# Biological impact of brake wear particles – aerosol exposures onto the surface of a 3D human epithelial tissue barrier

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## INTRODUCTION

Brake wear particles are a significant non-exhaust traffic-related source of air-pollution and may contribute almost equally to traffic-related $PM_{10}$ emissions in comparison to exhaust related sources [1,2]. The mass of brake wear particles in total traffic-related $PM_{10}$ emissions is estimated to be up to 21% [3]. There is, however, still a lack of restrictions regarding brake pad formulations and possible release of constituents generated by associated friction processes. The effect of brake wear particles exposure to humans via inhalation remains unclear and only few studies exist up to now. Therefore, the aim of this study was to mimic the inhalation of brake wear particles in vitro and to assess their possible adverse biological impact.

## METHODS

Brake wear particles released from commercially available “low-metallic” automotive brake pads were generated in a full scale automotive brake dynamometer simulating urban driving [4]. The collected fractions were analyzed using scanning electron microscopy (MIRA3, Tescan, CZ) with X-ray microprobe of an energy dispersion spectroscope (SEM-EDS) and Raman microspectroscopy (System XploRA$^{TM}$, HORIBA Jobin Yvon, France). Raman spectra were acquired with 532 nm excitation laser source, and 1200 g/mm grating.

Different concentrations ([0.5, 1 and 2 mg/ml] in cell culture medium) of brake wear particles were tested via the addition of 100 µl of the particle suspension onto the apical side of the in vitro triple cell co-culture model of the human epithelial airway barrier (consisting of A549 epithelial cells, human blood monocyte-derived macrophages and dendritic cells) [5] at the air liquid interface (referred to as pseudo-ALI) [6]. Cellular morphology was observed by laser scanning confocal microscopy (LSM), whilst biochemical effects associated with a (pro-) inflammatory response (TNF-α and IL-8) and oxidative stress response (GSH) were assessed.

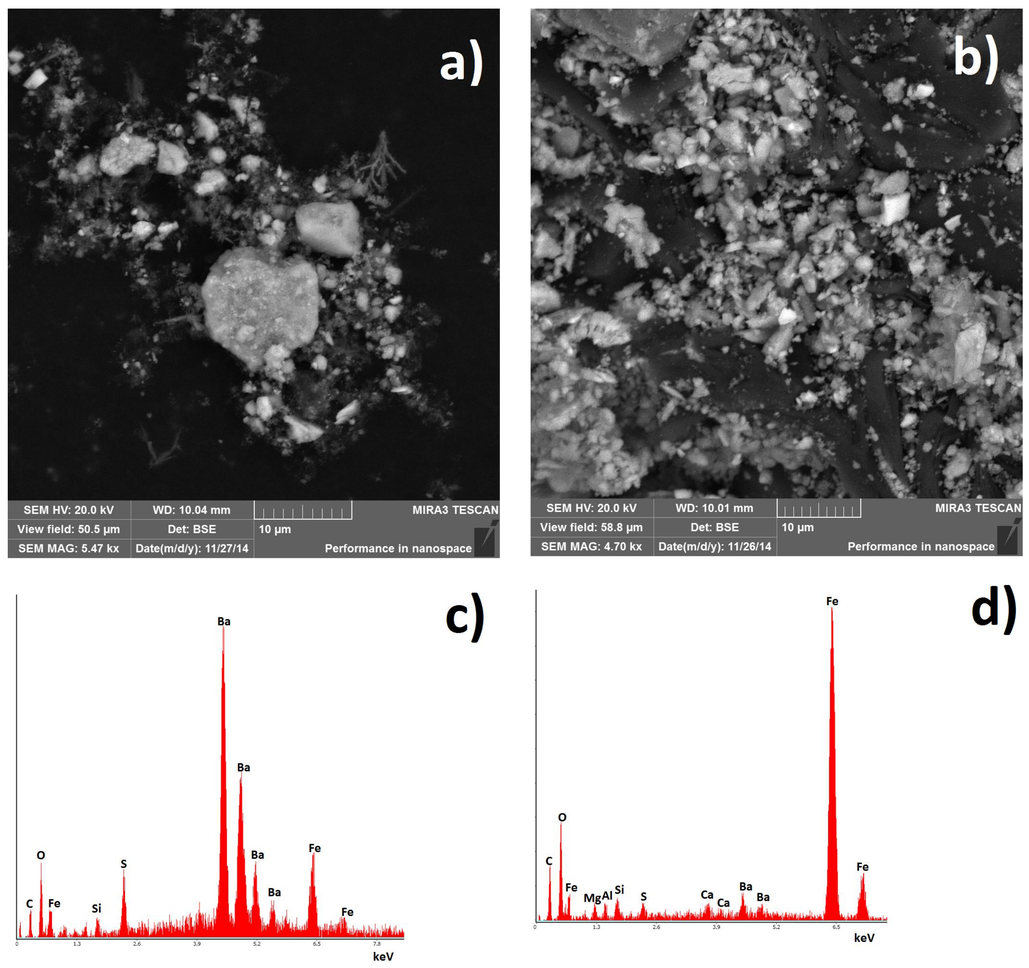


**Fig. 1: SEM image of nonairborne brake wear particles suspended in water (a); brake wear particles in powder form (b) and corresponding EDS spectra (c, d) obtained in all the sample.**

