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. Klein[1,2], Tommaso Serchi[1], Lucien Hoffmann[1], Brunhilde Blömeke[2] and Arno C. Gutleb[1]

[1]Dept. of Environment and Agro-biotechnologies (EVA), Centre de Recherche Public - Gabriel Lippmann, Luxembourg.
[2] Department 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 926) 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 SiO_2-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)

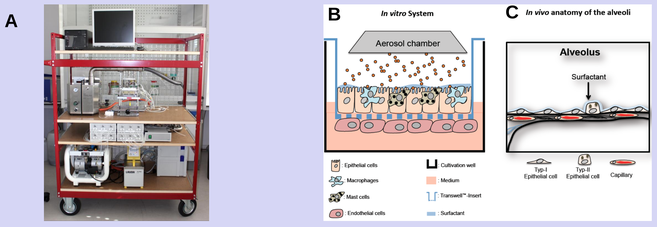

Figure 1: **A:** Setup to expose cells to particles in suspension or to chemicals. **B:** Organization and composition of the *in vitro* system compared to the *in vivo* anatomy of the alveolar barrier (**C**). The presence of immune cells on the alveolar surface was not considered in the *in vivo* scheme. Adapted and modified from Klein et al., 2011.

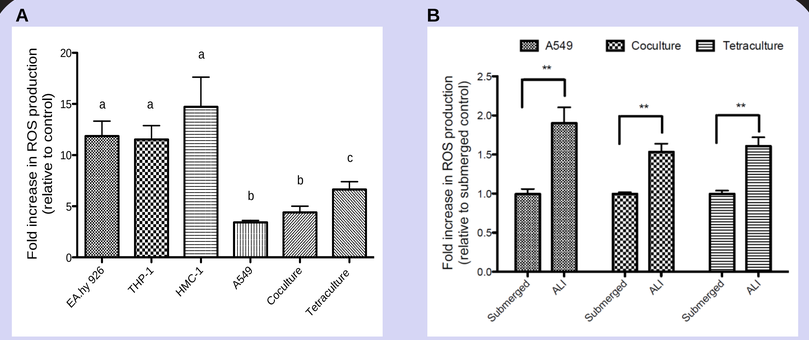

Figure 1: **DCFH-DA assay to analyze the behaviour of the different cultures in response to an oxidative stress inducer.**
A: Cultures were preloaded with DCFH-DA dye and subsequently exposed to 20 mM AAPH in medium for 2 h. Data represents the mean of four independent transwell inserts ± standard error of mean. Each letter corresponds to a group whose values are significantly different from other groups.
B: Cultures were cultivated at the air-liquid-interface and under submerged conditions. Samples were preloaded with DCFH-DA dye and the fluorescence of oxidized DCFH-DA was measured after 2 h . Data represents the mean of four independent transwell inserts ± standard error of mean, asterisks indicate significant differences from submerged controls (**p < 0.01).

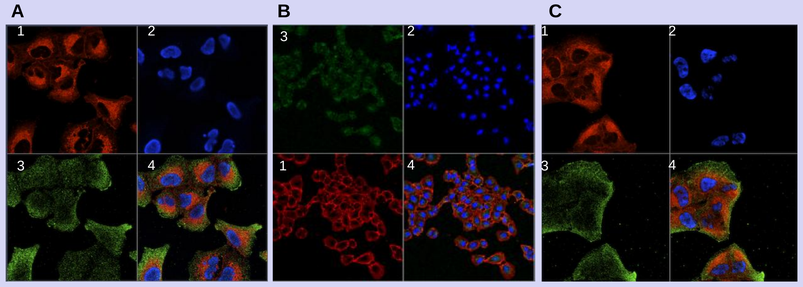

Figure 2: ***In vivo*, the production and secretion of surfactant creates an additional barrier, which can lead to modifications of particles due to the interaction with the surfactant proteins. Using confocal laser scanning microscopy we evaluated the production of surfactant in our A549 alveolar type II epithelial cells.**
Immunohistochemistry staining of surfactant protein A, B and C in A549 cells. A549 cells were grown in Labtek-II chambers for 48 h. Afterwards, cells were fixed, permeabilized and stained for cellular membranes, nuclei and surfactant protein**. 1:** membranes stained with cell mask deep red. **2:** Nuclei stained with DAPI. **3:** Surfactant protein stained with anti-surfactant-protein antibody (1:200). **4:** Overlay.

