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Functional Effects of Carbon Black Nanoparticles on Primary Airway Epithelial Cells *in vitro*

Health effects of carbon black and other nanoparticles in humans are not properly understood for risk analyses. Particles mainly enter our bodies via the respiratory tract, but effects on human airway epithelia or the extent of particle exposure are unclear.

To address this knowledge-gap, epithelial *in vitro*-models are generated from human nasal mucosa biopsy material [1]. Autologous fibroblasts are cultured basolaterally on transwell membranes, and epithelial cells are expanded and cultured apically. Co-cultures are transferred into air-liquid interface (ALI) conditions and further cultivated for approximately 4 weeks. Models are evaluated by light microscopy. The ALI-models are exposed to carbon black ultrafine particles (UFP). Particles are distributed onto cell cultures by nebulizing cell culture medium containing UFPs at different concentrations. (Geno-)toxicological assessments are performed using the comet assay and the MTT test. The transepithelial electrical resistance (TEER) is assessed as a proxy for barrier function.

After approximately 30 days, our co-cultures display a multilayered, stratified epithelial model with beating cilia and mucus production. A sufficient barrier is present, as measured by TEER (>400Ω). Our data suggest that carbon black exposure can disturb the barrier integrity from 4µg/cm^2 but with significant donor variation. No concomitant DNA damage or changes in cell viability was detected, which are traditional indicators of toxicity.

Thus, carbon black may induce barrier debilitation at non-toxic concentrations. Assuming a particle deposition of only 1%, as has been suggested to be more realistic [2], corresponds to the significantly lower concentration of 0.4µg/cm^2. Hence, this low concentration could induce barrier failure in some individuals. Further functional testing appears to be relevant for risk assessments. Comparing with city air concentrations of UFP around 5µg/m^3 [3], we lie two orders of magnitude higher with 1% of 4µg/m^2 (400µg/m^3), however such direct comparisons do not reflect the complex reality.

References

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