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Investigating the suitability of Co-cultures of human lung and endothelial cells for repeated exposure experiments with nano particle aerosols

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These days it becomes more and more important to establish long-term in-vitro models for the respiratory system, as for nanoparticles (NP) the respiratory system is the main port of entrance. NP can be found in various products of the daily use, e.g. nano-functionalized plastics and textiles. They can be emitted at different stages of the product´s life-cycle from manufacturing to recycling. We established a novel cell-culture exposure system on inserts (MatriGrid = MG) shaped like alveoles with co-cultures of primary peripheral lung cells (PLC) and an endothelial cell line (EA.hy926) as a model for the blood lung barrier.

Viability of Co-cultures was determined by Resazurin-assay and Lactatdehydrogenase-assay, Glutathione (GSH) was determined by HPLC after derivatization with monobromobimane. The confluency of the cultures was detected by TEER-measurement (transepithelial/transendothelial electric resistance). The size-distribution of the particles in the aerosols was determined by SMPS and OPS.

First of all we exposed Co-cultures 2 times to $BaSO_4$- and TiO_2-NP aerosols (washing & additive exposure). In Resazurin-assay exposure of Co-cultures to aerosols of $BaSO_4$ with averagely 5 x 10^4 particles/cm^3 decreases viability to 40-60 % (depending on patient) when cultures were washed before exposure. Exposure of Co-cultures to averagely 1 x 10^5 particles/cm^3 of TiO_2 shows a clear decrease to 0-30 % (depending on patient). LDH-assay shows a reduction of viability to 20-70 % (depending on patient) in the washed cultures, as well. For the GSH determination Co-cultures were incubated with $BaSO_4$- and TiO_2-aerosols for 1 h (postincubation 23 h and 71 h). The NP-aerosols show a reduction cellular GSH-levels for both NP when cultures were washed before exposure to the aerosols. Without washing (just additive aerosol exposure) there is a small increase in GSH. The particle size distributions of both aerosols were measured during every exposure. Based on the number size distribution most particles have a size below 100 nm. In the $BaSO_4$- and TiO_2-aerosols the median diameters were 57 and 76 nm, respectively.

The applied NP aerosols of $BaSO_4$ and TiO_2 cause more toxic effects in washed cultures than in just additively exposed cultures. These results show that Co-cultures (PLZ/EA.hy926) may build a barrier against the exposure stress. In the future we will also investigate the inflammatory response of this system after exposure to $BaSO_4$- and TiO_2-aerosols.