in the cultivation base module.

The impact of an acute 28-minute (112 puffs) exposure to three different dilutions of CS (from the 3R4F RC, University of Kentucky) and a 7- (28 puffs) or 28-minute (112 puffs) exposure to non-diluted aerosols from a novel EC device that uses MESH™ technology (IQOS® MESH, P4M3 generation 1.1, Classic Tobacco flavor, Philip Morris International) was assessed in human organotypic bronchial epithelial cell cultures (reconstituted from the bronchial epithelial cells of a 41-year-old male nonsmoker donor). A paired design was implemented: In parallel to the exposure to CS or EC aerosols, the cell cultures were also exposed to air in the same exposure module. A series of six experimental runs was conducted to increase the robustness of our assessment. Nicotine concentrations in phosphate-buffered saline were measured by liquid chromatography (LC) coupled to tandem mass spectrometry (MS). For histological analysis, cross-sections of the organotypic epithelium cultures were analyzed after hematoxylin and eosin (H&E) and Alcian blue staining. Ciliary beating frequency was measured by using the Sisson Ammons Video Analysis system on a total of 512 video frames recorded from the center of the insert surface. Concentrations of inflammatory mediators in the basolateral medium of the exposed cultures were measured by using Luminex® xMAP® technology and commercially available assay panels (EMD Millipore Corp). Targeted protein quantification was performed by parallel reaction monitoring, an LC-MS-based approach using a Q-Exactive™ Mass Spectrometer (Thermo Scientific). Messenger RNA microarray analysis was performed by using 100 ng of total RNA (per sample) that was reverse transcribed and amplified to cRNA by using the Affymetrix® HT 3' IVT PLUS kit and then hybridized to a GeneChip® Human Genome U133 Plus 2.0 Array (Affymetrix).

Conclusions

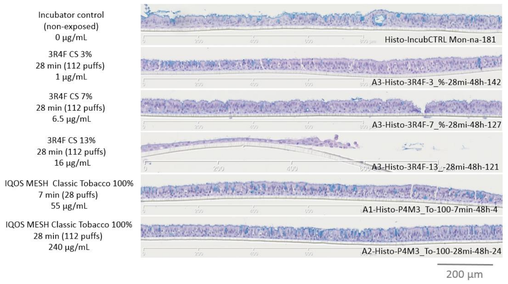
In contrast to 3R~~3~~4F CS exposure, exposure to *IQOS MESH*™ Classic Tobacco aerosols did not cause tissue damage or have an impact on ciliary beating functionality in bronchial epithelial cell cultures despite resulting in greater concentrations of deposited nicotine. Among the secreted proteins analyzed, many more regulatory mediators of inflammatory response were altered following CS exposure than after EC aerosol exposure. Cultures exposed to *IQOS MESH*™ Classic Tobacco aerosols showed fewer changes in proteins involved in xenobiotic metabolism than those exposed to CS. Global mRNA profiles pointed towards alterations in mechanisms related to cellular fate, proliferation, stress, and inflammatory response following CS exposure; these alterations were noticeably less in cultures exposed to the EC aerosol. Overall, using a systems toxicology approach, we detected molecular changes following *IQOS MESH*™ exposure. However, these molecular changes were minimal when compared with those observed following CS exposure.

References

BOUE, S., et al. 2015. Causal biological network database: a comprehensive platform of causal biological network models focused on the pulmonary and vascular systems. Database, 2015, bav030. **HOENG, J.**, et al. 2014. Case study: the role of mechanistic network models in systems toxicology. Drug Discovery Today, 19, 183-192. **MARTIN, F.**, et al. 2014. Quantification of biological network perturbations for mechanistic insight and diagnostics using two-layer causal models. BMC Bioinformatics,15, 238. **MARTIN, F.**, et al. 2012. Assessment of network perturbation amplitudes by applying high-throughput data to causal biological networks. BMC Systems Biology, 6, 54.

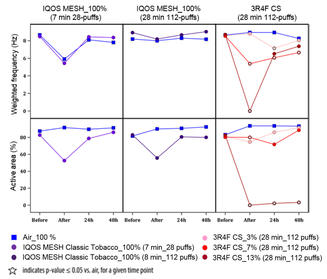
Results

Culture morphology



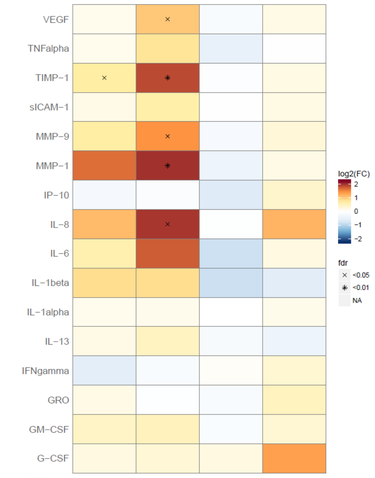
Representative images of H&E- and Alcian blue-stained sections are shown. Following a 28-minute (112 puffs) exposure to 13% 3R4F CS (corresponding to around 16 µg/mL deposited nicotine), bronchial cultures presented high levels of tissue damage. In contrast, exposure to undiluted IQOS MESH™ Classic Tobacco aerosols for the same duration did not alter the tissue morphology despite delivering higher nicotine concentrations (around 240 µg/mL).

