

## Biological effects in human lung cells exposed to platinum nanoparticle aerosol

S. Diabaté<sup>1</sup>, C. Weiss<sup>1</sup>, S. Mülhopt<sup>2</sup>, H.-R. Paur<sup>2</sup>, V. Niedetzky<sup>3</sup>, M. Seipenbusch<sup>3</sup>

<sup>1</sup> Institute for Toxicology and Genetics, Karlsruhe Institute of Technology, 76021, Karlsruhe, Germany

<sup>2</sup> Institute for Technical Chemistry, Karlsruhe Institute of Technology, 76021, Karlsruhe, Germany

<sup>3</sup> Institute for Mechanical Process Engineering and Mechanics, Karlsruhe Institute of Technology, 76131, Karlsruhe, Germany

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 are rarely 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 NP aerosol was diluted by the factor of 10 with synthetic air directly after the generation process. 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 Pt NP aerosol.

For the bioassay we employed the human alveolar epithelial cells A549 and the bronchial epithelial cells BEAS-2B, which was co-cultured with differentiated THP-1 macrophages, growing on Transwell inserts. The responses of the cells were analyzed by measuring viability (AlamarBlue assay), release of lactate dehydrogenase (LDH) as an indicator of membrane integrity, induction of heme oxygenase-1 (HO-1) as an indicator of an anti-oxidative response and release of Interleukin-8 (IL-8) as an indicator of a pro-inflammatory response. Additionally, Pt NPs collected on polycarbonate filters (pore diameter 0.4 µm) were used to study cell responses under submerged conditions.

**Results:** The aerosol was characterized by scanning mobility particle sizing (SMPS) and TEM. The exposure dose was determined to 8E+06 particles/cm<sup>3</sup> with a geometric mean diameter of 25 nm and a standard deviation of  $\sigma_g = 1.5$ . The mass concentration was 8.13 mg/m<sup>3</sup>.

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. 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.

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/cm<sup>2</sup>) and the lowest observed adverse effect level 1 µg/ml (0.31 µg/cm<sup>2</sup>) 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).

**Conclusion:** In summary, the exposure technique at the air-liquid interface and the bioassay described can be used for screening the toxicological potential of aerosols to identify health-related effects.

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