**Introduction**

Cigarette smoking is a documented risk factor for CVD, which includes atherosclerosis, ischaemic heart disease and acute coronary thrombosis [1]. However, the mechanistic link between cigarette smoking and CVD is yet to be fully elucidated. Several studies have reported the development and application of in vitro models of smoking-related diseases to help elucidate the mechanisms and key events, including cell migration inhibition that are associated with the development of atherosclerosis [2,3]. Over the last decade, the use of electronic cigarettes (e-cigarettes) has risen exponentially. E-cigarettes are seen as potentially safer alternative to conventional cigarettes [4].

This study reports the comparative effects of a commercial e-cigarette (Vype ePen) and a scientific reference cigarette (3RF4) on endothelial migration in vitro.

**Methods**

**Test products**

<table>
<thead>
<tr>
<th>Product type and manufacturer</th>
<th>Temperature (°C) of combustible during operation</th>
<th>Aerosol formation mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cigarette 3RF4 scientific reference cigarette, University of Kentucky</td>
<td>&gt;800</td>
<td>Pyrolysis and combustion of tobacco</td>
</tr>
<tr>
<td>Tobacco</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Paper</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Electronic cigarette (Vype ePen and eCigs) *(Studded Tobacco 1.6% Nicotine*, British American Tobacco)*

- E: Liquid
- N: Nicotine and co-
- C: Electronic and battery
- D: Debris body

**Figure 1.** Schematic representation of the test articles.

**Generation of test matrices**

These were produced according to the puffing regimes detailed in Table 1.

<table>
<thead>
<tr>
<th>Table 1: Aerosol generation regimes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Product</strong></td>
<td><strong>Puff regimen</strong></td>
</tr>
<tr>
<td>Cigarette</td>
<td>HCI</td>
</tr>
<tr>
<td>E-cigarette CRM 81</td>
<td>HCI</td>
</tr>
</tbody>
</table>

\* = HCI T-115 (5)

\* = CRM No 81 (6)

N/A = metric not applicable

**AqE**

Aqueous extracts (AqE) generated from 3RF4 cigarette or e-cigarette whole aerosols were produced as previously described [7]. Briefly, products were machine-puffed on a Borgwaldt-KC RM20H smoking engine, following puffing regimes (Table 1). AqEs were generated by bubbling 10 × 55 mL puffs through 29 mL of AqE capture media (VascuLifef media with added supplements and 0.1 % foetal bovine serum (FBS)) contained in an impinger. AqEs were generated on the day of each experiment and were used within 2 h post generation.

**Endothelial cell migration assay**

The scratch wound assay was utilized to detect and measure the inhibition of endothelial migration rates in vitro. Artificial wounds were created in monolayers of human umbilical vein endothelial cells (HUVEC). 24-well ImageLear plates (Essen Instruments) were seeded with HUVECs in complete VascuLife media and allowed to grow to confluency over a period of 24–48 h prior to performing the assay. Cells were treated with AqE and wound repair was assessed over 22 h using image analysis, as previously reported [8].

**Results**

**Figure 2.** Representative images from the scratch wound assay at 0, 5, 15 and 20 h timepoints in the presence of the AqE capture media or cytochalasin D (2 μM). Untreated HUVECs migrated into the wound over 20 h, which was not observed with cytochalasin D treatment.

**Figure 3.** Reference 3RF4 cigarette AqE inhibited HUVEC migration in a concentration-dependent manner (left). E-cigarette AqE from a closed modular device (Vype ePen) did not inhibit HUVEC migration (right). Data shown are mean wound widths as a % of the initial wound width (μm) from duplicate wells and from a minimum of 4 independent experiments.

**Figure 4.** Comparison of endothelial cell migration rates following exposure to AqE from 3RF4 cigarettes or AqE from cigarettes over a 20 h exposure period. 3RF4 AqE induced a concentration-dependent inhibition of HUVEC migration, compared to no inhibition of migration following exposure to e-cigarette AqE. Data are means ± SEM from duplicate wells and from a minimum of 4 independent experiments.

**Conclusions**

- 3RF4 extract induced a concentration-dependent inhibition of endothelial cell migration, with complete inhibition at concentrations >20% AqE.

- Exposure to e-cigarette extracts did not inhibit migration, even at 100% concentration, and cells could migrate into the wounded area.

- Our data demonstrate that e-cigarettes do not induce the inhibition of endothelial cell migration in vitro when compared to 3RF4.

- The scratch wound assay enables the comparative assessment between tobacco and nicotine products in vitro.

**References**


A comparative assessment of e-cigarette aerosols and cigarette smoke on in vitro endothelial cell migration

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The use of in vitro tests developed to model key endpoints associated with smoking-related disease can provide valuable insights into the disease mechanisms associated with tobacco use. E-cigarette use has increased significantly in recent years, and these endpoints may be suitable for the assessment of these next generation nicotine delivery products. One such test is the scratch wound assay, with which we have previously reported reduced migratory responses of endothelial cells following exposure to cigarette smoke extracts.

Aqueous aerosol extracts (AqE) were generated using the Health Canada Intense (HCI) regime for cigarettes and a modified HCI for e-cigarettes. Using human vein umbilical endothelial cells, we assessed cell migration rate following artificial wounding, prior to e-cigarette (Vype ePen) AqE exposure. Exposure to a scientific reference cigarette (3R4F) whole smoke AqE was conducted as a comparator. The rate of migration was assessed over a 20-hour period across a range of AqE concentrations.

3R4F extract induced a concentration-dependent inhibition of endothelial cell migration, with complete inhibition at concentrations >20% AqE. Exposure to e-cigarette extracts did not inhibit migration, even at 100% concentration, and cells could migrate into the wounded area.

Our data demonstrate that e-cigarettes do not induce the inhibition of endothelial cell migration in vitro when compared to 3R4F. The scratch wound assay enables the comparative assessment between tobacco and nicotine products in vitro.

Conflict of interest statement: This study was funded BAT, all the authors were employees of BAT when this study was conducted.