Effect of nanosilver and Tio_2 on MAPK activation: role of ROS and implication in DNA damage

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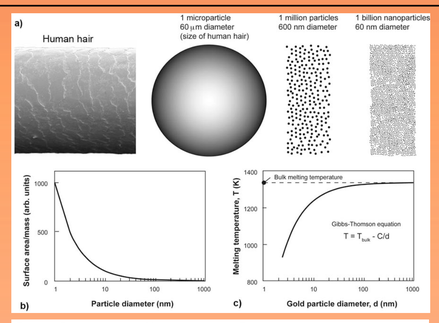
Abstract

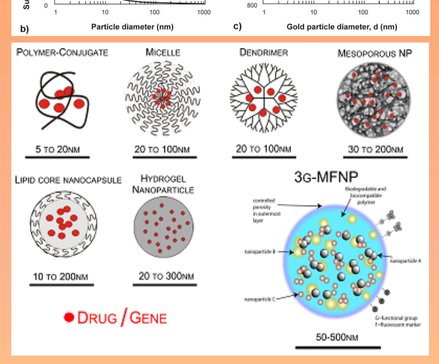
In recent years, large quantities of engineered nanoparticles (NP) have been rapidly produced and widely applied, leading to an increased exposure of workers and consumers to various kinds of manufactured NP. Many are the benefits of nanotechnology but there is increasing concern about potential adverse effects on humans and environment. Because of their size, they have unique physiochemical properties which may contribute to more aggressive forms of long-term toxicity. The interaction with several macromolecules could have many consequences such as mutational alteration, signalling effects, enzyme inhibition and oxidant injuries.

Although previous studies have proposed a role for ROS in NP-induced toxicity, the downstream pathways through which NP signal in human cells inducing cytokine production and DNA damage are unclear. Considering the increasing use of nanosilver (NS) and TiO_2 it has become crucial to develop a fundamental understanding of the cellular responses to these NP. Herein we looked at the possible mechanisms underlying the biological effects of NS and TiO_2. Both NS and TiO_2 exposure were able to induce ROS production in human embryonic epithelial cells (EUE) which could be inhibited by DPI. Furthermore we investigated MAPK activation induced by NP. We found that ERK and JNK activation was an early response but not sustained in time to NS exposure. Interestingly ROS generation resulted involved in JNK but not ERK activation as treatment with DPI and NAC could inhibit only JNK activation. In contrast ERK and JNK activation are an early response but sustained in time to TiO_2 exposure. We also investigated the role of MAPK in the DNA damage. Using a modified comet assay for the specific detection of oxidative DNA lesions, we showed that NS induce DNA oxidation. Moreover higher damage was achieved upon inhibition of ERK activation suggesting a role for this MAPK in DNA damage repair.

Activation of different MAPK might play an important role on the toxicity outcomes of NP and understanding this process may be helpful for the identification of NP toxicity biomarkers.

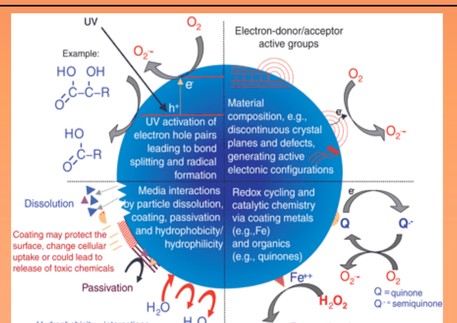
Introduction

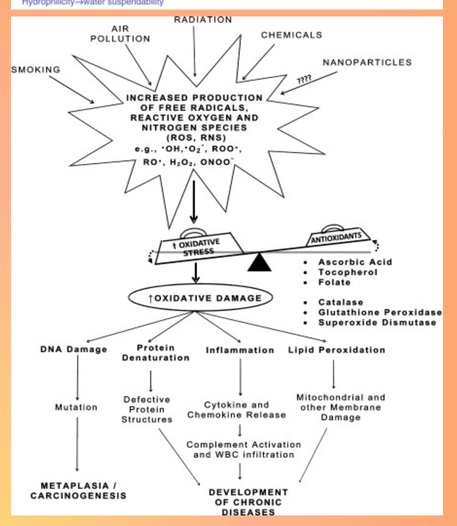




Nanoparticles - particle with one or more dimensions of the order of 100 (200) nm or less.

