NEXT GENERATION PRODUCTS

Organ-on-a-chip modelling of early atherosclerosis events reveals reduced activity of e-vapour products compared to cigarettes

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Matthew Stevenson[1]*, Jenna Rosano[2], Deborah Ramsey[2], Gwen Fewell[2], Fiona Chapman[1], Roman Wieczorek[3], Edgar Trelles Sticken[3], Sarah Jean Pour[3], Torge Evenburg[3], Liam Simms[1]|
[1]Imperial Brands PLC, 121 Winterstoke Road, Bristol, BS3 2LL, UK, [2]Synvivo Inc., 601 Genome Way, STE 2023E, Huntsville, AL 35806, USA, [3]Reemtsma Cigarettenfabriken GmbH, An Imperial Brands PLC company, Albert-Einstein-Ring-7, D-22761, Hamburg, Germany *Corresponding author's e-mail: Matthew.Stevenson@impbrands.com

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INTRODUCTION

Imperial Brands understands that smoking is a cause of serious disease in smokers including lung cancer, heart disease and emphysema. The greatest risk of smoking related diseases comes from burning tobacco and inhaling smoke containing around 7,000 chemicals. While science suggests that nicotine is addictive and not risk-free, Public Health experts worldwide have concluded that it is the toxicants in cigarette smoke generated by burning tobacco is the primary cause of smoking-related disease, not nicotine. Tobacco Harm Reduction (THR) refers to strategies designed to reduce the health risks associated with tobacco smoking. Next Generation Products (NGP), like E-Vapour Products (EVP), deliver nicotine without burning tobacco so have the potential to play a role in THR.

Atherosclerosis is reportedly responsible for the development of most cardiovascular diseases and develops through a pathway of endothelial dysfunction, lipid infiltration, macrophage recruitment and vascular remodelling[1,2].

Atherosclerosis takes 40-50 years to develop, therefore advanced models to accurately and quickly replicate key events of this condition are required.

METHODS

Test Articles

- 1R6F Reference Cigarette (University of Kentucky)
- E-vapour product (EVP), "blu 2.0 with Golden tobacco flavour 18mg/ml nicotine"

The EVP was obtained from the EU market.

Smoke / Aerosol Extract Generation method

Smoke and aerosol from test products were generated with a Vitrocell VC10s (Vitrocell, Munich, Germany) smoking machine. Smoke or aerosol extracts were prepared by bubbling the sample aerosol into 3 in-line Impingers each containing 10 mL of either Phosphate Buffered Saline (PBS) solution or cell media (See Figure 1). A total stock solution of 30 mLs per test article was used.

Figure 1: Bubbling smoke/vapour exposure system

Trapped nicotine was quantified within the aerosol and smoke bubbled PBS (bPBS) samples, using liquid chromatography with tandem mass spectrometry (LC-MS/MS) with an AB Sciex API 6500 QTRAP (SCIEX, Framingham, MA, USA) using nicotine-d4 as the internal standard. For the analysis of carbonyls, bPBS samples were diluted with 2,4-dinitrophenylhydrazine (DNPH). The carbonyl-DNPH derivates were then quantified using high performance liquid chromatography with a diode-array detector (HPLC-DAD, Agilent Technologies 1100 Series).

Biological Assessment

All the biological assessment was performed by SynVivo Inc.

The SynRAM™ IMN2 radial device (SynVivo Inc.) was used to assess the impacts of these exposures on endothelial cell viability, monocyte adherence and monocyte migration. The device was seeded with human umbilical vein endothelial cells under flow conditions (~4 x 10-2 dynes/cm^2), then cells were exposed to different concentrations of smoke / aerosol bubbled PBS (bPBS), for 4 or 24 hours, whereupon THP-1 monocytes were flowed through the chip. See Figures 2 & 3.

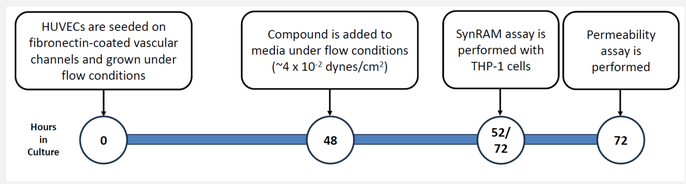


Figure 2: SynRAM experiment procedure

Cytotoxicity assessment

Functionalized devices were treated with the highest concentration (5%) of compound for 24 hours before performing a calcein AM/ethidium homodimer LIVE/DEAD assay to assess cytotoxicity. The LIVE/DEAD® Viability/Cytotoxicity Kit quickly discriminates live from dead cells by simultaneously staining with green-fluorescent calcein-AM to indicate intracellular esterase activity and red-fluorescent ethidium homodimer-1 to indicate loss of plasma membrane integrity. Percent viability was calculated using the following equation:

$$\%Viable\ Cells = \frac{Green\ Cells}{Red\ Cells + Green\ Cells} * 100\%$$

SynRAM Inflammatory Activity Assay and Immunohistochemical staining for ICAM-1

THP-1 monocytes are flowed into functionalized and treated devices, and the number of adherent and migrated THP-1 cells is assed to determine the level of inflammation. After inflammation assay, devices are fixed and HUVECs are stained for VCAM-1.

