**Mucociliary clearance** is an important defense mechanism that mediates removal of foreign particles and chemicals from the airways. Cilia beating thereby plays a key role that determines the rate of mucociliary clearance and thus constitutes a vital function of respiratory epithelia. Cigarette smoke has been reported to adversely impact cilia function and in vivo, by changing cilia beating frequency (CBF) or impairing ciliation genesis. To monitor CBF, semi-automated methods such as CiliaTA combine high-speed video recordings with the ability to determine CBF. Using CiliaTA, we were able to confirm that the MucilAir™ CBF can be modulated in vitro by either 185 µM isoproterenol or a temperature shift to 4°C. Moreover, in vitro exposure of MucilAir™ with whole smoke from conventional cigarettes (3R4F at 0 mg/L, 0.15 mg/L and 0.25 mg/L nicotine) caused a decrease in the total surface area of the culture showing active cilia beating. Cilia in the epithelial cell surface that were detected to be still active after exposure, showed variable beating frequencies, ranging from normal to decreased CBF. Compared to 3R4F, cigarette smoke exposure, the effect of equivalent concentrations (based on nicotine) of a candidate modified risk tobacco product (MRTP) THS2.2 was 2-fold less pronounced. The decrease in total surface area of cilia beating as well as beating frequency was less impacted by THS2.2. Overall, this study clearly discriminated the effects of THS2.2 from the deleterious impact of 3R4F on cilia beating.

**Materials & Methods**

Synchronized beating of cilia on epithelial cells of human airways mediates transport of inhaled pathogens and potential respiration particles trapped in the mucus layer (Figure 1) towards the pharynx, where they eventually become swallowed or expectorated [1]. Effective cilia beating is therefore a key requirement for functional mucociliary clearance in the lower (broncho-tracheal) and upper respiratory tract (paranasal – nose). Defects in cilia function as a consequence of disorders such as primary ciliary dyskinesia can lead to persistent respiratory infection [2]. In addition, exposure to cigarette smoke can impede mucociliary transport by inducing mucus hypersecretion and goblet cell hyperplasia and thus worsen airflow obstruction and predispose to bacterial infection [3]. Several studies showed that exposure of the cultured epithelium to particles of cigarette smoke results in a significant decrease in CBF [4]. Although other studies did not find any difference in ciliated beating frequency between smokers and nonsmokers, they confirmed that mucociliary transport was disrupted in regular smokers [5]. The following study was designed to investigate the effect of cigarette smoke on CBF in vitro, measured upon exposure of human nasal MucilAir™. Moreover, this study aimed to evaluate the impact of a MRTP aerosol on CBF. As not only beating frequency is an informative parameter to judge on the mucociliary clearance capacity, the overall activity of cilia on MucilAir™ was measured too (represented as Cilia power); this parameter was introduced as it is more sensitive compared to “% area active” shown in the CiliaTA report. Finally, general cytotoxicity as well as changes in the tissue morphology were assessed.

**Results**

**Modulation of CBF using known controls**

a) Experimental design/workflow. b) Exposure to isoproterenol (100µM) applied to the basal side of MucilAir™ for 3 hours produced an increase in CBF of approximately 5 Hz in 3D culture of nasal epithelial cells, while incubation at 4°C for 30 minutes resulted in a decrease of 4 Hz. c) The decrease in CBF correlated with a decrease in the total area with active cilia beating.

**Effect of aerosol exposure on CBF (I)**

Cilia of human MucilAir™ are less impacted by the exposure to the aerosol of a candidate modified risk tobacco product than to whole smoke from conventional cigarettes in vitro

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**Summary**

This study investigated the effect of cigarette smoke on CBF in vitro human nasal MucilAir™. Compared to the matching doses of 3R4F cigarette smoke, exposure to aerosol from THS2.2 shows only small effects, if any, on CBF and Cilia power after in vitro exposure of MucilAir™ cultures.

- The effect on CBF seems to be partially reversible for the low 3R4F dose, at 24-48h post-exposure.
- Cytotoxicity was investigated the effect of cigarette smoke on CBF in vitro human nasal MucilAir™. Compared to the matching doses of 3R4F cigarette smoke, exposure to aerosol from THS2.2 shows only small effects, if any, on CBF and Cilia power after in vitro exposure of MucilAir™ cultures.

**References**