TESTING NANOMATERIALS IN COMPLEX 3D IN VITRO LUNG MODELS

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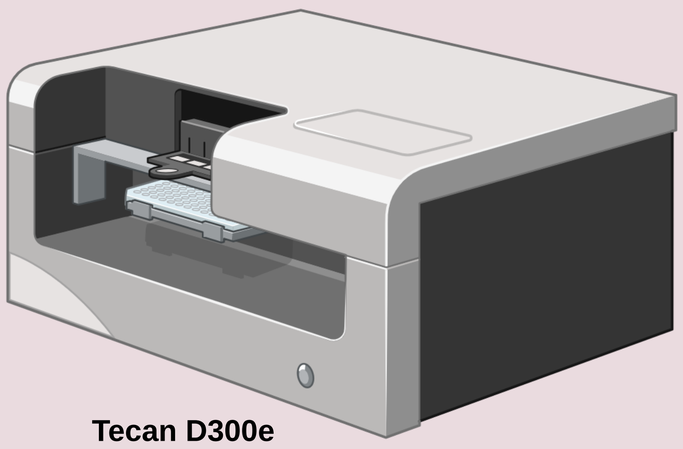
OBJECTIVES

Exposure of 3D models to NMs can be performed with multiple exposure strategies, all with advantages and disadvantages that should be considered to ensure the physiological relevance of the approach while having a high enough throughput. Here, we comment on exposure systems based on extensive experience of *in vitro* testing of NMs in a 3D alveolar model.

PRINTER TECHNOLOGY

+ Exposures at the ALI **(1.3 µL/cm²)**. Limited set-up time and keeps dead volumes to a minimum. The use of disposable materials result in little risk of cross-contamination while no cleaning between chemicals is required. Very high throughput.

− Disposable materials cause potentially high running costs and an increased environmental burden. Only water-compatible materials with <1 micron in diameter in stable suspension at concentrations ≤0.5% can be used, which severely limits its use.



Tecan D300e

SINGLE DROPLET CLOUD SYSTEMS

Cloud Alpha 6 **Cloud Alpha MAX** **PowderX**

+ High physiological resemblance, exposures at the ALI, available in multiple configurations, advanced technology allows exposure of dry powders thus bypassing the transfer of the powder in suspension (PowderX).

− Cleaning between deliveries is required to avoid cross contamination. It may be challenging to determine the administered concentration of certain NMs. Deposition efficiency varies between models - times of up to 30 minutes have been observed with individual materials.

increasing human physiology resemblance

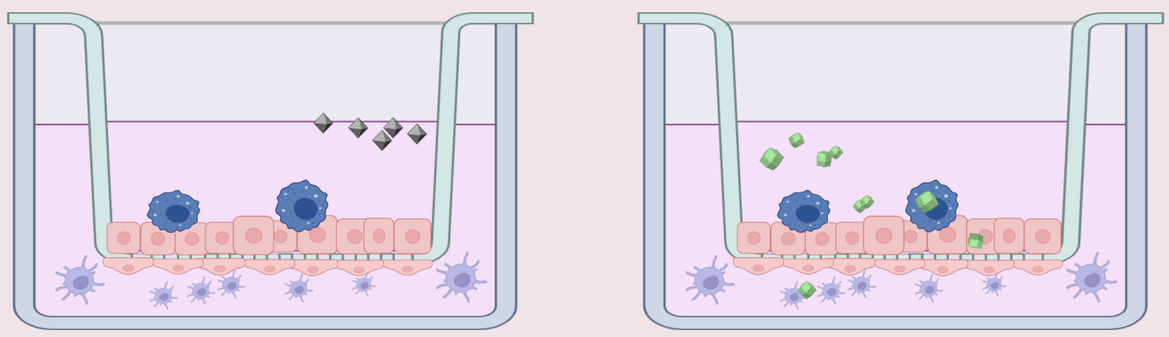
EXPOSURE STRATEGIES

SUBMERGED EXPOSURE

+ Speedy exposures, possibly high nominal concentration proximity.

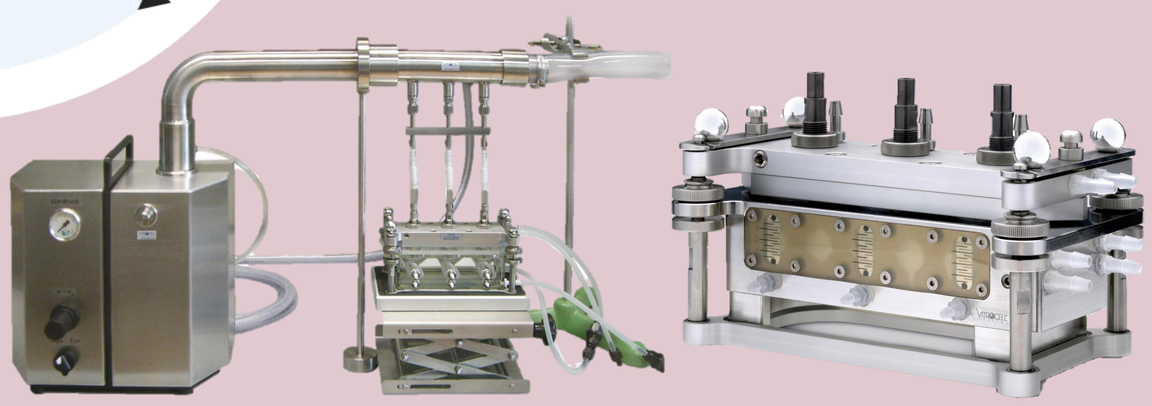
− Lacks human physiology resemblance as the system is never airlifted during preparation. The sedimentation and diffusion rates of NMs in cell culture medium must be determined.

As a compromise, **semi-ALI** involves airlifting the system but still delivering the NMs in limited amounts of medium **(65 µL/cm²)**, thus increasing the physiological resemblance as the ALI may be kept intact.



The sedimentation and diffusion rates of NMs suspended in cell culture media is largely dependent upon the effective density and diameter of formed agglomerates in suspension.

CONTINIOUS FLOW SYSTEMS



VAGF Nebulizing Generator **6/3 CF**

+ Direct exposures at the ALI to airborne substances with a possibility to mimic a long-term exposure duration very closely. Multiple methods of aerosol generation ensure that a wide range of materials can be used, including gases, complex mixtures, NMs and fibres.

− Has a much lower throughput compared to single droplet exposure systems unless large investments in equipment are made and may require large quantities of materials.

WHAT IS IN IT FOR YOU?

An accurate comprehension of the effective concentration is crucial for reliable outcomes, as overlooked deviations from the intended concentration introduce errors, thereby impacting the validity and reproducibility of the results.

Equally important is to consider the necessity of mimicking the true physiological scenario, *e.g.* ALI vs. non-ALI, and its' possible effect on the study outcome, and balance it against a high enough throughput and reasonable labour intensiveness.

Understanding the differences between exposure systems is key for FAIRification of data, comparison between studies using different exposure strategies, and for *in vitro* to *in vivo*-extrapolations.

ALI Air-Liquid Interface, **NMs** NanoMaterials, Figures created with Biorender.com

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