The Comparative Analysis of Cytokine Production by a Human 3D Tissue Model Following Exposure to Traditional Cigarette Smoke, Tobacco-Heated Product and E-Cigarette Aerosol

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Abstract

The battery of regulatory assays currently used to assess the toxicity of aerosol exposure are limited in their ability to identify changes at the cellular and molecular level. This has prompted a shift towards a more holistic systems biology approach when assessing the effects of exposure to potential toxicants.[1,2] For example, analysis of the inflammatory mediators produced may provide information on the toxicity-related mechanisms associated with such exposure.

In this study, we used the V-PLEX® human cytokine kit (MesoScale Diagnostics, LLC) to analyse a panel of 30 disease biomarkers following acute exposure of a human airway 3D tissue model (MucilAir™, Epithelix Sarl, Switzerland) at the ALI using a Vitrocell® VC10® to cigarette (3R4F) smoke, tobacco-heated product (THP) and E-cigarette aerosol. Following exposure, post-exposure and recovery (24 hour) medias were collected and biomarker levels quantified. Changes were observed for a number of biomarkers in both the post-exposure and post-recovery media including; IL-1β, IL-8, IL-10, IL12p70, IFN-γ and VEGF. For example, IFN-γ demonstrated ~two-fold increase in the post-recovery media following exposure to all test articles, VEGF was decreased following exposure to the THP and e-cigarette products but not 3R4F and IL-1β levels were increased following exposure to 3R4F but decreased following exposure to THP and e-cigarette.

Our observations demonstrate the potential of the MSD V-PLEX® human cytokine kit in assessing the effects of aerosol exposure on cytokine production, providing an insight to the different biological pathways affected by different commercially-available nicotine delivery systems.

Materials and Methods

MucilAir™ tissues (Epitheliix Sarl, Switzerland) were exposed to whole smoke from 3R4F Kentucky Reference cigarettes (University of Kentucky) and whole aerosol from THP and E-cigarette at the air-liquid interface (ALI) using a Vitrocell® VC10® smoking robot. 3R4F and THP were smoked according to the Health Canada Intense (HCI) and modified HCI (mHCI) regime's for 66 and 180 minutes respectively. E-cigarette was smoked according to a modified CORESTA Recommended Method No 81 (mCRM81) regimen for 180 minutes.

Following the exposure, measurement of nicotine delivery was performed by mass spectrometry and an aliquot of module media and 24 hour recovery media were stored at <-50°C with 0.1% bovine serum albumin (BSA) for cytokine analysis. Samples were subsequently analysed using a MesoScale Diagnostics (MSD) V-PLEX® human cytokine kit (MesoScale Diagnostics, LLC) according to manufacturers instructions. Briefly, samples were incubated in the 96-well V-PLEX® plate with agitation for one hour at room temperature. In addition, calibrator solutions composed of known concentrations of biomarker of interest were incubated in the same plate for purposes of constructing a standard curve. Plates were washed three times with MSD wash buffer (1X). Detection antibody solution was incubated in the 96-well plate for one hour with agitation. Plates were washed three times with MSD wash buffer (1X) and incubated in MSD read buffer (1X) for ten minutes prior to reading the plates using a MSD Sector 600 plate reader. All data analysis was performed using MSD Discovery Workbench. Results were normalised to the response observed for the ALI in which tissues were exposed to flowing air only.

Conclusions

- Exposure to 3R4F resulted in increased levels of IL-4, MDC, GM-CSF, IL-12/IL23p40, IL-10 and IFN-γ in the recovery media. Approximately three-fold increases in MDC, GM-CSF, IL-12/IL23p40 and IFNγ were observed whilst two-fold increases were observed for IL-4 and IL-10. In comparison, no marked effect was observed in the module media.
- In contrast to the response observed from 3R4F exposure, fewer changes in cytokine production were observed following THP and E-cigarette exposure. IFNγ demonstrated a two-fold increase in levels measured in the recovery media at doses 1 and 2 for THP. IL-12/IL23p40 also demonstrated a 1.5-fold increase in recovery media following exposure to THP. In addition, IFN-γ and IL-8 were increased following exposure to E-cigarette at dose 2. IL-1β also demonstrated a 1.5-fold increase in the recovery and module media following exposure to E-cigarette.
- A number of cytokines were reduced following exposure to THP and E-cigarette. For example, GM-CSF, MIP1α, VEGF and MCP-1.
- These results demonstrate the difference in cytokine profiles of MucilAir™ tissues following exposure to different nicotine-containing products.

References

1. Moffat, I. et al. Comparison of toxicogenomics and traditional approaches to inform mode of action and points of departure in human health risk assessment of benzo[a]pyrene in drinking water. *Crit. Rev. Toxicol.* **45**, 1–43 (2015).
2. Haswell, L.E., Corke, S., Verrastro, I. et al. In vitro RNA-seq-based toxicogenomics assessment shows reduced biological effect of tobacco heating products when compared to cigarette smoke. *Sci Rep* **8,** 1145 (2018).

