THE IMPACT OF EXPOSURE TO CIGARETTE SMOKE ON THE SECRETON OF INFLAMMATORY MEDIATORS: ASSESSMENT USING HUMAN ORGANOTYPIC SMALL AIRWAY EPITHELIAL CULTURES FROM FIVE DIFFERENT DONORS

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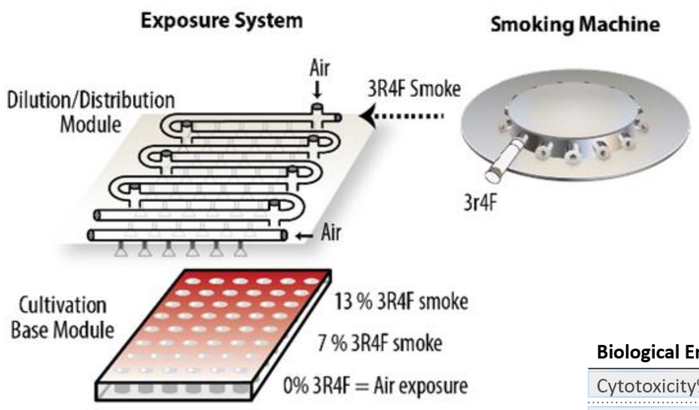
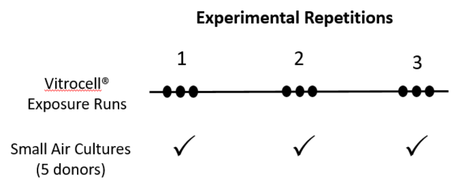
Introduction

The use of modified risk tobacco products (MRTP) could potentially offer a better alternative to smoking (Lopez and Eissenberg, 2015)[1]. Heated tobacco products, one type of potential MRTP, heat tobacco instead of combusting it, thus delivering an aerosol with fewer toxicants than are found in cigarette smoke (CS). Although clinical studies provide the most relevant data regarding the potential toxicity of MRTP aerosol exposure in humans, *in vitro* studies using human cells can help to uncover the associated mechanisms (cellular and molecular changes). The commercially available human small airway culture SmallAir™ (Epithelix, Geneva, Switzerland) is an organotypic culture model reconstituted from primary human small airway epithelial cells isolated from the distal lungs of donors. Cells are cultured at the air-liquid interface (ALI), and after approximately three weeks, the cultures are fully differentiated into a pseudostratified columnar epithelium exhibiting a morphology similar to their *in vivo* tissue counterpart, comprising basal cells, club cells, goblet cells, and ciliated cells (Huang et al., 2017)[2]. The culture conditions at the ALI allow for direct exposure to inhalable gases (e.g., CS, aerosols, airborne particles, or nanoparticles). Our previous study using the SmallAir™ model reported a dose-dependent secretion of pro-inflammatory mediators in response to the exposure to CS from the 3R4F scientific reference cigarette (Iskandar, 2017)[3]. The present study aimed to assess the profiles of inflammatory mediators in response to 3R4F CS using five different donors (including males and females, smokers and non-smokers). The cultures were exposed for 28 minutes to two dilutions of 3R4F CS (7% and 13%) or air using the Vitrocell® 24/48 exposure system. Nine independent exposure experiments were conducted to increase the robustness of the observations. The basolateral media of the cultures was collected at 72 hours following exposure, and a panel of several inflammatory markers was measured using Luminex® technology and Milliplex® kits (GRO, G-CSF, GM-CSF, IL-1α, IL-1β, IL-4, IL-6, IL-8, IP-10, MCP-1, RANTES, VEGF, TNF-α, MMP-1, MMP-9, sICAM-1, and TIMP-1).

Study Design





Post-Exposure Measurements

Biological Endpoint:	24 h PE	48 h PE	72 h PE
Cytotoxicity% (AK)	x	x	x
Secreted mediators			x

Donors Characteristics

	SA0664	SA0693	SA0610	SA0685	SA0665
Sex	Male	Male	Female	Female	Female
Age (years)	28	65	47	48	55
Smoking status	Smoker	Non-smoker	Non-smoker	Smoker	Non-smoker

Materials and Methods

3R4F reference cigarettes were purchased from the University of Kentucky, Kentucky Tobacco Research and Development Center. The reference item-derived smoke will be generated according to the Health Canada Intense (HCI) smoking regime. Cultures were exposed to a continuous aerosol for 28 minutes in a closed chamber using the Vitrocell® 24/48 exposure system (Vitrocell Systems GmbH, Waldkirch, Germany).

