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Exposure to an aerosol from a novel electronic cigarette using the *MESH*™ technology elicited reduced biological impacts than exposure to cigarette smoke on buccal and small airway epithelial cultures: a systems toxicology assessment

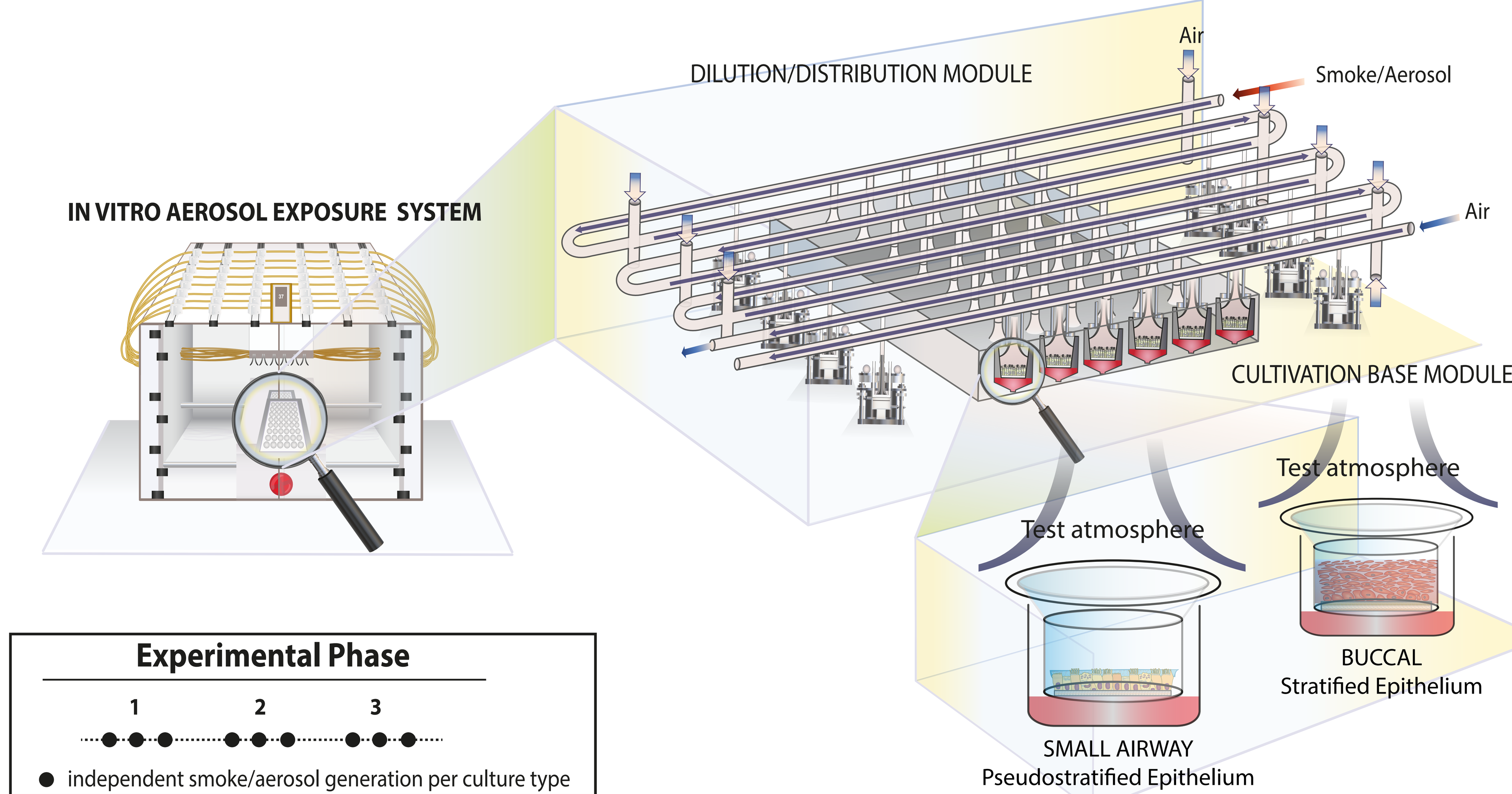
Anita R. Iskandar, Filippo Zanetti, Athanasios Kondylis, Florian Martin, Alain Sewer, Laura Ortega Torres, Shoaib Majeed, Sandro Steiner, Emmanuel Guedj, Celine Merg, Thomas Schneider, Keyur Trivedi, Stefan Frentzel, Nikolai V. Ivanov, Manuel C. Peitsch, Julia Hoeng
PMI R&D, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland

