# Testing particulate matter toxicity via *in vitro* methods: what should be tested?

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Keywords: particulate matter, nanoparticles, cytotoxicity, reactive oxygen species
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Unlike other toxic substances, usually of a known chemical formula, air particulate matter (PM) is a mixture of solid and liquid particles. The most frequently used tests are *in vitro* in nature and examine 'cell viability' following 24-hour exposure to PM. In most cases, PM induces sub-toxic viability responses but other key cell functions are not detected.

The aim of this study was to compare the toxicity profiles of engineered NPs: zinc oxide (ZnO), crystalline form of silicon oxide ($SiO_2$), and nickel (Ni), which are frequently present in ambient air pollution. Three different assays (acellular and cellular) were chosen to test PM biological targets: (1) plasmid scission assay – detecting DNA damage (indicative of the ability to produce reactive oxygen species; ROS; Figure 1); (2) haemolysis assay – informing about red blood cells (RBCs) membranes integrity; (3) proliferation assay inspected on HUVEC (human umbilical vein endothelial cells) at 24, 48 and 72 hours post-exposure to NPs.

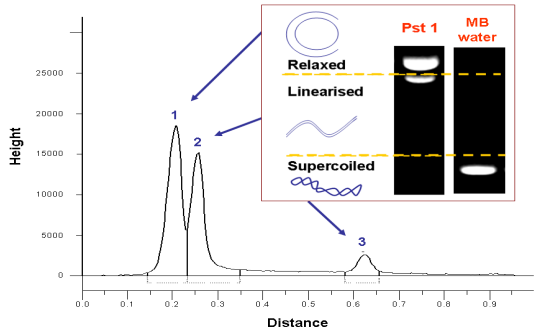


Figure 1. Identification and quantification of plasmid X174 RF DNA isoforms. ROS converts 'supercoiled' (undamaged) into 'relaxed' and 'linearized' isoforms.

DNA damage was observed at a dose of 1 µg/ml for ZnO and Ni NPs. Severe damage (90%) was caused by Ni NPs at a dose of 1 mg/ml. Silica particles caused no damage over the dose range (Figure 2).

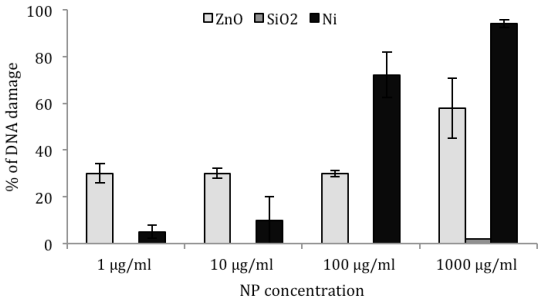


Figure 2. NPs impact on plasmid DNA.

Only $SiO_2$ NPs caused leakage of haemoglobin from RBCs (Figure 3).

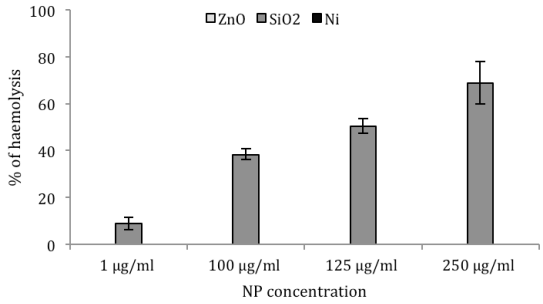


Figure 3. NPs disruption of RBCs membranes.

Results from proliferation assay showed that the most toxic NPs were ZnO, at a dose of 50 µg/cm$^2$, killing nearly all cells after 24 hours. The same dose of Ni NPs decreased the number of cells by 50% and significantly reduced cell proliferation in the second and third days of incubation. In contrast, $SiO_2$ NPs caused no change in cell viability after 24 hours of incubation. However, after 48 and 72 hours, proliferation was inhibited by 40% and 50%, respectively.

Concluding, exposure to NPs, which constitute PM, causes multi-factorial biological responses that may only be detected through a panel of bioassays that target key biological responses. For example, metal NPs (Zn, Ni) induce DNA damage and exert immediate endothelial cell death, whereas only crystalline quartz disrupts membrane integrity and its effect on cell proliferation could be seen in long-term exposure. Therefore, toxic effects of PM may be multi-directional, as different components of PM may influence different properties of a cell.

This work was supported by Doctus - Malopolski Scholarship Fund for PhD students co-funded by the European Social Fund, and PhD research project N N404 102 639

Wlodarczyk, A.J. (2012) (2012) *The influence of particulate matter nanoparticles on bronchial epithelium and vascular endothelium - new mechanisms of adverse health effects of industrial air pollution,* PhD dissertation, Jagiellonian University, Cracow, Poland