2191 Biointeractions of Aerospace Relevant Nanomaterials with Human Gut Microbiota in a Human Gut Simulator

R. Agans[1], A. Gordon[2], S. Hussain[3], and O. Paliy[2]. [1]*Henry M. Jackson Foundation, Wright-Patterson AFB, OH;* [2]*Wright State University, Fairborn, OH; and* [3]*Air Force Research Laboratory, Wright-Patterson AFB, OH.*

Broad inclusion and incorporation of engineered nanomaterials (ENMs) in consumer goods demands an understanding of the impact such products have on humans. Specific concern has risen regarding oral exposures to dietary ENMs and the subsequent impact on gastrointestinal health via microbial dysbiosis. Employing an *in vitro* Human Gut Simulator (HGS) system, we have examined interactions of dietary nano titanium dioxide (TiO_2) with human gut microbiota. Following HGS seeding and community stabilization, we administered TiO_2 over seven days, followed by a seven day recovery period. We measured changes to cell density, community structure, metabolic end products, and predicted community functional capacity. Addition of low dose TiO_2 resulted in a modest loss of cell density only in the transverse region of the HGS. High dose TiO_2, conversely, caused a rapid reduction in cell density as early as 24 hrs following exposure in the proximal vessel. Similarly, population density was also lost in the transverse and distal regions. Furthermore, cell density recovered to the original levels following cessation of TiO_2 addition. Microbiota profiling via 16S rRNA gene based high-throughput sequencing did not reveal any specific susceptibilities to TiO_2 exposure within individual HGS regions at the *Class* level. Comparison of the various ratios between bacterial abundance, and assessment of gram status differences during pre-TiO_2, and post-exposure periods; indicated a broad effect of TiO_2 on the microbiota. We also did not notice a specific change in measures of community diversity, with alpha diversity and evenness maintaining similar values during all measured periods. Predicted functional capacity of the microbiota also remained unchanged during TiO_2 exposures. Interestingly, Scanning Transmission Electron Microscopy (STEM) indicated close association of TiO_2 particles to bacterial cells, but no direct interaction. These results provide evidence for the negative impact of TiO_2 on the whole gut microbiota community independent from the host following recurring exposures.

2192 Impact of Various Surface Coatings on *In Vitro* Cell Uptake and Cytotoxicity of Ultrasmall Superparamagnetic Iron Oxide Nanoparticles (USPION)

T. Palacios-Hernandez, A. Nguyen, S. Skoog, Y. Wu, X. Tang, and P. Goering. *US FDA/CDRH, Silver Spring, MD.*

USPION are excellent candidates for medical applications, due to their unique physicochemical properties (e.g., nanoscale size, highly reactive surfaces, and superparamagnetism). However, the potential adverse health effects of USPION with different coatings, commonly employed to control their biological activity and stability, are not fully understood. Therefore, the goal of this study was to evaluate cellular uptake and cytotoxicity of carboxyl- and amino-coated USPION on human coronary artery endothelial cells (HCAEC) as a vascular cell model. Both types of USPION were spherical with average diameter of ~30 nm as assessed by transmission electron microscopy (TEM), hydrodynamic diameter of ~100 nm diameter as assessed by DLS, and negatively charged (-36.3 and -6.5 mV for carboxyl- and amino-coated USPION, respectively) according to zeta potential analyses. After heat sterilization, the size and surface charge were unchanged for carboxyl-coated USPION; however, surface charge for amino-coated USPION slightly increased (-0.66 mV) and evidence of aggregation was observed. A concentration-dependent cytotoxicity was observed using the Alamar Blue (AB) assay; cells exposed for 24 h to 25, 50 or 100 µg/mL of carboxyl-coated USPION exhibited viabilities of 100, 57, and 42 percent of control, respectively. Nanoparticle uptake assessed by confocal and phase contrast microscopy showed perinuclear accumulation inside cytoplasmic vesicles of carboxyl-coated USPION. In contrast, amino-coated USPION exhibited minimal cytotoxicity at all concentrations tested and negligible cell uptake. These findings indicate that HCAEC injury is directly proportional to USPION exposure concentration and cellular uptake; however, this response depends on the type and chemical properties of surface coatings on the particles. This data will help build upon the current toxicological profile of USPION employed in medical products.