Introduction and Objectives

The harm of cigarette smoke (CS) exposure to both the lower and upper respiratory tracts is widely known. Electronic cigarette (EC) exposure has been suggested to exert less harm compared with CS exposure. Many studies have assessed the potential toxicity of ECs in vitro. However, most studies tested the effects of the liquid formulations applied directly on cell cultures but not the effects of the formulations applied as a vapor/aerosol. In this study, using human organotypic buccal and small airway epithelial cultures, we examined the effects of an acute exposure to whole aerosol generated by a novel EC device, using *MESH*™ technology, and to whole mainstream CS.

Nine independent exposure experiments were conducted. In each experiment, cultures were exposed at the air-liquid interface to undiluted aerosol of "Classic Tobacco" flavor generated from the novel EC for 112 puffs or to diluted CS for the same puff number in Vitrocell® exposure systems. Deposited nicotine concentrations in the exposure chamber were measured as an exposure marker. Using systems toxicology, we complemented histological analysis with quantitative analysis of molecular changes within 48 hours following exposure (global expression profiles of both mRNA and miRNA and targeted protein profiles, including secretory proteins).

Methods



The impacts of an acute 28-minute (112-puff) exposure to CS (from the 3R4F reference cigarette, University of Kentucky) and to aerosol from a novel EC device using MESH™ technology (*IQOS® MESH*™ Classic Tobacco flavor, Philip Morris International) were assessed using human organotypic buccal epithelial cultures (reconstituted from the buccal epithelial cells of a 40 year-old male, nonsmoker donor) and small airway epithelial cultures (reconstituted from the small airway epithelial cells of a 72 year-old female, nonsmoker donor). A paired design was implemented: in parallel to the exposure to CS or EC aerosol, cultures were also exposed to air in the same exposure module. For each of the culture models, a series of nine experimental runs was conducted to increase the assessment robustness. For the histological analysis, the cross-sections of the organotypic epithelium cultures were analyzed after hematoxylin and eosin (H&E) and Alcian blue staining. Ciliary beating measurement was conducted 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 were measured from the basolateral medium of the exposed cultures using Luminex® xMAP® technology and commercially available assay panels (EMD Millipore Corp) according to the manufacturer's instructions. Concentrations of nicotine in phosphate-buffered saline (PBS) were measured using liquid chromatography coupled to tandem mass spectrometry. Messenger RNA microarrays were done using 100 ng of total RNA (per sample) that were reverse-transcribed and amplified to cRNA using the Affymetrix® HT 3' IVT PLUS kit.

EXPOSURE DOSE

Buccal Cultures

3R4F CS
24% for 112 puffs (28 min)
69% for 112 puffs (28 min)

***IQOS MESH*™ Aerosol**
100% for 112 puffs (28 min)

Small Airway Cultures

3R4F CS
7% for 112 puffs (28 min)
13% for 112 puffs (28 min)

***IQOS MESH*™ Aerosol**
100% for 112 puffs (28 min)

ENDPOINTS

	Time Point of Measurement				
	Pre-Exposure	Post-Exposure			
		0 h	4 h	24 h	48 h
Determined using exposed PBS samples					
Deposited nicotine in the exposure chamber	-	✓	-	-	-
Determined using the epithelial cultures					
Culture histology	-	-	-	-	✓
Transcriptomics	-	-	✓	✓	✓
Determined using the basolateral medium samples					
Secreted inflammatory mediators	-	-	-	-	✓

