3657 Proteinase-Activated Receptor-2 Regulates the Production of Fibrotic Mediators by Murine Bone Marrow-Derived Macrophages, *Ex Vivo* Alveolar Macrophages, and Mouse Lung Fibroblasts in Response to Multiwalled Carbon Nanotubes in an Asthma-Like Microenvironment

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Background and Purpose: Proteinase-activated receptor 2 (PAR2), a 7-transmembrane G protein-coupled receptor, is implicated in immune regulation and the pathogenesis of various inflammatory diseases, including pulmonary fibrosis and asthma. PAR2 is activated by proteolytic cleavage of the N terminus by serine proteases, including those found in house dust mite (HDM) extract. We previously reported that co-exposure to HDM extract and multiwalled carbon nanotubes (MWCNTs) synergistically enhanced allergic lung inflammation in mice and that *Par2-/-* mice had reduced airway fibrosis and arginase-1 (ARG-1) expression compared to wildtype (WT) mice. Additionally, ARG-1 was highly expressed in lung macrophages after co-exposure to HDM extract and MWCNTs. In the current study, we utilized bone marrow-derived macrophages (BMDM), murine *ex-vivo* alveolar macrophages (mexAMs), and mouse lung fibroblasts (MLF) isolated from WT or *Par2*-deficient (*Par2-/-*) mice to investigate the dynamic phenotype and functional changes of these cells exposed to MWCNTs in an asthma-like microenvironment consisting of the T_H2 cytokines IL-4 and IL-13. We hypothesized that PAR2 regulates MWCNT-induced macrophage differentiation to an M2 phenotype by regulating arginase-1. **Methods:** Wildtype (WT) C57BL/6 mice and PAR2-deficient (*Par2 -/-*) mice were used to isolate murine *ex-vivo* alveolar macrophages (mexAM), bone marrow-derived macrophages (BMDM), and mouse lung fibroblasts (MLF). MexAMs were generated by obtaining alveolar macrophages from mice by bronchoalveolar lavage and then cultured in complete culture media supplemented with recombinant human TGF-β1, murine GM-CSF, and rosiglitazone. The remaining lung tissue was proteolytically digested, strained, and used to isolate MLFs. BMDMs were obtained from the bone marrow of the hind legs of mice. Bone marrow was flushed from the femur and tibia and filtered. The resulting cell mixture was cultured in L929-conditioned media for 7 days. Mature cells were treated with MWCNTs, IL-4 and IL-13, or both. Recombinant murine IL-4 and IL-13 were used to simulate an asthma-like microenvironment. After cell treatments, samples were collected for immunoblotting, qRT-PCR, cytokine analysis, and flow cytometry. *In vivo* exposure experiments followed a 21-day protocol consisting of three exposure sessions in the sensitization and challenge phases. WT mice were exposed by oropharyngeal aspiration to the following treatments: vehicle solution control, MWCNTs, HDM extract, or both. Following our 21-day treatment protocol mouse lungs were digested and used for flow cytometry analysis. **Results:** In both *Par2-/-* BMDMs and MLFs, the secretion of profibrotic cytokines osteopontin (OPN) and transforming growth factor-beta 1 (TGF-β1) was enhanced compared to WT cells, suggesting a role of PAR2 in regulating profibrotic mediators. Additionally, the expression of collagen mRNAs (*col1a1* and *col1a2*) was upregulated in *Par2-/-* MLFs compared to WT MLFs stimulated with TGF-β1. This further suggests that PAR2 plays a role in collagen production and pulmonary fibrosis. Interestingly, treatment with MWCNTs increased the M2 polarization marker CD206 in WT and *Par2-/-* BMDMs compared to media control groups. This result suggests that MWCNTs are promoting the polarization of macrophages into an M2 or profibrotic phenotype. These results are further supported by Western blot analysis of ARG-1 in BMDMs and mexAMs. In the presence of IL-4 and IL-13, MWCNTs exacerbated ARG-1 production in WT and *PAR2 -/-* cells compared to IL-4 and IL-13-induced ARG-1 production alone. However, this exacerbation of ARG-1 was more prominent in WT cells than *Par2-/-* cells. Flow cytometry analysis on whole digested mouse lungs showed increased expression of CD206 on interstitial and alveolar macrophages in WT mice treated with MWCNTs compared to WT vehicle control mice. Additionally, mice treated with MWCNTs exhibited increased eosinophil and neutrophil population numbers compared to the vehicle control group. **Conclusions:** These findings indicate that PAR2 regulates the production of fibrotic mediators by MWCNTs in macrophages and fibroblasts in a cell-type-specific manner. The data suggests that PAR2 suppresses the production of profibrotic mediators and collagen production in mouse lung fibroblasts. However, PAR2 appears to function differently in macrophages. In BMDMs and mexAMs PAR2 regulates the production of ARG-1, which suggests that PAR2 helps regulate macrophage differentiation in addition to fibrotic mediators. Furthermore, MWCNTs exacerbate the production of profibrotic mediators as well as macrophage differentiation in an asthma-like microenvironment. Taken together, the profibrotic effects of MWCNTs are in part due to their polarizing effects on macrophages as well as PAR2's role in regulating the fibrotic pathway.

