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Biological effects in human lung cells exposed to platinum nanoparticle aerosol

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Platinum nanoparticles (Pt NPs) are under intense study because of their unique catalytic properties, however, Pt and other platinum group elements are released by cars at an increasing rate. Pt concentrations of 135-303 ng/g have been determined in street dust along highways in Germany (Djingova et al., 2003). Potential health effects of inhaled Pt NPs have not been studied so far. In order to evaluate the potential risk for human health *in vitro* cell-based assays have been performed under submerged conditions and at the air-liquid interface modelling the situation during inhalation.

Methods

A platinum nanoparticle aerosol was generated using a spark discharge generator in nitrogen as the carrier gas. The Pt nanoparticles were diluted by the factor of 10 directly after the generation process. Synthetic air was used for dilution so the oxygen necessary for the cells was added. The aerosol was directed to the Karlsruhe Exposure system (Paur et al., 2008; Diabaté et al., 2008) to analyze the toxicological potential of the freshly generated platinum nanoparticles.

For the bioassay we employed the human bronchial epithelial cell line BEAS-2B and the alveolar epithelial cell A549, both co-cultured with differentiated THP-1 macrophages, growing on Transwell inserts. The responses of the cells were analyzed by measuring the viability, the release of lactate dehydrogenase (LDH test) as an indicator of membrane integrity and release of Interleukin-8 (IL-8) as an indicator of a pro-inflammatory response.

Pt NPs collected on polycarbonate filters (pore diameter 0.4 µm) were used to study cell responses under submerged conditions. The particles were removed from the filters by carefully scraping and a stock solution of 1 mg/ml in cell culture medium containing 10% (v/v) fetal bovine serum was prepared. The solution was vortexed and sonicated to ensure complete dispersion. The cells were exposed to the indicated particle concentrations via the culture medium.

Results

The aerosol was characterized by scanning mobility particle sizing (SMPS 3071, TSI) and TEM (Fig. 1). The particle number concentration in the Karlsruhe Exposure system was determined to 8E+06 1/cm³ with a geometric mean diameter of 25 nm and a standard deviation of $\sigma_g = 1.5$. The mass concentration was 8.13 mg/m³.

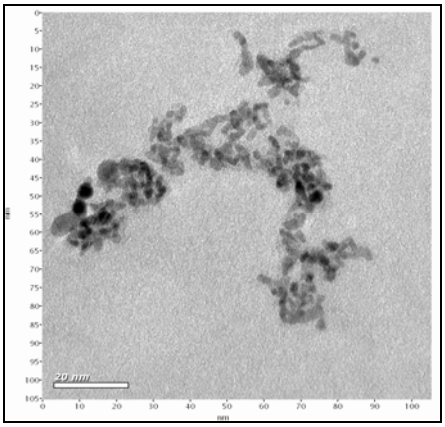

Figure 1. TEM image of Pt particles collected in one exposure chamber while a 2h experiment.

Submerged treatment of A549 cells for 24 h resulted in a loss of viability in dependence of Pt NP concentration. The no observed adverse effect level was 0.1 µg/ml (0.031 µg/cm²) and the lowest observed adverse effect level 1 µg/ml (0.31 µg/cm²) in the LDH as well as in the viability test. BEAS-2B cells seemed to be not as sensitive to Pt NPs as A549 cells (preliminary result).

Exposure to Pt NP aerosol for 2 and 4 hours with a post-incubation period of 20 h under submerged conditions resulted in a moderate increase of LDH leakage in comparison to clean air exposed controls of A549, not for BEAS-2B cells. The viability was not affected in both cell lines, however a remarkable increase of IL-8 release (up to 15 fold of the clean air exposed controls) was observed due to Pt NP exposure in both cell lines. The increase of IL-8 release was also dependent on the time of exposure which is related to the deposited mass.

Conclusion

In summary, the exposure technique at the air-liquid interface and the bioassay described can be used for screening the toxicological potential of a Pt NP aerosol to identify health-related effects.

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