PS 308 MULTIGENERATIONAL IMPACTS OF CARBON NANOMATERIAL EXPOSURE ON THE MODEL ORGANISM *DAPHNIA MAGNA.*

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We examined how carbon nanomaterial core structure and surface chemistry influence the toxicity of these materials to the progeny of an exposed F_0 generation of Daphnia magna. F_0 daphnids were exposed to various types of carbon nanomaterials with different core structures and functionalizations. Chronic toxicity was measured on the parent population by evaluating mortality and reproductive parameters over a 21-day period. In addition, the impact of carbon nanomaterials in the presence of additional environmental stressors was evaluated by running experiments under ideal population conditions or highly dense population conditions. The neonates produced by the F_0 generation were raised for an additional 21-day period and mortality and reproduction were assessed. This was repeated for the next 3 generations of Daphnia. Data indicate that some carbon nanomaterials can have an impact on the reproductive capacity of future generations of daphnids from exposed parents. However, preliminary data also indicate that daphnia populations can recover within three generations after the exposure has been removed. Future experiments will look at the mechanism by which nanomaterial exposure may impact these subsequent generations of daphnids after a parental exposure.

PS 309 THE BIOLOGICAL RESPONSE OF MULTIWALLED CARBON NANOTUBES IN DIFFERENT DISPERSANTS.

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To date, there are many reports about the cytotoxicity of multi-walled carbon nanotubes (MWCNTs). However, the results are still controversial. As the one reason, various dispersants are used by each researcher. Therefore, we clarified influence of the dispersants of MWCNTs on the cellular uptake and the cytotoxicity. First, we examined the cytotoxicity, MWCNTs uptake and cytokine secretion to MWCNTs in three different dispersants (gelatin, carboxylmethyl cellulose and 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine) on human bronchial epithelial cells (BEAS-2B). Cytotoxicity was measured by alamar blue assay and cellular uptake of MWCNTs and cytokine secretion were analyzed using flow cytometry. Next, we researched that the relationship between the cellular uptake of MWCNTs and cytotoxicity using different two cell lines, BEAS-2B and human malignant pleural mesothelioma cells. We found that the cellular uptake of MWCNTs was different for each of the three dispersants and that the level of cytotoxicity and inflammatory response correlated with the cellular uptake of MWCNTs. The relationship between the cellular uptake of MWCNTs and cytotoxic effects was observed in two different cultured cell lines. Notably, the cellular uptake of MWCNTs that induced cytotoxicity at each of the exposed IC_{50} values for the MWCNT-dispersant combinations were constant in the two cell lines. These results indicate that different dispersants affect MWCNT uptake into cells, and that cytotoxicity depends cellular uptake of MWCNTs, not depends exposed dosage such as IC_{50} value. It suggests a possibility that toxicity appear depending at time even if the exposure of MWCNTs is the low concentration because MWCNTs are biopersistent.

PS 310 THE *IN VITRO* EFFECTS OF SILICA NANOPARTICLES ON MOUSE MACROPHAGE MORPHOLOGY AND FUNCTION.

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Silica (SiO_2) nanoparticles (NPs) are hard core, durable NPs that are being proposed as drug delivery devices for cancer therapy. SiO_2 NPs accumulate in macrophages of clearance organs after systemic administration and may not be fully cleared after administration. We determined if accumulation of SiO_2 NPs (7 nm) in mouse macrophages impact their morphology and function *in vitro*. RAW 264.7 and J774.A cells were exposed to SiO_2 NPs (0.001 g/L- 0.1 g/L) for variable time intervals (24h – 72h) and their viability, proliferation, and function were evaluated. Our results show that exposure to high concentrations (0.01- 0.1 g/L) of SiO_2 NP induced cell death as measured by plate based and flow cytometry viability assays. Lower concentrations (0.001 to 0.005 g/L) of SiO_2-NPs significantly reduced cell proliferation by ~ 4 fold as compared to control cells as shown by Coulter counter measurements. Interestingly, SiO_2 NPs induced a heterogeneous cell size distribution in RAW cells. Exposure of RAW cells to 0.005 g/L SiO_2 NPs also induced a 2 fold increase in the number of macrophages larger than 20 μm as measured by Coulter counter. The SiO_2 NP induced cell size increase was also confirmed by confocal microscopy. Cell surface activation markers (CD40, CD80 and CD86) did not show significant changes in expression when dosed with SiO_2 NP (0.0025 to 0.01 g/L). Phagocytosis assays in SiO_2 NP-treated macrophages were performed using fluorochrome conjugated *E.coli*. Bacterial uptake was assayed by flow cytometry and results show that SiO_2 NPs did not impair phagocytosis at 0.01 g/L. The results indicate that SiO_2 NP exposure can impact macrophage cell morphology and proliferation *in vitro*, but does not appear to impact activation markers and phagocytosis as indicators of macrophage function.

