3662 Activation of THP-1 cells by mixed exposure to silicon dioxide nanomaterial and skin sensitizer or febrile substance

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Background and Purpose: Because nanomaterials (NM) are directly taken up into cells, there are strong concerns about related health risks, especially from their inhalation. For this reason, the Organisation for Economic Co-operation and Development is leading the effort to develop internationally standardized test methods that can efficiently and accurately evaluate the inhalation toxicity of various NM. Therefore, here we focus on the effects of NM on antigen-presenting cells, which play a fundamental role in eliminating foreign substances, and we are currently developing a method to evaluate immunotoxicity through NM inhalation exposure. Silicon dioxide NM are known to affect antigen-presenting cells such as the alveolar macrophages. We previously found that silicon dioxide NM (hereinafter nanosilica) significantly enhance CD54 expression in the THP-1 human monocytic cell line. This study investigated the effects of mixed exposure to nanosilica NM-204 along with skin sensitizer or febrile substance (lipopolysaccharide [LPS]), which have the ability to activate THP-1 cells. **Methods:** Regarding mixed exposure between sensitizer and nanosilica, the applied concentration of DNCB was fixed (4 μg/mL) and mixed exposure with NM-204 (various concentrations, 0.0316-100 μg/mL). Regarding mixed exposure between LPS and nanosilica, the applied concentration of LPS was fixed (1 ng/mL) and mixed exposure with NM-204 (various concentrations, 0.0316-100 μg/mL). **Results:** In the mixed exposure between sensitizer and nanosilica, the relative fluorescence intensity (RFI) for CD86 was 294.3-316.8%, comparable to that for DNCB alone of 318.4 ± 30.1 (n = 3). The RFI to CD54 was 462.8-518.3%, similar to that for DNCB alone (475.9% ± 176.3; n = 3). Therefore, no additive or synergistic effect due to mixed exposure was identified. On the other hand, in the mixed exposure between LPS and nanosilica, the RFI of CD86 was increased to over 150% at some concentrations of NM-204 (31.6 -100 μg/mL). Since neither NM-204 nor LPS increased CD86 expression when exposed alone, this was considered a priming effect due to mixed exposure. the RFI of CD54 was increased to maximum 1404.6% in the mixed exposure between LPS and nanosilica. This CD54 expression level exceeded the sum of the increasing effect by LPS and that by nanosilica. Therefore, a synergistic effect was observed in the mixed exposure of LPS and NM-204 (at 10 μg/mL or higher of NM-204). **Conclusions:** These results suggest that the activation of THP-1 cells by nanosilica occurred via a different mechanism than that of the activation by skin sensitizers and LPS.

3663 *In vitro* toxicity screening of different categories of two-dimensional (2D) nanomaterials for genotoxicity and activation of the NLRP3 inflammasome

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Background and Purpose: Two-dimensional (2D) nanomaterials are a large class of engineered nanoparticles with a multitude of applications in electronics, biosensors, and more. Increased demand for these materials, including graphene, montmorillonite nanoclay, transition metal dichalcogenides (TMDs), such as WS_2 and MoS_2, and hexagonal boron nitride (hBN), has elevated the potential for occupational exposures during manufacturing, notably respiratory exposure. Although graphene has been well investigated, there are relatively few toxicity studies of this class of materials as a whole. Existing studies indicate these materials may have the propensity to induce inflammation and cytotoxicity; however, some results are contradictory and comparison across the entire highly variable class remains difficult. The goal of the current study was to conduct a comparative toxicity study of representative 2D materials for the different categories listed above using high throughput *in vitro* screening assays. **Methods:** The five materials were thoroughly characterized including, but not limited to, size, density, surface area, and hydrodynamic diameter. A battery of toxicity assays was performed using human bronchial epithelial cells (BEAS-2B) and human THP-1 macrophages in doses ranging from 1-100 μg/ml for 24 hours. Cytotoxicity and cell viability were assessed using WST-1 and Alamar blue for each cell type. In Beas-2B cells, oxidative stress was assess using CellROX, and genotoxicity was assessed by quantifying changes in cell cycle, H2AX, comet assay, and micronuclei (cytokinesis block assay) in addition to a quantification of cytokine secretion. Using THP-1 cells, activation of the NLRP3 inflammasome was assessed by quantifying the secretion and/or activation of cytokines and caspases. Additionally, changes in phagocytic ability was measured using phrodoGreen uptake. **Results:** Significant reduction in cell viability was found to occur with graphene at doses ≥ 12.5 μg/ml. Nanoclay and hBN had significant changes at doses ≥ 25 μg/ml, while little to no changes were seen, even at the highest doses (100 μg/ml) for TMDs. No significant changes in DNA-associated oxidative stress or double stranded DNA damage (H2AX) were observed, though a trend for cell cycle arrest in G0/G1 phase was observed with high dose nanoclay exposure. While most cytokines measured were unchanged, nanoclay did induce a dose-dependent decrease in IL-12p70 in BEAS-2Bs. Furthermore, no significant changes in DNA damage or micronuclei formation was observed, suggesting these materials are unlikely to induce genotoxicity at the doses used in this study. Inflammasome activation was assessed in THP-1 cells. IL-1b was found to be significantly increased at an average of 3.6 (6.25 μg/ml) and 4.8 (25 μg/ml) times the control level following nanoclay exposure, and a significant 1.8-fold change occurred following hBN (6.25 μg/ml) exposure. A 4-fold change in Caspase-1 also resulted from exposure to 25 μg/ml nanoclay. However, no significant changes were found in cathepsin B or IL-18 release suggesting minimal activation of the NLRP-3 inflammasome for most materials with the exception of nanoclay. Finally, there were minimal difference in phagocytic capacity among treatment groups indicating no particle-specific effect on clearance capacity. **Conclusions:** These initial findings suggest that the TMD category is relatively less toxic than the other classes of 2D materials and nanoclay may be of greater toxicological concern compared to other materials in this study. However, minimal evidence suggest that these 2D nanomaterials are of overall significant toxicological concern.

