Nasal saline washing can attenuate air-pollution induced respiratory conditions

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Q Introduction/Rationale

- Air pollution is an environmental global threat to human health responsible for approximately seven million premature annual deaths worldwide. Its increasing level is one recognized risk factor for upper respiratory tract infections (URTI) and cardiovascular diseases^{1,2}
- Saline nasal washing has demonstrated efficacy as an adjuvant treatment for URTI symptoms.³ However, its effect on air-pollution induced respiratory conditions and the impact in nasal epithelium natural defense physiology, remains under-explored

Aims

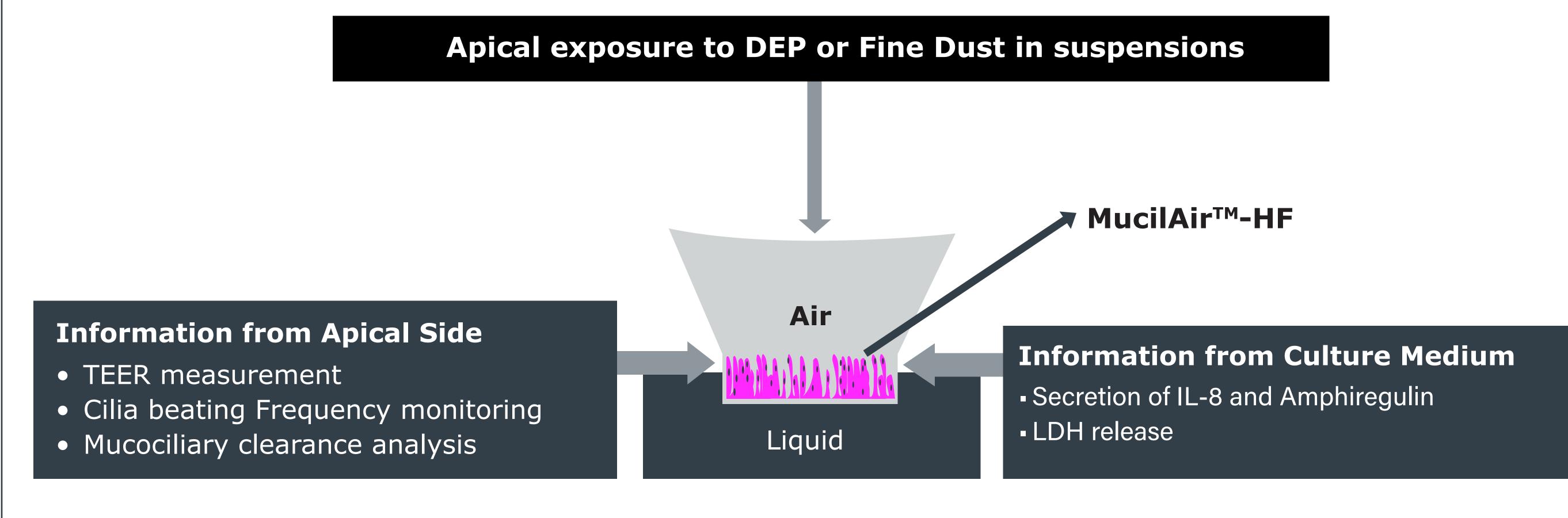
The aim of this study was to evaluate the capacity of mechanical washing with saline solution to revert the air-pollution induced deregulation of human nasal epithelial cells

