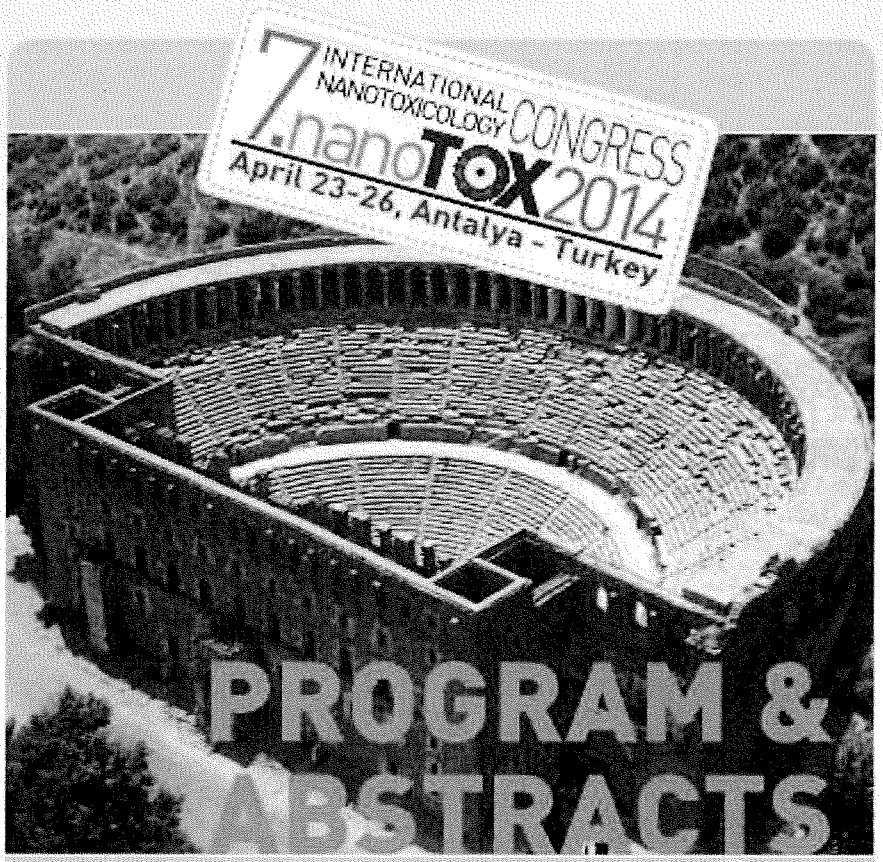

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TÜBİTAK

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Effects of Silver- and Gold Nanoparticles on the Retina – In Vitro and In Vivo

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PURPOSE: The goal is to explore the potential use of nanoparticles (NPs) as carriers in drug delivery of therapeutical agents to the retina. Nanomaterials are increasingly used in diagnostics, imaging and targeted drug delivery. In the clinic, Au-NPs are employed as e.g. anti-cancer agents and Ag-NPs are commonly used due to their antibacterial effects. Despite widespread use, the documentation is limited on the direct effect of Ag- and Au-NPs on eukaryotic cells, including human cells and the eye/retina. Here we investigate the uptake and distribution of especially Ag- and Au-NPs, as well as their possible toxic effect in a battery of assays ranging from human neural cells to the mouse eye in vivo.
METHODS: Low concentrations of Ag- and Au-NPs (0.022- 0.4 µg/ml) are studied at a cellular-, tissue and organ level, using a human neural stem cell line, organotypic cultures of the mouse retina and administration the mouse eye in vivo. Uptake and distribution of the NPs are analyzed using TEM. Using the human cells effects on viability (MTT and TUNEL assays), cell proliferation (Ki67 marker) and cell cycle analysis and phenotypic differentiation (GFAP (glial/neural) and DCX (neuronal) markers) were studied. In the retinal models adverse effects were studied using the parameters: gross morphology, glial- and microglial response, cytotoxic effects (apoptosis (TUNEL assay) and oxidative stress (AvidinD staining)). AgNO3 is used as a positive control for the reported toxic effect of Ag ions. Ag- and Au-NPs of two different sizes are included, 20 and 80 nm, respectively.
RESULTS: We demonstrate that 20 and 80 nm Ag- and Au-NPs are taken up into the cytoplasm, nucleus and mitochondria in the retina in vitro, using TEM analysis. Overall, adverse effects are caused by the NPs on the retina with changes in gross morphology and elevated glial- and microglial response. Moreover, neuronal toxicity (apoptosis and oxidative stress) was primarily seen in the outer nuclear layer, harboring the photoreceptors. In corresponding in vivo experiments preliminary results show no significant oxidative stress. Analysis is on-going revealing if the in vitro data can be extrapolated to the in vivo situation. Noteworthy, significant adverse effects on cell growth profile and morphology is documented and studies on the eventual effects on phenotypic differentiation are on-going.
CONCLUSIONS: In summary, our results strongly suggests that careful investigations is needed on the eventual adverse effects of Ag- and AuNPs if these are considered for usage in both daily consumer products and medicine.