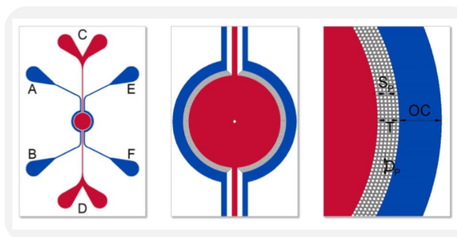


Figure 3: Schematic of the SynRAM model: Vascular channel A-B (outer channel, OC) is cultured with endothelial cells, which can be activated with pro-inflammatory compounds. A chemoattractant gradient is formed in channel E-F to attract adherent immune cells. Porous architecture made up of pillars with diameter Dp and space gaps Sp with a travel distance of T, enables migration of immune cells into the C-D channel.

RESULTS

Dosimetry: Nicotine and carbonyl levels in bubbled PBS

- Nicotine and eight carbonyls (Formaldehyde, Acetaldehyde, Acetone, Acrolein, Crotonaldehyde, n-Butyraldehyde, Propionaldehyde and Methylethylketone) were quantified in the bPBS matrix.
- The 1R6F cigarette bPBS sample contained the highest levels of nicotine to the PBS (253.83µg/ml), whereas the EVP extract contained 229.66µg/ml nicotine, despite the larger number of puffs required for the EVP sample.
- The 1R6F bPBS samples contained the highest levels of carbonyls (levels ranging from 1.6 – 148.61µg/ml). In contrast, marked reductions in carbonyls were observed for the EVP bPBS samples. Out of all the carbonyls assessed, only formaldehyde was quantifiable in EVP bPBS. This was at levels markedly lower than observed in 1R6F reference cigarette extracts (0.42 vs 7.81µg/ml). See Table 1.

Sample	Puffs per ml PBS	Nicotine (µg/mL)	Formaldehyde (µg/mL)	Acetaldehyde (µg/mL)	Acetone (µg/mL)	Acrolein (µg/mL)	Crotonaldehyde (µg/mL)	Butyraldehyde (µg/mL)	Propionaldehyde (µg/mL)	Methylethylketone (µg/mL)
1R6F Reference Cigarette	1.86	253.83	7.81	148.61	6.44	<0.38	1.62	1.96	7.11	1.55
EVP	4	229.66	0.42	<1.5	<0.75	<0.38	<0.38	<0.38	<0.38	<0.38

Table 1: Sample extract chemical characterisation

Cytotoxicity assessment

Sample	% Viability of HUVECs at 24 hours
Vehicle (5% PBS)	98.23%
1R6F Cigarette (5% PBS)	62.83%
EVP (5% PBS)	86.81%

- Compared to the PBS control, at 24 hours post-exposure with the highest test concentration (5% bPBS), cell viability was 62% for cigarette smoke and 86% for EVP extract. This is reflective of reduced toxicant content of EVP aerosol compared to cigarette smoke.

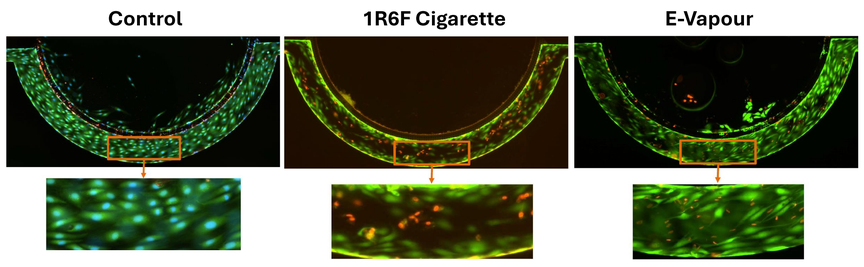
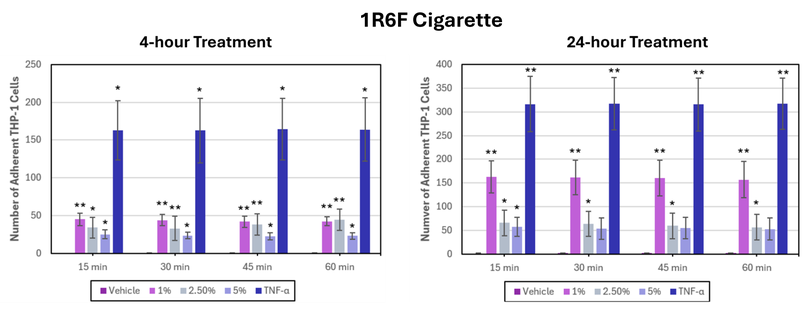