Methods

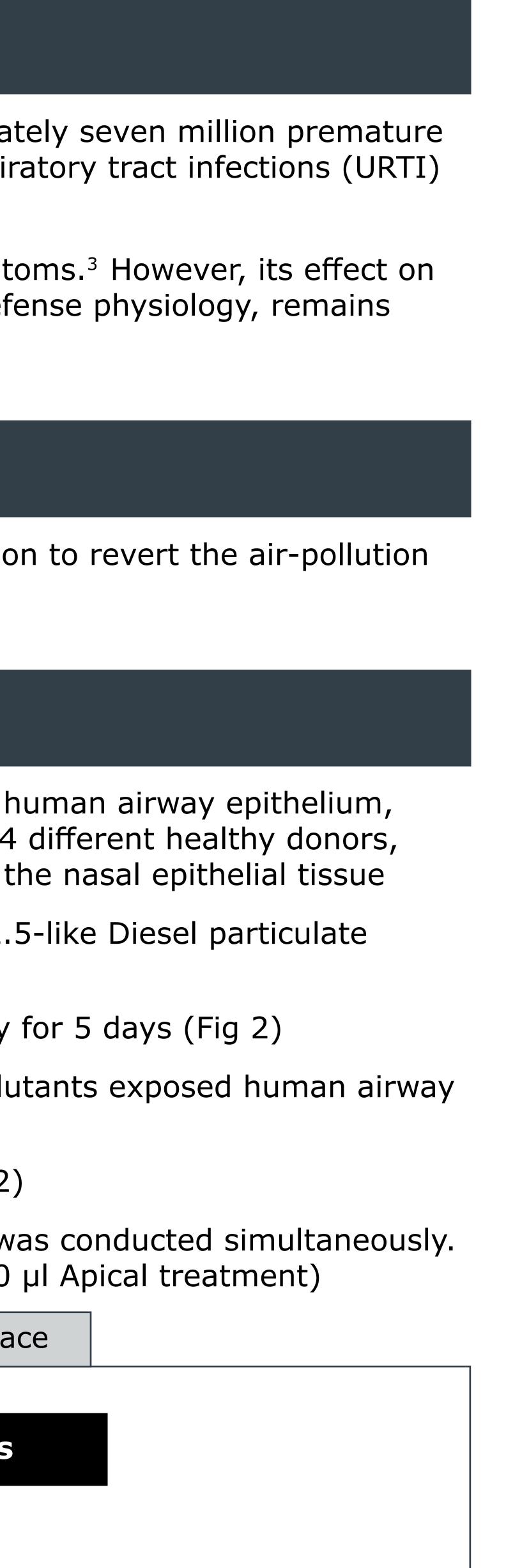
In this study, **MucilAir[™]-HF**, a pseudostratified and ready-to-use 3D in vitro cell model of human airway epithelium, constituted with epithelial cells freshly isolated, fully differentiated from nasal biopsies of 14 different healthy donors, co-cultured with human fibroblasts was used. This model replicates the full functionality of the nasal epithelial tissue

