# Establishment of a human pulmonary *in vitro* co-culture model for nanoplastic toxicological assessment: Calu-3 and THP-1 macrophages

**C. Anguita-Solé[1*], J. Gutiérrez-García[1], L. Rubio[1], & A. Hernández[1]**

[1] *Group of Mutagenesis, Department of Genetics and Microbiology, Faculty of Biosciences, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Barcelona, Spain*
* claudia.anguita@autonoma.cat

Plastic can be degraded with weathering into micro and nanoplastics (MNPLs) and be spread worldwide, ultimately reaching living organisms through air, water, and food sources. Apart from ingestion and dermal absorption, inhalation is one of the main routes of human exposure to MNPLs. Despite its particular concern, limited information has been collected regarding the interaction of MNPLs with the lung epithelium, leaving critical gaps in our understanding of their potential effects on respiratory health. Therefore, there is an urgent need to develop new approach methodologies (NAMs) capable of better mimicking human epithelia *in vitro* as a model to test MNPLs. Calu-3 is a lung adenocarcinoma cell line that can be cultured under air-liquid interface (ALI) conditions, exhibiting respiratory tract features that remain stable for extended periods. However, airway epithelial cells do not function in isolation, the immune system is also present in the lungs. To enhance the reliability of this model assessing MNPLs pulmonary effects, a co-culture of Calu-3 cells with THP-1 macrophages are established under ALI conditions. To test the new model robustness several endpoints were assessed, including the capability of generating stable tight junctions, barrier permeability, microvilli formation, macrophage-epithelial cell interaction and cytokine release. Following the standardization of the model, it was used to assess the effects of MNPLs on the barrier after two weeks of repeated exposures. Preliminary results demonstrated the impact of polyethylene terephthalate nanoplastics (PET-NPLs) on the barrier, highlighting the coculture model as a reliable NAM for testing the effects of repeated MNPL exposures on the lung epithelium.

**Funding:** This work received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 965196, the Generalitat de Catalunya (2021-SGR-00731), the ICREA-Academia programme (Ac2232418) to A. Hernández and the Universitat Autònoma de Barcelona (PPC2024_28). J. Gutiérrez-García holds a PIF Ph.D. fellowship from the UAB (B21P0042).