Presented at CORESTA Congress 2020

Results

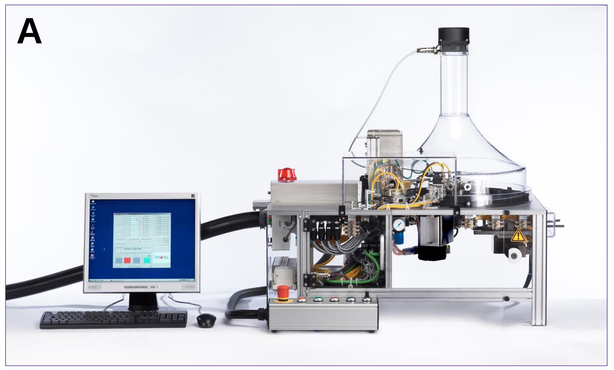
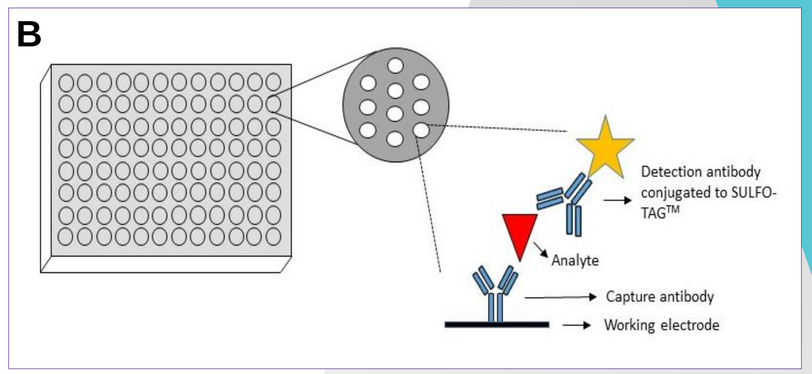
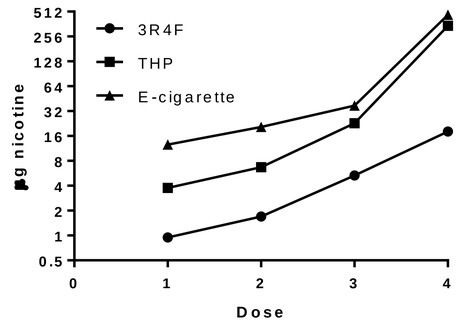


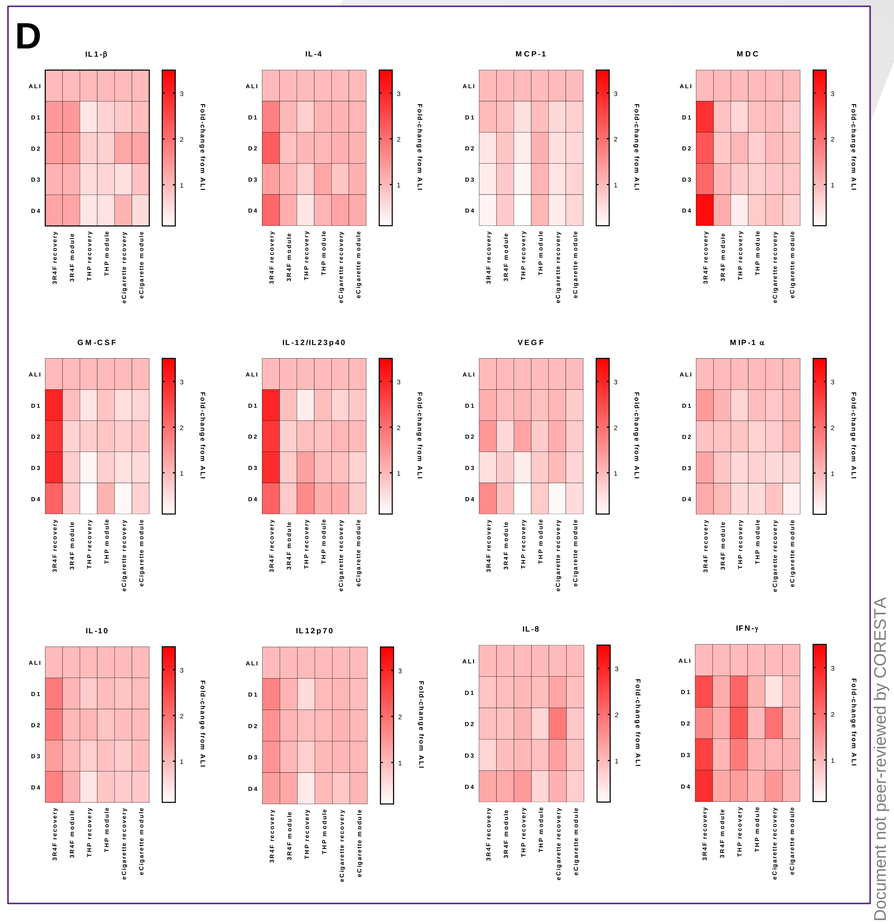
Image supplied by Vitrocell® Systems GmbH



C

	Airflow (L/min)		
Dose	**3R4F**	**THP**	**E-Cigarette**
D1	10	3	3
D2	6	1.5	1.5
D3	4	0.5	0.5
D4	1	0	0





A. Vitrocell® VC10® smoking robot.

B. Diagram of the Meso Scale Diagnostics (MSD) V-PLEX® methodology. Each well contains ten spots, each with a working electrode with a bound capture antibody specific for the analyte of interest. Addition of analyte is then followed by addition of a 'detection' antibody conjugated to a SULFO-TAG™. The application of voltage to the working electrode then results in emission of a chemiluminescent response proportional to the concentration of analyte captured.

C. Concentration of nicotine (µq) in dosimetry PBS is displayed for all doses of each of the three test articles. The table above provides dose information in airflow (L/min). A concentration-related increase in nicotine levels observed as airflow is reduced.

D. Cytokine levels from MSD quantification are displayed. Although 30 analytes were measured, only 12 were within the limit of quantification. Results demonstrate that a number of biomarkers were increased following exposure to 3R4F. In contrast, only a couple of biomarkers were observed at an increased level following exposure to THP and E-cigarette, for example, IFN-γ and IL-8.

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