2195 Label-Free 3D Raman Imaging of Carbon Nanotubes in Mammalian Cells

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Multi-walled carbon nanotubes (MWNTs) pose a respiratory hazard because they can cause pulmonary fibrosis, which may lead to mesothelioma. The Bionanosciences Group at UT Dallas is interested in understanding the interactions of triblock polymer Pluronic® F-108-coated MWNTs with macrophages that are the first responders to nanoparticles encountered in the body. This information is relevant for improving the biomedical efficacy of MWNT therapeutics, for understanding mechanisms of MWNT biocompatibility, and for developing methods to ameliorate MWNT toxicity. To better understand mechanisms of MWNT toxicity, it is important to know whether MWNTs physically enter cells and where they locate inside cells. We have developed procedures to detect internalized MWNTs and reconstruct 3D images of RAW 264.7 mouse macrophages with cell-associated MWNTs by laser scanning confocal Raman microscopy. 3D images of cells are reconstructed with stacks of optical sections from confocal planes to place the subcellular MWNT locations in the context of the intact cell. The 3D MWNT Raman images demonstrated that Pluronic® F-108-coated pristine MWNTs (P-MWNTs) and carboxylated MWNTs (C-MWNTs) exposed to RAW 264.7 cells for 24 h at 37 °C accumulated inside the cells within punctate vesicles, most likely in the endosome/lysosome system, but not in the cytoplasm. These results are consistent with our previous observations that RAW 264.7 cells accumulate ~100 times more C-MWNTs suspended in the surfactant Pluronic® F-108 than corresponding P-MWNTs during a 24 h incubation at 37 °C (Wang et al., 2018). Future work will involve assessing the intracellular distributions of various functionalized MWNTs and evaluating their fate after internalization, and whether they are degraded, secreted, or if they persist within cells. Also, Raman imaging with live cells will be used to access whether accumulation of MWNTs induce the release of cytokines that may cause lysosomal membrane damage and result in the redistribution of MWNTs to the cytoplasm. A better understanding of the mechanisms by which MWNTs interact with macrophages should lead to better rational designs of safer carbon nanomaterials. Wang et al., "Quantitation of cell-associated carbon nanotubes: Selective binding and accumulation of carboxylated carbon nanotubes by macrophages." *Nanotoxicology* (2018): 1-22.

2196 Titanium Dioxide Nanoparticle Induced AP-1 Activation via ERKs and p38 Kinase

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Titanium dioxide (TiO_2) is a white mineral used in the manufacturing of paint, paper, plastics, sun tan lotion, and other products. Recent studies indicated that TiO_2 nanoparticles cause chronic inflammation and lung tumor formation in rats. It is believed that the toxicity and carcinogenesis of TiO_2 is associated with particle size. Since activator protein-1 (AP-1) is known to play an important role in the induction of neoplastic transformation and regulation of multiple genes involved in cell proliferation and inflammation, we investigate the potency of TiO_2 nanoparticles on reactive oxygen species (ROS) generation and AP-1 signaling in a mouse epithermal cell line, JB6 cells. Incubation of JB6 cells with TiO_2 nanoparticles resulted in a dose dependent generation of •OH radicals. TiO_2 nanoparticles caused a 3-fold increase in AP-1 activity in the cells. The induction of AP-1 activity in cultured cells was dose-dependent. The signal transduction pathways for AP-1 activation were also investigated and the results demonstrate that TiO_2 stimulates mitogen-activated protein kinase (MAPK) family members, including extracellular signal-regulated protein kinases (ERKs), p38 kinase, and C-Jun N-terminal kinase (JNKs). Inhibition of ERKs, p38 kinase, but not JNKs with specific inhibitors SB203580, PD98059, and SP 600125, inhibited TiO_2 nanoparticles-induced AP-1 activation, respectively. These findings demonstrate that TiO_2 nanoparticles stimulated the generation of •OH radicals and induces AP-1 activation, which may be mediated through p38 kinase and ERKs pathways.

