Effects of Exposure to E-cigarette Aerosols Compared with Cigarette Smoke on 3D Human Buccal and Small Airway Cultures: A Systems Toxicology Assessment

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CORESTA Congress 2018, 22-26 October 2018, Kunming, China

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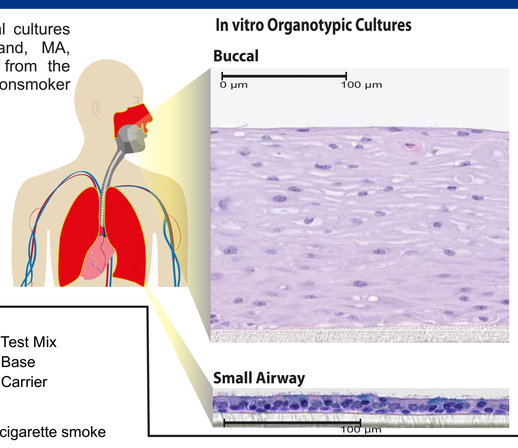
Introduction

Considerable attention has been given toward the potential reduced harm of e-cigarettes (e-cigs). Most in vitro studies have focused on testing e-liquid formulations directly on submerged 2D cultures. Here, we examined the effects of exposure to whole e-cig aerosols compared with mainstream cigarette smoke using human 3D organotypic cultures. Buccal and small airway cultures were exposed at the air-liquid interface over 28 minutes to 112 puffs of undiluted aerosols generated from an e-vapor product containing various e-liquids ("Carrier" containing humectants alone, "Base" containing humectants and 4% nicotine, and "Test Mix" containing humectants, 4% nicotine, and flavors) or to diluted CS in the Vitrocell® 24/48 exposure systems. Nine independent exposures were conducted for a robust assessment. Concentrations of the deposited nicotine and carbonyls in the exposure chamber were measured each time as markers of exposure. Biological endpoints investigated include histology, cytotoxicity, inflammatory mediators, and gene expression microarray.

Experimental Design

Human organotypic buccal cultures (EpiOral™, MatTek, Ashland, MA, USA) were reconstituted from the buccal epithelial cells of a nonsmoker donor (46-y, male).

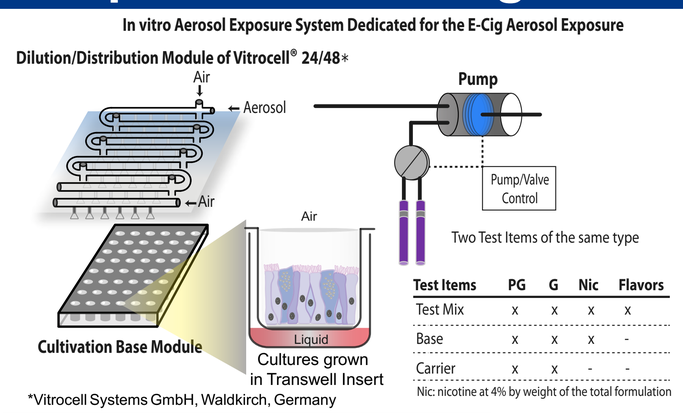
Human organotypic small airway cultures (SmallAir™, Epithelix, Geneva, Switzerland) were reconstituted from the small airway epithelial cells of a nonsmoker donor (65-y, male).



Exposure Duration
- 28 min exposure to 100% Test Mix
- 28 min exposure to 100% Base
- 28 min exposure to 100% Carrier
- 28 min exposure to 3R4F cigarette smoke at various concentrations:

Buccal Cultures
- 28 min exposure to 100% 3R4F cigarette smoke
- 28 min exposure to 24% 3R4F cigarette smoke
- 28 min exposure to 13% 3R4F cigarette smoke

