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Development of a 3D co-culture system to assess the respiratory sensitizing potential of chemicals

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Introduction

✧Chemical respiratory sensitization resulting from occupational exposure to synthetic compounds has increased over the last decades leading to important occupational health issues.

✧Complex *in vitro* co-culture systems represent valuable tools to understand the mechanisms involved in lung sensitization.

✧No validated *in vitro* model is currently available to assess the respiratory sensitization potential of chemicals.

✧An *in vitro* model developed by Klein *et al.*[1,2] combining alveolar type II epithelial cell line A549, acute monocyte cell line THP-1 cells differentiated into macrophage like cells, endothelial cells EA.hy 926 and human mast cell line HMC-1 in co-culture has been developed to assess the toxic effects of particles at the alveolar barrier. The co-culture was designed in a 3D-organisation to mimic at best the in vivo histology of the alveolar barrier and cultured at the air liquid interface (ALI) to mimic realistic exposure of inhaled compounds.

✧DCs, which have a crucial role in sensitization, need indeed to be included in such model to study the relevant process of sensitization. The co-culture was redesigned to address the sensitizing potential of inhaled compounds in a relevant way, including the possibility of migration of DCs to ensure to have a functional model.

Aim

✧Adapt the 3D-co-culture system previously developed by LIST (Klein *et al.*[1,2]) to allow the *in vitro* study of respiratory sensitization processes.

✧ Find markers for the assessment of the respiratory sensitizing potential of inhaled compounds by measuring the activation of DCs as well as the release of cytokines.