Figure 4: Images of SynRAM chips at 24 hours. Live, uncompromised HUVECs are Green, HUVECs undergoing cell death are Red

Monocyte adhesion assessment



Average ± SEM; n=3 per group except TNF-α (n=2); *p<0.05 & **p<0.01 using Student's t-test

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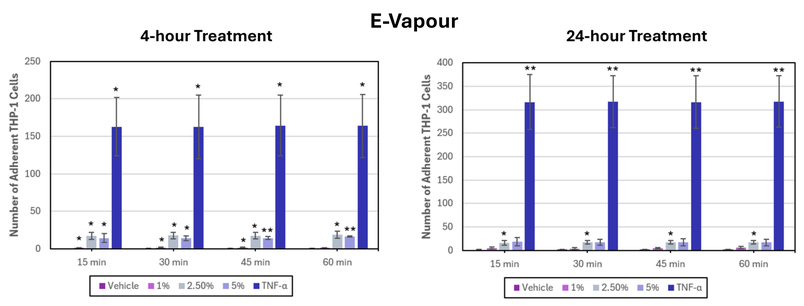

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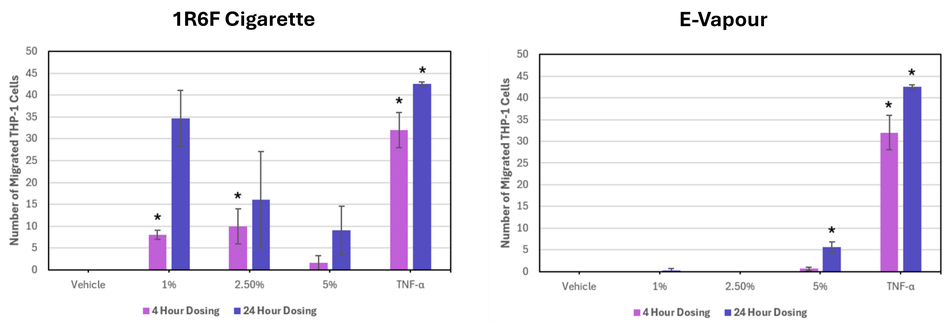
- Significant increases in monocyte adhesion compared to control was observed for the cigarette smoke extract for most test concentrations and exposure periods. Monocyte adhesion was also observed for the EVP but to a lesser degree than the cigarette smoke extract.

Monocyte migration assessment



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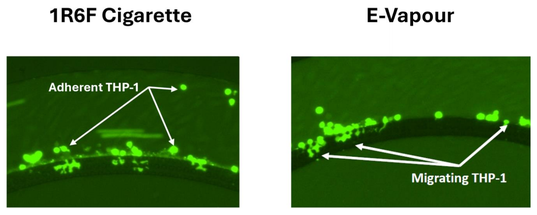
- Monocyte migration occurred following 4-hour exposure for 1% and 2.5% concentrations of cigarette smoke extract. Extensive variability was observed for the 1R6F cigarette extract likely due to the extensive cytotoxicity observed from this sample
- In contrast only the 5% concentration of EVP extract significantly induced migration compared to control at the 24-hour timepoint exposure.



CONCLUSIONS

- The results obtained from this study, demonstrate that the SynRAM model sensitively detected key events of atherosclerosis development using TNF-α positive control, with similar, albeit weaker, responses obtained for the 1R6F reference cigarette extracts.
- The EVP extracts exhibited reduced biological activity, in some of the key events of Atherosclerosis formation, when compared to cigarettes under the conditions of this model.
- These findings support the potential of EVPs to contribute positively to Tobacco Harm Reduction.

REFERENCES

[1] Björkegren, J. L. M., and Lusis, A. J. (2022). Atherosclerosis: recent developments. Cell 185 (10), 1630–1645. Epub 2022 May 2. PMID: 35504280; PMCID: PMC9119695. doi:10.1016/j.cell.2022.04.004
[2] Chapman et al. "Optimisation of an in vitro human cardiovascular model on-a-chip for toxicological assessment of nicotine delivery products." Frontiers in toxicology vol. 6 1395670. 13 Jun. 2024, doi:10.3389/ftox.2024.1395670

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