composition, and other physicochemical characteristics to these toxicity outcomes has resulted in a significant knowledge gap. The current study aimed to investigate two plastic nanoparticles, polystyrene (PS) and polymethyl methacrylate (PMMA) at two different particle sizes (15 nm and 1000nm diameters) for pulmonary genotoxicity and inflammation in vitro. **Methods:** Two sizes of two plastic microspheres, PS and PMMA, were used in the current study. These particles were fluorescently tagged (488/560) and were either 15 nm or 1000 nm in diameter. Human bronchiolar epithelial cells (BEAS-2B) were used to investigate the genotoxicity of the microplastics, while differentiated human monocytes (THP-1s) were used to assess inflammation. In both cell lines, cell viability and proliferation were assessed after 24-hour exposure to nano and microplastics at doses ranging 0-500 µg/ml using WST-1 and Alamar Blue in addition to lactate dehydrogenase (LDH) in THP-1 cells. DNA damage, cell cycle disruption, and oxidative stress were assessed in BEAS-2B cells while caspase-1 activity and IL-1β release was assessed in THP-1 cells. **Results:** Cytotoxicity occurred at lower doses after exposure to 15 nm particles when compared to their larger diameter counterparts. The 1000 nm diameter PS particle had minimal effect on cell viability, even at 500 µg/ml, while the 1000 nm diameter PMMA particle significantly reduced viability at approximately 300 µg/ml in the Alamar Blue assay. No significant changes in DNA damage or cell cycle occurred at 6.25 or 25 µg/ml for either particle; however, all materials and sizes induced varying degrees of oxidative stress with the 1000 nm PS particle inducing a more significant increase at the high dose. Each particle increased Caspase-1 activity at 6 hours beginning at the lower doses. Additionally, lower doses of 1000 nm PS increased IL-1B release at 24 hours of exposure. **Conclusions:** Overall, minimal to no change in cell viability occurs at doses lower than 180 µg/ml for both particles and sizes; however, the larger diameter particles were not as cytotoxic as the smaller particles at a given dose. Although there was minimal genotoxicity following exposure to any of the particles, oxidative stress was induced by all materials and inflammation occurred with all particles. These findings varied with chemical composition, size, and dose. Overall, PS1000 was the most toxic of the particles at the 25 and 6.25 µg/ml doses, though the smaller diameter particles affected cell viability and proliferation at lower doses compared to the larger diameter particles, while PMMA1000 was the least toxic. This data suggests that both particle size and chemical composition are prominent determinants of micro and nano plastic toxicity. Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention.

PS 4128 An Extensive Study Focusing on Quantum Dots and Their Interaction with Actin

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Background and Purpose: Quantum dots (QDs) are biocompatible nanoparticles with a unique range of optical and fluorescent properties which makes them a prospective diagnostic tool in nanomedicine applications for cell labeling, bioimaging, and drug administration. However, the integration of QDs in real life biological scenarios as a biomedicine carrier has been limited due to QDs' ability to non-specifically interact with the cells. As such, this study aims to study QDs' interaction with intracellular proteins, including actin and actin-binding proteins and the consequence of their interactions. **Methods:** We conducted spin-down assays to examine the effects of QDs on actin polymerization and depolymerization as well as the interaction between QDs and actin in the presence of alpha actinin. We then performed fluorometer-based assays to monitor pyrene labeled actin polymerization and depolymerization over time in the presence of varying concentrations of QD. We also carried out a wound-healing assay to investigate the effects of QD on the migration of HeLa cells over time. **Results:** Our spin-down experiment showed that high concentrations of QD hinder actin polymerization and promote depolymerization. Furthermore, our spin-down assay also demonstrated the ability of QD to cause bundling of actin filaments. However, in the presence of both alpha actinin and high QD concentration, actin bundling does not occur. This indicates an interaction between QD and alpha actinin. In addition, our fluorometer assay revealed that high concentrations of QD inhibit actin polymerization while lower concentrations stimulate polymerization. Correlating with these results, the wound-healing assay revealed that QD was able to hinder cell migration, likely due to the disruption of the actin cytoskeleton. **Conclusions:** Our study showcased a novel discovery in terms of QD' functionality: it interacts with actin and actin-binding proteins, resulting in defects in actin dynamics. These results provide insight and further emphasize on the importance of understanding the interactions between QDs and intracellular proteins to maximize their potential in biomedical applications while mitigating any side effects.