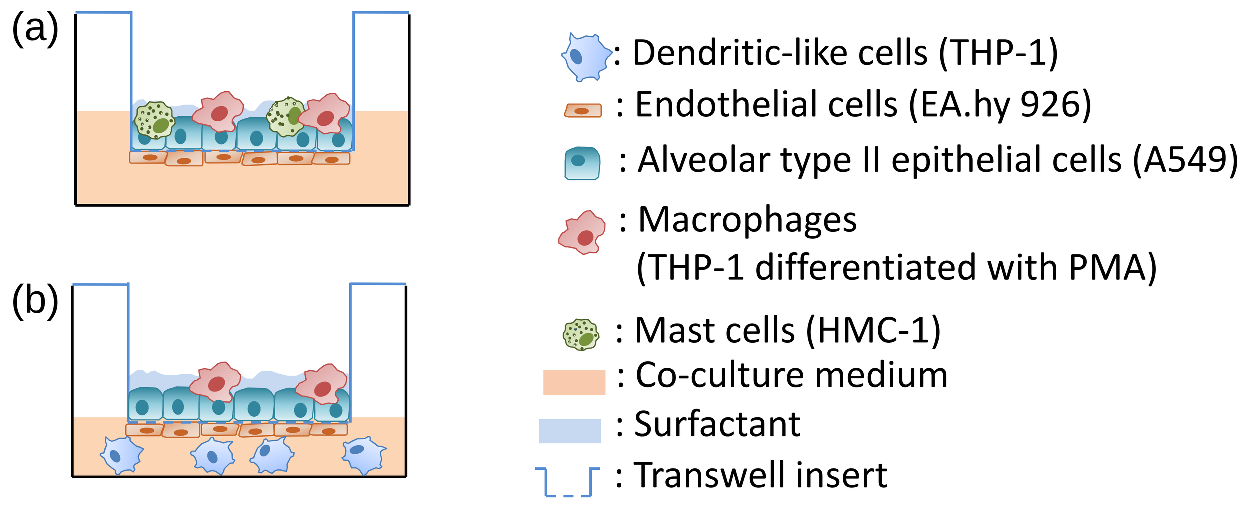
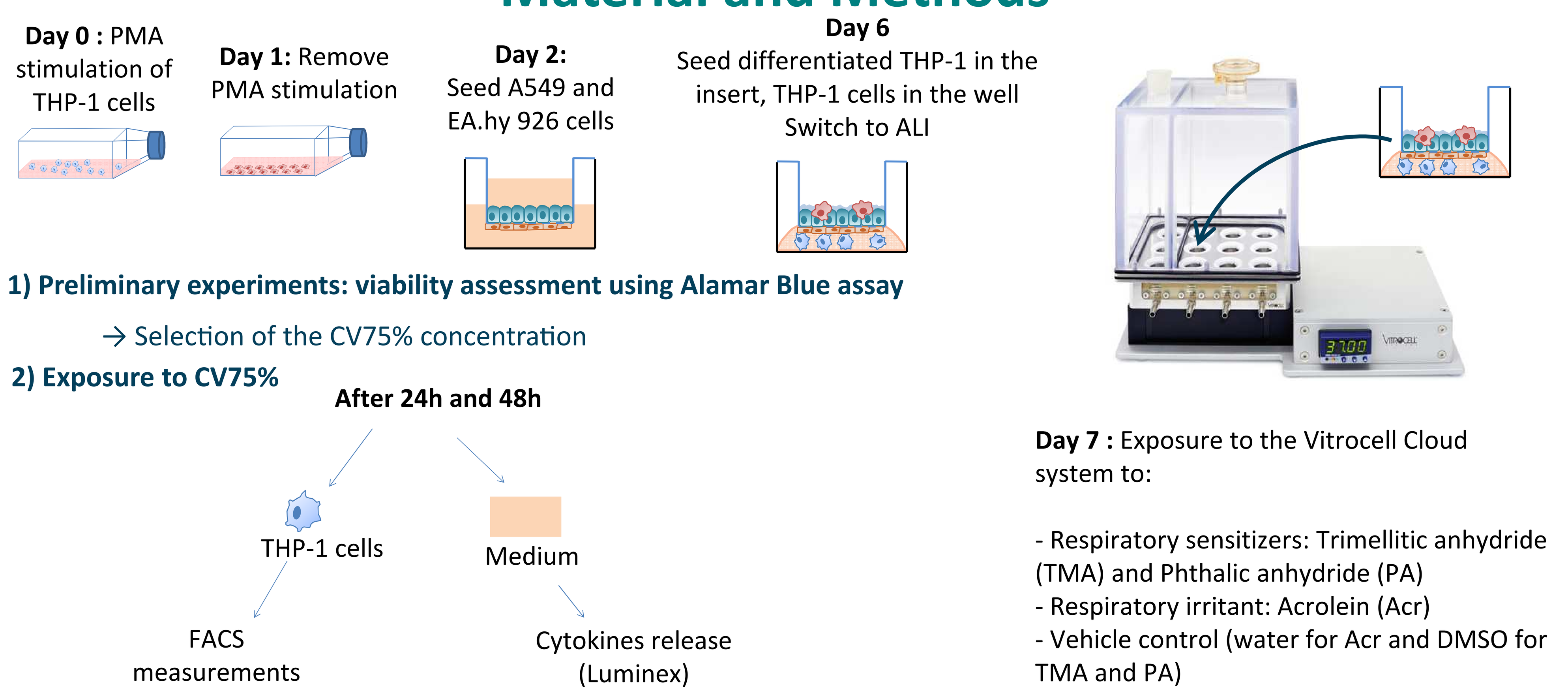


Figure 1: Co-culture system (a) mimicking the alveolar barrier to study cell-to-cell communication and inflammatory effects of NPs at the ALI (Klein *et al.* 2013, 2017) and its variant (b) of the system to study the sensitizing potential of chemicals at the ALI (Chary et al. Manuscript under submission)

Material and Methods



References

[1] Klein, S.G., *et al.* 2013. An improved 3D tetraculture system mimicking the cellular organisation at the alveolar barrier to study the potential toxic effects of particles on the lung. Part. Fibre Toxicol. 10, 31. doi:10.1186/1743-8977-10-31

[2] Keil, S.G. *et al.* 2017. Endothelial responses of the alveolar barrier in vitro in a dose-controlled exposure to diesel exhaust particulate matter. Part. Fibre Toxicol. 14(1),7 doi: 10.1186/s12989-017-0186-4

