2705 Toxicity Responses of Various Fibrillar and Crystalline Nanocellulose Materials: Differences and Similarities

E. R. Kisin[1], N. Yanamala[1], A. L. Menas[1], S. Friend[1], G. V. Shurin[2], U. B. Vogel[3], D. Beezhold[1], and A. A. Shvedova[1,4]. [1]*NIOSH, Morgantown, WV;* [2]*University of Pittsburgh, Pittsburgh, PA;* [3]*National Research Centre for the Working Environment, Copenhagen, Denmark; and* [4]*West Virginia University, Morgantown, WV.*

Extensive research of new nanostructured cellulosic materials has resulted in advances in material properties and unique product applications. New technologies facilitated the creation of bacterial, nanocrystalline and micro/nano-fibrillary cellulose derived from natural sources that are easily integrated into bio-based and recyclable products. The aim of the current study was to compare five different nanocellulose (NC) particles using *in vitro* approaches to determine if several physico-chemical characteristics (i.e. size, shape, origin) yield specific cytotoxicity effects. Human lung epithelial cells (A549) were exposed to NC for 24 and 72 h to determine how variations in the properties contribute to cellular outcomes, such as cytotoxicity, oxidative stress, and cytokine secretion. Our results showed that nanofibrillated cellulose (NCF) induced stronger cytotoxicity and oxidative stress responses compared to cellulose nanocrystals (CNC). CNC, on the other hand, caused a significantly stronger inflammatory response compared to NCF. Additionally, immunostaining indicated that only CNC particles were taken up by the cells. Clustering analysis of the inflammatory cytokines/chemokines revealed a similarity of NCF to the carbon nanofibers response, while CNC was akin to that of chitin, a known immune modulator and activator of innate cells. Taken together, these results indicate that size and shape of NC particles are critical to determining their toxicity: CNC and NCF induce distinctly different patterns of toxic and inflammatory response in lung cells.

2706 The Intracellular Fate of Multiwalled Carbon Nanotubes in Macrophages Using Laser Scanning Confocal Raman Microscopy

M. Huynh, C. Mikoryak, R. Wang, P. Pantano, and R. Draper. *University of Texas at Dallas, Richardson, TX.*

Both the production and use of multiwalled carbon nanotubes (MWNTs) are rapidly increasing world-wide despite the possible adverse effects they may have on human health. The Bionanosciences Group at UT Dallas is interested in understanding the interactions of polymer- and protein-coated MWNTs with macrophages that are the first responders to invaders in the body. This information is relevant for improving the biomedical efficacy of MWNT therapeutics, for understanding mechanisms of MWNT biocompatibility, and for designing methods to ameliorate MWNT toxicity. To better understand potential mechanisms of MWNT toxicity, it is important to know whether MWNTs physically enter cells and where they locate in cells. We have developed procedures to measure the subcellular location of MWNTs and reconstruct 3D images of cell-associated MWNTs cells at 37 °C and 4 °C 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 results at 37 °C show that polymer- and protein-coated carboxylated-MWNTs (C-MWNTs) are within punctate vesicles, most likely in the endosome/lysosome system. Conversely, at 4 °C, C-MWNT signals are only found at the periphery of the cells around the membrane, suggesting that the uptake of C-MWNT is through an energy-dependent receptor-mediated endocytosis pathway. Future work will involve cluster analyses and live-cell imaging to access whether MWNTs that induce cytokine release can be correlated with damage to the lysosomal membrane and redistribution of MWNTs to the cytoplasm. If successful, this will result in a relatively rapid technique that can be used to determine whether lysosomal damage has occurred because of MWNT exposure, and could also be applied to many other types of Raman-active nanoparticles that may induce pro-inflammatory responses.