Relatively large surface and and the number of particles per unit mass - increased interactions between NPs and biological tissue compared to larger particles.





❖Toxicology of a variety of particles including environmental and manifactured nanoparticles shows a clear link between oxidative stress and diseases including asthma, cancer and cardiovascular disfunction.

❖It has been suggested oxidative stress is a suitable measure for comparing and discriminating the toxic effects of different nanoparticles.

❖Identifying molecular an biochemical mechanisms by which nanoparticles induce toxic effects oxidative stress-mediated and, consequently, design tests that can be used for predict nanoparticle toxicity, would allow scientists to generate new and safer nanoparticle knowing structure-toxicity information.

Results

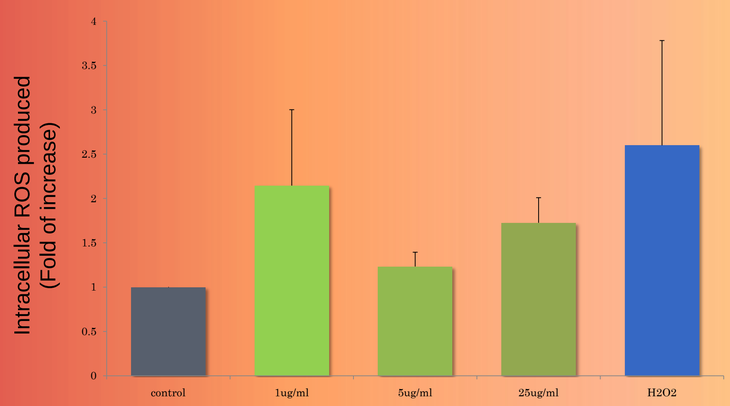


Fig. 1 Intracellular ROS production after treatment with Silver NPs. EUE cells were incubated for 40 min with 2μM cell permeable fluorophore dihydrodichlorofluorescein (DCFDH) before treatment with indicated concentration of silver NPs (30'). DCFDH is oxidized by ROS to dichlorofluorescein (DCF) which can be detected by fluorescence using an ex λ 488nm and em λ 510nm. 1mM H_2O_2 has been used as positive control.