PS 4129 The Transcription Factor PU.1 Mediates Multi-Walled Carbon Nanotubes-Induced Arachidonate 5-Lipoxygenase Expression and Proinflammatory Responses in Macrophages

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Background and Purpose: Exposure to fibrogenic multi-walled carbon nanotubes (MWCNTs) induces the production of proinflammatory lipid mediators (LMs) in myeloid cells to instigate inflammation. The molecular underpinnings of LM production in nanotoxicity remain unclear. **Methods:** Murine (J774A.1 cells) or human monocyte derived macrophages were treated with MWCNTs (Mitsui-7) or Carbon black (CB, Printex90), an amorphous carbon material control, at 2.5 or 10 µg/mL for 24 hours. The molecular induction of arachidonate 5-lypoxygenase (Alox5) and production of LMs were assessed by quantitative RT-PCR or ELISA assay, respectively, and the key transcription factor was identified by a series of luciferase reporter assay and chromatin immunoprecipitation assay after the exposure in macrophages. **Results:** MWCNTs induced the expression of Alox5 at both mRNA and protein levels in murine and human macrophages, accompanied by marked elevation of chemotactic LM leukotriene B4 (LTB4). Induction is comparable to those by potent M1 inducers. CB did not increase Alox5 expression or LTB4 production at equivalent doses. MWCNTs induced the expression of a heterologous luciferase reporter under the control of the murine *Alox5* promoter. Deletional analysis for the 5' upstream regulatory region of *Alox5* promoter uncovered multiple activation regions of which the activities were further increased by MWCNTs. We also found that the *Alox5* promoter contains four binding sites for PU.1, a ETS domain-containing master regulator of hematopoiesis, which was further increased by MWCNTs. Knockdown of PU.1 using specific small hairpin-RNA blocked the basal and induced expression of *Alox5* mRNA and the production of LTB4 as well as prostaglandin E2. **Conclusions:** The results demonstrate a critical role of PU.1 in mediating MWCNTs-induced expression of Alox5 and production of proinflammatory LMs, revealing a molecular framework where the hematopoietic transcription factor PU.1 is activated to orchestrate multiple proinflammatory responses to sterile particulate stimuli.

PS 4130 Physicochemical Properties and Functionalization Effects on the Bioactivity of Nickel (II) Oxide Nanoparticles in Macrophages

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Background and Purpose: Nickel (II) oxide nanoparticles (NiO NPs) have a wide array of uses, including applications in industry and medicine. The increased use of engineered nanomaterials such as NiO NPs is an emerging health concern as these particles can also cause chronic inflammation in a similar fashion to other environmental and endogenous particles such as crystalline silica or calcium pyrophosphate (CPP) crystals. Currently, there is evidence that particle surface properties contribute to the bioactivity of nanoparticles and define particle interactions with lysosomal membranes. Because lysosomal membrane permeability is a central factor in particle induced inflammation in macrophages, the analysis of NiO NPs' ability to initiate inflammation is necessary. Furthermore, the effect of Ni^{2+} on mitochondrial function should be considered as it relates to additional contributions to lysosome dysfunction and inflammation. In this study, nickel (II) oxide nanoparticles from three different manufacturers were compared. To further refine the understanding of functionalization to bioactivity of nickel oxide nanoparticles (NP), the three NiO NPs were functionalized with -COOH groups. **Methods:** Bare NiO NPs were analyzed with transmission electron microscopy to understand the morphology of the differing particles. Zeta potentials were calculated for each NiO NP to Bare NiO NPs (SIG, CHEM, and AA) were assessed for free Ni^{2+} ions after incubation for 1 hr or 72 hr at 37° C in synthetic lysosomal fluid (pH 4.7) using dimethylglyoxime (DMG), which forms a pink complex with free Ni^{2+} ions that can then be quantified with absorbance readings on a microplate reader. Bare and functionalized particles were assessed for cytotoxicity and inflammatory cytokine IL-1β in a macrophage model. Additionally, DMG was used to pretreat macrophages prior to NiO NP application to assess the role of Ni^{2+} ions in NiO NP bioactivity. Reactive oxygen species (ROS) were assessed in macrophages after 2 hours using a fluorescent indicator of ROS, MitoROS. Lysosomal membrane permeability due to NiO NPs was evaluated in macrophages after 4 hours. **Results:** TEM analysis of the three nickel oxide nanoparticles showed differences in morphology. SIG nanoparticles displayed a disordered mesoporous structure comprised of nickel oxide nanocrystals (~10 nm in diameter). CHEM nanoparticles ranged in size from 30-210 nm in diameter with an overall avg diameter of ~90 nm. AA nanoparticles had an average diameter of 12 nm. The Zeta potentials were measured at -12.7 (CHEM), -12.6 (SIG), and -9.08 (AA). NiO NPs incubated in synthetic lysosomal fluid for 1 hour demonstrated Ni^{2+} content that increased from CHEM to SIG to AA indicated by absorbance readings of 0.048, 0.143, and 7.88 respectively. At 72 hours incubation in synthetic lysosomal fluid, NiO NPs demonstrated a similar pattern of Ni^{2+} content with absorbance readings of 0.132 (CHEM), 1.49 (SIG), and 9.20 (AA). Functionalization of bare SIG and AA with -COOH groups reduced the respective Ni^{2+} ions released after 1 hr incubation with synthetic lysosomal fluid. Murine ex vivo alveolar macrophages treated with NiO NPs for 24 hr and assessed for cell death and IL-1β release demonstrated the greatest response with SIG particles, with AA and CHEM particles showing less cell death or IL-1β release in comparison. Functionalization with -COOH reduced the bioactivity of NiO NPs. Macrophages pre-treated with DMG Ni^{2+} chelator for 2 hr prior

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