2707 Differences in Multi- and Single-Walled Carbon Nanotube-Induced DNA Methylation: Alterations in Association with Nuclear Deposition

D. Oner[1], M. Ghosh[1], M. Ameloot[2], H. Bové[2], K. Van Landuydt[1], L. Godderis[1], and P. Hoet[1]. [1]*KU Leuven, Leuven, Belgium; and* [2]*UHasselt, Hasselt, Belgium.*

Subtle DNA methylation alterations mediated by carbon nanotubes (CNTs) exposure might contribute to pathogenesis and disease susceptibility. In order to understand the epigenetic toxicity, in particular DNA methylation alterations, of single-walled (SW)CNTs and short multiwalled (MW)CNTs, we performed global/genome-wide, gene-specific DNA methylation and RNA-expression analyses after exposing human bronchial epithelial cells (16HBE14o- cell line). In addition, the presence of CNTs on/in the cell nucleus was evaluated in a label-free way using femtosecond pulsed laser microscopy. Generally, a higher number of SWCNTs, compared to MWCNTs, was deposited at both the cellular and nuclear level after exposure. Nonetheless, both CNT types were in physical contact with the nuclei. No global (5-mC) DNA methylation alteration was observed for both CNTs. After exposure to MWCNTs, 2398 genes were hypomethylated (at gene promoters), and after exposure to SWCNTs, 589 CpG sites (located on 501 genes) were either hypo- (= 493 CpG sites) or hypermethylated (= 96 CpG sites). Cells exposed to MWCNTs exhibited a better correlation between gene promoter methylation and gene expression alterations. Differentially methylated and expressed genes induced changes (MWCNTs > SWCNTs) at different cellular pathways, such as p53 signalling, DNA damage repair and cell cycle. On the other hand, SWCNT exposure showed hypermethylation on functionally important genes, such as SKI proto-oncogene (*SKI*), glutathione S-transferase pi 1 (*GTSP1*) and shroom family member 2 (*SHROOM2*) and neurofibromatosis type I (NF1), which the latter is both hypermethylated and downregulated.After exposure to both types of CNTs, epigenetic alterations may contribute to toxic or repair response. Moreover, our results suggest that the observed differences in the epigenetic response depend on particle type and differential CNT-nucleus interactions.

2708 Cytokine Production by Rat Mesothelial Cells Exposed to Carbon Nanotubes as a Means of Assessing Long-Term Risks for Mesothelioma

S. Sridharan, A. J. Taylor-Just, and J. C. Bonner. *North Carolina State University, Raleigh, NC.*

Multiwalled carbon nanotubes (MWCNTs) are a type of highly durable engineered nanomaterial with many industrial applications but are structurally similar to asbestos fibers. It has been shown previously that MWCNTs cause fibrosis and inflammation in the lungs of mice. Prolonged exposure to MWCNTs could potentially cause neoplastic transformation of mesothelial cells in the pleural lining of the lungs leading to mesothelioma. However, it is not known whether MWCNTs causes mesothelioma in humans. A biomarker of human mesothelioma is osteopontin (OPN), a cytokine that produced by mesothelial cells that mediates cell migration. We hypothesize that long-term exposure to MWCNTs would cause transformation of normal rat mesothelial cells to mesothelioma cells *in vitro* that can be predicted by an increase in OPN. Normal rat mesothelial cells (NRM-2) were grown in six-well cell culture plates *in vitro* and exposed to tangled (t)- or rigid (r)-MWCNTs for 45 weeks. With each new cell passage, MWCNTs were re-introduced to the cell medium for a final concentration of 0.1 µg/ml. Expression of the mRNAs encoding OPN was measured using real-time qPCR, the concentration of OPN secreted protein *in vitro* was measured using an ELISA, and cell invasion assays were used to analyze the ability of cells to exhibit neoplastic characteristics. Rat pleural mesothelioma cells (ME1) were used as a comparison to NRM2 cells. Mesothelioma (ME1) cells spontaneously produced high levels of OPN whereas untreated control NRM-2 cells did not. There was a significant increase in OPN mRNA expression in NRM-2 cells after chronic exposure to r-MWCNTs but not t-MWCNTs. In the invasion assay, ME1 cells spontaneously showed increased invasion compared to untreated NRM2. Surprisingly, chronic exposure to t-MWCNTs but not r-MWCNTs caused increased invasion of NRM2 cells. Induction of OPN mRNA does not correlate with increased invasion of MWCNT-treated mesothelial cells. Further study is needed to assess reliable biomarkers of mesothelial cell transformation to predict the carcinogenicity of MWCNTs. *Funding: Supported by NIEHS grant R01-ES020897 and NIEHS P30-ES025128.*

