Unraveling the effects of real-life micro- and nanoplastics on the lung barrier using an ALI co-culture model

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Plastic materials undergo environmental weathering, leading to the formation of micro- and nanoplastics (MNPLs) that are widely dispersed and can enter living organisms through air, water, and food. Inhalation represents a major route of human exposure, yet interactions between MNPLs and the lung epithelium remain poorly understood. This underscores the need for advanced new approach methodologies (NAMs) that better recapitulate the complexity of the respiratory barrier.

In this study, a human-relevant *in vitro* lung model based on Calu-3 epithelial cells cultured under air-liquid interface (ALI) conditions and co-cultured with THP-1-derived macrophages is used to investigate the effects of nanoplastics on epithelial barrier function. ALI conditions promote epithelial differentiation, mucus production, and tight junction formation, closely resembling the human airway epithelium while enabling long-term exposure. In addition, the inclusion of THP-1-derived macrophages incorporates a key immune component, allowing evaluation of epithelial-immune interactions and inflammatory responses.

The established model is exposed to real-life MNPLs, including polyethylene terephthalate (PET), polylactic acid (PLA), and polytetrafluoroethylene (PTFE, Teflon), at two time points (24 hours and 1 week). Multiple endpoints are assessed, including barrier integrity (TEER), cellular internalization (confocal and TEM microscopy), mitochondrial function (Mitoprobe assay), oxidative stress (DCF assay), and genotoxicity (Comet assay). In addition, gene expression analysis by qPCR evaluates molecular responses related to inflammation, oxidative stress, and epithelial function.

Preliminary and ongoing results indicate that all tested real-life MNPLs are internalized, while no significant changes in barrier integrity are observed under the conditions tested. Early data suggest material-specific effects on mitochondrial function, with PET nanoplastics showing a greater impact at 24 hours compared to PLA and PTFE.

Overall, this approach enables comparative assessment of MNPLs with different physicochemical properties and supports the use of advanced co-culture systems as robust NAMs for studying inhalation toxicology.

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