PS 311 MODULATION OF IL-8 ACTIVITY UPON LIPID RAFT DISRUPTION AND NANOPARTICLE EXPOSURE.

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With the increase in production of engineered nanomaterials, researchers are discovering that there is a direct impact of these nanomaterials on the cell membranes. The cell membrane contains many different types of lipids and proteins responsible for numerous cell functions. The assembly of cholesterols and sphingolipids within the membrane forms microdomain structures known as lipid rafts. Lipid rafts contain many receptors that are involved in regulating cytokine production. Studies have shown that engineered nanomaterials can erode the membrane and create holes. We hypothesized that nanoparticles can interfere with cellular signaling by inhibiting the assembly of receptors on the lipid rafts. Using alveolar epithelial cells containing an Interleukin-8 promoter Luciferase construct, we were able to evaluate the ability of charged nanoparticles to alter cytokine gene expression elicited by a secondary stimulus. We exposed cells to charged nanoparticles, followed with stimulation of the IL-8 promoter with the proinflammatory cytokine tumor necrosis factor-α (TNF-α). We then used endocytic inhibitors and cholesterol depletors to determine if the nanoparticles rely on endocytosis and membrane cholesterol to alter the TNF-α stimulated IL-8 promoter. Cell lysis products were collected to assess for intracellular proteins and IL-8 promoter expression. Additionally, cells were exposed to nanoparticles after adding TNF-α to determine if the nanoparticles can disrupt cellular signaling upon receptor activation. We found that cationic nanoparticles are able to reduce the IL-8 promoter activity despite exposure to the endocytic inhibitors. When membrane cholesterol is removed from the cells, all the effects of the nanoparticles disappeared. Furthermore, these nanoparticles can only disrupt signaling prior to receptor activation with TNF-α. Our studies demonstrated that cationic nanoparticles can disrupt cellular signaling in a cholesterol dependent manner. It is also possible that cationic nanoparticles can interfere with the receptor assembly on lipid rafts, thereby disrupting cellular signaling.

PS 312 EVALUATION OF OXIDATIVE STRESS AND APOPTOSIS IN THE LIVER FOLLOWING A SINGLE INTRATRACHEAL INSTILLATION OF CERIUM OXIDE NANOPARTICLES IN MALE SPRAGUE DAWLEY RATS.

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Cerium oxide (CeO2) nanoparticles appear to exhibit antioxidant properties which have led some to suggest that these particles may be used to treat medical conditions that are associated with increases in oxidative stress. Conversely, other *in vitro* and *in vivo* work has suggested that exposure to CeO2 nanoparticles can, at least under certain conditions, lead to increases in oxidative stress. Herein we attempt to explore the underlying mechanism of this finding. To this end, 7-week old male Sprague Dawley rats (n=72) were randomized to one of two groups: CeO2 nanoparticle (20 nm diameter) instillation (7 mg/kg in 300 μl normal saline) or age-matched saline control (300 μl normal saline). After instillation, animals were sacrificed at 1, 3, 14, 28, 56 and 90 days (n=6/group). Compared to saline-control animals, the concentration of malondialdehyde (MDA) per gram of liver tissue in CeO2 exposed animals was 25%, 31% and 20% higher at days 1, 3, and 90 post exposure ($p<0.05$) but unchanged at days 14, 28 days and 56. The increases in lipid peroxidation at day 1 and 3 were associated with 32% and 10% increases in the Bax to Bcl-2 ratio ($P<0.05$) while the ratio of Bax to Bcl-2 was 55%, 62%, and 47% lower at days 14, 28 and 56 ($P<0.05$). Compared to saline-control, the levels of cleaved caspase-3 (17 kDa and 19 kDa fragments) were by 32% higher and 49%