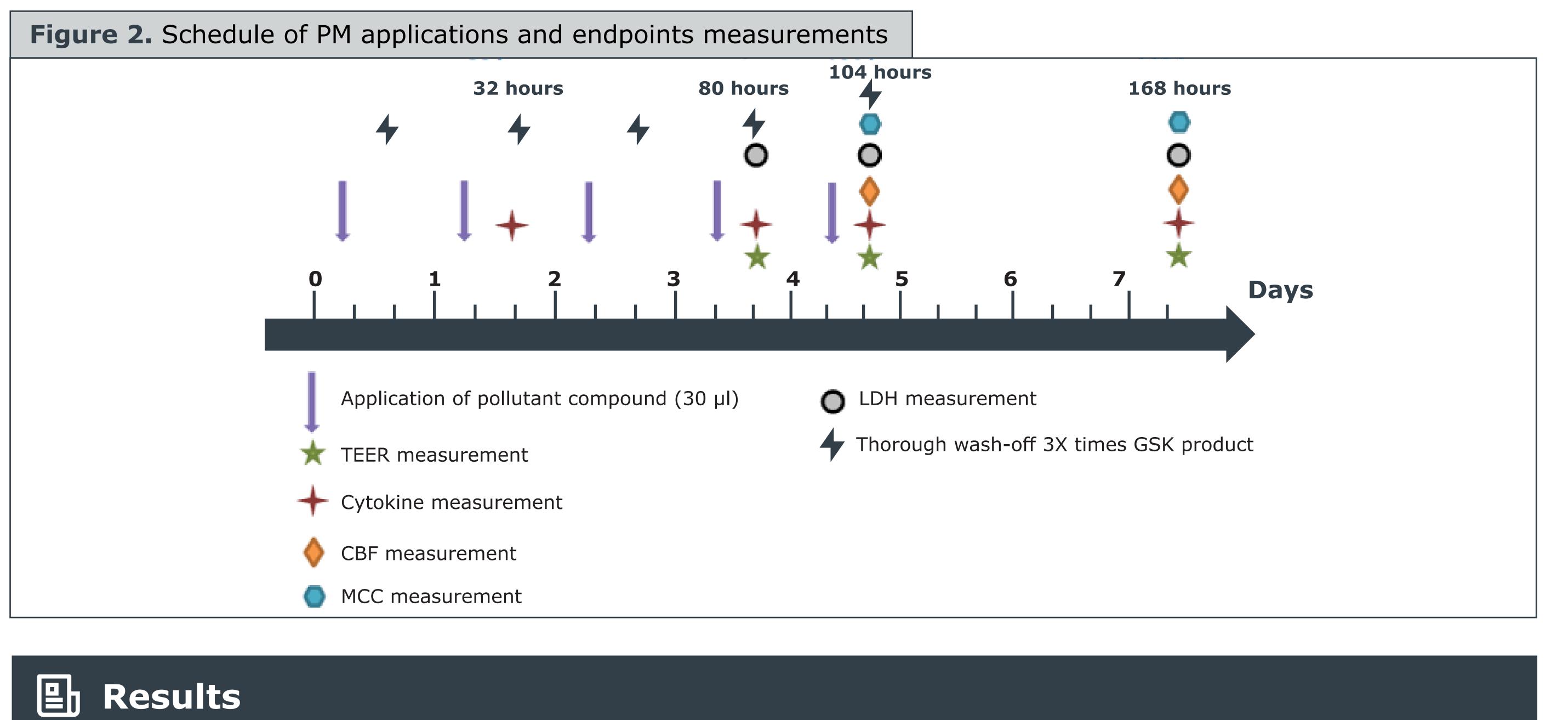
- MucilAir[™]-HF was exposed to repeated daily doses of Particle Mater (PM) pollutants- PM2.5-like Diesel particulate matter (DEP) and PM10-like Fine Dust (FD) in the air-liquid interface (Fig 1)
- Exposure dose of PM pollutants: DEP/FD 30μl equivalent to 150 μg/cm² for 8 hours/day for 5 days (Fig 2)
- The effect of saline wash-off, using a GSK product (Otrivin Natural Aloe Vera), on PM pollutants exposed human airway epithelial cells was then evaluated
- Exposure dose of nasal washing simulation: 3-times a day (3X 250 µl) for 5 days (Fig 2)
- Repeated dose exposure of DEP/FD on MucilAir[™]-HF with and without saline wash-off was conducted simultaneously. Control used was Vehicle control (0.04% dipalmitoyl lecithin in 0.9% saline solution, 30 µl Apical treatment)

Figure 1. Testing strategies of MucilAir[™]-HF exposed to PM pollutants in the air-liquid interface



- The endpoints assessed were:
- Cytotoxicity (Trans-Epithelial Electrical Resistance-TEER, Lactate Dehydrogenase-LDH)
- Airway epithelial functions (Cilia Beating Frequency-CBF, Mucociliary Clearance-MCC)
- Airway inflammation/remodelling (chemokine-Interleukin 8 and growth factor-Amphiregulin basal release)
- A multi-endpoints analysis was performed at 32, 80, 104 and 168 hours from apical wash, basolateral medium and optionally from MucilAir[™]-HF tissue (Fig 2)





