trations, MBAuNPs may induce inflammation and cellular proliferation, undesirable effects on any AuNPs that are intended for medical use. Long-term exposure studies with MBAuNPs need to be conducted to determine whether these effects are only transitory or if they induce long-lasting consequences on cells.

1516 An Integrated Approach for Assessing the Inhalation Toxicity of Nanomaterials

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Inhalation is the most prominent means of exposure to manufactured nanomaterials (NMs). While the current regulatory requirement for substances of concern in many jurisdictions is a 90-day rodent inhalation study, there are monetary, ethical, and scientific concerns associated with this test. Therefore, non-animal approaches are being sought to assess the hazard associated with these NMs. One such approach has been developed to assess the potential of multi-walled carbon nanotubes (MWCNTs) to cause pulmonary fibrosis, a critical adverse outcome linked to prolonged NM exposure. Relevant human lung cells are exposed to MWCNTs at suspension or at the air-liquid interface (ALI) while considering human-relevant dosimetry. Mono- and co-cultures of human cell lines–including alveolar epithelial cells (A549), fibroblasts (MRC-5), and macrophages (THP-1)–were exposed to two types of MWCNTs (Mitsui 7s and Nanocyl) at different concentrations (5, 10, and 20 µg/ml for suspension and 2-10 µg/cm^2 for ALI exposures) to assess the pro-fibrotic response. The results from the suspension experiments with mono-cultures demonstrate that acute (24 h) or prolonged (96 h) exposure to different concentrations of Mitsui-7 do not induce any cytotoxicity (assessed by lactate dehydrogenase release), however, an induction of pro-inflammatory (assessed by interleukin (IL)-8, tumor necrosis factor-alpha, and IL-1 beta levels) and pro-fibrotic response (assessed by osteopontin levels) was observed following the prolonged exposure of 96 h which might indicate the onset of pulmonary fibrosis. ALI exposures also revealed that prolonged exposures are more suitable to predict pro-fibrotic effects as demonstrated by the slight increase in osteopontin levels in the basal medium. This work will be complemented by comparative studies using a reconstructed primary human alveolar tissue model (EpiAlveolar, MatTek Corp). When combined with other *in vitro* and *in silico* methods in an integrated approach, this system could be used to predict pulmonary toxicity and to enable effective risk assessment of substances including MWCNTs.

1517 Signaling Pathways Involved in Nanosilica-Induced Chemokine Responses in Different Human Epithelial Lung Cells

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Non-crystalline (amorphous) silica particles of submicro- and nanosize are produced in large amounts with a wide range of applications for consumers, and also with potential medical applications. Different particle sizes of the same material may have different properties and induce toxicity via separate mechanisms. We have previously shown a role for EGF-receptors as silica nanoparticles (SiNPs) of 50 nm size (Si50) induced marked cytokine responses via a TACE/TGF-α/EGFR-cascade, acting in concert with the classical NF-κB pathway in the bronchial epithelial cell line, BEAS-2B. The aims of this study were to investigate and compare the acute chemokine responses of Si50 with a smaller SiNP (10 nm; Si10) in both BEAS-2B cells and another bronchial cell line, HBEC, and to determine the role of signaling pathways in these two different epithelial cell lines. BEAS-2B and HBEC were grown in LHC-9-medium and substituted with DMEM/F12-medium 1 day prior to particle exposure. The cells were exposed with SiNPs of two sizes Si10 and Si50. 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 expression and release of the chemokine CXCL8 were analyzed by real time PCR and ELISA. Involvement of signaling proteins were studied by using chemical inhibitors, gene silencing (siRNA) together with activation/ phosphorylation of the signaling proteins (MAPKs and NF-κB) by Western blotting. The pro-inflammatory responses and cytotoxicity varied between the two SiNPs. The expressions and releases of CXCL8 were much higher for Si10 than for Si50. The chemokine responses were similar in the two epithelial lung cell cultures. For both SiNPs the CXCL8 responses seemed to be mediated partly through epidermal growth factor receptor (EGFR) and the MAPKs p38 and JNK. Thus, Si10 and Si50 are suggested to mediate chemokine responses by the same signaling proteins, but to different extent. In conclusion, particle sizes together with other physical/ chemical characteristics seem important for the chemokine responses in bronchial epithelial cell lines.