higher (P< 0.05) at days 1 and 3 before being decreased by 35%, 25%, 20%, and 7% at days 14, 28, 56, and 90 respectively (P<0.05). Taken together, these data are suggest that the initial response of the liver to intratracheal instillation of CeO2 nanoparticles is characterized by increased oxidative stress and caspase-3 activation at days 1 and 3 post exposure. Whether these events are associated with hepatic apoptosis and subsequent tissue remodeling is currently under investigation.

PS 313 AFTERGLOW ZNS:CU, AG CONJUGATED TO PHOTOSENSITIZE NANOPARTICLES INDUCES CYTOTOXICITY AND DNA DAMAGE ON HUMAN BREAST CANCER CELLS.

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BACKGROUND: Photodynamic therapy (PDT) has been widely used for skin cancer treatment, but rarely for deep tumor due to the issue of light penetration. A new PDT-agent system in which the light is generated by afterglow nanoparticles (NP) with attached photosensitizers, which making PDT impossible for deep cancer treatment. Data on the toxicological properties of the afterglow NP (such as, ZnS:Cu, Co and ZnS:Cu,Ag) are incomplete. The aim of the current study was to evaluate these afterglow NP-induced cytotoxicity and genotoxicity in human breast cancer cells following 4, 24 or 48 hours exposure to afterglow-NP. METHODS: Water soluble afterglow nanoparticles are synthesized by colloidal chemistry methods and the quantum yields and emission spectra will be measured. The NP- photosensitizers conjugates are coated with biodegradable polymers (DL-lactide-co-glycolide; PLGA) and the tumor destruction are monitored using bioluminescence imaging. Its potential cellular uptake by the tumor cells and assess their resultant toxicity will investigate with human breast cancer cell line (MCF-7) using MTT assay and CometAssay. RESULTS: The NP and photosensitizers were localized into the cells in the perinuclear region after a 24-hour incubation period. The NPs or photosensitizers only have no cytotoxicity. However, cytotoxicity was increased significantly when both NPs and photosensitizers were encapsulated into PLGA NPs using MTT assay and CometAssay. Further observations using a multiparameter cytotoxicity assay indicate that the NPs/photosensitizers-loaded PLGA NPs may induce the effect of toxic oxidative stress on cancer cells. CONCLUSIONS: The PLGA coated only ZnS:Cu,Ag and the free photosensizer have no cytoxicity effect using MTT assay and CometAssay. The ZnS:Cu,Ag- photosensizer -loaded PLGA microsphere induced the effect of toxic oxidative stress in MCF-7 cell line. The results of this study suggest that ZnS:Cu,Ag- photosensizer afterglow NPs PLGA microspheres represent potential and promising photodynamic therapy agents for deep cancer treatment.

PS 314 PROTEIN EXPRESSION PROFILES OF INTESTINAL EPITHELIAL COCULTURES AFTER LOW-LEVEL EXPOSURE TO FUNCTIONALIZED CARBON NANOTUBES.

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To assess the biological effects of dispersible, functionalized carbon nanotube (CNT) exposure in an *in vitro* model simulating the digestive tract, Caco-2/HT29-MTX cell protein expression was quantified and compared using label-free quantitative mass spectrometry (LFQMS). Co-cultured cells (75% Caco-2, 25% HT29-MTX) were exposed to well-characterized carboxylated single-wall carbon nanotubes (SWCNT-COOH), carboxylated multi-wall carbon nanotubes (MWCNT-COOH), and poly(vinylpyrrolidone) (PVP) polymer functionalized multi-wall carbon nanotubes (MWCNT-PVP) for 48 hr at 500 pg/mL & 10 µg/mL. Proteins were extracted from the cells, reduced, alkylated, and proteolyzed. Tryptic peptides were analyzed by LC-MS/MS, acquired data searched against the International Protein Index (IPI) human database using SEQUEST algorithms in Bioworks, and peptide/protein identities validated by the Trans-Proteomic Pipeline. Protein quantitation and statistical analyses were performed using the LFQMS platform IdentiQuantXL™. Of the 5,008 proteins that were globally identified with >90% confidence, the expression of 2,444 unique proteins was compared across the dose groups. Among these, 428 proteins were differentially expressed (P<0.01). At the high dose, the extent of differential protein expression was CNT-specific and directly related to CNT colloidal stability. Surprisingly, cells responded to low dose MWCNT-PVP exposure with 3-fold greater differential expression than the high dose. Bioinformatic analysis indicated significant and CNT-specific effects on relevant functional networks and canonical pathways, with little overlap across CNT type and in the absence of overt toxicity. Supported by NIEHS RC2ES018025.