2193 Incinerated Carbon Nanotube-Enabled Thermoplastics Enhance Cytotoxicity in Human Airway *In Vitro* Models

J. Coyle[1], R. Derk[1], T. Kornberg[1,2], D. Singh[3], T. Stueckle[1], P. Demokritou[3], Y. Rojanasakul[2], and L. Rojanasakul[1]. [1]*NIOSH, Morgantown, WV;* [2]*West Virginia University, Morgantown, WV; and* [3]*Harvard University, Boston, MA.*

Engineered nanomaterials (NMs) are increasingly being incorporated into synthetic materials as fillers and additives. However, the potential pulmonary exposure effects of NM-enabled composites (NECs) during recycling and disposal have not been adequately addressed. The current investigation aims to characterize the cytotoxicity of incinerated NM-enabled thermoplastic composites (iNECs) on two *in vitro* pulmonary models. Ultrafine particles released from thermally decomposed pristine PC and carbon nanotube-containing polycarbonate (PC/-CNT) and polyurethane (PU/-CNT) were captured on inline filters, extracted, and suspended in sterile water. Incinerated thermoplastics were ultrasonicated and diluted in culture medium for acute *in vitro* exposure to primary small airway epithelial (pSAE) and BEAS-2B cells. The Harvard DG model was utilized to estimate the particle settling into the cellular microenvironment to characterize *in vitro* deposited dose. After exposure, both cell lines were assessed for cytotoxicity, ROS, and mitochondrial membrane potential (ΔΨm). BEAS-2B demonstrated significant dose-dependent sensitivity to iNECs than incinerated pristine counterparts. In pSAE cells, cytotoxicity, enhancement in ROS, and dissipation of ΔΨm caused by PC, PC-CNT, and PU-CNT were generally lower in magnitude compared to BEAS-2B cells at treatments examined, and is likely attributable to differences in depositional characteristics between the respective culture media for both respective cell lines. Whilst the effect of iNECs on the distal respiratory airway epithelia remains limited in interpretation, the current *in vitro* model of the respiratory bronchial epithelia demonstrated profound sensitivity to iNECs at depositional doses plausibly relevant for occupational cohorts, indicating potential risk to occupational cohorts with direct exposure to incinerated thermoplastics NECs during disposal.

2194 The Influence of Fluid Dynamics on Nanomaterial Delivery and Toxicity: Elucidating the Roles of Particle Size and Cell Model

K. Comfort, K. Burns, and M. Jewett. *University of Dayton, Dayton, OH.*
Sponsor: L. Braydich-Stolle

Colloidal silver nanoparticles (AgNPs) are being increasingly utilized in biomedical applications. However, the effectiveness of these procedures are dependent upon sustained, strong interactions between AgNPs and the surrounding environment. Therefore, prior to the development of effective NP-based therapeutics an accurate means of assessing NP delivery must be established. Both *in vitro* and *in vivo* methodologies are being utilized, however, limited correlation exists between these models. One way to overcome this limitation is through the development of enhanced *in vitro* environments that retain the advantages of cellular systems but more accurately mimic true physiology. In this work, a dynamic *in vitro* environment was utilized to characterize the AgNP deposition efficiency. Dynamic flow was generated through the use of a peristaltic pump, operating at a flowrate equivalent to known capillary rates. To better understand how dynamic flow impacted deposition, two cell models were utilized; an adherent lung epithelial model (A549) and a suspension monocyte model (U937). Additionally, as bio-transport mechanisms are a function of particle size we included two experimental, citrate-coated AgNPs - 5 and 50 nm. AgNP deposition was evaluated and found to vary as a function of flow environment, cell model, and primary particle size. Dynamic flow significantly decreased the delivered dose of AgNPs to the adherent lung cells; with the 5 nm AgNPs experiencing the greatest drop in deposition. However, AgNP delivery was increased within a dynamic environment for the monocytes, due to increased nano-cellular interactions. For both cell models, the subsequent cytotoxicity, stress, and inflammatory responses correlated to delivered NP dosages, as assessed via reactive oxygen species production, p53 levels, and cytokine secretion. This work highlights the need for NP deposition and safety evaluations to be carried out in a physiologically relevant exposure system.

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