UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

DOTTORATO DI RICERCA IN Tecnologie Convergenti per i Sistemi Biomolecolari – XL CICLO

Research Topic ID: TECSBI.1

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Project Title: New approach methodologies to assess the biological safety and mechanisms of action of advance (nano)materials

(based on the Horizon Project INTEGRANO - Multidimensional Integrated Quantitative Approach To Assess Safety And Sustainability Of Nanomaterials In Real Case Life Cycle Scenarios Using Nanospecific Impact Categories)

Scientific background and 'open issues'

The Green Deal aims at advancing the European economy, while preserving the natural resources, reducing emission of greenhouse gases, protecting health and promoting social sustainability. In these perspectives, special attention is devoted to sustaining the production of greener chemicals and materials. In 2022, the EU launched the "Strategic Research and Innovation Plan for safe and sustainable Chemicals and Materials" while the EU-Joint Research Center (JRC) published a technical report entitled "Safe and Sustainable by design chemicals and materials"[1]. In these documents, special attention is payed to the safety assessment of the new materials or products, by emphasizing also the need to develop new advanced predictive biological models to reduce or even replace the use of animals in experimentation, in compliance with the 3R principle.

Many newly developed materials are synthesized and incorporated in nanoforms (NFs) and human risk mainly derive from inhalation exposure to the nanoparticles (NPs) intentionally or unintentionally released into the atmosphere. The field of inhalation toxicology is still dominated by the use of laboratory mammals, exposed through aerosolization or instillation of chemicals or (nano)particles. In vitro systems, based on mono and co-culture of human lung cells in submerged conditions are also largely used, but they are not able to represent the complexity of the lung responses and usually not adequate to test the chronic respiratory effects. In the last years, huge efforts have been devoted to obtain 3D reconstructed tissues or organoids of the human lungs and to develop more advanced exposure systems mimicking the inhalation exposure at the air-liquid interface (ALI)[2]. Starting from this state of the art, new approach methodologies (NAMs) for inhalation exposure and hazard assessment are being developed to address the needs of the SSbD framework and in general to study the health impact of airborne pollutants.

In this context the research activities pertain to the project INTEGRANO (Multidimensional Integrated Quantitative Approach to Assess Safety and Sustainability of Nanomaterials in Real Case Life Cycle Scenarios Using Nanospecific Impact Categories) funded in the framework of the Horizon Europe (HORIZON-CL4-2023-RESILIENCE-01-22: Integrated approach for impact assessment of safe and sustainable chemicals and materials)

Objectives

The project is intended to develop new advanced in vitro models of the lung barrier and exposure systems at the ALI, to better mimicking the physiological conditions of exposure and responses to airborne pollutants, and in particular to NPs. The new biological systems, based on the interplay of lung epithelial, endothelial and/or immune cells, also in combination with a reconstructed extracellular matrix will be used to implement an adverse outcomes pathways (AOPs)-based approach to characterize the cytotoxic and proinflammatory effects of new nano(bio)materials developed for different purposes, as antimicrobials or depolluting agents.

In particular, by investigating the properties and mode-of-action of advance (nano)materials (NMs), like inorganic-organic hybrid NPs (Ag and TiO_2-based) or functional bio-based NPs (silica- and/or lignin-based), the project aims at establishing NAMs combining advanced exposure systems, complex predictive biological models and molecular/omics, coupled with morphological approaches. A strong scientific contribution is expected in the fields of environmental health and safety assessment of new chemicals and materials.

Methodologies

Different scenarios of inhalation exposure to different new advance NMs and environmental particles, will be considered and human adverse health outcomes will be investigated, following an AOPs-oriented strategy focused on specific diseases (e.g. COPD; lung fibrosis). The physico-chemical properties of the NPs will be characterized in cell culture media and in relevant biological fluids, using dedicated techniques.

In vitro models of the lung epithelium will be developed using human bronchial or alveolar epithelial cells (Calu-3, BEAS-2B, A549), possibly in co-culture with cells of the immune system (THP-1 derived macrophages) or of the lung micro-circulation (HPMEC cells). To mimic responses during chronic pathological conditions, advanced complex models with collagen/hydrogels films scaffolds will be developed and used to assess the contribution of the extracellular matrix (ECM) in the development of fibrotic processes due to NMs exposure. 3D bioprinted reconstructed tissues on transwell inserts and, possibly, lung organoids will be considered to define the most reliable biological models for the scope of the project.

Besides preliminary testing in classic submerged conditions, the co-cultures will be differentiated and exposed at the air liquid interface (ALI). This action will take advantage from the use of recent technologies, like the Vitrocell-Cloud α or the Cultex RFS systems, which respectively allow the NP nebulization and the direct air flux on the top of cell cultures differentiated at the ALI.

The exposed cells will be then analysed by exploiting the molecular initiating events, key events and adverse outcomes representative of the identified AOPs. In this respect, cell viability, cell death, oxidative stress and genotoxicity markers will be analysed by biochemical, (immuno)cytological and molecular techniques. The release of inflammatory mediators will be studied by ELISA or Multiplex kits. The analyses will be also paralleled

with light and electron microscopy analyses to morphologically characterize the biological systems and the bio-interactions with the NMs at subcellular level.

Collaboration / Co-tutoring opportunities

The project will take advantage from the collaboration between the Cell Biology and Toxicology group at the Department of Earth and Environmental Sciences and the Organic Chemistry group at the Department of Medicine and Surgery.
All the research activities will be performed in strict collaboration with the INTEGRANO consortium partners and with research groups involved in associated international projects.

Project's Sustainability & Mobility

Lab competences

The proposing labs have several years-experience in human and environmental nano-bio-technology and nanotoxicology and are fully equipped with basic and advance instruments to perform the research.

Pertinent research papers

- Motta et al. 2023. An integrated new approach methodology for inhalation risk assessment of safe and sustainable by design nanomaterials. Environment International 183 (2024) 108420
- Motta et al. 2023. Preliminary Toxicological Analysis in a Safe-by-Design and Adverse Outcome Pathway-Driven Approach on Different Silver Nanoparticles: Assessment of Acute Responses in A549 Cells. Toxics 11, 2: 195

Foreign institutions for mobility

- Institute of Lung Health and Immunity (LHI), Comprehensive Pneumology Center (CPC-M), Helmholtz Zentrum München (Dr. Tobias Stoeger)
- ERIN Department, Environmental Health, Luxembourg Institute of Science and Technology (Prof. Arno Gutleb, Dr. Tommaso Serchi)
- Norwegian Institute for Air Research (NILU) (Dr. Eleonora Longhin)

References

[1] AAVV. Safe and Sustainable by Design chemicals and materials. JRC technical Report, 2022. doi:10.2760/487955
[2] Bedford et al., 2022. Recent advancements and application of in vitro models for predicting inhalation toxicity in humans. Toxicology in Vitro 79 (2022) 105299