PS 315 COMPARATIVE STUDY ON ANTIBACTERIAL EFFICIENCY AND CYTOTOXICITY OF VARIOUS TYPES OF ION-DOPED TITANIUM DIOXIDE NANOPARTICLES.

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Visible light absorbing titanium dioxide (TiO2) has been widely accepted photocatalyst in harnessing solar energy for photocatalysis process. Doping TiO2 with non-metal or metal ions is one of the most efficient methods to shift the spectral response of TiO2 from UV to visible light region. Efficiency and safety of the these materials are important selection criteria for further commercial uses. In this study, chromium- (Cr-TiO2), nitrogen- (N-TiO2) and carbon-doped TiO2 (C-TiO2) nanoparticles were investigated on their antibacterial efficiency in S. aureus and E. coli and their toxic effects in human skin epithelial (A431) cells. The nanoparticles were firstly subjected to physical and chemical characterizations such as light absorption by UV spectrophotometer, morphology by TEM, chemical composition by EDX, crystal structure by XRD, hydrodynamic diameter by DLS as well as photocatalytic activity by DPPH assay. The results showed that all types of ion-doped TiO2 nanoparticles can absorb both UV and visible light. N-TiO2 nanoparticles showed the highest photocatalytic activity in correlation to their antibacterial effects against S. aureus and E. coli under visible light followed by C-TiO2 and Cr-TiO2, respectively. Using CCK-8 and DCF assays, N-TiO2 and C-TiO2 demonstrated their ability to reduce cell viability and to generate ROS in A431 cells, respectively, in a concentration-dependent manner. The higher effects can be seen in Cr-TiO2. Beside their photocatalytic ability under visible light, it is interesting that non-metal-doped (N-TiO2 and C-TiO2) showed higher antibacterial efficiency but lower toxicity to A431 cells than metal-doped (Cr-TiO2) particles. The results from this study can be used as a selection guidance of ion-doped TiO2 nanoparticles for specific applications.

PS 316 DEVELOPMENT AND *IN VITRO* BIOACTIVITY PROFILING OF ALTERNATIVE SUSTAINABLE NANOMATERIALS.

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Sustainable, environmentally benign nanomaterials (NMs) are being designed as alternatives based on functionality to conventional metal-based nanomaterials (NMs) in order to minimize potential risk to human health and the environment. Development of rapid methods to evaluate the potential hazard of alternatives before entering the marketplace is critical for informing material design and utilization. Cellular based high-throughput screening (HTS) assays, currently being utilized in the ToxCast chemical screening project, are valuable to evaluate the differences in the bioactivities of conventional NMs and their alternatives. Preliminary research has focused on development of nanoparticles (NPs) from natural biopolymer materials that will maintain integrity for intended applications and then rapidly degrade post-use. These NMs, infused with active components, would become inert after use and could serve as novel NM platforms in oral drug delivery or environmental remediation. Biodegradable cellulose and lignin NPs have been synthesized by an environmentally-friendly water-based antisolvent precipitation process based on pH-jump. The hydroxpropyl methylcellulose phthalate NPs (~200-300 nm in diameter) dissolve above pH ~ 5.5 limiting their potential applications. However, the synthesized lignin NPs (~30-100 nm in diameter) have been stabilized up to pH ~ 9, allowing them to be stable in physiological conditions for their intended use and in the HTS assays (pH ~7.2). The next phase of research will focus on further characterizing lignin NPs and evaluating bioactivity of infused particles pre- and post-use using ToxCast assays. This research takes an innovative and

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