PS 591 Determination of Stoichiometric ROS Degeneration and Relationship between Redox Potential and Bioavailability to Design Safe CNTs

S. Tsuruoka[1], H. Matsumoto[2], K. Takeuchi[3], K. Koyama[1], N. Saito[5], Y. Usui[1], S. Kobayashi[5], E. Akiba[4], D. W. Porter[6], V. Castranova[6], F. R. Cassee[7] and M. Endo[1, 3]. *[1]Research Center for Exotic Nanocarbons, Shinshu University, Nagano, Nagano, Japan, [2]Tokyo Institute of Technology, Meguro, Tokyo, Japan, [3]Institute for Carbon Science & Technology, Shinshu University, Nagano, Nagano, Japan, [4]Kuraray Living Co., Ltd., Osaka, Osaka, Japan, [5]School of Health Science, Shinshu University, Matsumoto, Nagano, Japan, [6]NIOSH, Morgantown, WV and [7]RIVM, Bilthoven, Netherlands.*

An important goal is to design safe carbon nanotubes (CNTs) by controlling their properties. Biological evaluations have been conducted with individual CNTs, and such results have been used for risk assessment (NIOSH CIB 65, 2013). However, information is lacking to define the relationships between the chemical properties of CNTs and their bioavailability. The present work aimed to obtain a stoichiometric expression of ROS degeneration by CNTs, which predicted ROS scavenging results with CNTs. ROS degeneration rate was measured with an ESR-DMPO method. The Fenton reaction was used to generate OH radicals. To evaluate CNT surface reactions, surfactant concentration was minimized to eliminate its influence, and reactivity of CNTs was determined. Results indicate that this allows kinetic quantification of the relationship between degeneration reactions and CNT morphology. Since the degeneration of ROS is attributed to a redox reaction according to the hypothesis in reaction kinetics on CNT surface, CNT bioactivity is defined as a redox potential that can be used to elucidate the relationship between bioactivity and CNT physicochemical properties. In conclusion, redox potential will be used to predict CNT bioactivity using its surface morphology and to design safer CNTs. [This work was supported by the Exotic Nanocarbon Project, Japan Regional Innovation Strategy Program by the Excellence, JST(Japan Science and Technology Agency)(ST, KK, YU, ME)].

PS 592 Development of Determination Method of Single-Walled Carbon Nanotubes in the Lung of Intratracheal-Instilled Rat

N. Shinohara[1, 2], K. Uchino[2], K. Fujita[1, 2], S. Endoh[2], J. Maru[2] and H. Kato[1, 2]. *[1]National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan and [2]Technology Research Association for Single Wall Carbon Nanotubes (TASC), Tsukuba, Ibaraki, Japan.*

Novel method to determine single-walled carbon nanotubes (SWCNTs) in rat lung was developed to evaluate the pulmonary SWCNTs clearance after intratracheal instillation. First, rat lung homogenate containing SWCNTs was treated with nitric acid (HNO3) under gradual heating using microwave digestion system. After the acid treatment, SWCNT adsorbed on the PFTE tube or glass tube was desorbed with ethanol using ultrasonication. Secondly, acid-digested samples and washed ethanol were filtrated with silica wool filled in stainless tube or silica glass tube. Wool and tube had been preheated at 900 °C in a muffle furnace to reduce the contamination. The wool containing the SWCNTs was washed with pure water 5 times, and then heated gradually to 900 °C using pyrolyzer under O2 gas flow. CO2 outgas from the pyrolyzer was determined over time with IR (infrared spectroscopy) using the calibration curve created with glucose. In the IR analysis, CO2 derived from single biological carbon without SWCNTs and SWCNTs without biological carbon were separately detected under 500 °C and 500 to 900 °C, respectively. In the case acid treatment was not conducted for the biological carbon sample containing SWCNTs, however, the tailing of the IR peak of CO2 derived from biological carbon interfered the IR peak of CO2 derived from SWCNTs. After the acid treatment to the biological carbon sample containing SWCNTs, the IR peak of CO2 derived from residual biological carbon were completely separated from the IR peak of CO2 derived from SWCNTs. SWCNTs in the powder, SWCNTs in the suspension, and SWCNTs spiked to rat lung homogenate could be recovered at higher than 90% between several μg and 100 μg of SWCNTs. This developed method will be applied to determine the pulmonary SWCNTs burdens after intratracheal instillation of SWCNTs. This research was funded by NEDO, Japan (P10024).