Conclusions

Tissue damage was not seen in cultures exposed to the *IQOS MESH*™ Classic Tobacco aerosol despite resulting in greater concentrations of deposited nicotine. Among the secreted proteins analyzed, greater alterations in the levels of proteins regulating inflammatory response were detected following exposure to CS than to the *IQOS MESH*™ Classic Tobacco aerosol in small airway epithelial cultures. In buccal cultures, CS and *IQOS MESH*™ Classic Tobacco aerosol elicited different inflammatory response. The global mRNA profiles pointed toward alterations in mechanisms related to cellular fate, proliferation, stress, and inflammatory response following CS exposure that were noticeably less following exposure to the *IQOS MESH*™ Classic Tobacco aerosol. Overall, the findings suggested that relative to the impacts of CS exposure, much smaller impacts were detected following undiluted *IQOS MESH*™ Classic Tobacco aerosol exposure in buccal and small airway epithelial cultures.

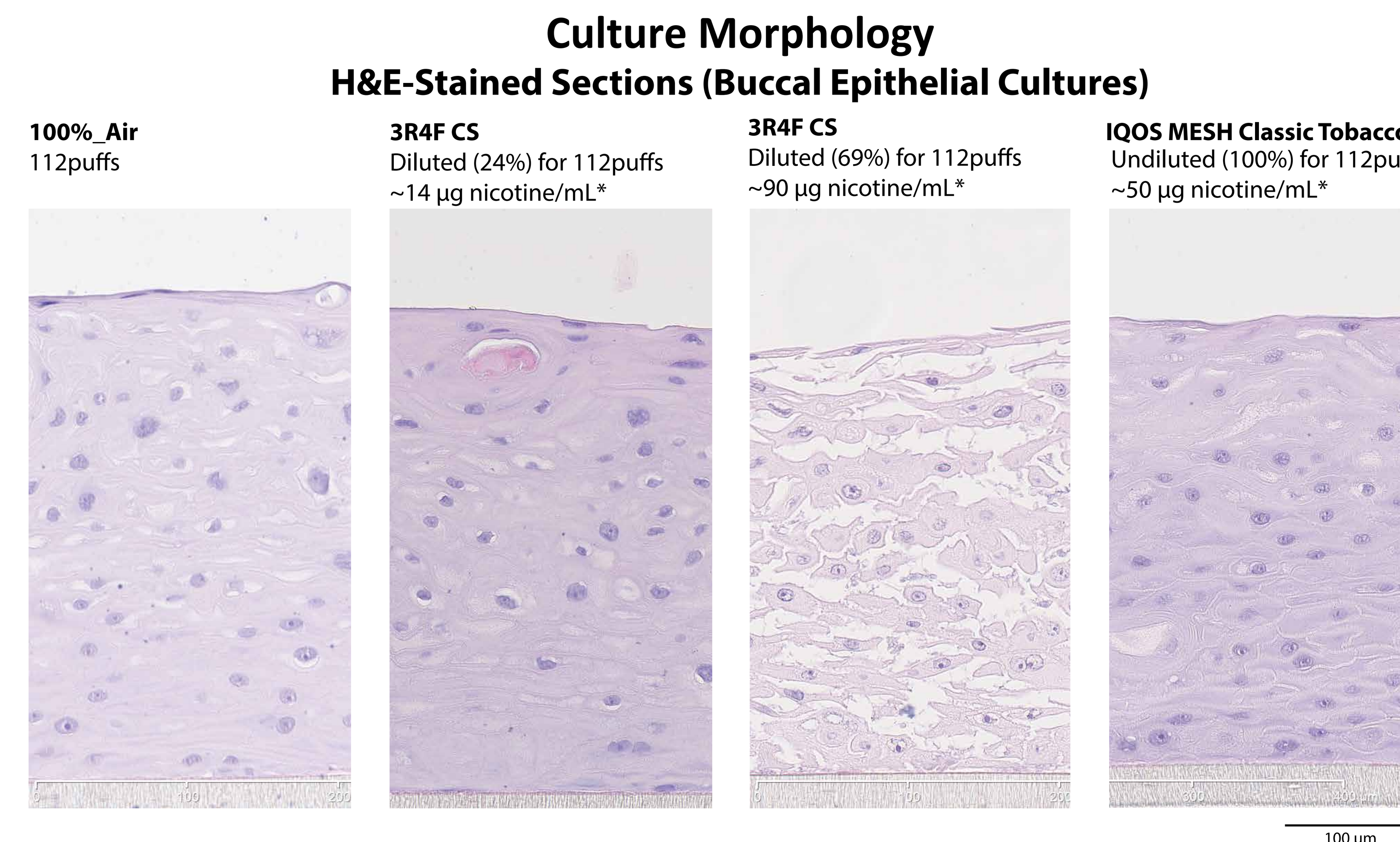
References

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.

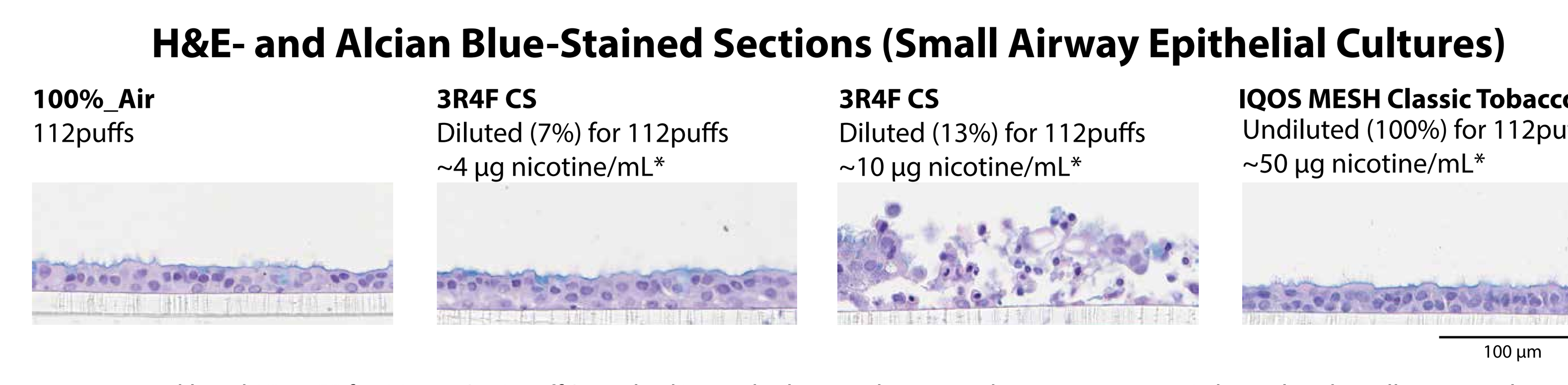
Results

Culture Morphology

H&E-Stained Sections (Buccal Epithelial Cultures)

