Development of an experimental cell-based approach to evaluate biological effects of aerosols from the application of hair-straightening products *in vitro* using relevant product application and cell exposure conditions

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Introduction

Aerosols might be generated during the application of hair-straightening products by professional hairdressers. Such aerosols can include a considerable amount of inhalable particles. Occupational safety assessment for the gaseous phase can be conducted based on known constituents, which are toxicologically characterized such as aldehydes, acetone and siloxanes. However, this is not possible for the particulate phase of the aerosol, since composition and combinatory biological effects of the native aerosol mixtures compounds are largely unknown.

Objectives

The aim of the studies was to set up a cell-based *in vitro* model to evaluate the applicability of an *in vitro* strategy for a relative classification of biological effects from this kind of consumer products using (1) real hair strands and straightening product usage under simulated application conditions and (2) a relevant *in vitro* setting based on human lung cells and a testing of the native aerosol in a dose dependent manner.

Materials and Methods

Aerosol generation and cell exposure

An aerosol box was constructed and optimized by computational fluid dynamics (CFD) simulations, in which natural hair strands could be treated with hair-straightener according to the instructions of the manufacturer. The box included an optimized shape and a fan to facilitate sampling of aerosols from the hair treatments to the cell exposure. Hair treatment included a washing procedure before the application of the hair-straightener and a heating of the treated hair using a flat iron procedure inside the aerosol box (Fig.1). 1, 5 or 10 hair treatments were conducted following to each other to generate varying aerosol concentrations. Air-lifted interface cultures from A549 cells (human lung cell line) were exposed using the P.R.I.T.® ExpoCube® under thermal precipitation conditions to enhance particle deposition from aerosols. Aerosols from positive or negative substances were generated by nebulization of lactose, $CuSO_4$ or SDS (sodium dodecyl sulfate) solutions (Aeroneb® pro, Aerogen) inside the box. Control exposures included concurring clean air exposures during each single experiment and empty box exposures without aerosol generation.

Aerosol characterization

Aerosols were characterized by chemical analysis (HPLC, gravimetry). Particulate concentrations were monitored continuously during cell exposure by light scattering photometry, particle sizes were controlled using a particle spectrometer (Grimm, WiniWRAS).

Cellular effects

Cells were analyzed with regard to viability (WST-1) and interleukin secretion (IL-8) after a 24 hours post-exposure re-incubation period under cell-specific conditions inside an incubator.

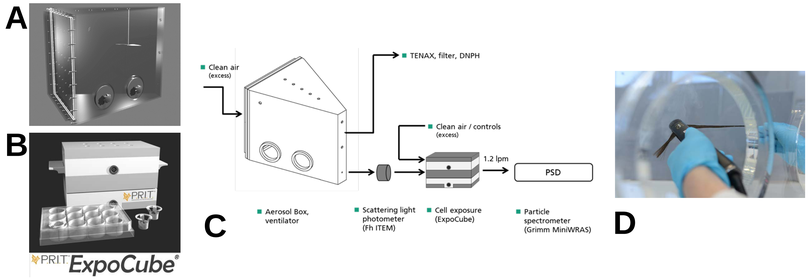
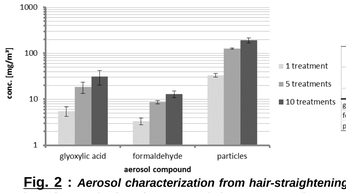


Fig. 1 : ***Experimental setup.*: (A)** Aerosol generation and sampling box, **(B)** P.R.I.T. ® ExpoCube ® for ALI cell exposure, **(C)** complete setup, also including scattering light photometer for online monitoring of particle concentrations and a particle spectrometer (Grimm MiniWRAS), **(D)** Aerosol generation from hair-straightening product using natural hair and a flat-iron procedure.