1518 Impact of Long-Term Exposure to Nanocellulose Materials on the Morphological Transformation of Human Lung Epithelial Cells

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The wide use of nanocellulose (NC) materials in industrial applications requires the investigation of their effects on human health, especially their long-term effects that have not been addressed. In this context, the aim of the present study was to evaluate the potential carcinogenic effect of cellulose nanocrystals (CNC) - one of the major forms of NC, using epithelial cells of pulmonary origin (BEAS-2B and A549). We demonstrated here that prolonged exposure (4 weeks) of the cells to occupationally relevant sub-toxic concentration (30 µg/cm^2) of two forms of CNC (powder and gel) derived from wood, enhanced neoplastic-like transformation. It is evidenced by increased cell proliferation, anchorage-independent growth, migration and invasion. A strong activation of cells resulted in the high number of cytoplasmic vacuoles, finger-like protrusions, lipid droplets and multi-nucleation. Additionally, a significant depletion of antioxidants, accumulation of oxidatively modified proteins and an increase in lipid peroxidation products were found in the cells exposed to CNC. Apoptosis analysis displayed no effect in A549 and inhibitory effect of CNC in BEAS-2B cells. Furthermore, the signaling mechanisms of the cells were precisely defined by their cytokine responses. The increased proliferation was synergistic and mediated by both pro-inflammatory and pro-carcinogenic cytokines. Overall, our results provide new evidence to suggest the potential carcinogenic effect of CNC.

1519 Utilizing the Asialoglycoprotein Receptor to Produce Targeted Hepatocellular Toxicity through Magnetically Mediated Energy Delivery Using Iron Oxide Nanoparticles

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Iron oxide nanoparticles (IONPs) have shown promise for use in cancer therapies by producing selective toxicity in cancer cells. Selective toxicity is achieved through targeted uptake of IONPs by cancer cells and delivery of energy with an alternating magnetic field (AMF). The magnetic properties of superparamagnetic IONPs allow for magnetic mediated energy delivery, causing the activation of cell death pathways; however, the mechanism by which this occurs is unclear. One proposed mechanism is through permeabilization of lysosomes, releasing digestive enzymes and ROS. The goal of this research was to create stabile colloidal IONPs that are sequestered by lysosomes and induce cell death in human hepatocellular carcinoma (HCC) cells. This study utilizes the asialoglycoprotein receptor (ASGr) to target galactose modified IONPs to hepatocytes through receptor mediated endocytosis, which results in an increased IONP concentration in the lysosomes. IONPs were synthesized using a co-precipitation method and modified by using functional amines to bind to lactose, exposing a galactose saccharide. IONPs with capped amines and no terminal galactose were used as control particles with respect to receptor-mediated uptake. Transmission electron microscopy (TEM) showed the average core size to be 16.6±2.1 nm, and dynamic light scattering showed the average hydrodynamic radius to be 28.2±3.1 and 295.3±62.6 nm for capped and galactose modified particles, respectively. When IONPs in cell media were exposed to AMF (19.9 kA/m, 110.1 kHz) for 10 minutes, temperature increased 17.7°C for capped and 18.9°C for galactose modified IONPS, indicating that the functional coatings did not impair response to AMF. Using a human liver cancer cell line (Huh7), IONPs uptake increased by 2.1 fold utilizing the galactose targeting ligand. Increased uptake of IONPs was confirmed by ICP-MS. Increased IONP uptake was not observed in a human kidney cell line (HEK293) without ASGr, or when the ASGr competitive inhibitor α-lactose was added. TEM analysis showed that sequestering of IONPs occurred within the lysosome. These results demonstrate the ability to increase delivery of superparamagnetic particles to the lysosomes within liver cancer cells through the ASGr receptor. Future experiments will examine whether the cytotoxicity of these particles can be enhanced by delivery of energy through AMF.


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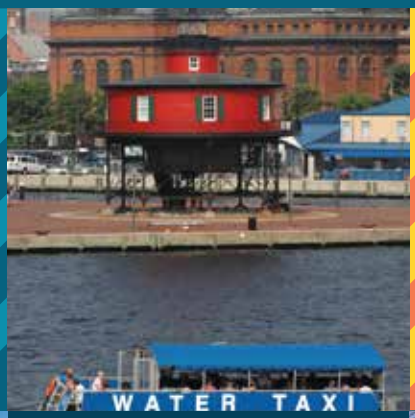

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