Exposure of Engineered Nanoparticles to Human Lung Epithelial Cells:

Influence of Chemical Composition and Catalytic Activity on Oxidative Stress

EMPA
Materials Science & Technology

Ludwig L. Limbach[1], Peter Wick[2], Pius Manser[2], Robert N. Grass[1], Arie Bruinink[2], Wendelin J. Stark[1]

1 Department of Chemistry and Applied biosciences, Institute for Chemical and Bioengineering, ETH Zurich, CH-8093 Zurich

2 Empa, Swiss Federal Laboratories for Materials Testing and Research, Laboratory for Materials-Biology Interactions, CH-9014 St. Gallen

contact: peter.wick@empa.ch

Introduction

The chemical and catalytic activity of nanoparticles has strongly contributed to the current tremendous interest in engineered nanomaterials and often serves as a guiding principle for the design of functional materials. Since it has most recently become evident that such active materials can enter into cells (Figure 1) or organisms, the present study investigates the level of intracellular oxidations after metal-doped silica nanoparticles and the corresponding pure oxides in vitro. The resulting oxidative stress was quantitatively measured as the release of reactive oxygen species (ROS) by the conversion of the sensitive probe HDCF-DA to the fluorescent DCF (Figure 3).

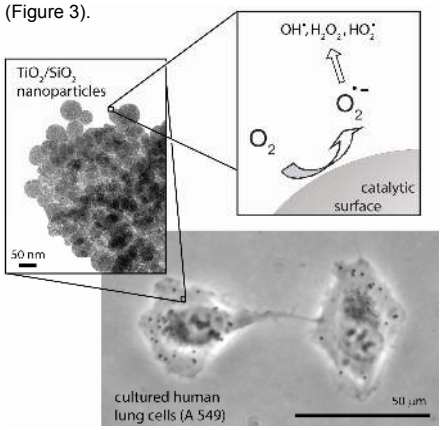


Figure 1: *Scheme of catalytic active nanoparticles in human lung epithelial cells*

Materials and Methods

The present study used two sets of materials: (1) silica doped with 0.5 and 1.6 wt % of iron, cobalt, manganese, and titanium and the corresponding pure oxide; and (2) a series of eight iron-containing silica nanoparticles (0-10 wt% Fe/SiO_2) to systematically investigate the role of catalytic effects (Figure 2).

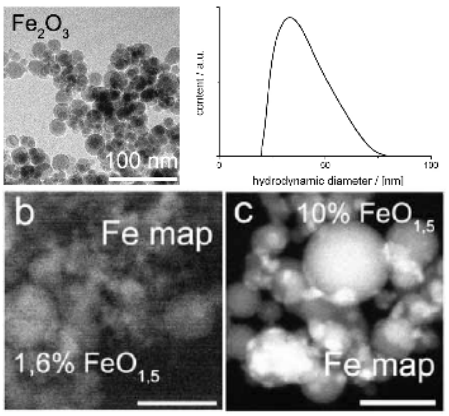


Figure 2: *Morphology of applied nanoparticles: (upper Figure) representative transmission electron micrograph of ironoxide and a typical log-normal particle size distribution as measured by X-ray disk centrifugation (b) Scanning transmission electron micrograph of 1.6% $FeO_{1.5}$ on SiO_2 and (c) 10% $FeO_{1.5}$ on SiO_2. Size bar 50 nm*