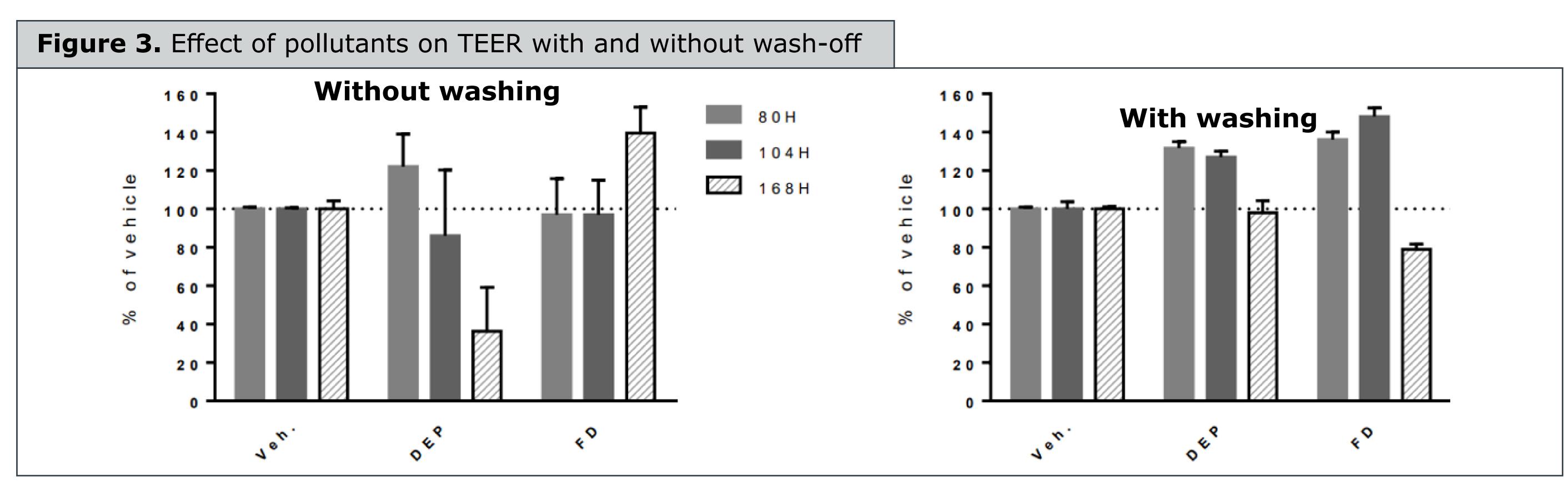
The study demonstrates, that mechanical saline washing induces benefits on PM pollutants induced deregulation of MucilAir[™]-HF

Cytotoxicity

Saline washing prevents DEP induced decrease of Tissue Integrity (TEER)

- Compared to vehicle, repeated DEP exposure induced toxic alterations in the epithelia causing disruption of barrier function at 104 hours and 168 hours in without washing condition

- Repeated mechanical washing prevents toxic effects of DEP exposure on TEER. However, a general decrease of TEER was observed at 168 hours, but TEERs were >100 Ω .cm² (TEER values below 100 Ω .cm² represents disruption of epithelium)
- FD exposure do not induce major toxic alterations in the epithelia. TEER was however, increased in without washing conditions at 168 hours which was further potentiated by repeated washing, visible at 80 and 104 hours (Fig 3)



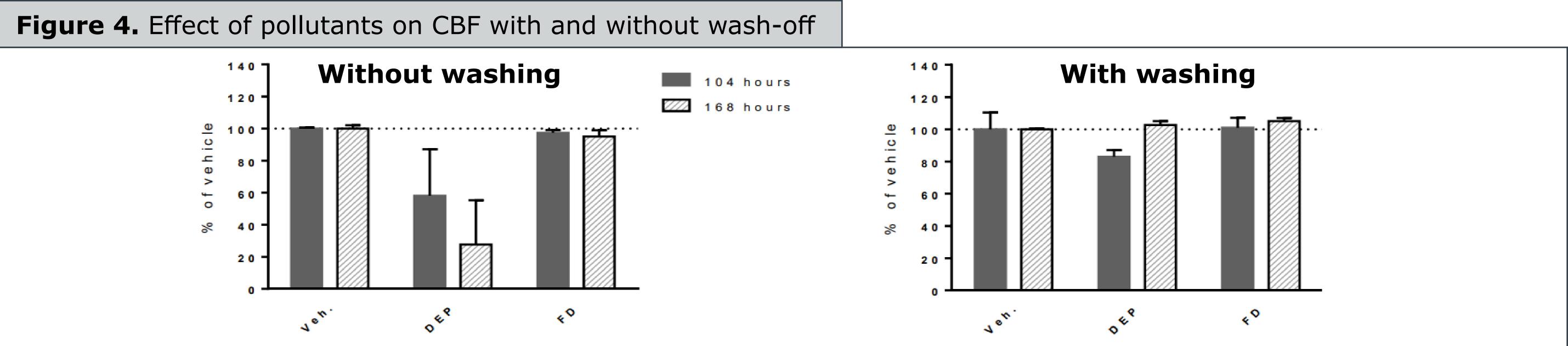
Saline washing prevents pollutant induced cytotoxicity (LDH release)

LDH is a stable cytoplasmic enzyme that is rapidly released into the culture medium upon rupture of the plasma membrane. For LDH, threshold limit value was 5% cytotoxicity, which corresponds to a physiological LDH release in MucilAir^M-HF. Cytotoxicity was <5 % at all time points with washing conditions.

