1520 Role of Scavenger Receptors in Silica Nanoparticle-Induced Cytokine Formation in Bronchial Epithelial Cells

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A major challenge in nanoparticle (NP) research is to elucidate critical initial targets that mediate NP signalling events which lead to cytotoxicity and inflammation. The aim of the present study was to elucidate the roles of scavenger receptors (SRs) in silica (Si)-NP-induced cytokine responses. The human bronchial epithelial cell line, BEAS-2B, was used, grown in LHC-9-medium and substituted with DMEM/F12-medium 1 day prior to SiNP (0-200 µg/ml) exposure. The cells were exposed with SiNPs of 10 nm (Si10) and 50 nm (Si50) sizes. The SiNPs were characterized with respect to size (by transmission electron microscopy), surface area and chemical composition, and dynamic light scattering (DLS) in exposure media. The release of CXCL8 and IL-6, and the growth factor TGFα, were analyzed by ELISA. The expressions of the scavenger receptors; SR-B1, LOX-1 and CXCL16, were determined by real time PCR before or after gene silencing with siRNAs. The phosphorylation of the signaling proteins (MAPK p38 and NF-κB) were analysed by Western blotting. The roles of the respective SRs in cytokine responses were examined by using siRNA and a chemical SR-B1-inhibitor, BLT-1(block lipid transport). Cells exposed to the SiNPs, showed marked cytokine responses (CXCL8, IL-6 and TGFα) after 20 h exposure, with Si10 as most potent. The BEAS-2B cells showed expression of SR-B1, LOX-1 and CXCL16, which was substantially knocked down by the siRNAs. Notably, siRNA against SR-B1, partially reduced all the SiNP-induced cytokine responses. The siRNA against the scavenger receptors LOX-1 and CXCL16 also reduced the SiNP-induced cytokine responses, but to a less extent than siRNA against SR-B1. BLT-1 partially reduced the levels of IL-6 and CXCL8 induced by SiNP. Furthermore, siRNA against SR-B1 in Si10-exposed cells, did not affect p38- and p65 (NF-κB)-phosphorylation. In conclusion, our data show that the scavenger receptor SR-B1 seems to have an important role in mediating IL-6 and CXCL8 responses by SiNPs of different sizes in BEAS-2B cells. Notably, SR-B1 seems to act via TACE-linked EGF-R activation, but not involving p38- and presumably not NFkB pathways. To which extent SR-B1 mediates the effects of other types of NPs, and whether this is a direct or indirect effect, needs to be examined, in addition to the relationship between NP structure and effect

1521 Amorphous Silica Coating Potentially Protects against Iron Homeostasis Disruption Induced by Nano-Iron Oxide in Acute and Sub-Chronic *In Vitro* Exposure Models

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Nano-scaled iron oxide (nFe_2O_3) has unique physicochemical properties which allow for its use in a wide range of applications, including advanced drug delivery systems and environmental catalysts. However, the existing literature is conflicting as to this particle's ability to induce adverse effects via inhalation - a particular concern for those exposed in an occupational setting. Our previous work showed that a continuous delivered dose (0.18 µg/cm^2) of nFe_2O_3 induced a neoplastic-like transformation in human primary small airway epithelial cells after 10 weeks exposure, with evidence suggesting that this transformation is related to disruption of iron homeostasis in the exposed cells. Herein, we utilized a sub-chronic exposure model to investigate the potentially protective qualities of an amorphous nano-silica ($nSiO_2$) coating on nFe_2O_3-induced bio-effects using a human lung bronchial epithelial cell line. Starting at 2.5 months of continuous exposure (administered dose of 0.6 µg/cm^2), nFe_2O_3 treated cells displayed significantly elevated mitochondrial activity and reactive oxygen species (ROS) generation, as compared to non-treated controls, and this activity became dramatically heightened starting at 4 months of exposure. Cells sub-chronically exposed to $nSiO_2$-nFe_2O_3, however, showed no significant mitochondrial activity or ROS generation increase compared to non-treated cells. Furthermore, starting at 3 months continuous exposure, nFe_2O_3 exposed cells possessed elevated intracellular iron levels compared to both $nSiO_2$-nFe_2O_3 treated and non-treated cells, indicating that $nSiO_2$ -coating has the potential to reduce or prevent particle solubilization, thus reducing iron homeostasis overload and subsequent adverse outcomes. Overall, our data suggests that $nSiO_2$ coating has the potential to protect against nFe_2O_3-induced iron homeostasis disruption and subsequent cellular transformation, supporting a strategy for safe-by-design nanoparticle manufacturing.