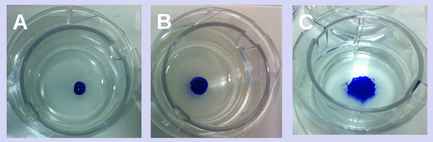

Figure 3: **Results for the surfactant droplet test. In figure 2, the production of surfactant proteins in A549 cells was evaluated. By using the surfactant droplet test, the secretion of surfactant can be visualized indirectly as the secretion lowers the surface tension of the epithelial layer.**
Briefly, the medium was removed from the apical compartment of the transwell and the cells were cultivated at the air-liquid-interface for 24 h. Controls were kept under submerged conditions.
In order to determine the surface tension, DMP/O droplets were placed on the cell surface. Droplet diameters, d0 and d, were measured before and after deposition. A large diameter indicates a high surface tension, e.g. of cells grown under submerged conditions. A small diameter of the drop indicates a lower surface tension, e.g. of cells grown under ALI conditions with the possibility to secrete surfactant.
A: A549 cell exposed for 24h at the air-liquid-interface; **B:** A549 kept under submerged conditions; **C:** EA.hy 926 cells kept under submerged conditions

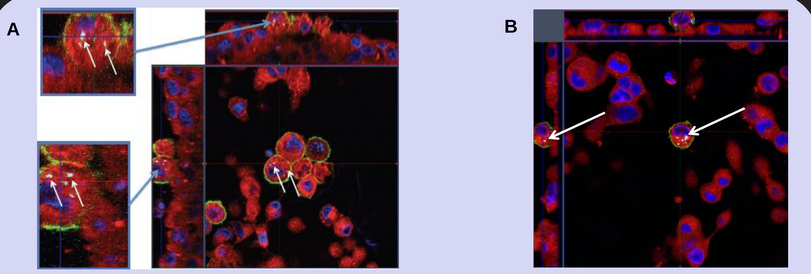

Figure 4: **Z-stack image series to analyze the phagocytic activity of THP-1 macrophages in the triculture present in the apical compartment of the system after exposure to 50 nm SiO_2-Rhodamine nanoparticles with the Vitrocell system and submerged exposure.**
A: Tetracultures of A549, differentiated THP-1, HMC-1 and EA.hy 926 exposed to an aerosol of 50 nm SiO_2-Rhodamine particles for 30 minutes using the Vitrocell aerosol exposure system. Signals of ingested SiO_2-Rhodamine particles were detected in differentiated THP-1 cells (white arrows), but not in A549 or HMC-1 cells. The image shows an x–y projection with the respective side views.
E: Tetracultures of A549, differentiated THP-1, HMC-1 and EA.hy 926 were exposed to cell culture medium containing 10 mg/L of 50 nm SiO_2-Rhodamine particles for 24 h. Signals of ingested SiO_2-Rhodamine particles were detected in differentiated THP-1 cells (white arrows), but not in A549 or HMC-1 cells. The image shows an x–y projection with the respective side views.
Cellular membranes stained with cell mask deep red dye (red) and nuclei with DAPI (blue) are shown. Macrophage cells are counterstained with an anti-CD11b-antibody.

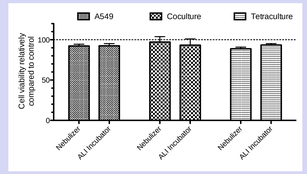

Figure 5: Cell viability of cultures exposed to an aerosol of PBS and clean air at the ALI by the Vitrocell exposure system. Data represents the mean of four independent transwell inserts ± standard error of mean.

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 1 A).
- 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 SiO_2-Rhodamine NPs (Figure 4).
- The exposure conditions are not affecting 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

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