

# Effect of cigarette smoke and e-liquid vapour on ciliary beat frequency (CBF) of freshly isolated human nasal epithelial cells

Peter C. Dartsch<sup>1</sup>, Thomas A. Mrva<sup>2</sup>, Adrian Morski<sup>3</sup>, Bernhard Olzowy<sup>3</sup>

<sup>1</sup>Dartsch Scientific GmbH, Germany; <sup>2</sup>Happy People GmbH, Germany; <sup>3</sup>HNO Zentrum Landsberg, Germany

**Keywords:** ciliary beat frequency, nasal epithelial cells, cigarette smoke, e-liquid vapour

## Background

Ciliary beat is one of the most important defense mechanisms in the respiratory tract with its frequency and coordination depending on many factors. A chronic inhalation of cigarette smoke is associated with a decrease or even paralysation in ciliary beat activity [1-4]. The epithelial cells line the lumen in a unique position to interact directly with inhaled cigarette smoke. This study was undertaken to examine freshly isolated human nasal epithelial cells for alterations of ciliary beat frequency after exposure to cigarette smoke and e-liquid vapour extracts.

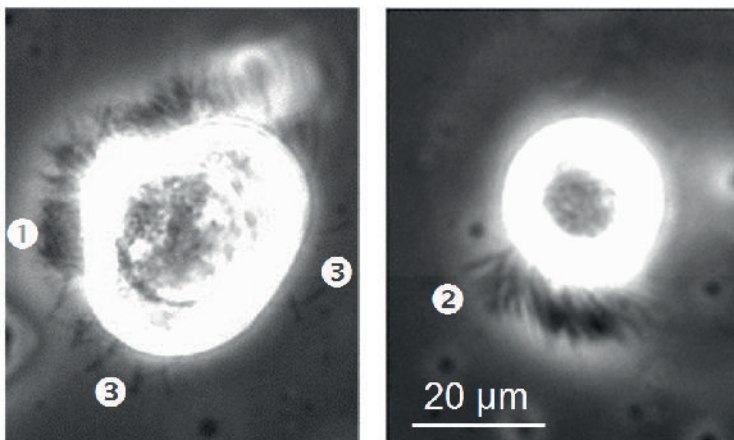
## Materials and methods

Cells were obtained from 3 male and healthy volunteers at the age from 35 to 59 years on different experimental days. Volunteers had been free of respiratory infection for at least 2 weeks. Ciliated human nasal epithelial cells were obtained with a cytology brush from the inferior turbinate of the volunteers by an otorhinolaryngologist and dispersed in Airway Medium (Promocell, Heidelberg, Germany) containing 100 µg/mL penicillin and 100 IU/mL streptomycin and were buffered with 10 mM HEPES buffer to avoid pH changes during transportation. Isolated epithelial cells were transported to the laboratory immediately after retrieval within 60 minutes.

In order to simulate the conditions in reality, a special smoking apparatus was constructed which allows to vary the frequency, length and the depths of the puffs. For smoking a cigarette, 10 puffs with a duration of 3 seconds and a pause of 30 seconds between two puffs was presumed. The same conditions were applied for the e-cigarette (EVOD, vaporiser 2,2 Ω and rechargeable battery 3,7 V; KangerTech). The smoke of the cigarette and the vapour of the e-cigarette were aspirated by a suction pump and bubbled into 10 ml of cell culture medium. The resulting primary extracts had a neutral pH value of  $7.4 \pm 0.3$ .

The investigations were done by using one common cigarette brand of medium strength with 10 mg tar, 0,8 mg nicotine und 10 mg carbon monoxide, and two e-liquids of the brand Happy Liquids: (1) „Apple“ with 6 mg/ml nicotine, and (2) „Strawberry-Menthol“ with 6 mg/ml nicotine.

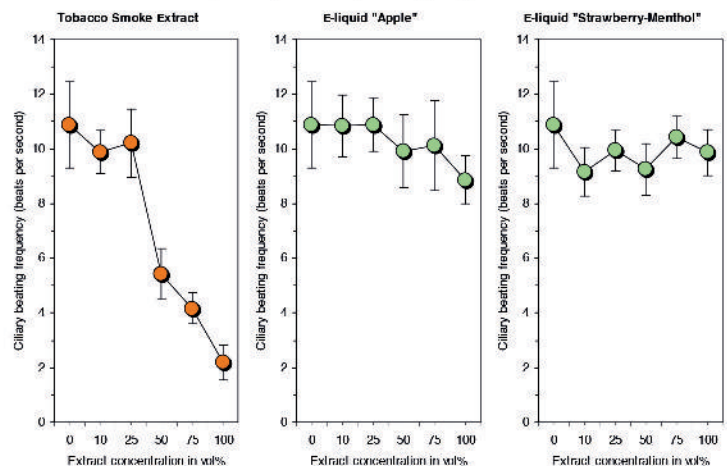
For measurement of ciliary beat frequency, increasing amounts of cigarette smoke and e-liquid vapour extracts were pipetted to 250 µl of suspended nasal epithelial cells and incubated for 15 minutes in a temperature-controlled chamber at 37 °C (ibidi, München, Germany) mounted on the stage of an Olympus IX50 inverted microscope. The ciliary beat was recorded by a Basler high-speed video camera acA640-120um operated by pylon camera software 4.2 from Basler (Rauscher, Olching, Germany) at a speed of 100 frames per second with an Olympus 40x planachromate objective. Beat frequency was calculated afterwards by visual examination of the beats from the single pictures recorded by the high-speed camera.



**Figure 1:** Examples for the cell morphology of freshly isolated and free-floating human nasal epithelial cells with cilia which can be observed in arrays (1), bundles (2) or in singular form (3). Phase contrast microscopy of vital cells with beating cilia.

## Results

Ciliary beat frequency (CBF) of freshly isolated human nasal epithelial cells was in the range of 10 beats per second for untreated cells. Treatment with cigarette smoke extracts caused a dose-dependent decrease in beat frequency which became significant ( $p < 0.01$ ) at a primary extract concentration  $> 25$  vol% according to the two-tailed Wilcoxon-Mann-Whitney U test as a nonparametric test for non-normal distributions. E-liquid vapour did not cause a statistically significant decrease at all tested concentrations of the primary extract (see Figure).



**Figure 2:** Graphical presentation of the ciliary beating frequency (CBF) with different extract dilutions of tobacco smoke and e-liquids after 15 min of application. Data represent mean values  $\pm$  standard deviations of 3 donors.

## Conclusion

The results show that vaping has much less harmful effects on epithelial cells located in the respiratory tract and their defense and clearing function than cigarette smoke which accounts for a number of frequently observed respiratory infections and airway diseases.

## References

- Dalhamn T (1959): The effect of cigarette smoke on ciliary activity in the upper respiratory tract. *AMA Arch Otolaryngol* **70**:166-168.
- Stanley PJ, Wilson R, Greenstone MA, MacWilliam L, Cole PJ (1986): Effect of cigarette smoking on nasal mucociliary clearance and ciliary beat frequency. *Thorax* **41**: 519-523.
- Dye JA, Adler KB (1994): Effects of cigarette smoke on epithelial cells of the respiratory tract. *Thorax* **49**: 825-834.
- NA Cohen, S Zhang, DB Sharp, E Tamashiro, Chen B, Sorscher EJ, Woodworth BA (2009): Cigarette smoke condensate inhibits trans-epithelial chloride transport and ciliary beat frequency. *Laryngoscope*, **119**: 2269–2274.