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

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1. Introduction

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.

2. Method

In this study, a monoculture of A549 cells grown on the membrane of transwell inserts at the Air Liquid Interface (ALI) was used as a model for the alveolar barrier. Given the technical challenges and reduced relevance to in vivo conditions associated with dispersing advanced materials in liquid such as cell culture medium, the PowderX device (Vitrocell®), which allows aerosolization of dry powders, was evaluated.

The cells were exposed to varying doses of reference materials (Quartz DQ12, Corundum nanoparticles, and multi-walled carbon nanotubes (NM-401 from JRC)) at the ALI using the PowderX device, which was then compared to semi-ALI exposure. Semi-ALI exposure utilizes a reduced volume of medium, unlike the traditional submerged exposure, ensuring that it just covers the entire surface of the membrane insert, which then evaporates to restore ALI conditions.

The metabolic activity of the A549 cells was evaluated using the Alamar Blue assay, while the production of reactive oxygen species was quantified via flow cytometry. The production of key cytokines was evaluated by Luminex in both the apical wash and in

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the basolateral compartment. The cellular localization of the particles was determined using light microscopy on Richardson-stained cell sections.

3. Conclusion

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. In contrast, the semi-ALI exposure method is more cost-effective for such applications.