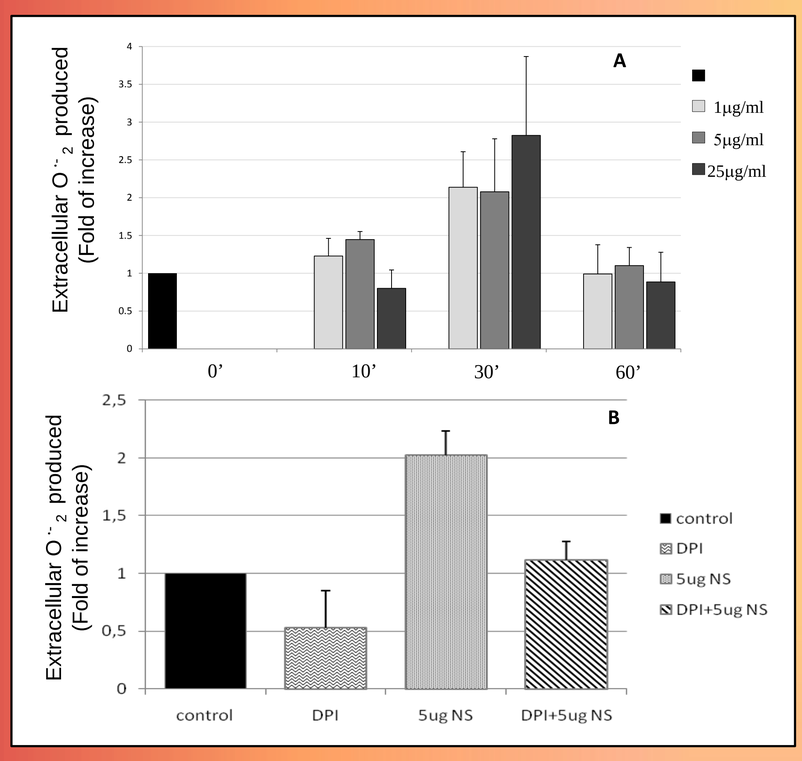


Fig. 2 Extracellular superoxide production after treatment with different concentration of NanoSilver. A) EUE cells were incubated for 30 min with 2.5mM nitrotetrazolium blue (NBT), a cell not permeable compound, before treatment with different concentrations of Silver nanoparticles. NBT reacts specifically with extracellular produced-superoxide resulting in the formation of insoluble blue formazan deposit. The insoluble blue formazan was solubilised by adding 2 M KOH, and dimethyl sulphoxide and measured at 630nm by spectrometry. **B)** Incubation with 2.5μM DPI, followed by the procedure described above, inhibited superoxide production showing that superoxide production silver-induced is completely dependent from NOX protein activation.

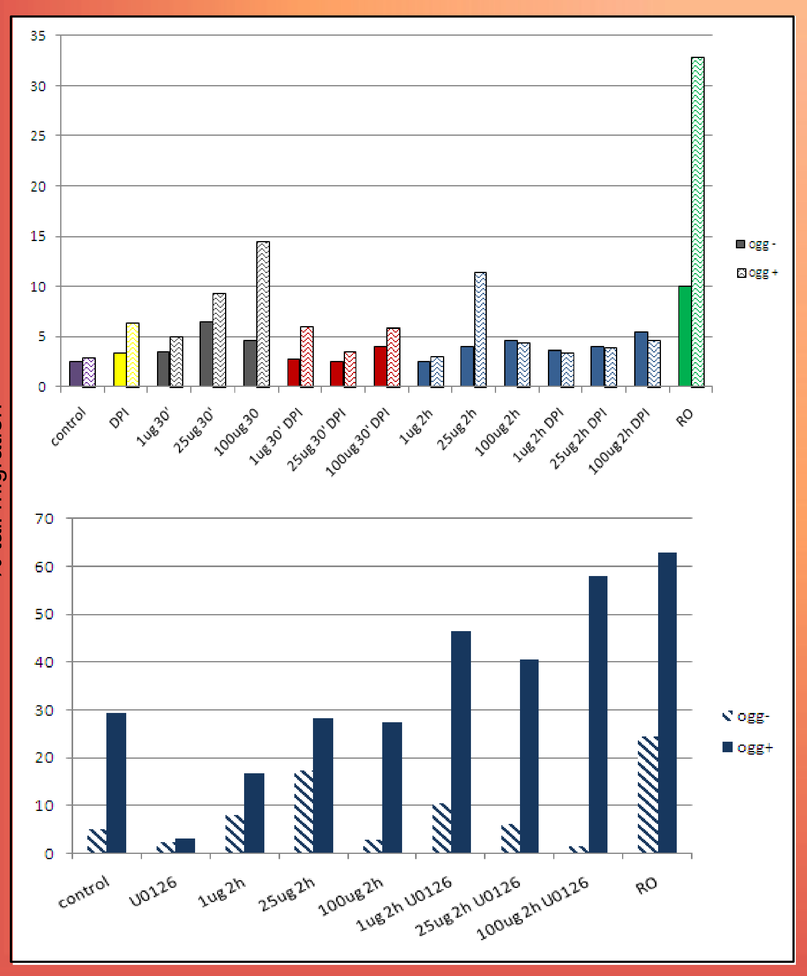
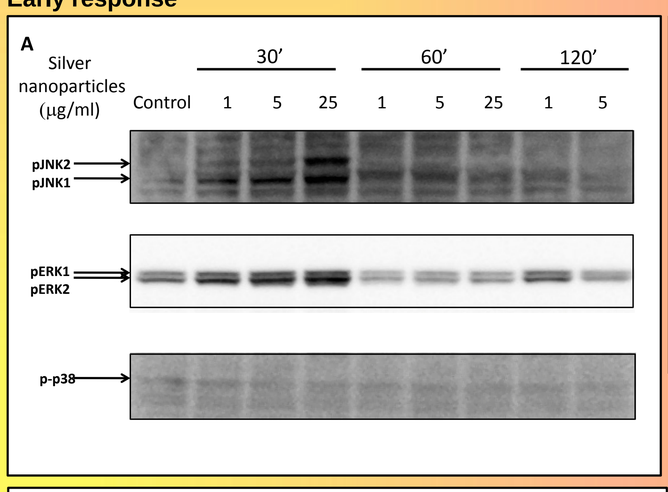
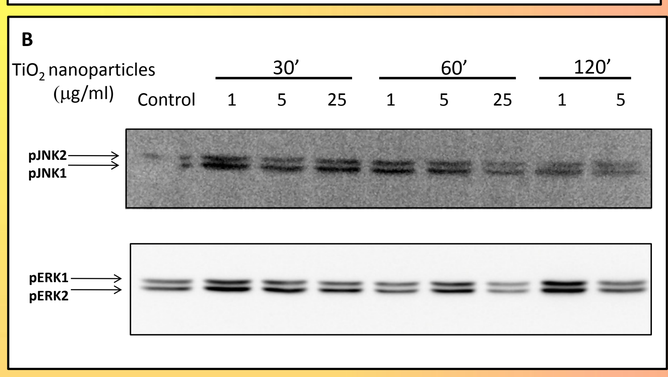


