in the HEK after UVB exposure, but ferroptosis, a non-apoptotic programmed cell death promoted by excessive lipid peroxidation, plays a significant role in initiating UVB-induced inflammation in the skin. Using different cell death inhibitors (zVad-fmk, ferrosatatin-1 and necrostatin-1), apoptosis, ferroptosis and necroptosis in SFNPs alone or co-exposed with UVB keratinocytes were evaluated. **Results:** Exposure to both SFNPs used in the study induced dose-dependent loss of viability, cell damage, ROS accumulation, release of inflammatory mediators, increases in oxidative stress markers and DNA damage. Pre-exposure to UVB caused significant increase in observed responses. Based on the hierarchical clustering analysis of the inflammatory cytokine/chemokine responses, SFNPs pretreated with UVB alone were segregated from UVB control and no UVB exposed samples. IL-8 and RANTES were upregulated in response to SFNPs exposure alone while IL-1β, IL-6 and TNF-α were significantly increased with UVB pre-treatment. Moreover, we have found that all three inhibitors of apoptosis, ferroptosis and necroptosis could prevent cell damage when exposed to SFNPs alone in this study. However, zVad-fmk (zVad), pan-caspase inhibitor, demonstrated the strongest preventive effect when cells were pre-treated by UVB prior to SFNPs exposure. Ferrosatatin-1 (Fer-1) or necrostatin-1 (Necr-1), ferroptosis and necroptosis inhibitors, also rescued a significant percentage of HEK. Further, HEK exposure to SFNPs alone led to generation of lipid peroxidation products (4-hydroxynonenal, 4-HNE), accumulation of intracellular ROS and depletion of glutathione which were inhibited by Fer-1 and zVad but not by Necr-1. UVB co-exposure significantly intensified the effect of SFNPs inhibited by Fer -1 or zVad only. **Conclusions:** Overall, our data indicate that SFNPs alone or combined with UVB induced oxidative stress, release of inflammatory mediators and DNA damage. Additionally, our results show that following exposure to SFNPs alone or combined with UVB, majority of damaged HEK undergo either caspase- or lipid peroxidation-dependent type of regulated cell death, thus suggesting the involvement of apoptotic and ferroptotic mechanisms. Additional studies are required to better understand the precise molecular mechanisms of programmed cell death induced by SFNPs alone or co-exposed to UVB.

PS 3660 *In Vitro* Toxicity Evaluation of Wood Dust Released from Sanding Wood with and Without a Wood Sealant Containing Zinc Oxide Nanoparticles

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Background and Purpose: Nanotechnology enabled products are currently being deployed in the construction industry to improve durability, strength, and life span. One such application that is finding widespread usage is the incorporation of zinc oxide (ZnO) into sealants to enhance the durability of wood and reduce use of harmful chemicals. The International Agency for Research on Cancer has classified wood dust as a Group 1 carcinogen and is known to cause cancer in the nasal cavity, paranasal sinuses, and nasopharynx. ZnO nanoparticles are known to cause pulmonary irritation, metal fume fever and systemic toxicity. We Investigated whether coating wood with a sealant containing ZnO alters the release characteristics of wood dust particulates during product handling and if ZnO nanoparticle coating amplified wood dust toxicity. **Methods:** Particles were collected from three sanding operations performed by a tradesperson using a random orbital sander on a plywood board, with and without a ZnO-sealant coating. To further determine the influence of size fraction on the toxicity profile, the collected dust was separated into PM_5 and > PM_5 particulate using a custom dust separation system equipped with a cyclone separator and an acoustical aerosol generator. The dose-response toxicity of the four-wood dust particulate (PM_5 Wood Dust, PM_5 Zinc Wood Dust, >PM_5 Wood Dust and >PM_5 Zinc Wood Dust) was evaluated using human bronchial epithelial cells (BEAS-2B) and differentiated human macrophages (THP-1) over a wide dose range of 0–156.25 μg/cm^2 or 0-500 μg/ml. **Results:** Scanning mobility particle sizer measurements during the sanding operation showed highest differential particle number concentrations in the size range below 100 nm. Sanding treated wood did not release excess particle number compared to untreated wood. A Microorifice Uniform Deposit Impactor was used to monitor the aerodynamic size of the collected particles and found 96.8% of the particles to be ≤ 10μm and 75% of the particulate ≤ 5.6 μm. Electron microscopy revealed that the collected wood dust particles were in the micron size range and had an irregular morphology. The presence of zinc on the surface of wood dust was confirmed by energy-dispersive X-ray spectroscopy. Dynamic light scattering showed that both types of PM_5 particulates had an average hydrodynamic aggregate diameter of 650 ± 400 nm and no significant alteration with the coating. The uptake of both respirable wood dusts was confirmed in both cell types using transmission electron microscopy. The ferric reducing ability of the serum assay showed PM_5 particulates to be more reactive than > PM_5 particulates, but there was no change in reactivity between the zinc-coated and uncoated wood dust. Cytotoxicity in the epithelial cells did not alter with the zinc sealant coating compared to uncoated wood dust and the IC50 of PM_5 Wood Dust, PM_5 Zinc Wood Dust, >PM_5 Wood Dust and >PM_5 Zinc Wood Dust particulate is 7.1 (3-11), 7.5 (4 -11), 54.5 (12-97), 41.4 (21-62) μg/cm^2, respectively. PM_5 wood dust was 5-6-fold more toxic than > PM_5 particulates. Membrane damage measured by lactate dehydrogenase (LDH) release showed a similar trend with PM_5 particulates showing greater membrane damage compared to > PM_5 particulates and no alteration due to coating. The differentiated macrophages were less sensitive than the epithelial cells but showed similar responses in cytotoxicity and membrane damage. **Conclusions:** Overall, the PM_5 wood dust samples had greater toxicity than the larger particulate sample. Coating wood with a ZnO containing sealant did not change the toxicity of the wood dust *in vitro* in either of the size fractions.