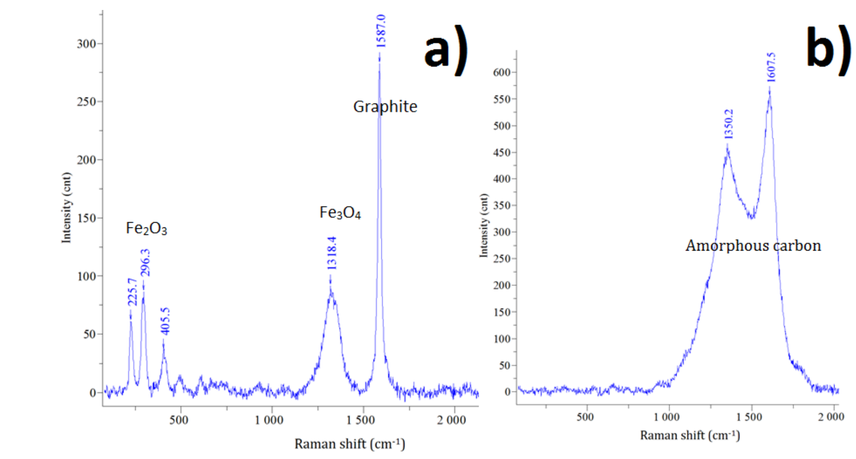


**Fig.2: Raman spectra of mixture of iron oxides and graphite (a) and amorphous carbon (b).**

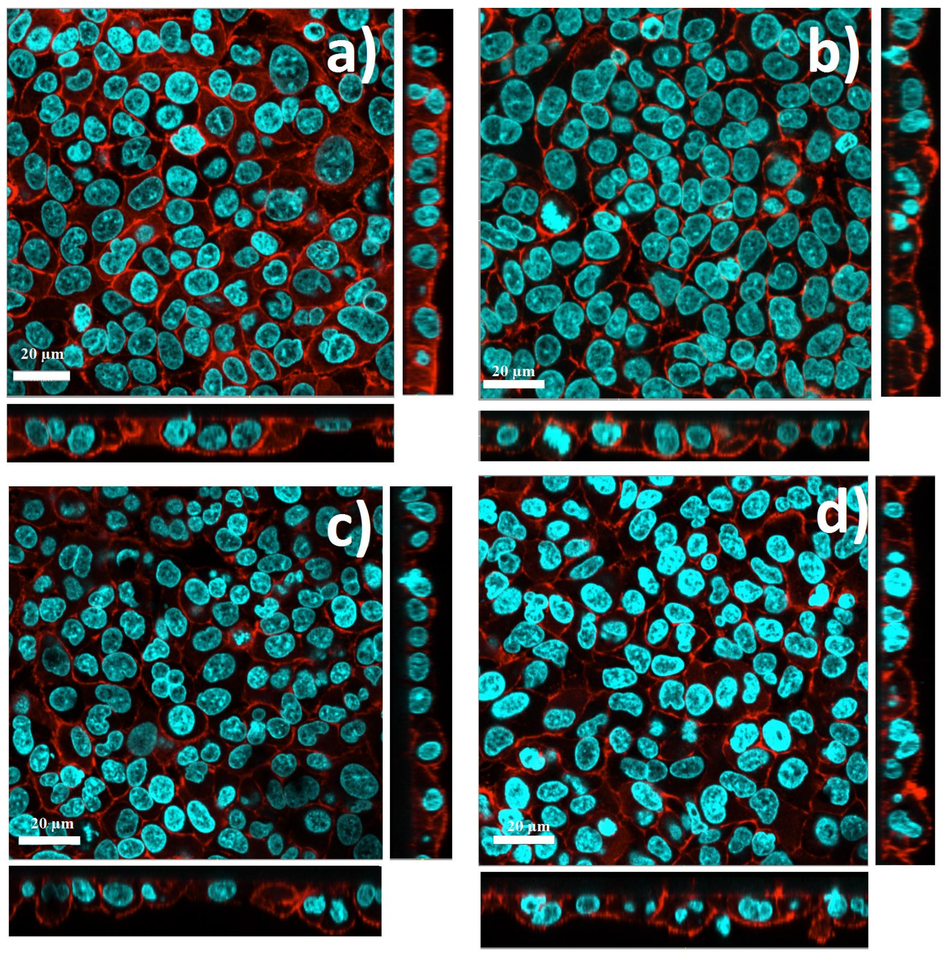


**Fig.3: LSM images of triple cell co-culture model - negative control (a) and cells exposed to brake wear particles with concentration 0.5 mg/ml (b), 1 mg/ml (c) and 2 mg/ml (d). Blue color represents nuclei and red color represents actin cytoskeleton.**

## RESULTS

The mixture of all released fractions of brake wear particles was analyzed by SEM-EDS (fig. 1) and Raman microspectroscopy (fig. 2). No change in cell morphology was observed for any condition, *i.e.* negative control and particle exposed cells (see fig. 3). In addition, no significant effects ($p$>0.05) were observed for the release of (pro-)inflammatory mediators tumor-necrosis factor-α (fig. 4a) and interleukin-8 (fig. 4b), as well as in the intracellular antioxidant glutathione (fig. 4c) compared to the negative control.

