Exposure of an alveolar model to aerosolised dry materials at the Air-Liquid Interface (ALI)

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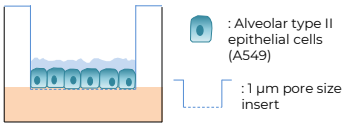
Background

The MACRAMÉ project (for Advanced Characterisation Methodologies to assess and predict the Health and Environmental Risks) is dedicated to creating a structured approach for studying in vitro inhalation toxicology through a series of in vitro and ex vivo models that mimic the biological complexity of the human respiratory system, including both the upper and lower airways. This project will conduct rigorous testing, validation, and implementation of various biological models that reflect the human respiratory system, focusing on the bronchial region (upper airways) and the alveolar region (lower airways). Currently, there exists a noticeable data gap concerning the suitability of these models for nanomaterials and advanced materials. MACRAMÉ seeks to validate the efficacy of these proposed methodologies in assessing such inhalable pollutants. In this context, it is essential to assess various exposure devices to establish a decision tree for selecting the most suitable device and biological system depending on the nature of the material and biological endpoint of interest.

Material & Method

Cell model: A549 cells at the Air Liquid Interface (ALI)



Particles:

- Quartz DQ12
- Corundum
- Multi-walled carbon nanotubes NM401

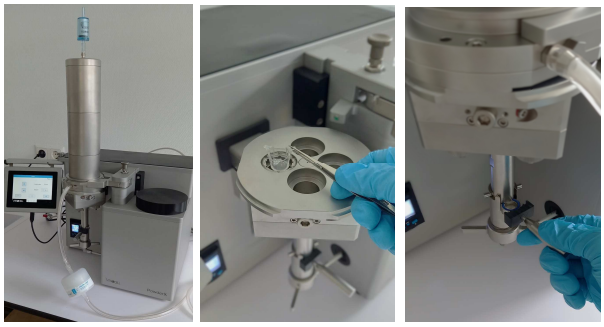
Exposure:

- Vitrocell® PowderX at the ALI
- Semi-ALI

Endpoints:

- Light microscopy imaging
- Viability after 24h exposure (Alamar Blue assay)

Vitrocell® PowderX exposures



PowderX aerosolizes small dry powder samples (1-100 mg) for deposition on cell cultures in 12- or 24-well inserts.

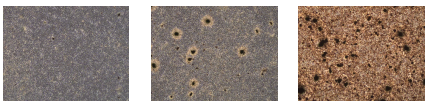
Working Principle:
1 - Load the powder sample.
2 - Seal the chamber.
3 - Aerosolize the powder under high pressure for homogeneous dispersion.
4- Powder settles on cell cultures.

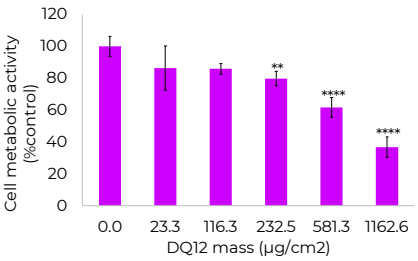
Parameter settings:

Nozzle size = 1 mm, P = 2 bar, Settling time = 15 min, Extraction time = 2 min

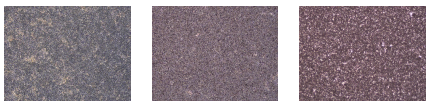
Particle	Mass in powder sample (mg)	Nominal dose (µg/cm2)
DQ12	1 to 50	23.3 to 1162.6
Corundum	1 to 50	23.3 to 1162.6
NM401	0.5 to 8	11.6 to 186

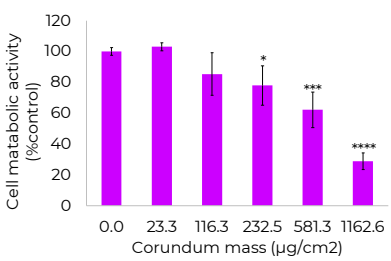
DQ12



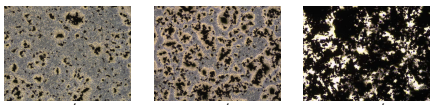


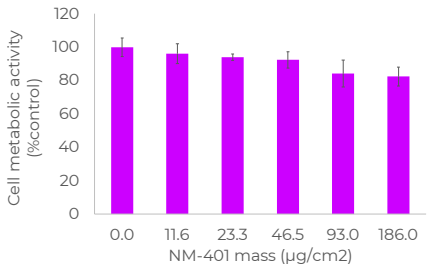
Corundum





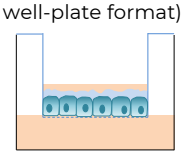
Multi-walled carbon nanotubes NM401

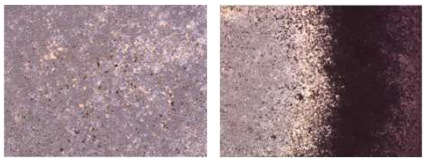




Semi-ALI exposures

65 µL of particles suspension / insert (12 well-plate format)





Light microscopic imaging after the exposure to particles, particles are not homogeneously distributed on the insert.

Conclusion and next steps

Optimizations are needed for the semi-ALI exposure to ensure a homogeneous distribution of the particles.

The use of the PowderX facilitates the exposure to dry materials, eliminating the need for a dispersion protocol and allows to obtain dose-response curves for each tested material. However, since PowderX can only accommodate exposure to four wells simultaneously, it does not support high-throughput screening.

Additional experiments will be performed to evaluate the production of key cytokines by Luminex in both the apical wash and in the basolateral compartment and the particle localisation will be performed using Richardson stain.