The CULTEX® RFS Compact:

Ciliatoxicity in immortalized human primary bronchiolar epithelial cells after repeated air-liquid interface exposure to e-cigarette vapor and native mainstream smoke of K3R4F cigarettes

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Introduction

E-cigarettes are described as "reduced-risk" products or alternatives to combustible cigarettes. There is only limited information about the biological effects of the e-liquid aerosol especially on human lung cells and their function. The mucociliary clearance for example is the primary physical mechanism to protect the human airways against harmful effects of inhaled particular atmospheres.

Cigarette smoke is discussed to be one of the clinically most important causes of impaired mucociliary clearance. The dysfunction has been connected to changes such as decreasing number, altered structure and beat frequency of ciliated cells. Clinical studies have shown that cilia length is reduced in healthy smokers and that long-term exposure to cigarette smoke leads to reduced numbers of ciliated cells in mice.

We present an *in vitro* model of immortalized human primary bronchiolar epithelial cells with *in vivo* like morphology to study the influence of e-liquid vapor on ciliated and mucus secreting cells.

Materials and Methods

Normal human bronchial epithelial (NHBE) cells, isolated from bronchus samples of a male patient (age 75) with a non-small cell lung cancer (NSCLC) (Scheffler et al., 2015), were transduced with the third generation state-of-the-art lentiviral constructs containing cyclin-dependent kinase 4 (CDK4) and human telomerase reverse transcriptase (hTERT). This immortalized cell line CL-1548 exhibit *in vivo* like mucociliary differentiation and was used for the repeated exposure studies. Cl-1548 cells, cultivated in regular flasks by using AEGM medium incl. supplements, G418 (50 g/ml) and Puromycin (0.3 µg/ml) were seeded on collagen IV coated cell culture inserts after reaching 80 – 90 % confluency. After 11 days of cultivation at the air-liquid interface, the cells were exposed repeatedly to clean air (10 times), mainstream cigarette smoke (4 × K3R4F cigarettes per run according to ISO 3308, University of Kentucky, Lexington, KY, USA) and e-liquid vapor without nicotine (50 puffs with a puff volume of 35 ml in 2 seconds, a blow-out time of 7 s and an inter-puff interval of 10 s; Tennessee Cured, Johnsons Creek, Hartland, WI, USA) using the CULTEX® RFS – Radial Flow System. Two systems were operated in parallel: one contained the smoke-exposed cell culture inserts, the other one held cell culture inserts which were exposed to clean air (DIN 12021; process control). During the exposure period samples were taken after 0, 4, 8 and 10 smoke exposure repetitions and analyzed microscopically. The Figures show cultures after 10 smoke exposure cycles by using hematoxylin/eosin stained sections and sections marked with antibodies against MUC5AC and MUC5B.

Results I

Throughout the whole exposure experiment, the incubator control cultures showed a pronounced mucociliary differentiation (IC). Cilia bearing as well as mucus secreting cells were distributed homogeneously within the culture. The clean air control cultures revealed a comparable morphological differentiation pattern until the end of the experiment (CA).

Figure 1 shows the morphological changes within the culture after 10 exposure repetitions to the different test aerosols. The number of cilia bearing cells as well as the cilia length was comparable in control cultures that remained in the incubator and cultures, which were exposed to clean air (CA). Cultures exposed to mainstream cigarette smoke (CS) showed a clear reduction in mucus production and cilia bearing. A comparable but less pronounced effect could be observed for the cells treated with the e-liquid aerosol (EC).

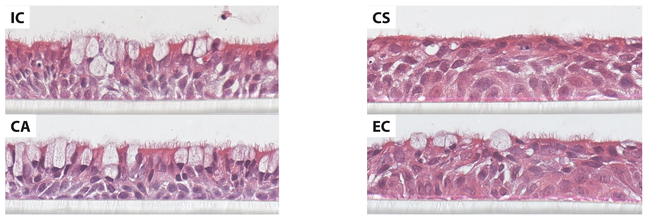

Figure 1: Cross section of a cell culture insert membrane with HE (Hematoxylin and Eosin) stained NHBE cells after the exposure to clean air (CA), cigarette smoke (CS) and e-cigarette vapor (EC). Non-exposed cells were used as an incubator control (IC).

Results II

Figure 2 and 3 show the changes in the population of mucus producing cells (immunohistochemical staining of MUC5AC and MUC5B) within the cultures after 10 exposure repetitions. The number of mucus secreting cells was comparable in control cultures that remained in the incubator (IC) and cultures, which were exposed to clean air (CA). Cultures exposed to mainstream cigarette smoke (CS) and e-cigarette vapor (EC) exhibited a clear reduction in mucus secreting cells and their secretion activity, whereby the effect less pronounced for the cells treated with the e-liquid aerosol.

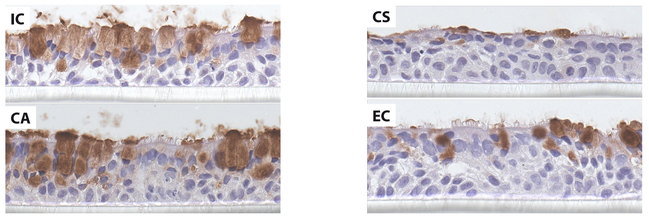

Figure 2: Cross section of a cell culture insert membrane with immunohistochemically (MUC5AC)stained NHBE cells after the exposure to cleaan air (CA), cigarette smoke (CS) and e-cigarette vapor (EC). Non-exposed cells were used as an incubator control (IC).

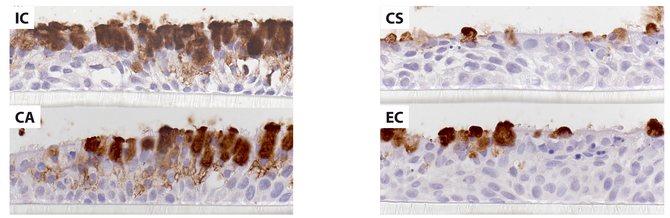

Figure 3: Cross section of a cell culture insert membrane with immunohistochemically (MUC5B) stained NHBE cells after the exposure to clean air (CA), cigarette smoke (CS) and e-cigarette vapor (EC). Non-exposed cells were used as an incubator control (IC).

Conclusions

- The immortalized human primary bronchiolar epithelial cell line (CL-1548) shows a comparable mucociliary differentiation as their parent cells (Scheffler et al. 2015)
- The direct exposure of the cultures at the air-liquid interface to mainstream smoke of K3R4F research cigarettes resulted in a reduction of cilia bearing and mucus secreting cells – a comparable effects as already observed in experiments with NHBE cells (Aufderheide et al. 2015).
- The direct exposure (air-liquid interface) to the e-liquid aerosol induced comparable effects, that means the exposure of the immortalized cells resulted in a reduction of cilia bearing and mucus producing cells including their secretion activity.
- In comparison with untreated or clean air exposed human lung cells, the exposure to e-cigarette vapor affects the number of cilia bearing and mucus producing cells (defense mechanisms) thus interfering with normal cell morphology and function.

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