Organotypic small airway cultures were purchased from Epithelix (Geneva, Switzerland). They were reconstituted from the primary small airway epithelial cells of five different donors.

Adenylate Kinase (AK) Cytotoxicity was measured in the basolateral media using the ToxiLight™ bioassay kit (Lonza, Rockland, MA, USA)

Concentrations of pro-inflammatory mediators were measured from the basolateral medium of the exposed cultures using xMAP® technology (Luminex® Corp., Austin, USA). Four commercially available assay panels (Merck© KGaA, Darmstadt, Germany) were used. Twenty-five microliters (µL) of standards, controls, and samples (undiluted and diluted) were added in duplicates to the plate along with 25 µL of bead cocktail and 25 µL of their respective matrix solution. The plate was incubated for two hours at room temperature (RT) for HYCTOMAG-60K, HMMP2MAG-55K, and HTMP2MAG-54K and overnight at 4°C for HSP1MAG-63K-01. The plate was washed three times with 200 µL of wash buffer using a magnetic plate washer (405 TS, Biotek® Instruments Inc., Winooski, USA). Twenty-five µL of detection antibodies were added and incubated for one hour at RT followed by the addition of 25 µL of Streptavidin-Phycoerythrin incubated for 30 minutes at RT. Then, the plate was washed, as previously described, and 100 µL (for HMMP2MAG-55K, HTMP2MAG-54K, and HSP1MAG-63K-01) and 150 µL (for HCYTOMAG-60K) of sheath fluid were added. The plate reading was done using FLEXMAP 3D® equipped with xPONENT® software (Luminex Corp., Austin, USA). A non-linear calibration model (five-parameter logistic model) was used to predict (quantify) the concentrations of the pro-inflammatory mediators.

Results

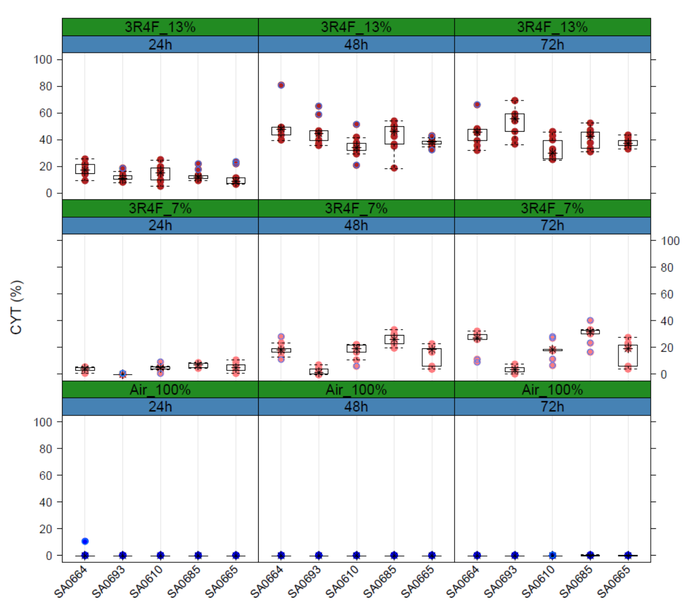


Figure 1. Cytotoxicity elicited by 3R4F CS exposure in small airway cultures obtained from five different donors (AK assay). Results are illustrated using boxplots. Dots are simultaneously depicted over the boxplots.

Cytotoxicity was assessed by measuring the activity of AK released by non-intact cells into the basolateral medium at 24, 48, and 72 hours post-exposure. Relative cytotoxicity was calculated as a percentage of the cytotoxicity induced by Triton X-100 treatment during 24 hours (considered as 100% cytotoxicity). Cytotoxicity increased in a dose- and time-dependent manner following exposure to 3R4F CS.