1522 Effect of Tin Dioxide Nanoparticles on Cell Viability, Morphology, Size, Granularity and Migration in Lung Epithelial Cells

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Engineered nanomaterials (EMN) are widely used for biomedical and industrial applications, which has highlighted the warning of possible side effects, specifically by inhalation in environmental settings. Some of the most produced ENM belong to the metal oxide nanoparticles category and they have oxidative and pro-inflammatory properties but also can induce cell death after internalization. Tin dioxide nanoparticles (SnO_2-NPs) is one of the newest metal oxide nanoparticles which are nowadays under research, specifically to produce nanocomposites, however, the toxicity studies of tin dioxide are limited and there is only information about possible cytotoxicity on a hepatocellular carcinoma cell line. For this reason, in this study we evaluated the effect of SnO_2-NPs exposure on lung epithelial cells, which can be the primary target by inhalation. SnO_2-NPs were characterized by scanning electron microscope and cell cultures were exposed to 1, 10 and 50 µg/cm^2 of SnO_2-NPs for 24 h. Then, the morphology, the cell viability, the size, granularity and cell migration was evaluated and results were analyzed by non-parametric test (>0.05) using GraphPad Prim 6. The primary size of SnO_2-NPs was <100 nm of diameter and cell cultures showed nanoparticles internalized but morphology, cell viability, cell size, granularity and cell migration were unaffected. Exposure to SnO_2-NPs (1, 10 and 50 µg/cm^2) had no evident cytotoxicity effects on lung epithelial cells after 24 h. These results highlight the evidence that toxicity of each nanoparticle must be analyzed independently, since some of the evidence of toxicity of some other metal oxide nanoparticles cannot predict the toxicity of tin dioxide.

1523 Effect of Substrate Stiffness and Topography on Nanoparticle-Induced Toxicity and Endocytosis

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Substrate mechanics and topography play a major role in lung health and pathophysiology. Exposure to nanoscale particles by inhalation have been shown to induce acute and chronic toxicity effects often causing a significant inflammatory response. Continued exposure producing damage to the lung tissue can result in a chronic conditions including excessive matrix deposition, cellular proliferation, and disorganized remodeling of the extracellular matrix leading to pathological microstructures and increased tissue moduli. Several studies have proven the importance of substrate modulus, but none have investigated the effect substrate mechanics and topography have on nanoparticle induced toxicity and up-take. In this study Sylgard 184 and 527 polydimethylsiloxane (PDMS), a biocompatible elastomeric polymer, were used to create cell substrates ranging in modulus from 5 - 5,000 kPa simulating a wide range of physiological tissue stiffness. In addition, electrospun biomimetic nanofibers were fabricated from polycaprolactone (PCL) and employed as a physiological microstructure functioning as topographical substrates. Combinations of soft vs. stiff and nanofiber covered engineered substrates were utilized to evaluate the cytotoxicity, endocytosis, and cell morphology in response to 30 nm Zinc Oxide nanoparticles. Caspase 3/7, lactase dehydrogenase (LDH), and an Alamar Blue viability assay were conducted to determine cytotoxicity. Dark-field microscopy and inductively coupled-mass spectrometry was conducted to evaluate the up-take of nanoparticles on the different engineered substrates. The soft ~1-5 kPa and stiff 5,000 kPa substrates displayed the most sensitivity to nanoparticle exposures, resulting in 40-50% loss of viability. The substrates ranging in moduli values from 50 kPa - 500 kPa displayed statistically reduced apoptosis, LDH release, and reduction of viability, with 23 ± 4% less nanoparticle uptake. Future analysis will evaluate the feasibility of using substrate modulus and topography to create simulated acute vs. chronic lung models for toxicological assessment of nanoscale particles.


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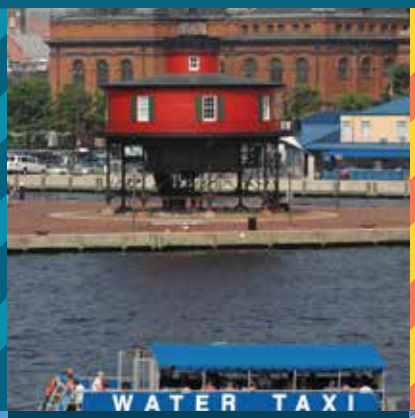

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