PS 593 Acute Inhalation Toxicity of Graphene Oxide and 5-Day Repeated Inhalation Toxicity of Graphene

J. Kim[1], J. Shin[2], J. Hwang[2], J. Lee[2], T. Kim[1], J. Lee[1], Y. Kim[6], H. Lee[3], N. Song[4], K. Ahn[5] and I. Yu[1]. *[1]Institute of Nanoproduct Safety Research, Hoseo University, Asan, Republic of Korea, [2]Occupational Lung Diseases Institute, KCOMWEL, Ansan, Republic of Korea, [3]Donga University, Busan, Republic of Korea, [4]KRISS, Daejeon, Republic of Korea, [5]Hanyang University, Ansan, Republic of Korea and [6]Korea Ginseng Corporation, Daejeon, Republic of Korea.*

Hazard of graphene exposure to human health, however, has not been known well. Acute pulmonary toxic effect of graphene oxide was evaluated via nose-only inhalation for 6 hours to experimental animals. Male Sprague-Dawley rats were divided into 3 groups (4 rats in each group), the fresh air control (0 mg/m^3), low (0.46 ± 0.06 mg/m^3), and high (3.76 ± 0.24 mg/m^3) concentration group, respectively. Lactate dehydrogenase (LDH), microprotein (mP), and microalbumin (mALB) levels were evaluated from the bronchoalveolar lavage (BAL) fluid on day 1, 7, and 14 after 6 hours exposure to graphene oxide. In addition, 5-day repeated inhalation toxicity of graphene was conducted to rats by assigning 3 groups (20 rats in each group); the control (0 mg/m^3), low dose (0.68 ± 0.14 mg/m^3) and high dose (3.86 ± 0.94 mg/m^3). The rats were exposed to graphene for 6 hr/day for 5 day. The exposed rats were allowed to recover for 14 to 28 days to evaluate the biopersistent effect of graphene oxide and graphene on the lungs after the acute and 5-day repeated exposure. The ingestion of graphene oxide and graphene in the alveolar macrophages was also evaluated. There was no statistical significant difference in average concentrations of LDH, mP, and mALB in the exposed groups when comparing with the unexposed control in all post-exposure groups (1, 7, and 14 days). The ingestion of graphene oxide in the alveolar macrophages was observed in all post exposure groups of the high dose group, but it was difficult to observe the graphene oxide in the control and low concentration group. Graphene oxide did not induce acute pulmonary toxic effect in the lungs of experimental animals. Graphene also did not induce any significant body weight, organ weight and lung weight changes after 5 day exposure and during 28 days of recovery.

PS 594 Modulation of Toll-Like Receptor Activity by Pristine Single-Walled Carbon Nanotubes with Distinct Chiral Enrichment

X. Zheng[1], N. Afrooz[2], N. B. Saleh[2], J. Bisesi[1] and T. Sabo-Attwood[1]. *[1]University of Florida, Gainesville, FL and [2]University of South Carolina, Columbia, SC.*

The rapid expansion of nanomaterial use in industrial, consumer and medical sectors has raised concern regarding associated health effects from unintended exposure. There is exceptional interest in single-walled carbon nanotubes (SWNT), as they are used in many common applications. As SWNT share a resemblance to asbestos, there is concern regarding inhalation exposure and associated lung diseases. Although consequences of SWNT exposure in the lung include pulmonary injury and fibrosis, few studies have focused on the ability of these particles to modulate infections by pathogenic agents. Recognition of invading pathogens occurs through interaction with toll-like receptors (TLRs) that stimulate the immune system through activation of nuclear factor kappa beta (NF-κB). Based on this knowledge, we hypothesized that SWNT alter TLR activity and the production of pro-inflammatory cytokines through induction of NF-κB. Furthermore, SWNT of distinct chiral enrichment (SG65, SG76 and CG200) would differentially impact TLR activity. Using human embryonic kidney (HEK) cell lines which overexpress TLR2 or TLR3 and an NF-κB-driven reporter gene, we show that all of the SWNT tested failed to modulate activity of either TLR. However, SG65 SWNT significantly suppressed TLR2 activation by the agonist zymosan while significantly enhancing TLR3 activation by the agonist poly (I:C). Neither SG76 or CG200 SWNT nor the particle control carbon black (CB) had any influence on TLR2 or 3 activities. Characterization of SWNT by dynamic light scattering (DLS) reveal SG65 SWNT formed smaller aggregates and were more stable compared to the other SWNT tested. Finally, utilization of near-infrared fluorescence (NIRF) suggests that the SG65 SWNT interacted with the TLR agonists. Overall, these data suggest that chirality, aggregate size and stability of SWNTs may influence the activity of TLRs and have implications for interfering with normal pathogen defense systems.

The Toxicologist

Supplement to *Toxicological Sciences*

53rd Annual Meeting and ToxExpo™

March 23–27, 2014 • Phoenix, Arizona

An Official Journal of the Society of Toxicology

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OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 138, Issue 1
March 2014

www.toxsci.oxfordjournals.org