[3] Patent LU93401

Acknowledgment

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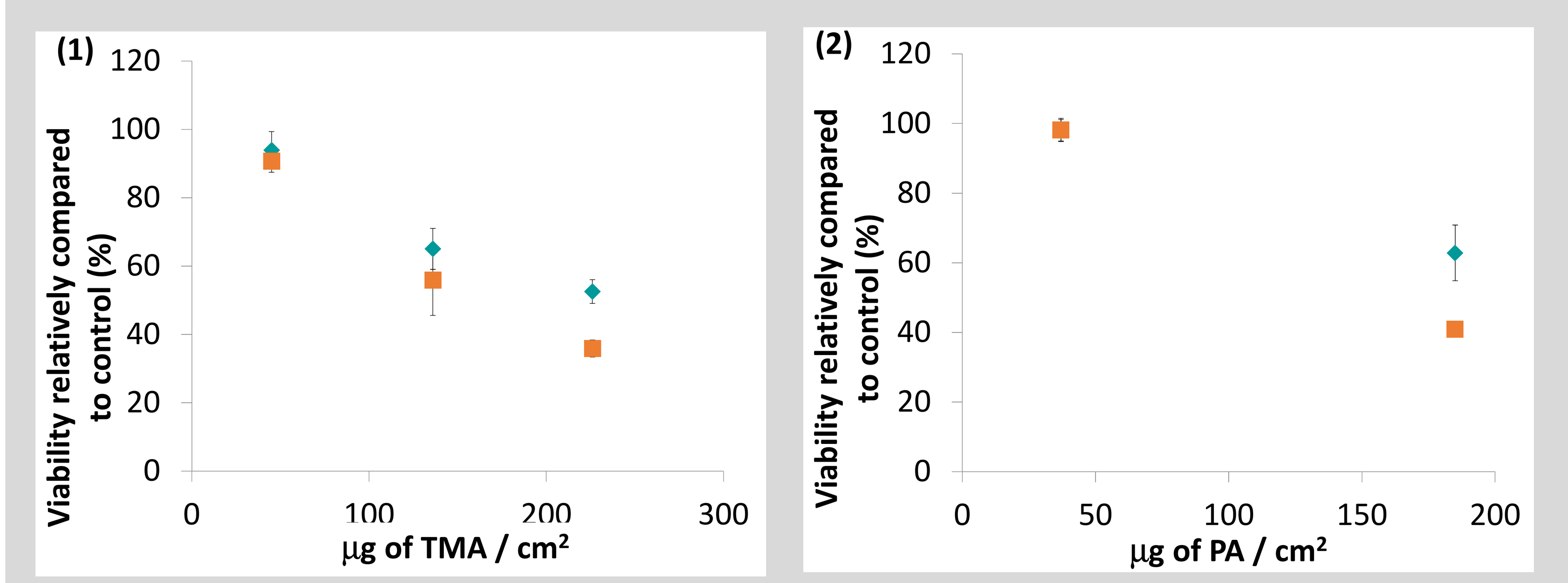
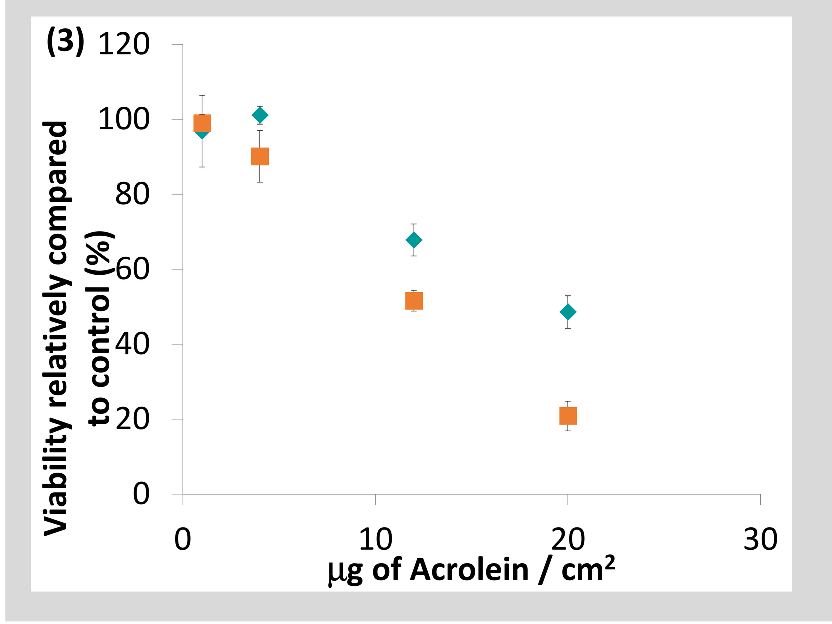
✧We thank the LCSB Luxembourg for the help and support with cytofluorimetry analysis.

Results

✧ Tissue viability





◆ 24h ■ 48h

Figure 2: Viability oft the co-culture compared to negative control after exposure for 24h and 48h to **(1)** TMA, **(2)** Pa and **(3)** Acr (Mean +/- standard error) (n=3).