PS 3661 Aerosolized nano-zinc oxide (nZnO) exposure alters the cellular composition of the fully differentiated human airway epithelium

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Background and Purpose: Engineered nanomaterials such as metal-oxide nanoparticles (NP) are widely used in numerous industrial and daily use applications, which makes their exposure a common occupational hazard. Nano-zinc oxide particles (nZnO) are used in many industries such as food packaging, rubber, textiles, and leather. Exposure leads to pulmonary inflammation and oxidative stress, which may result in development of lung disease or worsening of pre-existing lung disease. The adult human airway epithelium is comprised of four major cell types, basal, club, goblet, and ciliated cells. Any change in the numbers of these cell types is termed as 'airway remodeling', which is a disease-related phenotype in several lung diseases. Thus, the aim of this study was to investigate if aerosolized nZnO exposure leads to changes in the cellular composition of the airway epithelium, using air-liquid interface (ALI)-differentiated primary human bronchial epithelial cells. **Methods:** Normal human bronchial epithelial cells (NHBEpC) (at ≥ 90% viability) were seeded on 12-well porous 0.4 μm- cell culture inserts in basal cell culture medium. On the next day, the media from the apical and basal chambers was removed, and 1 mL complete ALI Medium containing necessary supplements was added to the basal chamber. On the same day, the cells in the apical chamber were exposed to air (airlift) to create the ALI and allowed to differentiate for 28 days. Optimal differentiation was verified by qPCR and immunofluorescent (IF) staining of cell-type specific markers. Stock suspensions of nZnO were sonicated to produce a stable suspension and characterized for hydrodynamic diameter using dynamic light scattering (DLS) analysis. Fully differentiated cells were exposed to aerosolized nZnO for 6 minutes, at the indicated doses, using the VitroCell® Cloud Alpha 12 system. Deposited dose was measured using a quartz crystal microbalance (QCM) integrated in the VitroCell® system. Transmission electron microscopy (TEM) was used to demonstrate dispersion of aerosolized NP on the inserts using TEM grids. Cells were harvested at 24 hours or 7 days post exposure, to investigate changes in (a) inflammation, (b) oxidative stress-related, and (c) cell-type specific markers using qPCR and/or IF staining. Cellular toxicity was quantified by measuring lactate dehydrogenase (LDH) activity in the 'apical wash' and basolateral chamber media. **Results:** qPCR analysis of the differentiation time-course shows a steady increase in markers of terminally differentiated cells, such as club (SCGB1A1), goblet (MUC5AC) and ciliated cells (FoxJ1). The basal cell (stem/progenitor cell) marker (KRT5) shows an initial increase in expression, but did not vary much during differentiation. The presence of all four major cell types at protein level was confirmed by IF staining of the fully differentiated airway epithelium. DLS analysis showed that nZnO particles suspended in water (vehicle), had a mean hydrodynamic size (Z avg) of 236.6 ± 18.8 (nm±SD), and a Polydispersity index of 0.27 ± 0.03 (PdI±SD), suggesting a constant uniform dispersion. Cellular toxicity analysis showed that the higher dose of nZnO (Mean ± SD) 4.25 ± 0.41 μg/cm^2 induced a significant increase in LDH activity (in apical wash and basolateral chamber media) at both 24 hours and 7 days' time points, as compared to the low dose nZnO (0.514 ± 0.09 μg/cm^2), and vehicle control. Exposure to high dose of nZnO induced a significant decrease in expression of club (SCGB1A1), goblet (MUC5AC) and ciliated cells (FoxJ1) markers at mRNA levels. This effect was observed at both 24 hours and 7 days' time points, suggesting an early blockage in the cellular differentiation process, which requires further evaluation. In parallel, the expression of basal cell marker(KRT5) was partially modulated by nZnO exposure. Since these cells reside at the bottom of the pseudostratified airway epithelium, it's possible that they were not directly impacted by nZnO exposure, at the doses/time points used. Mechanistically, we found that expression of oxidative stress related gene, heme-oxygenase-1 (HMOX-1), was significantly elevated in nZnO-exposed cells at both doses and time points. Additionally, nZnO exposure induced a significant increase in expression of interleukin-1β, a pro-inflammatory cytokine. **Conclusions:** These results suggest that acute exposure to aerosolized nZnO could alter the cellular composition of the adult airway epithelium, possibly via oxidative stress and inflammation-related mechanisms. Further studies are underway to decipher the specific mechanisms which might regulate nZnO-mediated changes in the numbers of different cell types of the adult airway epithelium, airway remodeling, and the potential for lung disease development upon chronic exposure.

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