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Exposure of A549 cells to SiO_2-NPs under submerged conditions and at the air-liquid interface using the Karlsruhe exposure system (KIT-TAF)

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State-of-the-art cellular nanotoxicology assays operate by exposure of submerged lung epithelial cells using nanoparticles (NPs) in suspension. However, cell exposure to aerosols at the air-liquid interface (ALI) is described to more closely represent the situation of the lung *in vivo*, as it allows a more realistic interaction of cells and airborne NPs than the conventional method.

In the first part, stably transfected human lung epithelial A549 cells containing an IL-8-luciferase reporter construct [Oostingh et al., 2008] and wild type cells were exposed under submerged conditions to SiO_2-NPs (Aerosil®200, primary particle size 12 nm) and tumour necrosis factor-alpha (TNF-α). Cytotoxicity in terms of LDH release and immune responses in terms of IL-8 release and IL-8-promoter induction were investigated.
In the second part, A549-IL-8-luc reporter cells were exposed to SiO_2-NPs at the ALI using the Karlsruhe exposure system [Mülhopt et al., 2009] and the endpoints were compared to control cells exposed under submerged conditions. The aerosol was generated by dispersion of an aqueous 1 mg/ml solution using an atomizer. Particle number concentration and size distribution measured with a Scanning Mobility Particle Sizer were stable throughout exposure. The NP agglomerates were deposited on A549-IL-8-luc cells seeded on 24 mm Transwell® membranes at a flow of 100 ml/min with or without electrostatic force. The dose was determined by transmission electron microscopy after deposition on TEM grids. As controls, cells were exposed to filtered air and under submerged conditions to SiO_2-NPs and TNF-α.

In both cell lines the SiO_2-NPs induced dose-dependent cytotoxicity and IL-8 release under submerged serum-free conditions. Moderate luc activity was observed in A549-IL-8-luc cells which decreased without serum at high cytotoxic SiO_2-NP concentrations. For the aerosol-exposed cells, an increase in LDH release and IL-8 promoter activity was observed; however, also the airflow seemed to moderately induce IL-8.

In summary, A549-IL-8-luc reporter cells in submerged culture proved to be suitable for testing Aerosil®200-induced IL-8-promoter activity. Furthermore, it was shown that the ALI system is in principle suitable in combination with these reporter cells; however, the practical application of exposing cells at the ALI remains a highly demanding task. In order to resolve the initial problems, ranging from well-to-well variability to cell detachment from the Transwell® membrane, further experiments need to be performed.

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