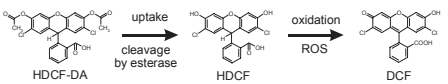
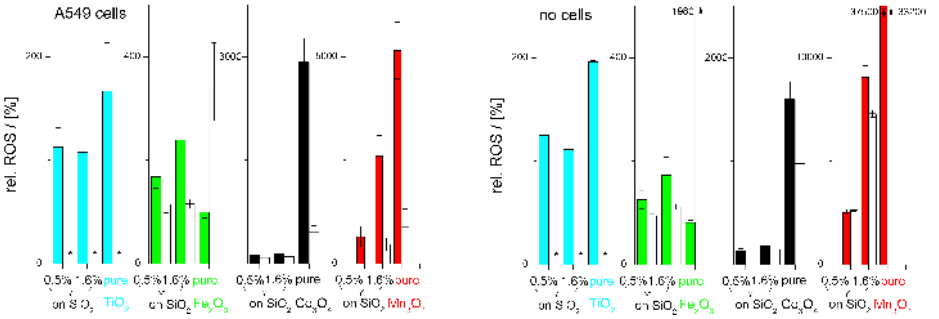
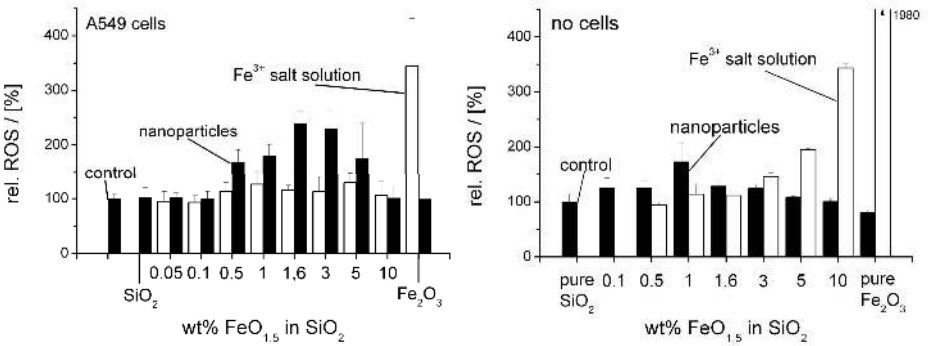


Figure 3: *reaction sequence of the non-fluorescent HDCF-DA to the fluorescent DCF*

Results



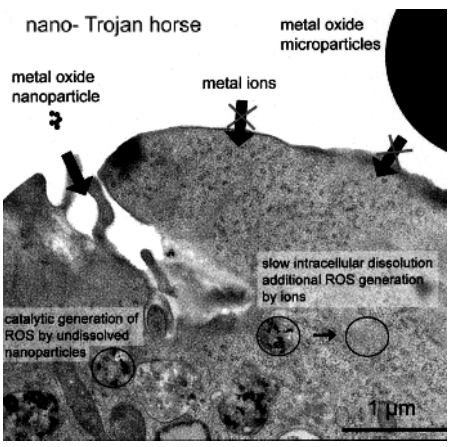
ROS concentration in human lung epithelial cells (left) after 4 h nanoparticles exposure (full columns) relative to reference cultures without particle exposition. Empty columns depict cultures only exposed to the corresponding amount of metal salts as aqueous solution. Comparison of ROS production in cell free (right) culture medium for heavy metals at 30 µg/ml medium. Exposure to particles (full columns) stimulates few ROS for iron oxide and titania. Dissolved iron ions (empty bars), however, promote a 20 times higher ROS production than exposure to the same amount of iron in the form of Fe2O3 nanoparticles. This proves that the FeO3 particles did not significantly dissolve to iron within the duration of the exposure. Note the different scale bares.



ROS production of 30 ppm iron/silica nanoparticles exposed to A549 cells (left) relative to saline controls exactly follows the activity pattern of FeO_x/SiO_2 known for heterogeneous catalysts. Exposure to nanoparticles (full columns) results indifferent ROS levels than exposure to iron ions (empty bars) at the same concentration. A cell free control experiment in medium (right) showed no statistically relevant dependence of ROS levels on the nanoparticles composition (full columns). Exposure to iron salts at the same iron dose did provoke some ROS above 5 ppm $(Fe)_{aq}$. Much less ROS was formed with A549 cells exposed to 30 ppm aqueous iron (left, empty bar, about 320%) if compared to the cell free control (right, empty bar, about 1980%) corroborating the barrier function of cell membranes for ions.

Conclusions

The present investigation has shown that the **chemical composition** of nanoparticles is a most **decisive factor** determining the **formation of ROS** in exposed cells. Beyond mass-based chemical effects where a toxic substance enters and damages a tissue or a cell, the size and mobility of nanoparticles give rise to two other effects: Partially **soluble materials** such as cobalt oxide may be taken up into cells by a **Trojan-horse type mechanism** which can significantly **increase the damaging action** of such materials. Catalytically active nanoparticles can give rise to **prolonged damaging action** in a cell since the material is not degraded during its interference with intracellular constituents.



Literature:
Limbach LK, Wick P, Manser P, Grass RN, Bruinink A, WJ Stark, Environ. Sci. Technol., (2007) 41(11)pp4158-63