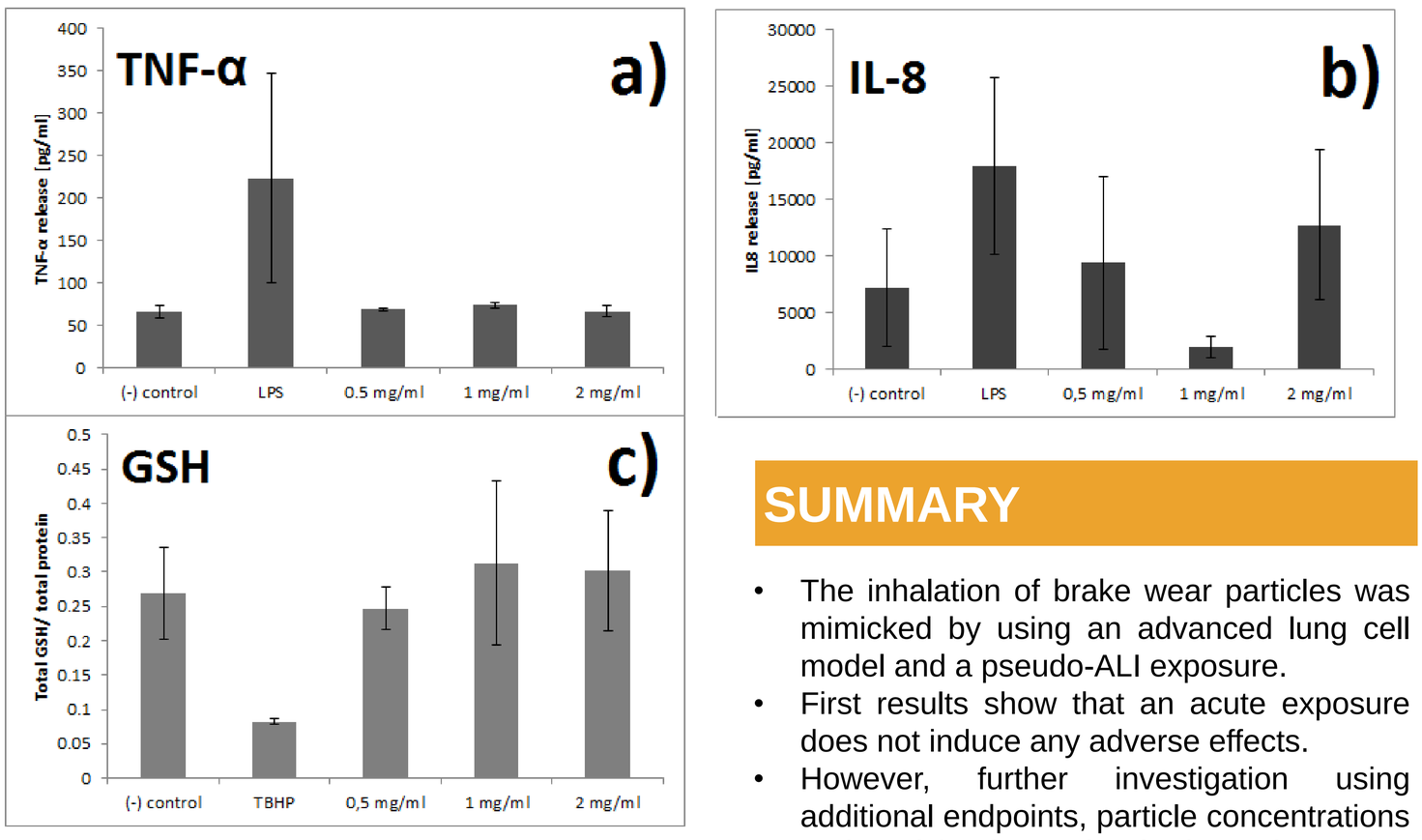


**Fig.4: Levels of released pro-inflammatory mediators TNF-α (a) and IL-8 (b) and oxidative stress response (c). Error bars represent SEM, data were obtained after 3 repetitions.**

## SUMMARY

- The inhalation of brake wear particles was mimicked by using an advanced lung cell model and a pseudo-ALI exposure.
- First results show that an acute exposure does not induce any adverse effects.
- However, further investigation using additional endpoints, particle concentrations and particles from other brake pads need to be tested to support this finding.

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**References:** [1] Harrison R.M. et *al*., *Atmos Environ*, 2001. [2] Querol X. et *al*., *J Aerosol Sci,* 2004. [3] Grigoratos T., Martini G., *Environ Sci Pollut Res,* 2015. [4] Kukutschova J. et *al.* , *Wear*, 2009. [5] Endes C. et *al., Part Fibre Toxicol.,* 2014. [6] Rothen-Rutishauser et *al., Am J Respir Cell Mol Biol*., 2005.

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