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An *in vitro* exposure method to assess adverse effects of ambient air using human lung cells

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Introduction

Ambient air consists mainly of nitrogen and oxygen, additionally it is polluted by particulate matter (PM), nitrogen oxides (NO_x), and ozone (O_3). Epidemiological studies have associated these air pollutants with cardiovascular and pulmonary diseases (e.g. asthma) [1-3]. Complementing these studies with *in vitro* or *in vivo* models is important to gain a better understanding of the potential hazard at the cellular level and to eliminate confounders (*e.g.* smoking).

Our aim was to evaluate the usability of a portable *in vitro* exposure system to detect adverse effects of ambient air (in summer and winter), where a multi-cellular human lung model was directly exposed at the air-liquid interface (proof of concept).

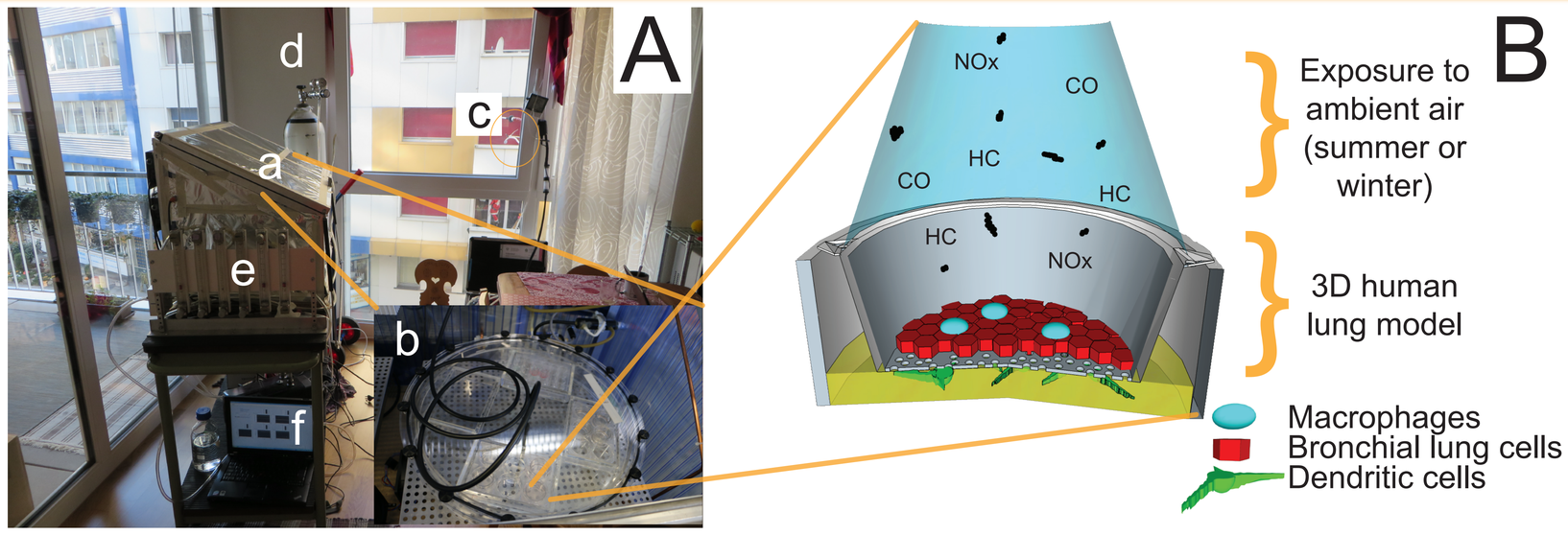

Fig. 1 Exposure system.
[A] The exposure box [a], wrapped in aluminium foil to protect the cells from light, is placed on a balcony (in summer) or inside the apartment (winter). The exposure box contains two chambers ([b], one for ambient air [c] and another for filtered medicinal air as a control [d]). Conditions inside the exposure box were controlled by flowmeters [e] and monitored in real time [f].
[B] Scheme of the human lung model composed of three different human cell types. The cells are cultured at the air-liquid interface, ambient air (or filtered medicinal air) on the top, and cell culture medium (yellow) at the bottom. Cells were exposed for 12 hours [Figure adapted from 5].

Results

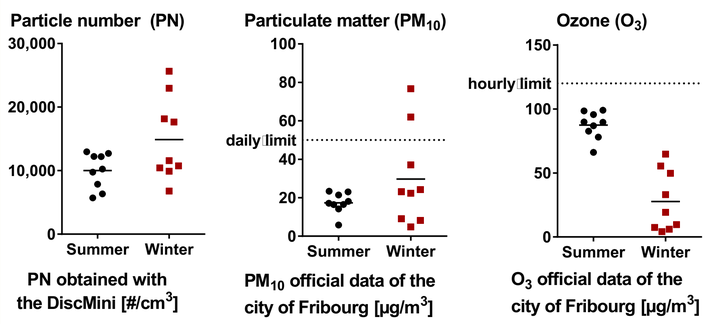

Fig. 2 Ambient air characterisation. Shown are 12h averages on exposure days.
- Higher PN in winter ambient air than summer
- On two days PM_{10} exceeded daily Swiss limit (50 µg/cm³)
- Higher ozone levels in summer