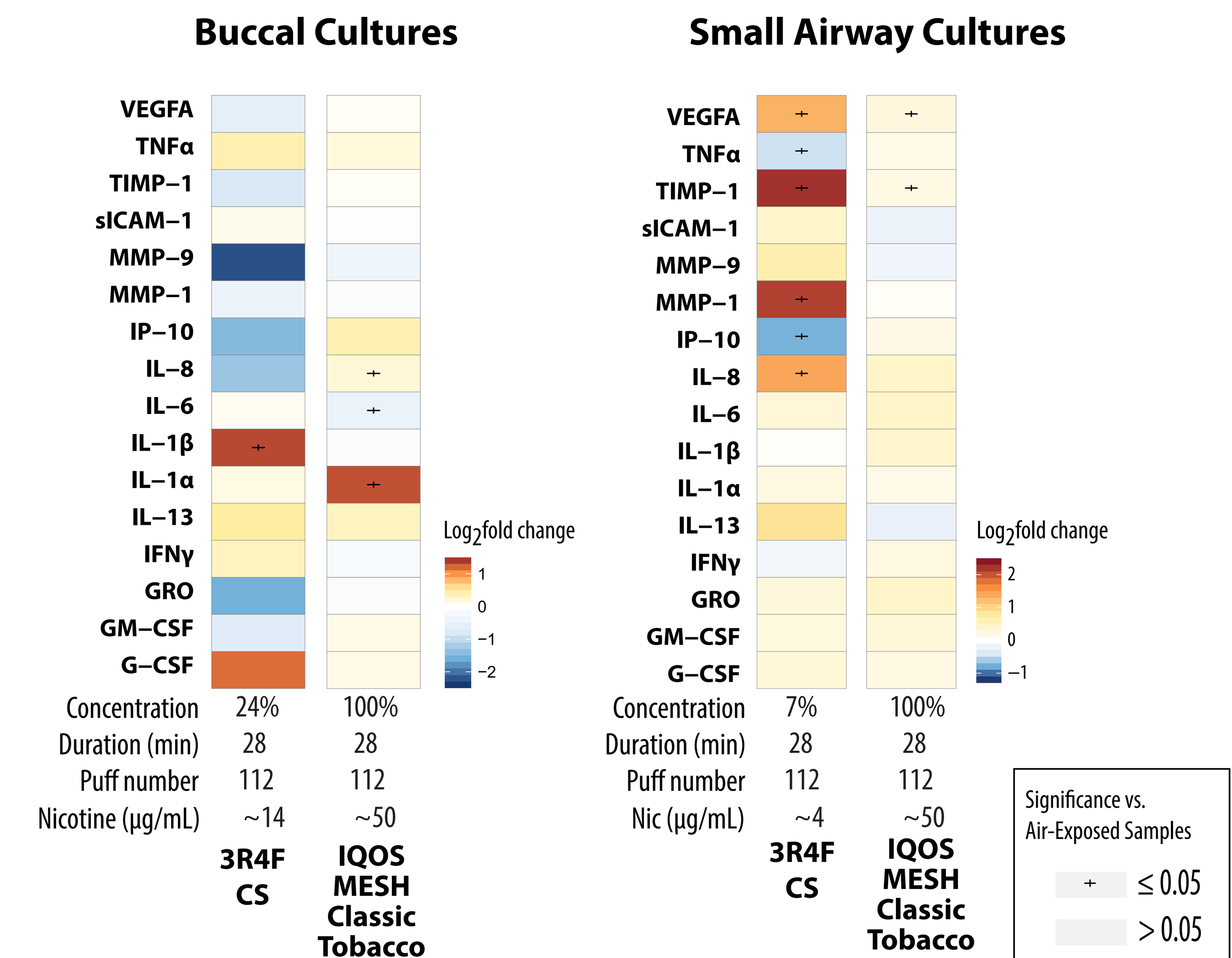


H&E- and Alcian Blue-Stained Sections (Small Airway Epithelial Cultures)



