3495 Utilization of Near Infrared Fluorescence Imaging to Track and Quantify Pulmonary Retention of Single-Walled Carbon Nanotubes in Mice

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Adequate detection techniques to examine the biotoxicity from pulmonary exposures of single-walled carbon nanotubes (SWNTs) present a unique challenge. Traditional analytical methods that effectively assess their distribution *in vivo* lack spatial resolution for differentiating carbon nanomaterials from the carbon in the biomass of living cells. Near infrared fluorescence (NIRF) imaging has emerged as a valuable tool for utility in studies for detection and quantification of single-walled carbon nanotubes (SWNTs) *in vivo*. The objective of this study was to develop and optimize NIRF-based imaging and quantitation methods for tracking and quantifying SWNTs in a murine pulmonary exposure model. We designed a custom NIRF system that allows for visualization, semi-quantitation and spectroscopic analysis of SWNTs in whole organs and histological tissue sections. To assess biopersistence *in vivo*, C57BL/6 mice were challenged with a single dose of SWNTs by pharyngeal aspiration (20 ug/mouse), and their distribution was tracked using NIRF imaging at 3, 7 and 14 days. Spectroscopic analysis revealed 90% recovery of SWNTs from normal lung tissues. Semi-quantitative analysis of images showed strong and innate SWNT-derived fluorescence throughout whole lungs tissues at all-time points measured. This was consistent with data from spectroscopic quantitation analysis; overall results showed a steady level of SWNT at each time-point with minimal clearance observed. Additionally, histopathological examination of airway perfused lungs revealed that SWNTs were present in the terminal bronchioles, alveolar ducts and proximal acinar alveoli and associated with inflammatory cell infiltrates dominated by macrophages in bronchiolar and alveolar spaces. Adverse impacts on viability and health condition of exposed mice were not observed during the study. Results of this study show pulmonary retention of SWNTs up to day 14 post initial exposure and highlight the utility of NIRF as a tool to assess clearance and distribution of SWNTs *in vivo*. This work is imperative as processes of clearance are vital in understanding long-term health impacts observed in other particulates.

3496 Characterization of Lung Toxicity Following Pulmonary Exposure to Graphene Nanoparticles in Different Oxidized Forms

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As the manufacturing of graphene nanoparticles (GNP) increases, there is concern for the health effects that due to lung exposure in workers. This study evaluated lung toxicity of different oxidized forms of GNP: non-oxidized graphite nanoplates (Gr), an oxidized intermediate (GO), and the reduced form of GO (rGO), with multi-walled carbon nanotubes (MWCNT, Mitsui-7) as a particle control. Male C57BL/6J mice received 4 or 40 µg of Gr, GO, rGO, MWCNT, or dispersion medium (DM) by oropharyngeal aspiration. Bronchoalveolar lavage (BAL) was performed at 4 hr, 1 d, 7 d, 1 m, and 2 m post-exposure. In separate mice, lungs were preserved for pathology and RNA analysis. Lung injury was elevated in MWCNT and rGO at 40 µg when compared to all groups at all times, with the greatest increase in the MWCNT group at 1 and 7 d, and in the rGO group at 1 and 2 m. Gr and GO at 40 µg, and MWCNT at 4 µg increased injury at early time points only. Alveolar macrophages (AM) in BAL were elevated in all 40 µg groups at 7 d, with the greatest increase in the rGO animals at 7 d and 1 m. This response persisted for the rGO and MWCNT 40 µg groups up to 2 m. BAL neutrophils were increased in all 40 µg groups up to 7 d, and persisted in the MWCNT and rGO groups up to 2 m. 4 µg doses of rGO and MWCNT also caused increased neutrophils at early time points. Acutely, all 40 µg exposures increased relative mRNA expression of Cxcl2, Ccl2, Il6, and Ccl22. The effects persisted 1 m in the Gr and GO 40 µg dose and MWCNT 4 µg dose, and 2 m for rGO and MWCNT 40 µg groups. Histopathology showed fibrosis at 7 d in MWCNT and rGO 40 µg groups scored as minimal, which persisted throughout the time course, and mild type II epithelial hyperplasia and inflammation, which resolved over time. Gr and GO caused transient inflammation/injury, whereas rGO caused comparable toxicity to MWCNT. Differences in toxicity may be due to chemistry, shape, and/or density of materials, with a higher volume load per mass delivered for the low density particles, MWCNT and rGO.