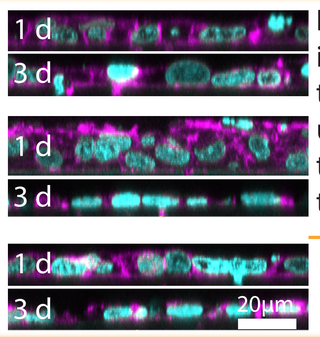

Fig. 4 Microscopy images. XZ cross-sections (winter). Nucleus (cyan), F-Actin cytoskeleton (magenta).
- Cell morphology not impaired for any condition

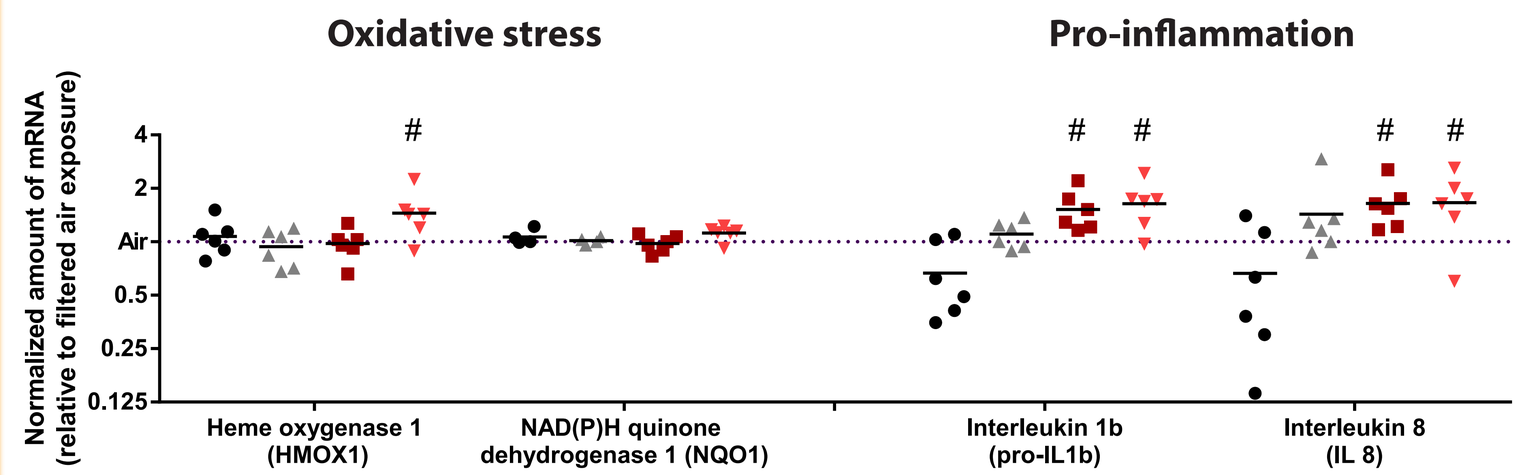

Fig. 3 Gene expression analysis of four selected genes. Oxidative stress related genes (HMOX1 and NQO1) as well as pro-inttflammatory genes (pro-IL1b and IL8) are shown. Normalized to filtered air control and GAPDH (Air line=1, ddCt method), n=4-6. #: Statistically different from the filtered medicinal air, two-way ANOVA, $p < 0.05$

Summer ambient air
- No oxidative stress
- No pro-inflammation

Winter ambient air
- Oxidative stress (3 days only)
- Pro-inflammation

Materials and Methods

Exposure setup
- 12 hours exposure to filtered medicinal air or ambient air per day (07.00-19.00).
- One (1 day) to three (3 days) exposure (12 h/day).
- Summer ambient air: three repetitions in Aug '16
- Winter ambient air: three repetitions in Dec '16 and Jan '17

Fig. 6 Analysis of ambient air. Left: the official analysis station of the city of Fribourg (Perolles). Right: DiSCmini of Testo.

3D human lung epithelial tissue model is composed of
- Bronchial epithelial cells (16HBE14o-)
- Macrophages (from human monocytes)
- Dendritic cells (from human monocytes)
- The cells are grown at the air liquid interface (air on top, medium on bottom).

Endpoints
- Gene expression analysis (qPCR).
- Confocal microscopy images. DAPI stained the nucleus and Phalloidin Rhodamine F-actin cytoskeleton.

Abbreviations
IL8= interleukin 8; HMOX1= heme oxygenase 1; NOx= nitrogen oxides; NQO1= NAD(P)H quinone dehydrogenase 1; pro-IL1b= pro-form (inactive) of interleukin 1b

Conclusion

- Proof of concept of direct exposure of ambient air confirmed
- Repeated exposure on sub-sequent days possible
- Pro-inflammatory response higher in winter correlates with higher PN/PM_{10}
- Effects already noticeable after one exposure day and similar after three days, sensitive method
- Mobile exposure system can be implemented at other locations (work place, nanomaterial facilities)

[1] WHO. http://www.who.int/mediacentre/factsheets/fs313/en/. 2014; **[2]** Dockery, D.W., et al., N Engl J Med, 1993; **[3]** Pope, C.A., et al., Circulation, 2004; **[4]** Blank et al., Am J Respir Cell Mol Biol. 2007; **[5]** Bisig, C.J. et al., CHIMIA Intern J for Chem, 2015.

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