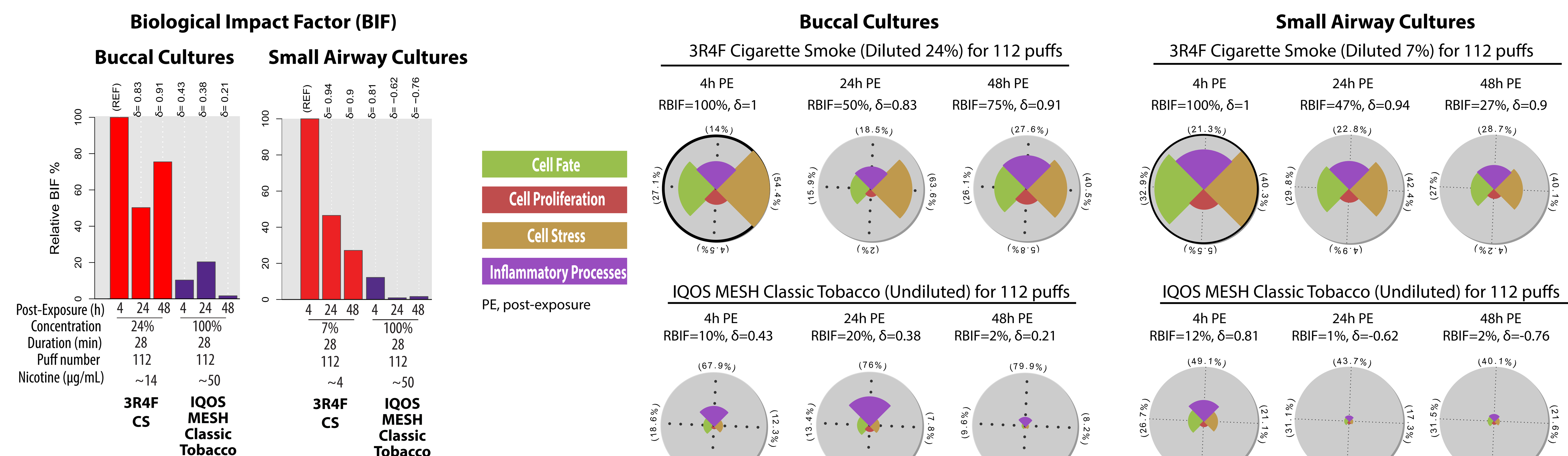
Exposure to diluted 3R4F CS for 28 min (112 puffs) resulted in marked tissue damage in human organotypic buccal and small airway cultures. In contrast, exposure to undiluted *IQOS MESH*™ Classic Tobacco for the same duration did not. *Concentrations were determined in PBS located in the well in the exposure chamber.

Secretion of Inflammatory Mediators



Fold changes of the concentrations of secreted inflammatory mediators are shown. Exposure to 3R4F CS for 28 minutes (112 puffs) markedly increased the concentrations of the mediators in the basolateral medium of small airway cultures compared to the levels following exposure to air; however, smaller changes were detected following exposure to *IQOS MESH*™ Classic Tobacco for the same duration. In buccal cultures, the profile of inflammatory mediator secretion that was induced by 3R4F CS was different from that induced by *IQOS MESH*™ Classic Tobacco at these tested doses.

Network Enrichment Based on the Transcriptome Changes in Human Organotypic Buccal and Small Airway Epithelial Cultures



Causal network enrichment analysis using the Network Perturbation Amplitude methodology (Hoeng et al., 2014; Martin et al., 2012; Martin et al., 2014) was used to contextualize high-dimensional transcriptomics data by combining gene expression log2fold-changes into fewer differential node values (one value for each node of a causal biological network model). The collection of causal biological networks used in the study was the human network suite CBN v1.3 (Boué et al., 2015). The transcriptome profiles following exposure to diluted 3R4F CS for 28 minutes (112 puffs) resulted in greater overall biological impacts on various biological processes (Cell Fate, Cell Proliferation, Cell Stress, and Inflammatory Processes) than the profiles following exposure to undiluted *IQOS MESH*™ Classic Tobacco aerosol exposure for the same duration. The impacts of exposure to *IQOS MESH*™ Classic Tobacco aerosol returned to the levels similar to the air-exposed cultures at the 48 hours post-exposure time point (for buccal cultures) and at the 24 hours post-exposure time point (for small airway cultures).

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