Airway epithelial functions

Saline washing restores airway epithelial functions by preventing DEP induced decrease of Cilia Beating Frequency

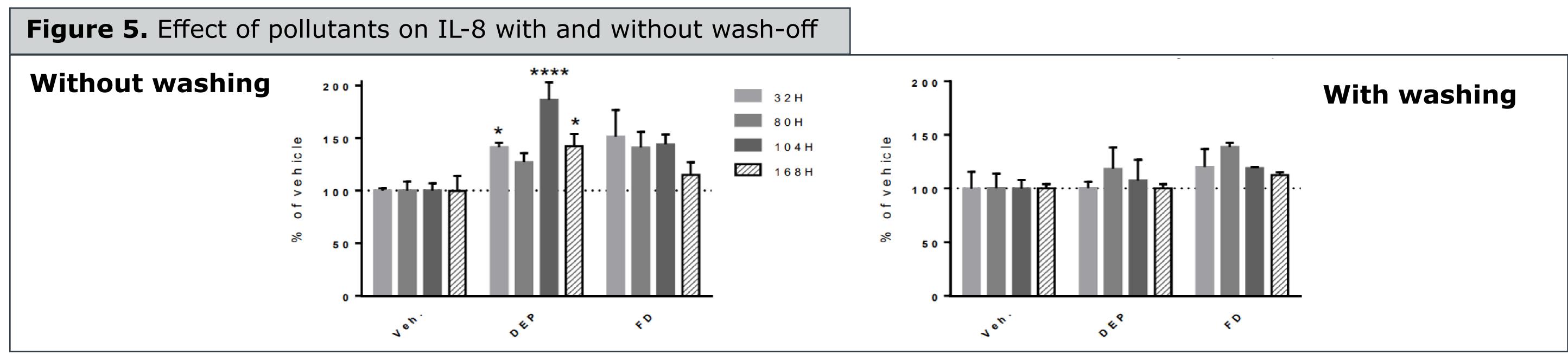
DEP exposure induced non-significant decrease of CBF at both time points (104 and 168h) by 42% and 72% respectively, while exposure to FD did not modify CBF versus vehicle during without washing. Mechanical washing prevented this DEP induced decrease of CBF, mean 83% and 103% respectively (Fig 4)



Mucociliary Clearance deregulation was prevented by saline washing The effect of mechanical washing was less marked on DEP induced deregulation of MCC, but still can be observed. DEP decreased the MCC at 104 and 168 hours time points by 36% and 54% respectively, which was prevented with washing at 104 hours, mean 87%. Exposure to FD induced rather an increase of MCC at both time points which was further increased at 104 hours with washing as compared to vehicle

Airway inflammation/remodelling

Saline washing prevents DEP induced increase of airway inflammation (IL-8) DEP induced significant increase in IL-8 release at 32, 104 and 168 hours, corresponding to 141, 186 and 142 % respectively. Through mechanical washing it was decreased to 100, 107 and 100 % respectively. FD induces moderate, non-significant increase of IL-8 release which was attenuated with repeated mechanical washing (Fig 5)



Statistical comparison was performed using two-way ANOVA with Dunnett's post-tests comparing all conditions to vehicle (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

DEP induced a 2-3 fold increase of Amphiregulin release at 80 and 104 hours, which was only slightly attenuated with washing (1.9-1.6 fold of vehicle). FD induced a non-significant, small increase of Amphiregulin release (average of 1.6 fold increase) at all the four time points, which was not modified with repeated mechanical washing (average 1.5 fold of vehicle)

The study demonstrates, DEP exposure induces a significant increase of IL-8, decreases CBF, TEER and MCC, and induces a non-significant increase of Amphiregulin release which was prevented with repeated mechanical washing of GSK product. Repeated exposure to FD induced only modest modifications on MucilAir[™]-HF. Therefore, it was difficult to evaluate the effects of the mechanical washing

Conclusions

References

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Abstract # 367 **Category: Scientific Research Abstracts**

Saline washing improves airway inflammation by attenuating DEP induced increase of Amphiregulin release

• Mechanical washing of pollutants may revert their toxic alterations by restoring mucociliary clearance, maintaining tissue integrity, and lowering inflammatory mediators in the nasal epithelium

• These findings suggest nasal washing may have a role in restoring nasal epithelial functionality after pollution exposure. Further clinical research is needed to confirm these findings.

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