Results



	1 treatment				5 treatments				10 treatments			
	mean [µg/216l]	s.d. [µg/216l]	mean [mg/m³]	s.d. [mg/m³]	mean [µg/216l]	s.d. [µg/216l]	mean [mg/m³]	s.d. [mg/m³]	mean [µg/216l]	s.d. [µg/216l]	mean [mg/m³]	s.d. [mg/m³]
glyoxylic acid	1193,75	285,61	5,53	1,32	3984,67	1088,59	18,45	5,04	6703,75	2343,54	31,04	10,85
formaldehyde	727,50	123,23	3,37	0,57	1868,67	150,00	8,65	0,69	2807,00	473,92	13,00	2,19
particles	7177,08	697,48	33,23	3,23	27500,00	901,85	127,31	4,18	41475,00	5342,52	192,01	24,73

Fig. 2 : ***Aerosol characterization from hair-straightening products:*** Glyoxylic acid, formaldehyde and particle concentrations inside the aerosol box after conducting of 1, 5 or 10 hair-treatments using 2g natural hair strains. 5 or 10 hair-treatments were designed as "worst-case" scenarios to reach highest technical possible aerosol concentrations and clearly overestimated real application conditions.

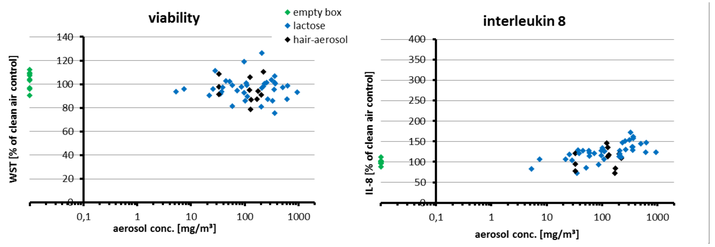


Fig. 3 : ***Cellular responses to negative controls (empty box, lactose) in compare to aerosol from hair-treatments:*** Results of viability assays (WST-1) and analysis of interleukin secretion 24 hours after exposure as a percentage of control (clean air). Results of single exposure runs.

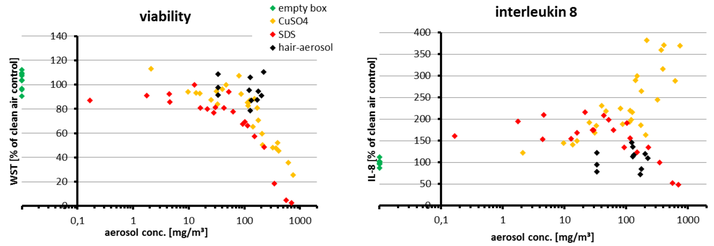


Fig. 4 : ***Cellular responses to positive controls ($CuSO_4$, SDS) in compare to aerosol from hair-treatments:*** Results of viability assays (WST-1) and analysis of interleukin secretion 24 hours after exposure as a percentage of control (clean air). Results of single exposure runs.

- Product application inside the aerosol box using a product relevant procedure resulted in a reproducible generation of aerosols with respect to components of the particulate and vapor phase. (**Fig. 2**). Particle sizes were < 4.5 µm and therefore in the respirable size range. It was possible to increase the aerosol concentration up to a successive treatment of 10 hair strains for a "worst case" testing scenario.
- Empty box exposures (control of procedure) and exposure to lactose aerosol as negative control did not induce significant effects in compare to concurrent clean air controls. Results from hair treatment aerosols were comparable to negative controls (**Fig. 3**).
- Exposures to positive controls $CuSO_4$ and SDS clearly induced cell toxicity in a dose-dependent manner. Highest concentrations of aerosols from hair treatments differed from viability results of positive controls. Positive controls clearly induced dose dependent effects in interleukin secretion which were significantly different from aerosols from hair treatment (**Fig. 4**).

Conclusions

- Reproducible aerosol generation from a consumer product use and sampling to a cell-based *in vitro* testing platform using human lung cells in a relevant culture setup (ALI culture) was possible.
- Application of a set of positive and negative control substances which had been generated under (1) comparable conditions and (2) sharing similar particle characteristics (size) in compare to the aerosol from hair treatments induced cellular effects in a dose dependent manner in a relevant concentration range with respect to the application of the test aerosol.
- The relative classification of effects from the test aerosol, positive and negative controls will be used in the elaboration of a strategy to draw conclusions with respect to safety assessment approaches from this kind of experimental *in vitro* data.

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