3658 Gold Nanoparticles Disturb Mitochondrial Oxidative Phosphorylation Machinery at Low Doses

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Background and Purpose: Gold nanoparticles (AuNPs) have gained considerable attention in biomedical applications due to their unique properties, making them valuable for diagnostics, drug/gene delivery, imaging, and therapy. Despite their tremendous potential for diverse medical applications, the impact on AuNPs on biological systems, particularly at low doses under chronic conditions, is not well-understood. Our previous research demonstrated that a very low dose of AuNPs (0.1 nM) significantly impact gene pathways associated with mitochondria in human dermal fibroblasts (HDF). Mitochondria, the most studied organelle in biomedical sciences, are best-known for its role in oxidative phosphorylation (OXPHOS), a process where energy is harnessed through respiratory chain complexes and ATP synthase to create adeno-sine triphosphate (ATP), used as a source of chemical energy in cells. Loss in the OXPHOS efficiency can lead to mitochondria dysfunction, which is implicated in the pathophysiology of several diseases, such as cancer, neurodegenerative diseases, cardiovascular diseases, and metabolic disorders. Mitochondria dysfunction is the mode of toxicity of many chemicals, including drugs and environmental pollutants. Yet, the effects of AuNPs on the OXPHOS machinery, particularly in realistic low-dose scenarios, remain largely unexplored. Herein, we report the immediate effects of low doses of AuNPs with different shapes and surface chemistries on the OXPHOS system, providing insights into their biological effects in human cells, crucial to understanding the fundamentals of nanotoxicity. **Methods:** Spherical (AuNSs) and rods shaped AuNPs (AuNRs) were synthesized in our lab and their surface were modified with coatings largely applied for biomedical applications. Citrate-capped spherical AuNPs were produced using the Turkevich method, while gold nanorods were synthesized through a seed-mediated approach. Poly(acrylic acid) (PAA), poly(allylamine hydrochloride) (PAH), and poly(ethylene glycol) methyl thiol (PEG-SH) were used to coat both spheres and rods. All the colloidal systems obtained were well-characterized by UV-vis spectroscopy, dynamic light scattering (DLS), zeta potential in water and cell media, and transmission electron microscopy (TEM). HDF cells, primary cells, were exposed to varying concentrations of AuNPs (ranging from 0.1 nM to 2 nM). Cell viability was determined using trypan blue staining. The interaction of AuNPs with cells were investigated by Enhanced Darkfiled Hyperspectral Microscopy (CytoViva). The impact of AuNPs on the OXPHOS machinery was evaluated using the gold standard SeaHorse. Moreover, cellular reactive oxygen species (ROS) levels were quantified by measuring the fluorescence of the CellROX probe normalized by the protein count. **Results:** Among all particles tested, Citrate AuNSs (negatively charged) and PAA (negatively charged), PAH (positively charged) and PEG (neutral) AuNSs and AuNRs, only the PAH-coated AuNRs induced cell death, even at a concentration as low as 0.5 nM. Enhanced Darkfield Hyperspectral microscopy confirmed these particles' interaction with HDF cells. Interestingly, PEG-coated AuNSs exhibited less interaction with cells than the other particles. Distinct outcomes were revealed when the bioenergetic parameters of cells exposed to AuNPs (0.1 and 1nM) were analysed by SeaHorse: while citrate-coated AuNSs showed no impact on the OXPHOS machinery, PAA and PEG-coated AuNSs displayed a significant decrease in the basal and ATP-linked respiration. AuNRs presenting the different surface chemistries led to a significant reduction of basal and ATP-linked respiration with PAH-coated AuNRs significantly decreasing spare respiratory capacity. The assessment of cellular ROS indicated a substantial increase in ROS levels provoked by PEG-coated AuNS and PAH-coated AuNRs in HDF cells. The ROS data correlate with the OXPHOS machinery results, indicating that PEG-AuNSs (1 nM) and PAH-AuNRs (0.1 nM) had a more pronounced impact on cells compared to other AuNPs. Reduced oxygen consumption in the mitochondria can trigger different mechanisms of toxicity, including ROS generation, increasing the chances of cell injury by oxidative stress and potentially leading to cell death. **Conclusions:** Our findings indicate that even at low doses, AuNPs have the potential to disrupt mitochondrial OXPHOS function, and this impact is correlated to specific physicochemical properties of the AuNPs (in this case, surface chemistry). Our research highlights how the surface chemistry of AuNPs influences the mitochondrial OXPHOS machinery and consequently, cell energy production, and prompts concerns about their safety. Further research is crucial to comprehensively understand the biological effects of nanoparticles and guarantee the safe utilization of these nanomaterials for biomedical applications.

3659 *In vitro* toxicity assessment of spinel ferrite nanoparticles and UVB co-exposure in human epidermal keratinocytes

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Background and Purpose: Spinel ferrite nanoparticles (SFNPs) have attracted significant attention due to their unique characteristics that make them promising candidates for diverse applications in biomedicine, water treatment, catalysts, energy fields and industrial electronic devices. However, before these materials can be considered for potential uses, investigation of their toxicity is needed. Additionally, it is important to address the combined effect of SFNPs with ultraviolet light B (UVB) as it is known that UVB-induced skin inflammation contributes to a number of cutaneous diseases. **Methods:** This study was carried out to assess the ability of two SFNPs, $NiFe_2O_4$ and $CoFe_2O_4$, alone (1.25-30 μg/cm^2) or in combination with UVB (1-2 kJ/m^2) to induce cell cytotoxicity, oxidative stress, inflammation, and DNA damage in human epidermal keratinocytes (HEK). In addition, modes of cell death (apoptosis, necroptosis, ferroptosis) in response to SFNPs alone or with UVB pre-treatment were investigated. It is known that not only apoptosis, the most studied and best characterized programmed cell death, occurs

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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