2197 Particle Size and Surface Charge Dependent Toxicity of PAMAM Dendrimers in Cultured Endothelial Cells

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Different nanomaterials are under development for various biomedical applications in which nanoparticles contact blood and vasculature. Therefore, investigating the interactions between nanomaterials and vascular endothelial cells is of great importance. Here, we show the effects of polyamidoamine (PAMAM) dendrimers of two different sizes, generation 2 (G2; approximately 3 nm diameter) and generation 7 (G7; 9 nm), with neutral (OH-terminated), anionic (COOH-terminated) and cationic (NH_2-terminated) surface modifications on cultured human umbilical vein endothelial cells (HUVECs). HUVECs and extracellular membrane vesicles (EVs) were characterized by flow cytometry (FC), nanoparticle tracking analysis (NTA), laser scanning confocal microscopy (LSCM) and electron microscopy (SEM, TEM). We found that only cationic dendrimers (5-100 µg/mL G7-NH_2 and 100 µg/mL G2-NH_2) and not anionic or neutral dendrimers were cytotoxic to HUVECs. In addition, cationic dendrimers at low concentrations (5 µg/mL) markedly increased the HUVEC surface expression of the proinflammatory activation marker ICAM-1 and phosphatidylserine (PS). Both G2-NH_2 and G7-NH_2 dendrimers caused g1 arrest, but only G7-NH_2 dendrimers induced significant HUVEC apoptosis. G7-NH_2 interacted strongly with HUVEC plasma membranes and mitochondrial membranes, and phospholipid vesicles containing G7-NH_2 formed, which resulted in extensive plasma membrane blebbing and disintegration. Furthermore, flow cytometric analysis showed that G7-NH_2-treated HUVECs released large numbers of extracellular vesicles (EVs) positive for CD105 and PS. A notable population of EVs positive for the mitochondrial marker TOM20 but negative for the autophagosome marker LC3 was found. In summary, large cationic PAMAM dendrimers (G7-NH_2) showed both proinflammatory and proapoptotic effects in endothelial cells; at high dendrimer concentrations, these effects were accompanied by necrotic cytotoxicity. G7-NH_2 caused plasma and mitochondrial membrane disintegration and the release of EVs, including EVs of mitochondrial origin that were not associated with mitophagy. *The findings and conclusions in this study have not been formally disseminated by the US FDA and should not be construed to represent any Agency determination or policy.*

2198 Developing a Protocol for Observing the Effects of Sex Differences on Macrophage Polarization

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Macrophages are the primary innate immune cells in the lungs. They protect the lungs by phagocytizing inhaled foreign particles and organisms. Macrophages exhibit several different phenotypes with distinct functions—the "classically activated" M1 phenotype, which is associated with inflammation, or the "alternatively activated" M2s, which can be divided into a variety of subsets. There is evidence that estrogen receptor alpha (ERα) signaling promotes M2 macrophage development, contributing to sex differences seen in human respiratory disease prevalence. For example, it may help explain why asthma, an M2-driven disease, occurs more often in women than in men. However, the exact role that sex hormones play in respiratory pathology is still unknown. Understanding how sex hormones influence macrophage phenotype development in the lungs will help us address this gap of knowledge. The goal of this project was to develop a protocol for macrophage phenotype and ERα expression analysis via flow cytometry. Developing an effective protocol for cytometric assessment of these proteins is challenging due to our need to simultaneously assess both cytosolic and nuclear proteins, as well as extracellular markers. Murine bone marrow-derived macrophages and primary alveolar macrophages (AMs) were polarized into M1 and M2 phenotypes and analyzed for ERα, M1 markers CD38 and Ly6C, and the M2 marker YM1. After establishing that our fixation and permeabilization protocol was suited for all markers of interest, we determined effective concentrations for each antibody. Expression of the M1 and M2 phenotype markers corresponded appropriately to the M1- and M2-polarized macrophages, respectively. ELISA measurement of cytokines was used to corroborate our cytometric analysis of M1 and M2a macrophages. ERα was expressed at a similar basal level in both male and female mice. Interestingly, in AMs exposed to multi-walled carbon nanotubes, the M1 marker CD38 was expressed more highly in M1 macrophages from male mice compared to M1s from female mice. This suggests that male mice may experience a more significant M1-like response compared to females. Overall, this project improved our protocols for studying macrophages in order to better understand how sex hormones affect phenotype development. In the future, we will apply these protocols to *in vivo* studies assessing sex differences in the immune response to inhaled particles. *This project was supported by NIH grants R25 ES022866 and R01 ES023209.*

2199 Evaluating the Toxicity of Silver Nanoparticles in ARPE-19 Cells through High-Content Morphometric Analysis

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Efficient methods are needed to evaluate the human and environmental health effects of silver nanoparticle (AgNP) exposure. A high content imaging-based phenotypic profiling approach was used to determine the effects

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