3483 Differential Analysis of the Effects Induced by Cellular Exposure to Pristine and Acid Treated Single Walled Carbon Nanotubes

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Despite the unique properties of single walled carbon nanotubes (SWCNTs), their implementation in a variety of biomedical and industrial applications had been hindered by many concerns associated with potential risks imposed by their exposure. Previous reports have shown that CNTs toxicity can be attributed to their length, surface chemistry, aggregation or metal impurities, however, no systematic investigation exists that correlates changes in the cellular dynamics with CNTs properties. Herein, we provide a comprehensive analysis of the induced cyto- and genotoxic effects associated with human lung cells exposure to pristine and acid treated SWCNTs. Specifically, by employing an electrical cell impedance sensing (ECIS) platform, we measured changes in the cellular behavior, attachment and cell-cell interactions as well as the overall cellular fate upon exposure to SWCNTs with different properties. We further complemented our real-time studies with conventional microscopy and flow cytometry techniques to derive structure function relationships that correlate the changes in the cellular dynamics at a molecular level with the SWCNTs' physico-chemical properties. Our results showed functional differences in the cellular behavior based on the nanotube's length, surface chemistry, purity and agglomeration state. Specifically, while all SWCNTs samples showed significant reduction in cellular viability, proliferation and migration, exposure to pristine SWCNTs lead to significant generation of ROS, the largest reduction in cellular migration and a cell cycle arrest at the G1/S phase. In contrast, exposure to the acid treated SWCNTs did not lead to ROS generation or major effects on cellular migration, however; it lead to more significant changes in the expression of structural and adhesion proteins and a higher percentage of cells arrested at the G2/M phase. Our results provide mechanistic insights into the induced cyto- and genotoxic effects associated with SWCNTs exposure as a function of their physical and chemical properties and further help define novel means to use electronic platforms and single cells as biosensors to assess the toxicity of different types of nanomaterials in real time.

3484 Detection of Transcriptomic Signature for a Senescence-Associated Secretory Phenotype (SASP) in Human Peritoneal Mesothelial Cells Exposed to Multiwalled Carbon Nanotubes

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Multiwalled carbon nanotubes (MWCNTs) have been shown in *in vivo* studies to cause mesothelioma, a tumor frequently associated with asbestos exposure. For instance, intraperitoneal treatment in rats with a tailor-made MWCNT (e.g. 'MWCNT A', geometric mean length: 8.57 µm, and diameter: 0.085 µm of WHO fibers weighted by mass) led to high-tumor incidence and early fatality (Rittinghausen et al. 2014 Part Fibre Toxicol 11:59). To identify molecular cues leading to MWCNT-induced mesothelioma, we carried out a parallel *in vitro* study on the same MWCNT using classical and molecular genotoxicity approaches in primary human peritoneal mesothelial LP9 cells. Findings included markers for cellular senescence, e.g. inhibition of cell division, nuclear fragmentation, chromatin condensation, senescence-associated heterochromatin foci (SAHFs), pan-nuclear staining with an anti-γH2AX antibody, and enlarged cells, being positive for senescence-associated β-galactosidase activity. Using microarray technology, we also analyzed MWCNT-treated (3 µg/cm2, 24 h incubation) LP9 cells, in which amosite asbestos served as positive, while milled MWCNT as material control. We found many differentially regulated genes in MWCNT A-treated cells, which were many-folds higher up- or downregulated, than in cells exposed to amosite or milled MWCNT. There were common biomarkers in MWCNT A and amosite, but also exclusive ones. Notably, expression of genes associated with senescence-associated secretory phenotype (SASP) was differentially regulated especially in MWCNT A, but not in asbestos- or milled MWCNT-treated LP9 cells. The detection of SASP signature (chemokines, cytokines, metalloproteases, and growth factors) supports our previous results to suggest for a potential role of cellular senescence in mesothelioma development after exposure to certain long and straight MWCNT.

3485 Functional and Molecular Responses to Inhalation of MWCNT From the Perspective of Occupationally-Relevant Depositions

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In order to assess the toxic response to occupationally-relevant depositions of multi-walled carbon nanotubes (MWCNTs), a dose- and time-dependent 4-week inhalation exposure (0.5-5 mg/m3) to MWNCTs (Mitsui-7) was conducted to model a worker exposed to 76 (40 µg alveolar deposition), 7.6 (4 µg) and 0.76 (0.4 µg) years at average inhalable workplace concentrations of 10.6 µg/m3. Mice were sacrificed at 0, 28, and 84 d post-exposure with lung, liver, and aorta collected for microarray-based gene expression profiling analyzed in conjunction with alterations in lavage proteins, alveolar macrophage function, histopathology, extrathoracic translocation, and systemic effects. Differentially expressed genes, upstream transcriptional regulators, and responses corresponding to inflammation/immune function and pathological changes (e.g. fibrosis) were persistent in the high dose but transient in the middle dose. The responses reflected the 58 lavage proteins measured and morphometric analysis of alveolar fibrosis which was increased in the high dose only. Similarly, isolated alveolar macrophages challenged with LPS (1 ug/ml) *ex vivo* had enhanced cytokine production that was sustained only in the high dose. At all doses, MWCNT translocated to extrathoracic organs indicating a dependence on physicochemical properties instead of a specific molecular mechanism. Analysis of systemic effects showed minimal to no changes in liver or aorta transcriptomics, 58 analyzed plasma proteins, or liver and renal histopathology. Endothelial cells challenged with plasma collected from the high dose group showed enhanced expression of adhesion molecules. In summary, altered molecular pathways, histopathology changes, and systemic effects occur primarily at depositions (or dose rates) predicted to be significantly higher than what was measured in 8 MWCNT workplaces.

3486 Toxicity Assessment and Bioaccumulation in Zebrafish Embryos Exposed to Carbon Nanotubes Suspended in Pluronic® F-108

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Carbon nanotubes (CNTs) are often suspended in Pluronic® surfactants by sonication, which may confound toxicity studies because sonication of surfactants can create degradation products that are toxic to mammalian cells. Here we present a toxicity assessment of Pluronic® F-108 with and without suspended CNTs using embryonic zebrafish as an *in vivo* model. Pluronic® sonolytic degradation products were toxic to zebrafish embryos just as they were to mammalian cells. When the toxic Pluronic® fragments were removed, there was little effect of pristine multi-walled CNTs (pMWNTs), carboxylated MWNTs (cMWNTs), or pristine single-walled carbon nanotubes (pSWNTs) on embryo viability and development, even at high concentrations. A gel electrophoretic method coupled with Raman imaging was developed to measure the bioaccumulation of CNTs by zebrafish embryos, and dose dependent uptake of CNTs was observed. These data indicate that embryos accumulate pMWNTs, cMWNTs, and pSWNTs yet there is very little embryo toxicity.

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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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