Concentration used for cytokines and surface markers measurements:

- Respiratory sensitizers: 93 µg/cm² of TMA and 74 µg/cm² PA
- Respiratory irritant: 10 µg/cm² of Acr
- Vehicle controls

✧ FACS measurements

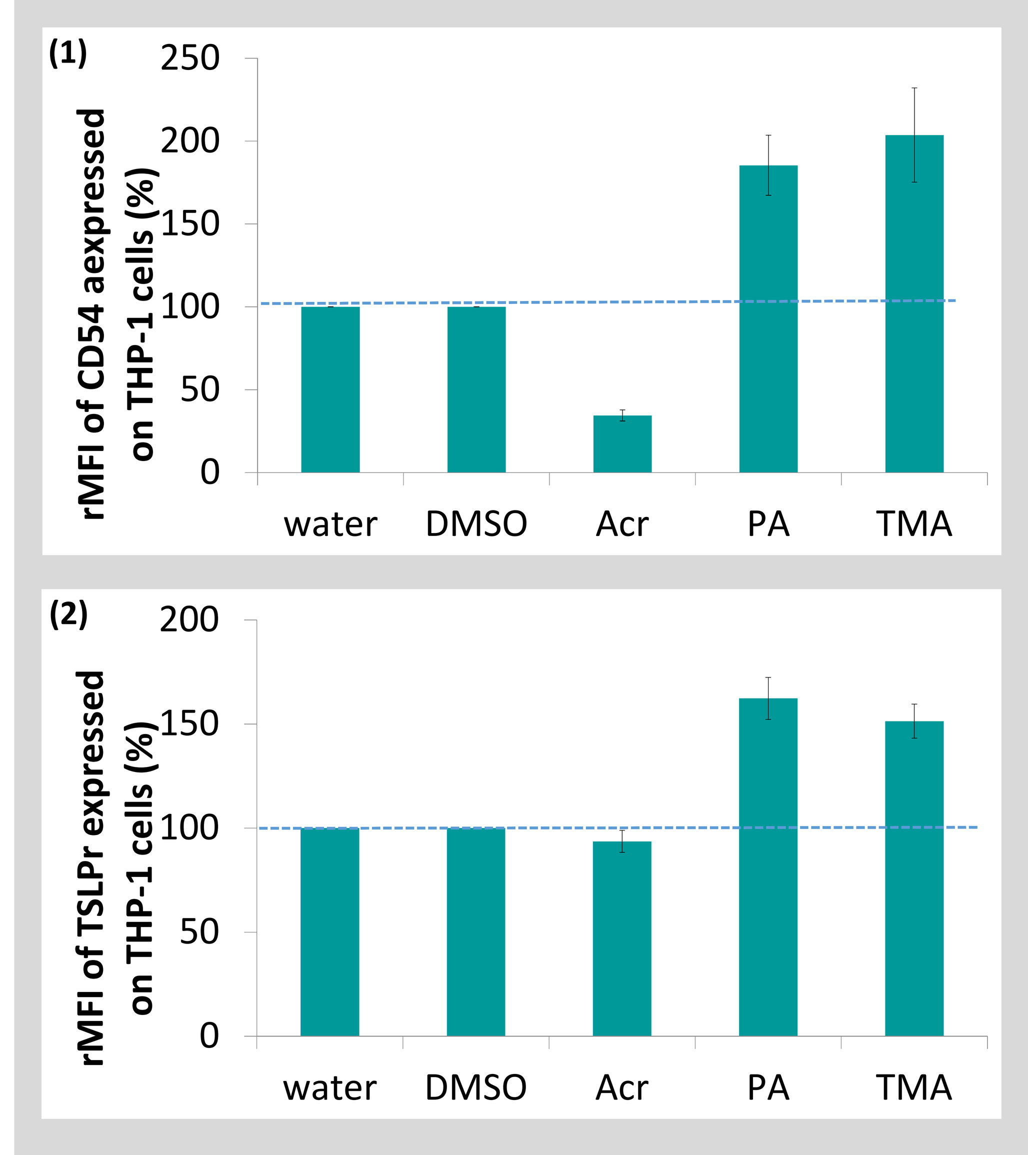


Figure 3: Cell surface marker expression of **(1)** CD54 and **(2)** TSLPr on THP-1 cells in the co-culture after exposure to chemical sensitizers and irritant for 24h (Mean +/- standard error) (n=3).

