Advanced models for *in vitro* nanotoxicology

Elisa Moschini
Luxembourg Institute of Science and Technology - University of Milano-Bicocca

The new challenges related to the advent of nanomaterials (NMs) call nano-toxicologists to a change in the assessment approach and to move toward the use of more advanced models in order to mimic human exposure and to investigate the effects of pollution, allergens and other relevant NMs under realistic environmental concentrations.
The response triggered by the inhalation of air pollutants is not limited to local effects of the respiratory system but is often systemic, resulting in endothelial dysfunction or other secondary effects.
The use of *in vitro* models that mimic the main biological barriers (skin, lung and intestine) in healthy or diseased conditions allows to better characterize low-level chronic exposure scenarios, to determine the local toxicity, to evaluate the translocation potential (internal dose) and to understand the effects of the exposure at a more complex hierarchical level.
A complex tetra-culture system was developed by Klein *et al.* 2013 and 2017 to better investigate the secondary effects induced by the exposure to diesel exhausted particulate matter (DEPM) on endothelial cells contributing to increase our knowledge on the link between air pollution and cardiovascular diseases.
The model consists of alveolar type-II cell line (A549), differentiated macrophage-like cells (THP-1), mast cells (HMC-1) and endothelial cells (EA.hy 926), seeded in a 3D-orientation on a microporous membrane to mimic the cell response of the alveolar surface *in vitro* in conjunction with naïve aerosol exposure (Vitrocell^{TM} chamber) at the air liquid interface (ALI). After exposure to DEPM the expression of different anti-oxidant target genes and inflammatory genes and endothelial markers was evaluated. The exposure triggered a response in the endothelial cells after indirect exposure of the tetra-culture system to low doses of particles, underlining the sensitivity of ALI exposure systems.
In a recent research the same approach was applied to study the biological effects induced by different sized and shaped Au and Ag NPs using the Cloud system (Vitrocell^{TM}) to aerosolize the particles.

Recently we increased the level of complexity of the model in order to adapt it to the study of the respiratory sensitization potential of allergens. The modification consisted in introducing dendritic like cells (non-differentiated THP-1 cells). The preliminary results obtained testing different chemicals testify that this model can discriminate *in vitro* between respiratory sensitizers and irritants making it a potential candidate for the study of allergenic NPs.