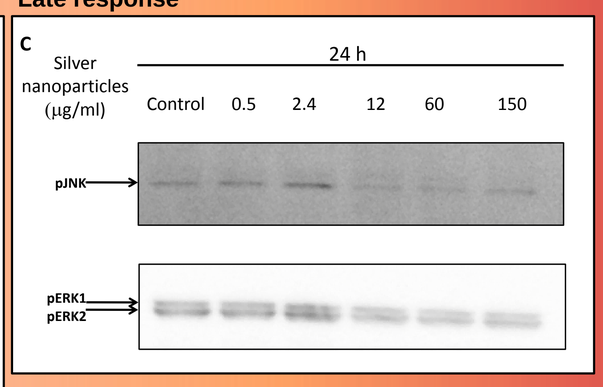
Fig 5 Oxidative DNA lesions induced by silver nanoparticles and role of ERK in the repair mechanism.
$1x10^5$ cells were seeded 24h before treatment with silver. After 24h the media was replaced and EUE, near confluence, were incubated either with 2.5 mM DPI or 10mM of U0126 followed by treatment with silver NPs for 30' or 2h. Cells were embedded in a low-melting-point agarose suspension, lysed and half of the samples were incubated for 30' with Ogg1 at 37 ˚C. Electrophoresis of the suspended lysed cells were performed followed by visual analysis with staining of DNA and calculating fluorescence to determine the extent of DNA damage by the Comet assay IV imaging software. Results are express in percentage of tail migration.

Early response





Late response



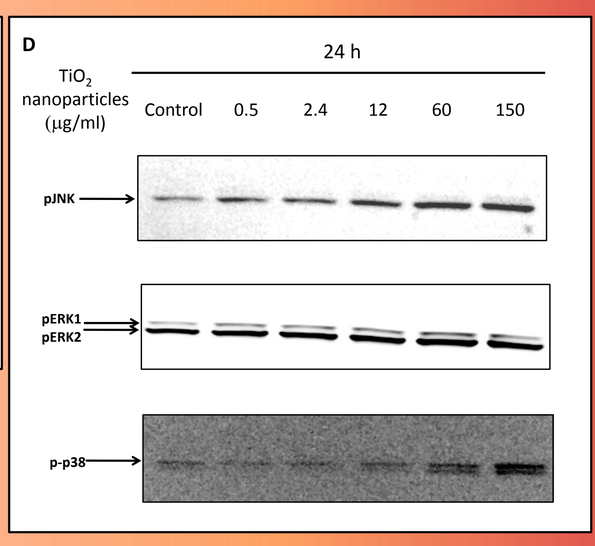


Fig. 3 Mapks activation upon treatment with Silver nanoparticles and TiO_2 Cells were seeded 24h before treatment with NPs. After 24h the media was replaced and EUE, near confluence, were treated with different concentration of NPs for the indicated time, and JNK, ERK and p38 phosphorylation levels were determined by western blot with the appropriate antibody (A-D).