✧ Cytokines pattern

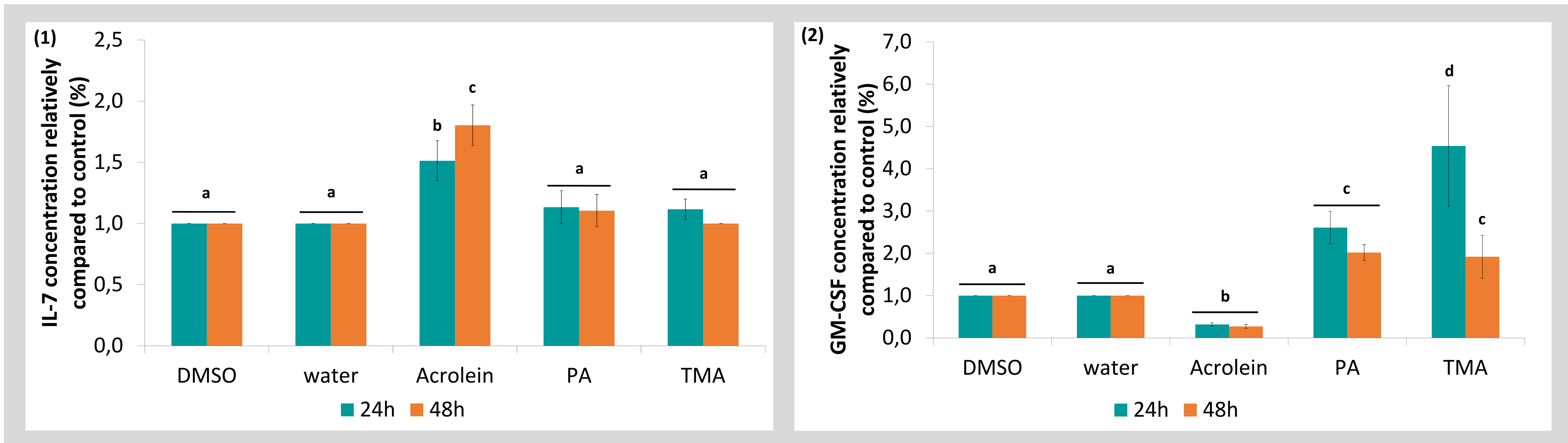


Figure 4: Cytokines released in the co-culture after exposure to chemical sensitizers and irritant for 24h and 48h **(1)**: IL-7 basal levels: DMSO basal level for 24h: 18,2 ± 0,0 pg/mL; for 48h: 19,5 ± 1,2 pg/mL; Water for 24h: 18,6 ± 0,3 pg/mL; for 48h: 18,2 ± 0,0 pg/mL **(2)** GM-CSF basal levels: DMSO basal level: DMSO: for 24h: 32,4 ± 6,0 pg/mL; for 48h: 31,0 ± 3,7 pg/mL; water: for 24h: 47,0 ± 9,2 pg/mL; for 48h: 43,1 ± 5,4 pg/mL (Mean +/- SE) (Letters illustrate significant differences (Factorial ANOVA + Fisher LSD post hoc test at P<0,05 level of significance, n=6).

	Acrolein		PA		TMA	
	24h	*48h*	*24h*	*48h*	*24h*	*48h*
MCP-1	↘	=	=	↗	=	=
MIP-3a	↘	↘	↗	↗	↗	↗
IL-6	↘	↘	↗	↗	↗	↗
IL-7	↗	↗	=	=	=	=
RANTES	=	↗	↗	↗	↗	↗
GM-CSF	↘	↘	↗	↗	↗	↗
IL-10	↘	↘	↗	↗	↗	=

Table 1: Summary of the cytokines released in the co- culture after exposure to chemical sensitizers and irritant for 24h and 48h

Summary and Conclusions

✧CD54 could be used as a marker to discriminate irritants from chemical respiratory sensitizers.

✧TSLPr could be used as a marker to identify chemical respiratory sensitizers.

✧A selected panel of cytokines allows discrimination of respiratory sensitizers and respiratory irritants.

✧The proposed model with the selected biomarkers is able to predict respiratory sensitization *in vitro* and to discriminate chemical respiratory sensitizers from respiratory irritants.

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