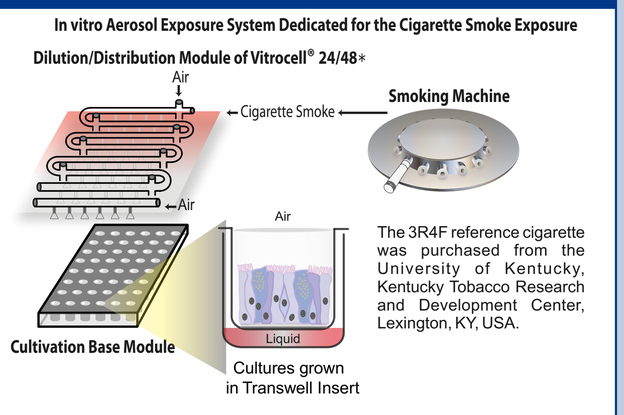
Small Airway Cultures
- 28 min exposure to 13 % 3R4F cigarette smoke
- 28 min exposure to 7 % 3R4F cigarette smoke
- 28 min exposure to 3 % 3R4F cigarette smoke



Test Items	PG	G	Nic	Flavors
Test Mix	x	x	x	x
Base	x	x	x	-
Carrier	x	x	-	-

Nic: nicotine at 4% by weight of the total formulation

*Vitrocell Systems GmbH, Waldkirch, Germany



The 3R4F reference cigarette was purchased from the University of Kentucky, Kentucky Tobacco Research and Development Center, Lexington, KY, USA.

Smoke/Aerosol Generation Parameter:
-55 mL puff volume, 5 s puff duration, 2 puffs /s, 8 s exhaust time, square-shaped
-55 mL puff volume, 2 s puff duration, 2 puffs/s, 8 s exhaust time, bell-shaped

Exposure Characterization:
- Deposition of nicotine in the exposure chamber
- Deposition of carbonyl compounds in the exposure chamber

Measured 0 h Post-Exposure

Post-Exposure Measurements

Biological Endpoint:	2 h	24 h	48 h
Culture histology	-	-	x
Secreted mediators	-	-	x
Transcriptomics	x	x	-

Experimental Repetitions

Year of 2017

Number of Exposure Run for Each Culture Type			
3R4F smoke exposure	3	3	3
E-Cig aerosol exposure	3	3	3

Materials & Methods

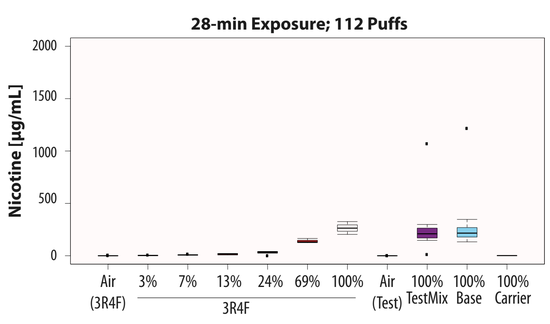
HISTOLOGY – Cross-sections of the organotypic epithelium cultures were analysed after hematoxylin and eosin (H&E) (for both cultures) and Alcian blue staining (only for small airway cultures), as described in [1, 2].

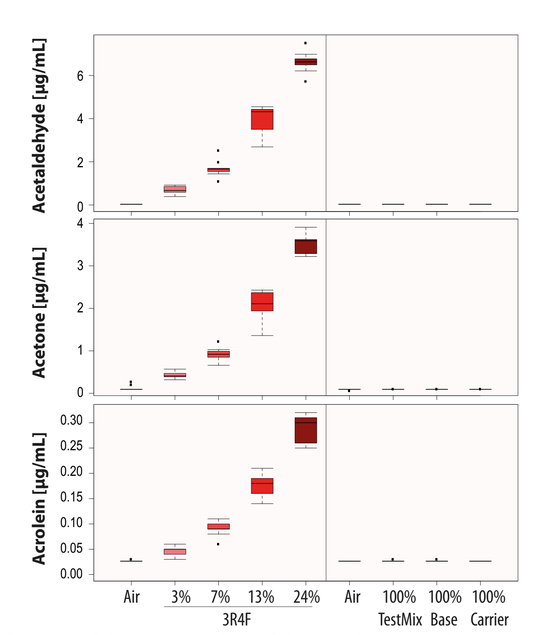
INFLAMMATORY MEDIATOR CONCENTRATIONS – Concentrations of inflammatory mediators were measured from the basolateral medium of the exposed cultures using Luminex® xMAP® technology and commercially available assay panels (EMD Millipore Corp.) according to the manufacturer's instructions, as described in [1, 2].

