THP-1 and HMC-1 cell interaction with epithelial cells in a 3D tetraculture system of the alveolar barrier modulates the response to oxidative stress

Sebastian G. Klein1,2, Tommaso Serchi1, Lucien Hoffmann1, Brunilde Blömeke2 and Arno C. Gutleb1
1Dept. of Environment and Agro-biotechnologies (EVA), Centre de Recherche Public - Gabriel Lippmann, Luxembourg. 2Department of Environmental Toxicology, University Trier, Germany.

Introduction
Exposure to fine and ultra-fine ambient particles is still a problem of concern in many industrialised parts of the world and the intensified use of nanotechnology may further increase exposure to small particles. Among the mechanisms proposed for the adverse effects of NPs and PM, the induction of oxidative stress seems to be the most important one. Oxidative stress plays a role in many diseases, such as asthma, atherosclerosis, etc. and its impact on the development and the progression of such diseases was underestimated in the past.

While monocultures of cells that serve as potential targets for pro-oxidant stimuli, such as macrophage-like cells (THP-1) or human mast cells (HMC-1), show a strong response when exposed to oxidative stress, they behave differently in a coculture together with other cell types. Here we present a tetraculture system originally developed by Alfaro-Moreno et al. (2008) that we adapted and modified to study the toxicity of NPs and PM by using a native aerosol exposure system (Vitrocell™ chamber). The system is composed of an alveolar type-II cell line (A549), differentiated macrophage-like cells (THP-1), mast cells (HMC-1) and endothelial cells (EA.hy 928) seeded in a 3D-orientation on a microporous membrane. The tetraculture system, together with a realistic exposure scenario will allow a more realistic judgement about the hazard of new compounds.

To show the functionality of the modified system, we evaluated the response of different cultures (monocultures, coculture and tetraculture) to an oxidative stress inducer. Besides this, the production of surfactant as an important in vivo barrier was studied in vitro, as well as the potential of macrophage-like cells to serve as phagocytes in the system.

Material and Methods
- The spatial distribution of the cells in the tetraculture was analyzed by confocal laser scanning microscopy (CLSM).
- To evaluate the response to oxidative stress, the DCFH-DA assay was used together with AAPH as inducer of oxidative stress.
- To evaluate cell viability, we used the Alamar Blue assay in an adapted version for the transwell inserts.
- The tetraculture was exposed to an aerosol of 50 nm SiO2-Rhodamine NPs in PBS using the Vitrocell™ system.
- The distribution of the NPs in the tetraculture after exposure was evaluated by CLSM (Zeiss LSM 510 META).
- Digital image restoration and evaluation was done using ImageJ and Zen 2011 (Zeiss).

Conclusions
- The interplay of model cells for the immune system (THP-1 and HMC-1) with A549 epithelial cells influences the behaviour of the system, resulting in an alleviative effect for oxidative stress compared to the monocultures (Figure 1A).
- ALI cultures have already a significantly elevated level of oxidative stress compared to submerged controls, which is probably due to the higher availability of oxygen under ALI conditions (Figure 1B).
- The surfactant layer can be considered as an important source of proteins with which NPs can interact in vivo. A549 cells grown under submerged conditions produce surfactant, but the secretion is lower than under ALI conditions (Figure 2 and 3).
- Macrophage-like THP-1 cells are efficiently intercepting the SiO2-Rhodamine NPs (Figure 4).
- The exposure conditions are not significantly affecting the viability of the different cultures (Figure 5).
- The system can be used in conjunction with a native aerosol exposure system and may finally lead to a more realistic judgement about the hazard of new compounds and/or new nano-scaled materials in the future.

References