Ciliary beating function



Compared with exposure to air, exposure to 3R4F CS resulted in a reduced frequency and percentage of active area of ciliary beating. In particular, cultures exposed to the highest dose of 3R4F CS (13%, 28-min, 112 puffs) presented a massive decrease in the active area of ciliary beating, demonstrating severe tissue damage. Exposure to undiluted IQOS MESH™ Classic Tobacco aerosols did not cause a decrease in frequency but led to a transient decrease in the percentage of active area of ciliary beating immediately after exposure. However, the active area recovered to basal levels at the 24-hour post-exposure time point.

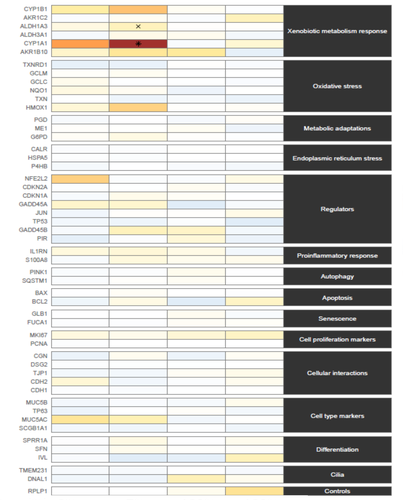
Secretion of inflammatory mediators



Concentration (%)	3%	7%	100%	100%
Duration (min)	28	28	7	28
Puff number	112	112	28	112
Nicotine (µg /mL)	~1	~6.5	~55	~240
	3R4F CS		IQOS MESH Classic Tobacco	

Fold changes of concentrations of secreted inflammatory mediators 48 hours after exposure are shown. Compared with exposure to air, exposure to 7% 3R4F CS for 28 minutes (112 puffs) markedly increased the concentrations of the mediators in the basolateral medium of bronchial organotypic cultures. In contrast, exposure to IQOS MESH™ Classic Tobacco for the same duration produced smaller changes despite presenting much higher nicotine concentrations.

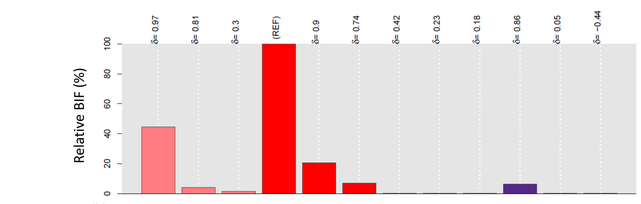
Targeted proteomics analysis



Concentration (%)	3%	7%	100%	100%
Duration (min)	28	28	7	28
Puff number	112	112	28	112
Nicotine (µg /mL)	~1	~6.5	~55	~240
	3R4F CS		IQOS MESH Classic Tobacco	

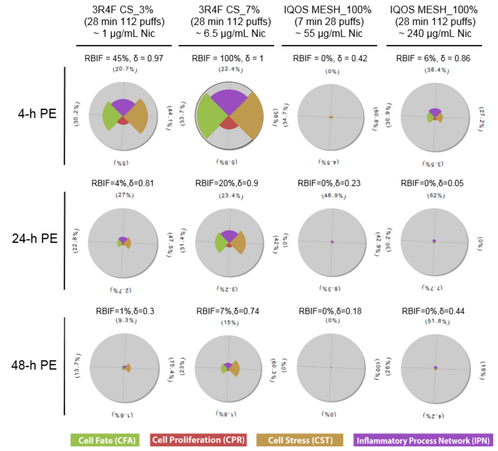
Fold changes of protein abundance in bronchial organotypic cultures 48 hours after exposure are shown. Exposure to 7% 3R4F CS for 28 minutes (112 puffs) markedly increased the concentrations of some proteins involved in the metabolism of xenobiotics. In contrast, despite resulting in much greater deposited nicotine concentrations, exposure to undiluted IQOS MESH™ Classic Tobacco aerosols for the same duration resulted in fewer changes in protein abundance, with none of the differences reaching statistical significance.

Biological impact factor (BIF)



Post-exposure (h)	4	24	48	4	24	48	4	24	48	4	24	48
Concentration (%)	3%			7%			100%			100%		
Duration (min)	28			28			7			28		
Puff number	112			112			28			112		
Nicotine (µg /mL)	~1			~6.5			~55			~240		
	3R4F CS						IQOS MESH Classic Tobacco					

Network enrichment based on transcriptomic changes



Causal network enrichment analysis was performed by using the Network Perturbation Amplitude (NPA) methodology (Hoeng et al., 2014; Martin et al., 2012; Martin et al., 2014) in order to contextualize high-dimensional transcriptomics data by combining gene expression log2-fold-changes into fewer differential node values (one value for each node of a causal biological network model). The human network suite CBN v1.3 (Boué et al., 2015) was used as the collection of causal biological networks in this study. The transcriptome profiles following exposure to diluted 3R4F CS for 28 minutes (112 puffs) showed a greater overall biological impact on various biological processes (cell fate, cell proliferation, cell stress, and inflammatory processes) than those following exposure to undiluted IQOS MESH™ Classic Tobacco aerosol exposure for the same duration. The changes in cultures exposed to IQOS MESH™ Classic Tobacco aerosols normalized and returned to those similar to air-exposed cultures 24 hours after exposure, indicating the recovery of the cultures. PE, post-exposure; BIF, biological impact factor; Nic, nicotine.

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