3497 Atomic Layer Deposition Coating of Multi-Walled Carbon Nanotubes with Metal Oxides Alters Pro-Fibrogenic Responses in an *In Vitro* Tri-Culture System of the Pulmonary Alveolus

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Introduction. Multi-walled carbon nanotube (MWCNT) inhalation exposure is a potential risk to human health due to their increasing use in consumer products. Atomic layer deposition (ALD) is a method for applying conformal nanoscale coatings to enhance surface properties of materials. In rodent models, pulmonary exposure to MWCNTs causes increased inflammation & fibrosis. Recently, we have determined that ALD coating of MWCNTs with aluminum oxide decreased fibrosis and pro-fibrogenic cytokines (OPN & TGF-β1) induced by uncoated MWCNTs in lungs of mice. The purpose of this study was to determine whether ALD coating with metal oxides such as aluminum (A), zinc (Z) or titanium oxide (T) would alter the fibrogenic response to MWCNTs. Methods. Uncoated (U-) MWCNTs or ALD-coated MWCNTs (A-, Z-, T-MW) were incubated with THP-1 macrophages, BEAS-2B epithelial cells, & HLF-19Lu fibroblasts alone or in co-/tri-culture. Pro-fibrogenic mediator analysis included: TGF-β1, OPN, and collagen mRNAs (Col1a1 & Col1a2). qRT-PCR was performed to assess mRNA expression. Results. With THP-1s alone, all MWCNTs increased Col1a1 mRNA at 48 & 72hr but only Z-MW increased Col1a2, OPN, & TGF-β1 at 72hr. U-MW induced Col1a1 & Col1a2 mRNA at 24hr at the highest dose (50 & 100µg/ml). High doses of Z-MW (10, 50, 100µg/ml) increased Col1a2 substantially but decreased OPN & TGF-β1 mRNAs. In BEAS-2Bs, U-MW, A-MW and Z-MW exposure increased TGF-β1 mRNA with U-MW having the most effect. Changes in mRNA seen in cells cultured alone were dampened when in co-culture with the exception of OPN where mRNA levels were increased when THP-1s were in co-culture with HLFs. In the tri-culture model, Z-MW increased Col1a1 & Col1a2 mRNA at 24hr. A-MW & T-MW had no effect on Col1a1 & Col1a2 in tri-culture. Conclusions. hese findings indicate that ALD coating of MWCNTs alters pro-fibrotic mediators *in vitro* with Z-MW increasing fibrogenic activity and A- or T-MW decreasing fibrogenic activity. Funding: Supported by NIEHS grant R01-ES020897

3498 Hyaluronan Functionalization Reduces Lung Inflammatory and Fibrotic Responses of Multi-Walled Carbon Nanotubes

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Multi-walled carbon nanotubes (MWCNT) have promising applications in the fields of nanotechnology, nano diagnostics and nanomedicine. However, there are possibilities of human health risk especially for lung diseases as a result of inhalation exposures. Hyaluronic acid/hyaluronan (HA), a high molecular weight oligosaccharide, is one the major components of lung extracellular matrix. The high molecular weight isoform of HA has anti-inflammatory properties. We hypothesized that grafting high molecular weight HA on MWCNTs can reduce their toxicity. We further aimed at comparing the toxicity of commercially available (as prepared), purified and functionalized nanotubes with hyaluronan grafted MWCNT (MWCNT-HA). Nanotube were characterized by transmission electron microscopy, FTIR, ICP-OES and Raman spectroscopy while their suspensions were analyzed using Dynamic Light Scattering. Toxicity of nanotubes was evaluated in human lung fibroblasts and in mouse lungs (after oropharyngeal aspiration of 1.5mg/Kg nanotubes). Mice were sacrificed either at day 1 or day 21 after nanotube exposure; lungs were lavaged and collected for histology and protein/RNA isolations. MWCNT-HA induce lower cellularity, TNF-alpha production and HA levels in BAL of mice than all other MWCNT preparations. Histological scoring further indicated reduced perivascular and particle-associated inflammation, collagen deposition and epithelial proliferation in MWCNT-HA treated mouse lungs compared to other MWCNT preparations. In human lung fibroblasts, MWCNT-HA exposure results in reduced pro-fibrotic gene expression however it induces proliferation.

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Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 55th Annual Meeting of the Society of Toxicology, held at the New Orleans Ernest N. Morial Convention Center, March 13–17, 2016.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 603.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 629.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence. Author names which are underlined in the author block indicate the author is a member of the Society of Toxicology. For example, J. Smith.

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