MRNA MICROARRAY – 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, as preously reported [1, 2]. Causal network enrichment analysis was done using the Network Perturbation Amplitude (NPA) methodology [4] and [5] was used to contextualize high dimensional transcriptomics data by combining gene expression (log)2fold-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(s) was the human network suite CBN v1.3 [5].

Results

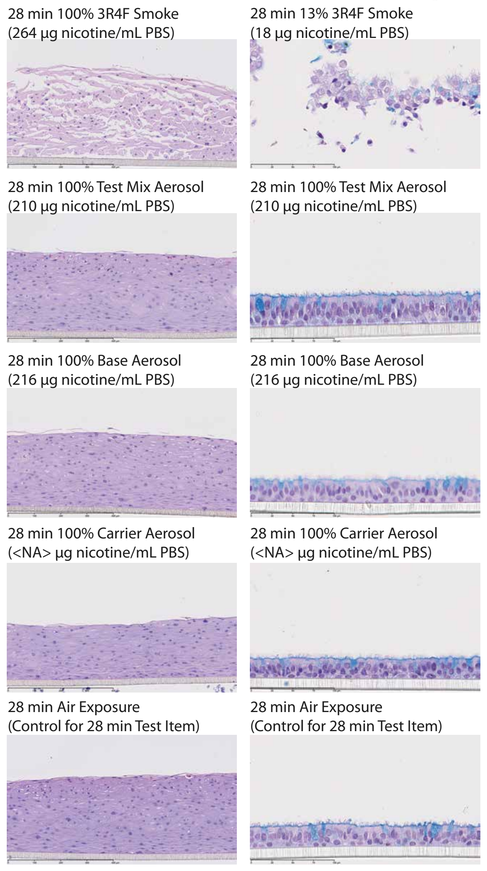
Deposition of Nicotine and Carbonyls in the Exposure Chamber





Boxplots of the concentrations of deposited compounds are shown. Exposure to various concentrations of 3R4F cigarette smoke resulted in increased concentrations of nicotine and carbonyl compounds (acetaldehyde, acetone, and acrolein) deposited in the exposure chamber. Exposure to undiluted Test Mix, Base, or Carrier aerosols resulted in negligible concentrations of deposited carbonyl compounds.

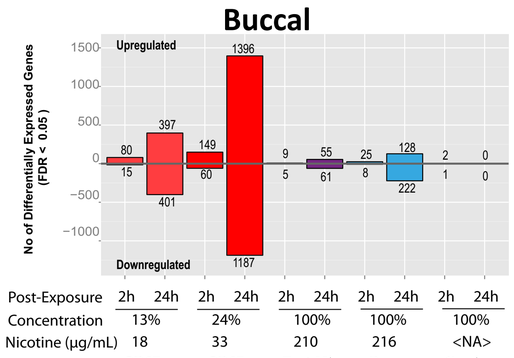
Culture Morphology Following Exposure



Representative images of the H&E-stained sections (and Alcian blue for small airway cultures) are shown. Tissue damage was observed in buccal cultures exposed to 100% 3R4F cigarette smoke for 28 min (112 puffs), corresponding to around 264 g/mL deposited nicotine. In small airway cultures, tissue damage was seen in following exposure to the 28-min (112-puffs) 13% 3R4F cigarette smoke, corresponding to around 18 g/mL deposited nicotine.

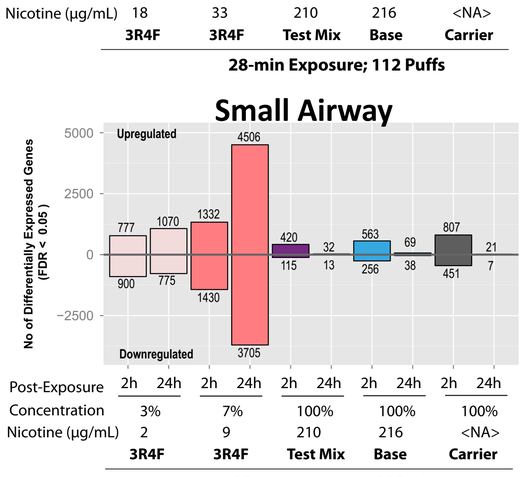
In contrast, even following exposure to the Test Mix or Base aerosols at higher concentrations of deposited nicotine, the culture morphology was not altered.