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How differently charged nanoparticles affect the human lung epithelium: a comparative study in three different cell types

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At present a number of characteristics of nanoparticles (NPs) emerge to be crucial for the induction of cytotoxicity. One important factor appears to be surface charge. More data on the potential toxicity and the induction of oxidative stress (OS) via the production of reactive oxygen species (ROS) in cells relevant to human exposure is therefore needed. In order to assess cytotoxicity and ROS production mediated by the surface charge of NPs, three different lung epithelial cell systems were compared using a panel of differently charged NPs in the present study.
Au and Ag NPs with a size range of 7 to 10 nm were coated with either sodium citrate or different concentrations of chitosan, resulting in charge range from -50mV to +70 mV. Cytotoxicity was measured by means of the CTB and LDH assay, whereas cellular ROS production was measured using the DCFH-DA assay. Three different lung epithelial cell types were examined in this study, the A549 and BEAS-2B cell lines and primary lung epithelial cells (NHBE cells). To further understand the NPs' behaviour, cell-free ROS production was measured. In order to model normal and high exposure situations, different doses of NPs were used for exposure.
There were no apparent cytotoxic effects after exposure to negatively or low positively charged silver or gold NPs. However, an increase in surface charge to +65- 70 mV resulted in a large increase in cytotoxicity, especially at high particle concentrations. This trend was also observed in

respect to cell-mediated ROS production induced by the NPs. Furthermore, cell-free ROS measurements showed that the higher the surface charge the more ROS is produced by the particles. When NPs were incubated in different cell culture media, their surface charge changed and led to an abrogation of ROS production in some conditions. In contrast, using the same medium in cell culture resulted in ROS production, which was thus derived from the cells.
In conclusion, the study shows that the surface charge plays an important role in cytotoxicity and induction of OS by the NPs. The data of this study suggests a safety threshold in respect to surface charge after which NPs, irrespective of their core, become cytotoxic and produce higher amounts of ROS. Understanding this threshold could lead to safer NPs and consumer products.

P147
Multiwall carbon nanotubes promote pulmonary fibrosis through activation of TGF-β/Smad signaling pathway

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Multiwall carbon nanotubes (MWCNTs) have been widely used in many disciplines due to their unique physical and chemical properties, but have also raised great concerns about their possible negative health impacts, especially through occupational exposure. Although recent studies have demonstrated that MWCNTs induce granuloma formation and/or fibrotic responses in the lungs of rats or mice, their cellular and molecular mechanisms remain largely unaddressed. Here, it is reported that the TGF-β/Smad signaling pathway can be activated by MWCNTs and play a critical role in MWCNT-induced pulmonary fibrosis. Firstly, in vivo data show that spontaneously hypertensive (SH) rats administered long MWCNTs (20-50 μm) but not short MWCNTs (0.5-2 μm) exhibit increased fibroblast proliferation, collagen deposition and granuloma formation in lung tissue. Secondly, the in vivo experiments also indicate that only long MWCNTs can significantly activate macrophages and increase the production of transforming growth factor (TGF)-β1, which induces the phosphorylation of Smad2 and then the expression of collagen I/III and extracellular matrix (ECM) protease inhibitors in lung tissues. Thirdly, MWCNTs can also activate fibroblasts in vitro through activation of TGF-β/Smad signaling pathway. Finally, the present in vitro studies further demonstrate that the TGF-β/Smad signaling pathway is indeed necessary for the expression of collagen III in fibroblast cells. Together, these data demonstrate that MWCNTs stimulate pulmonary fibrotic responses such as fibroblast proliferation and collagen deposition in a TGF-β/Smad-dependent manner. These observations also suggest that tube length acts as an important factor in MWCNT-induced macrophage activation and subsequent TGF-β1 secretion. These in vivo and in vitro studies further highlight the potential adverse health effects that may occur following MWCNT exposure and provide a better understanding of the cellular and molecular mechanisms by which MWCNTs induce pulmonary fibrotic reactions.

P148
Kinetic Studies of Apoptosis on Single Cell Arrays in High-Througput

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Dynamics of molecular processes in living cells appears to be heterogeneous at the single-cell level. Hence time-lapse microscopy becomes increasingly important as it allows measurement of e.g. cell fate decisions and cellular responses in general. Nanoparticles show cytotoxicity in many circumstances. Yet the signaling pathway is not known. In order to resolve the pathway we developed a high-throughput single cell platform that allows to follow the time course of several markers in thousand of cells in parallel. Events like lysosomal break, loss of mitochondrial outer membrane permeabilization, increase of ROS level, exposure of phosphatidylserine to the outer membrane and loss of membrane integrity can be monitored simultaneously.

P149
Comparing the effects of multi-walled carbon nanotubes, asbestos and glass wool on mitochondrial function and mitochondria-related gene expression in human lung cells

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The application of multi-walled carbon nanotubes (MWCNTs) in nanotechnology has rapidly been developing during the last decades. Multiple in vivo and in vitro studies have indicated that certain types of MWCNTs induce asbestos-like