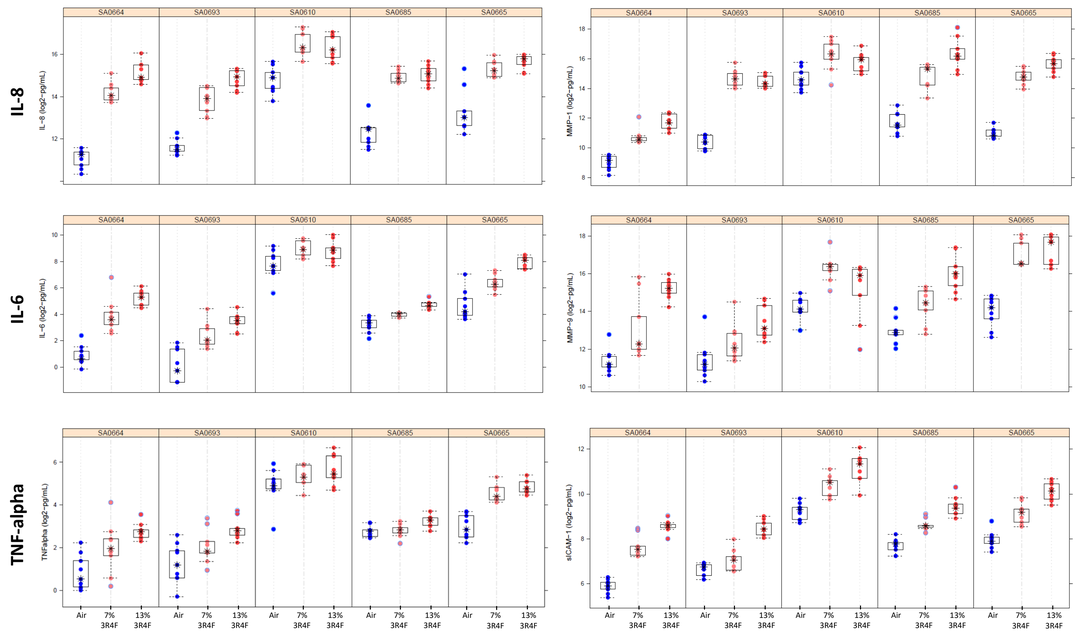


Figure 2. Pro-inflammatory response for six selected analytes of small airway cultures obtained from five different donors exposed to 3R4F CS. Results are plotted in different panels for each tissue donor and are depicted using boxplots. Dots are simultaneously used to depict actual sample values using colors depending upon the experimental stimuli.

The secretion of a set of cytokines, chemokines, and growth factors in response to 3R4F CS exposure was evaluated in the cultures obtained from different donors at 72 hours following exposure. Different donors presented variable basal levels of pro-inflammatory mediators. However, a dose-dependent secretion of all the pro-inflammatory mediators analyzed was preserved across the different donors in response to 3R4F CS exposure.

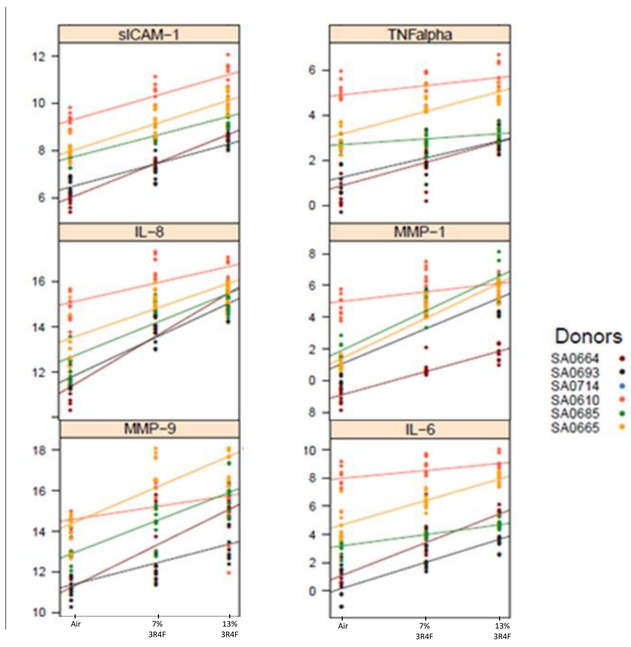


Figure 3. Linear trends of secretion of pro-inflammatory mediators in response to 3R4F CS exposure in small airway cultures obtained from five different donors. Data points are colored according to the different tissue donors. Lines connect the average levels of the quantified analytes for each tissue donor. They depict the linear trend observed for increasing stimulus concentration

Conclusions

Cultures obtained from the different donors presented variable basal levels of the cytokines analyzed. However, in response to 3R4F CS, a dose-dependent secretion pattern of most of the inflammatory cytokines was preserved across the different donors. Altogether, our results show that the secretion of inflammatory mediators in response to CS is highly reproducible among human organotypic small airway epithelial cultures. The results suggest that a multi-analyte profiling analysis is a robust endpoint to evaluate the inflammatory responses following exposure.

References

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