An investigation into the effects of E-cigarette aerosols using a physiologically relevant *in-vitro* model

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Although widely popular as smoking cessation tools, limited information is available on the health effects of E-cigarette (EC) use. An urgent requirement of EC research is a standard testing method to investigate possible adverse effects. The present study aims to assess EC cytotoxicity using an *in-vitro* multicellular human airways model.

Human bronchial epithelial cells (CALU3) and pulmonary fibroblasts (MRC5) were co-cultured on permeable membranes for 11–14 days at air–liquid interface. A bespoke smoking machine was used to deliver air, whole cigarette smoke (WCS) or EC vapour (ECV) to the airways model under standard ISO:3308 conditions for 7 m. Considering the prolonged vaping habits of EC users compared to cigarette smoking, ECV exposure was additionally investigated at 1 h, 2 h, 3 h, 4.5 h and 6 h time points.

24 h post exposure, XTT cell viability analysis showed that while WCS had the expected detrimental impact on cell viability, air exposure had no effect at any time point. Interestingly, a steady decrease in the viability of ECV exposed cells was observed at times greater than 2 h. Viability was 61.31 ± 5.75% control, 51.11 ± 5.56% control and 42.10 ± 2.69% control after 3 h, 4.5 h and 6 h respectively. Furthermore, ELISA analysis of supernatants revealed an increase in IL-6/IL-8 pro-inflammatory cytokines at 3 h post ECV exposure, despite the increased cell death.

Results indicate that extended EC exposure (≥3 h) under these conditions has a detrimental impact on cell viability and leads to exaggerated cytokine production in the airways model.