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# Developing a model to test microplastic impact on lung epithelial barriers formation and functionality

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## Abstract

Nano- and microplastics (NPs/MPs) are plastic particles smaller than 5 mm. They can be manufactured for a specific purpose or released through the degradation of plastics and NP/MP-containing products, and are thus widely distributed in the environment. The two major routes of exposure for humans are ingestion through food and inhalation of indoor air. Studies have already reported that airborne particles can deposit or accumulate in the lungs causing occupational diseases such as lung cancer in textile workers.

The aim of this study is to develop and test a model for the evaluation of the impact of PET and PS MPs on human health, with particular attention to the integrity and functionality of the lung barrier.

To mimic lung tissue, A549 cells were grown on PET inserts, either in submerged or air-liquid interface (ALI) conditions. The latter being more similar to the natural environment in the alveoli.

We then characterized our model in detail using various methods prior to MP exposure. In particular, by performing time-course experiments, we observed that transepithelial electrical resistance (TEER) and epithelial permeability (estimated by the Lucifer Yellow assay) increased and decreased, respectively, over time and reached a plateau 10 days after seeding, indicating the formation of an epithelial barrier.

In subsequent experiments, we examined and compared surfactant production and tight junction expression by A549 cells grown either in submerged or ALI conditions. This showed that ALI produced more surfactant (drop spreading method) and occludin (confocal microscopy)

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compared to submerged cells, with no significant difference in TEER and permeability values, suggesting that ALI-grown cells represent a more realistic model for lung exposure. Finally, the exposure of A549 cells to MPs was tested using this model and our preliminary results showed no clear effect of MPs on cell viability.

**Keywords:** microplastics, A549, inhalation exposure, lung epithelial barrier