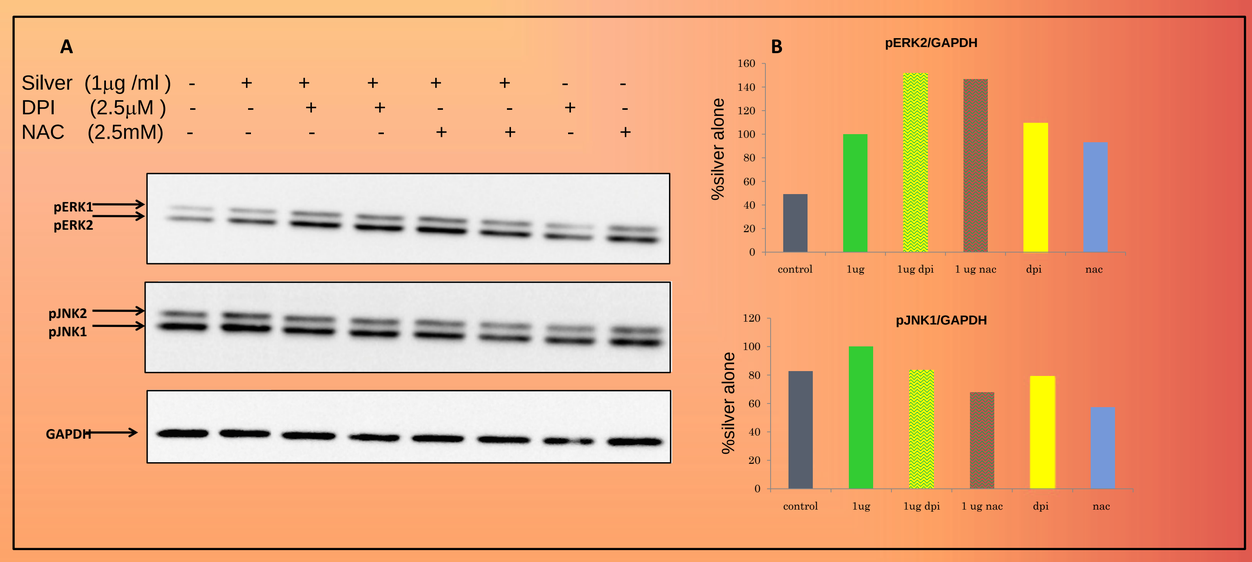


Fig. 4 ROS-dependent activation of JNK by Silver nanoparticles esposure. Cells were seeded 24h before treatment with silver. After 24h the media was replaced and EUE, near confluency, were incubated with DPI and NAC followed by treated with silver NPs for 30'. JNK and ERK phosphorylation levels were determined by western blot with the appropriate antibody (**A**). The bands of interest were imaged with and quantified by photon counting using the charged-coupled device camera of a Kodak Image Station 2000R and Kodak 1D 3.6 Image Analysis Software. Photon counting was used for creating the bar graph (**B**).

Conclusions

- Exposure to both NS and TiO_2 were able to induce ROS production in human embryonic epithelial EUE cells.
- Extracellular superoxide production was an early event after the exposure to silver nanoparticles in EUE and dependent to NOX activation.
- ERK and JNK activation was an early response but not sustained in time in nanosilver exposure. ROS generation resulted involved in JNK activation but not in ERK.
- In contrast JNK and p38 activation was sustained in time in TiO_2 exposure.
- Activation of different MAPKs might play an important role on the toxicity outcomes of nanoparticles and to understand this process may be helpful for the identification of nanoparticles toxicity biomarkers.
- Silver nanoparticles induced oxidative DNA lesions and superoxide plays a crucial role in such lesions.
- Protection against oxygen-induced DNA modifications induced by nanosilver occurred through an ERK activation-dependent pathway.

Supported by EC FP7 [Health-2007-1.3-4], Contract: 201335, EEA Research Fund, Contract: PNRF- 122- AI-1/07 and by EU FP7 [PEOPLE-2009-IEF], Contract: 252858.