Global Gene Expression Alterations Following Exposure



Post-Exposure	2h	24h	2h	24h	2h	24h	2h	24h	2h	24h
Concentration	13%		24%		100%		100%		100%	
Nicotine (µg/mL)	18		33		210		216		<NA>	
	3R4F		3R4F		Test Mix		Base		Carrier	

28-min Exposure; 112 Puffs

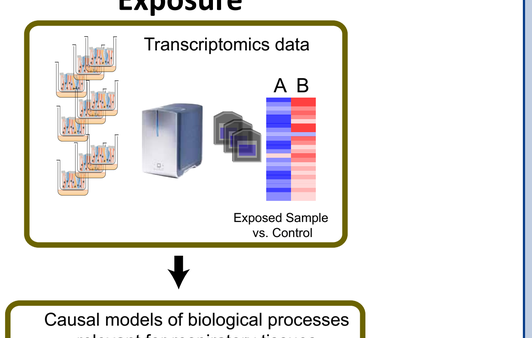


Post-Exposure	2h	24h	2h	24h	2h	24h	2h	24h	2h	24h
Concentration	3%		7%		100%		100%		100%	
Nicotine (µg/mL)	2		9		210		216		<NA>	
	3R4F		3R4F		Test Mix		Base		Carrier	

28-min Exposure; 112 Puffs

Exposure to 3R4F cigarette smoke resulted in a greater number of differentially expressed genes than exposure to Test Mix, Base, or Carrier aerosol at any post-exposure time point tested. In small airway cultures, greater number of differentially expressed genes was observed at an earlier post-exposure time point (2 h) following exposure to the Test Mix, Base, or Carrier aerosol than at the later time point (24 h). Differently, in buccal cultures, greater number of differentially expressed genes was seen 24 h following exposure to the Test Mix, Base, or Carrier aerosol than 2 h following exposure.

Network Enrichment Based on the Transcriptome Changes Following Exposure



The NPA score serves as quantitative measures of the exposure impact on a given biological process modeled in the network, which allow for a comparative analysis between various exposure conditions.

Secretion of Inflammatory Mediators Following Exposure

Boxplots of the concentrations of mediators in the culture medium are shown. Exposure to 3R4F cigarette smoke increased the concentrations of the mediators in the basolateral medium compared with the levels following exposure to air. Exposure to the Test Mix, Base, or Carrier aerosol elicited a similar prole in the mediator concentrations, which were not markedly different from the concentrations following air exposure. LOD, lower limit of detection; LOQ, lower limit of quantication. *p-value ≤0.05 compared with the corresponding air-exposed samples.

In both culture types, the NPA scores elicited following exposure to the Test Mix, Base, or Carrier aerosol were lower than those following exposure to 3R4F cigarette smoke at a given post-exposure time point.

References

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Competing Financial Interest: The research described in this poster was sponsored by Philip Morris Products SA

Conclusions

In conclusion, exposure to the Test Mix, Base, or Carrier aerosol resulted in a consistently lower impact than exposure to cigarette smoke on in vitro human organotypic buccal and small airway cultures. Overall, marked differences in the measured endpoints (culture morphology, secretion of inflammatory mediators, and global gene expression changes) were not observed following exposure to Test Mix, Base, or Carrier aerosols. Minor differences in the effects were detected across the Test Mix, Base, or Carrier aerosol exposure; however, the effects, if observed, were not consistent across all endpoints. The observation further suggested the lack of specific effects of nicotine or flavor ingredients in the alterations of various biological endpoints measured in the study. The data also indicated that cellular adaptation following exposure was dose- and tissue-specific.