3664 Direct Binding of Quantum Dots to Monomeric Actin; A New Insight of Quantum Dot Toxicity

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Background and Purpose: Recently, nanoparticles have gained attention worldwide due to their potential to revolutionize modern medicine and technology. Among these nanoparticles, quantum dots (QDs) stood out due to their vast potential in biomedical applications. According to the NIH, there are currently five clinical trials that use quantum dots for drug delivery or disease detection purposes. In these types of applications, quantum dots are required to enter the human body and are eventually taken up by cells. Yet, the interaction between quantum dots and intracellular proteins has not been explored. As such, our study aims to identify intracellular proteins that can bind to quantum dots and characterize the interaction between quantum dots with the identified intracellular proteins. **Methods:** To achieve our aims, we used mass spectrometry to identify quantum dots binding proteins. Next, to characterize the interaction between quantum dots and one representative quantum dots-binding protein, we performed a series of biochemical experiments, including native gel assay, intrinsic fluorescence measurement, thermodynamics calculation, UV-Vis, dynamic light scattering, and circular dichroism. **Results:** Our mass spectrometry data revealed several quantum dots binding proteins, including proteins that are associated with mitochondrial function, transcription, translation, heat shock protein, vesicular trafficking, and the actin cytoskeleton. Among these proteins, we chose monomeric actin, an abundant intracellular protein essential for many key cellular activities, as a representative to investigate the interaction between QDs and intracellular proteins. Using a series of biochemical experiments, we found that CdSe/ZnS quantum dots spontaneously bind to monomeric actin in a 1:2.5 ratio and cause a shift in the position of fluorescence amino acids during complex formation. Furthermore, we found an alteration in the secondary structure of monomeric actin by quantum dots. **Conclusions:** Overall, our study proposed direct interaction with intracellular proteins as a new mechanism for quantum dots' toxicity. Through our research, we hope to provide a more rounded understanding of QDs' toxicity so that we can develop safer quantum dots for biomedical applications.

3665 *In vitro* actin dynamics impacted by quantum dots

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Background and Purpose: Quantum dots (QDs) are biocompatible nanocrystals with unique optical and electronic properties. QDs possess a broad range of bright and stable fluorescence, which makes them useful in a wide range of applications. Particularly, QDs have been recognized for their ability to play a critical role as a diagnostic tool in nanomedicine applications for cell labeling, bioimaging, and drug administration. Even with such immense potential, the use of quantum dots in biological settings has been restricted due to concerns regarding their nonspecific interactions in the cell. Our previous studies revealed that QDs interact with monomeric actin and impair its function. We further investigated the effects of quantum dots on the rate of actin polymerization/depolymerization upon actin-QD interaction. **Methods:** To investigate the impact of QDs on the rate of actin dynamics and the binding of QDs to filamentous actin, we performed a series of spin-down assays. We then used a fluorometer-based assay to monitor the pyrene fluorescence and follow actin polymerization/depolymerization over time. Different concentrations of QDs were then added to the reaction mixture to study the effects on actin dynamics. **Results:** Our spin-down experiment showed that high concentrations of quantum dots negatively influence the actin polymerization process. We also found that quantum dots can bind to filamentous actin and cause bundling of actin filaments. In addition, our data also indicate that high concentrations of quantum dots cause depolymerization of actin filaments. Furthermore, our fluorometer-based assay revealed that high concentrations of QDs inhibit actin polymerization while the lower concentrations of QDs stimulate actin polymerization. **Conclusions:** Our study concluded that quantum dots alter the actin dynamics by affecting actin polymerization in a biphasic manner as well

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