The Toxicologist

Supplement to
Toxicological Sciences

57th Annual Meeting and ToxExpo™

San Antonio, Texas
March 11–15, 2018

OXFORD
UNIVERSITY PRESS

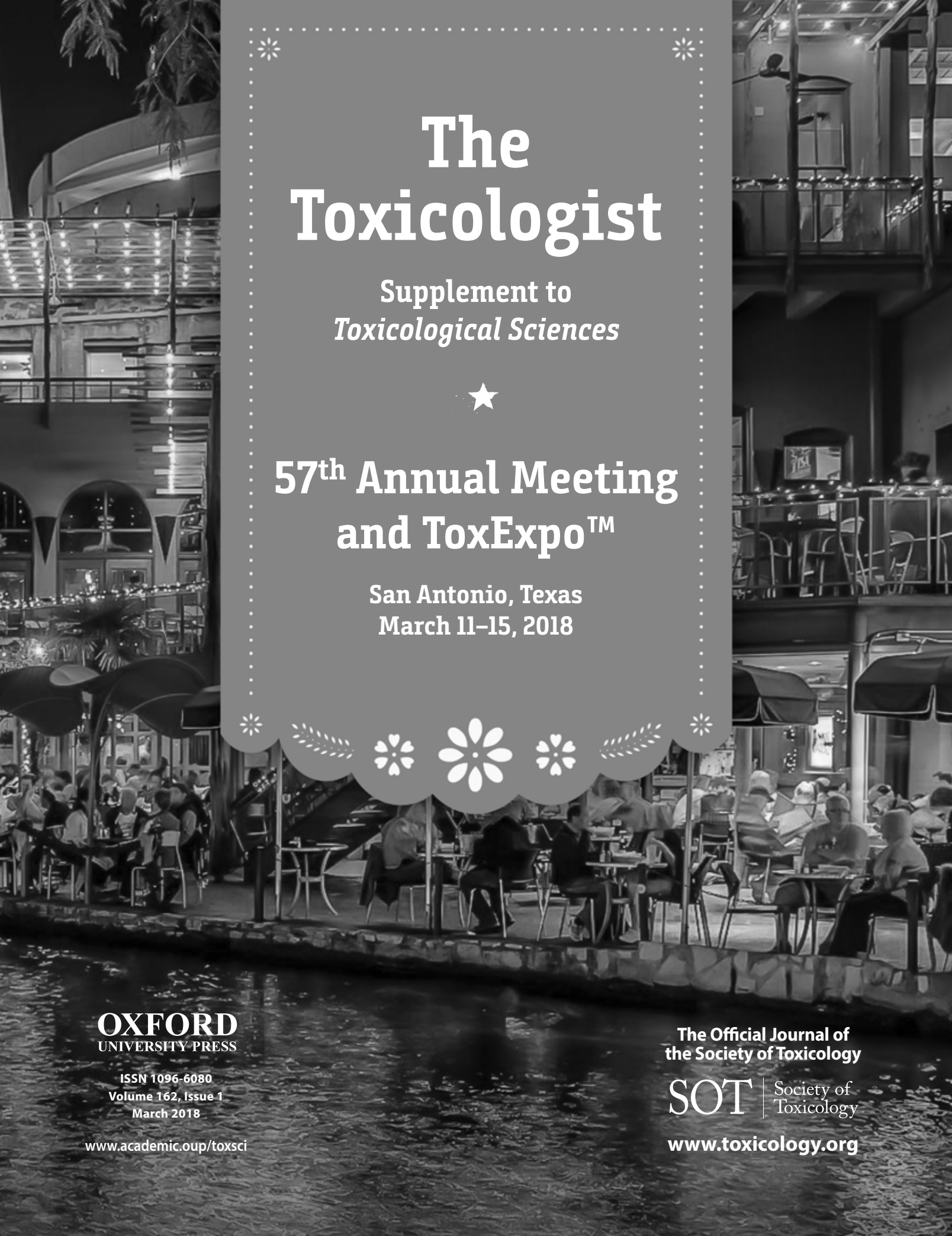
ISSN 1096-6080
Volume 162, Issue 1
March 2018

www.academic.oup/toxsci

The Official Journal of
the Society of Toxicology

SOT | Society of Toxicology

www.toxicology.org



The Toxicologist

Supplement to
Toxicological Sciences

57th Annual Meeting and ToxExpo™

San Antonio, Texas
March 11–15, 2018

OXFORD
UNIVERSITY PRESS

ISSN 1096-6080
Volume 162, Issue 1
March 2018

www.academic.oup/toxsci

The Official Journal of
the Society of Toxicology

SOT | Society of Toxicology

www.toxicology.org