Testing nanomaterials in complex 3D *in vitro* lung models

Emma Arnesdotter[1], Pamina Weber[1], Charlotte Stoffels[1], Sebastian Klein[1], Ionel Fizeşan[2], Sebastien Cambier[1], Elisa Moschini[1,3], Tommaso Serchi[1], Arno C. Gutleb[1]

1. Introduction

The potential health and environmental hazards linked to nanomaterials (NMs) present an obstacle to their widespread adoption and impede public perception of the advantages of nanotechnologies. DIAGONAL aims to advance Safe by Design expertise and resources to a stage where they can be integrated into the development process of industries dealing with MultiComponent NanoMaterials (MCNMs) and High-Aspect Ratio Nanoparticles (HARNs). The project relies in part on *in vitro* research to study specific hazard and exposure properties of MCNMs and HARNs. LIST investigates their effects upon inhalation exposure, which is one of the scenarios raising most NM safety-related concerns. To this end, an advanced *in vitro* 3D co-culture alveolar model (Chary et al. 2019) is used where the basolateral side is in submerged conditions while the apical side allows exposure at the air-liquid interface (ALI), resulting in a close replication of the human physiology. Exposure of 3D models to NMs can be performed with multiple exposure strategies, all with advantages and disadvantages which should be considered to ensure its relevance with a high enough throughput and reasonable labour intensiveness. Here, we share tips and tricks on common exposure systems used for *in vitro* testing of NM in the 3D alveolar model.

2. Exposure strategies in 3D *in vitro* lung models

Submerged exposure, where the system is never airlifted during preparation, offers speedy exposure and nominal concentration proximity but lacks human physiology resemblance. Importantly, the sedimentation and diffusion rates of NMs suspended in cell culture media, which is largely dependent upon the effective density and diameter of formed agglomerates in suspension (DeLoid et al. 2014), should be determined. As a compromise, semi-ALI involves airlifting the system but still delivering the NM in limited amounts of medium (65 µL/cm^2). Aerosol-based strategies like Vitrocell Cloud single droplet exposure systems mimic *in vivo* scenario closely but require extensive cleaning between materials to avoid cross contamination, limiting daily usage. Continuous-flow systems allow ALI exposures that closely resemble long-term exposures but obviously, results in a lesser throughput compared to single droplet exposure systems. Albeit being

[1] Environmental Research and Innovation (ERIN) Department, Luxembourg Institute of Science and Technology (LIST), 41 Rue du Brill, L-4422 Belvaux
[2] Department of Toxicology, Faculty of Pharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy, 8 Victor Babeş Street, 400012 Cluj-Napoca, Romania
[3] Institute of Biological Chemistry, Biophysics and Bioengineering, EPS, Heriot-Watt University, Edinburgh

primarily developed for chemicals, high-precision printers like Tecan D300e dispense suspended NMs up to 100 nm at ALI (1 µL/cm^2) with minimal setup time and low risk of cross-contamination, yet size restrictions limit its' versatility.

3. Conclusions

Reaching the nominal concentration in the *in vitro* system is crucial to ensure reliable experimental outcomes. Deviating significantly from the intended concentration can introduce errors and impacts the reproducibility of the results. Equally important is to consider the necessity of mimicking the true physiology and its' possible effect on the study outcome. Moreover, understanding the differences between these exposure systems is key to facilitate comparison and extrapolations between studies using different strategies.

4. References

Chary, A., Serchi, T., Moschini, E., Hennen, J., Cambier, S., Ezendam, J., Blömeke, B., & Gutleb, A. C. (2019). An in vitro coculture system for the detection of sensitization following aerosol exposure. ALTEX - Alternatives to Animal Experimentation, 36(3), 403–418. https://doi.org/10.14573/altex.1901241

DeLoid, G., Cohen, J. M., Darrah, T., Derk, R., Rojanasakul, L., Pyrgiotakis, G., Wohlleben, W., & Demokritou, P. (2014). Estimating the effective density of engineered nanomaterials for in vitro dosimetry. Nature Communications, 5(1). https://doi.org/10.1038/ncomms4514