2769 Assessment of Nanoparticle Toxicity in the Context of Virus Infection

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Titanium dioxide nanoparticles (TiO2) are increasingly used in the manufacturing of dietary supplements and cosmetics. Accumulation of nanoparticles in the intestine has been reported as a result of consumption of nanoparticles used as food additives, which could impart toxicity to the host by altering immune response and cellular metabolism. Mammalian intestine serves as a resourceful host for a variety of enteroviruses, and therefore the presence of viral toxins could exacerbate host response and immune toxicity from nanoparticles. To address this issue, we utilized murine norovirus infection of RAW 264.7 macrophages as a model system to investigate the toxicity of nanoparticles in the context of viral infection. The size, distribution, surface charge, stability, purity and endotoxin levels of the nanoparticles were determined prior to the study. RAW 264.7 cells were treated with TiO2 in anatase and rutile forms at concentrations of 20ug/ml and 2ug/ml for 3h and then infected with Murine norovirus at a multiplicity of infection (MOI) of 5, 0.5 and 0.05 particles. Plaque assay and real-time RT-PCR results indicated that TiO2 nanoparticles inhibited, or did not alter, norovirus replication even at MOI 5. Interestingly, pretreatment of cells with anatase and rutile forms increased viral replication up to seven-fold when infected at lower MOI. While preincubation of TiO2nanoparticles did not impact cytopathic effects to RAW macrophages, virus-induced cytopathicity was not prevented by nanoparticles. Cytokine analysis indicated that there was an increase in Tumor Necrosis Factor Alpha and Interleukin (IL)-12 due to the presence of nanoparticles during virus infection, while IL-6 was found to be reduced or unaltered. Comet assays and further gene expression profiling are underway to identify the genotoxic potential of nanoparticle accumulation in the context of viral infection. This study will elucidate safety and toxicity hazard profile prediction of nanoparticles exposed to the gastrointestinal tract in individuals infected with enteric viruses.

2770 Cytotoxic Effects Induced by Titanium Dioxide Aerosol Nanoparticles

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Titanium dioxide (TiO2) has widely been used such as cosmetics and industrials. However, investigations about toxic effects of TiO2 have been discovered. In particular, toxicity of TiO2 under aerosol exposure needs to be more demonstrated. The aim of this study is to elucidate the cytotoxicity effects of TiO2 aerosol nanoparticles by using the Cultex system. Before exposure, the calibration for the airway flow in cultex system was performed. The size distribution of TiO2 was measured by scanning mobility particle sizer (SMPS). Inductively coupled plasma mass spectrometry (ICP-MS) was used for the measuring a deposition efficiency. In addition, MTS assay was implicated as a cytotoxicity test. The initial input of air flow is total 2.3 liter per minute (LPM) in cultex system. In that amount of it, air flow of sampling pump and CO2 part is setted at 0.9 LPM, 0.05 LPM, respectively. Our air flow of total, sampling pump and CO2 part was 2.33 ± 0.009 LPM, 0.90 ± 0.001 LPM, 0.051 ± 0.001 LPM, respectively, which satisfied with all the set up value. Moreover, the average size was 59 ± 2.2 nm by SMPS. When exposed the TiO2 aerosol nanoparticles, the deposition rate was below 10% demonstrated by ICP-MS. Furthermore, cell viability was not diminished below 80% in MTS. After all, no cytotoxicity effect of TiO2 was observed at the aerosol exposure.

2771 Toxicity of Nano- and Ionic Silver to Embryonic Stem Cells - A Comparative Toxicogenomic Study

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The widespread application of silver nanoparticles (AgNPs) and silver-containing products has raised public safety concerns about their adverse effects on human health and the environment. To date, *in vitro* toxic effects of AgNPs and ionic silver (Ag+) on many somatic cell types are well established. However, only limited studies have been conducted in embryonic stem cells (ESCs) to evaluate their potential of developmental toxicity. In this study, we characterized transcriptomic changes induced by 5.0 µg/ml AgNPs during spontaneous differentiation of mouse ESCs, and compared to those induced by Ag+ under otherwise identical conditions. After 24 h exposure, 101 differentially expressed genes (DEGs) were identified in AgNP-treated cells, whereas considerably more (400) genes responded to Ag+. Despite the large differences in the numbers of DEGs, functional annotation and pathway analysis of the regulated genes revealed overall similarities between AgNPs and Ag+. In both cases, most of the functions and pathways impacted fell into two major categories, embryonic development and metabolism. Nevertheless, a number of canonical pathways related to cancer were found for Ag+ but not for AgNPs. Conversely, it was noted that several members of the heat shock protein and the metallothionein families were upregulated by AgNPs but not Ag+, suggesting specific oxidative stress effect of AgNPs in ESCs. Taking together, these results suggest that both AgNPs and Ag+ have the potential to cause developmental toxicities, and that although transcriptomic responses to AgNPs and Ag+ were substantially similar, AgNPs may exert specific effects on ESCs due to their nanosized particulate form.

2772 Assessing Genotoxicity of Iron Oxide Nanoparticles within an *In Vitro* Liver 3D Model

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Nanomaterials have demonstrated their applicability in various fields. Risk evaluations are still required for their safe application in order to assess and understand possible toxicity effects. The hepatic system is where these effects might possibly be observed as it is highly involved in the clearance of nanomaterials. The liver 3D *in vitro* model used was developed in order to evaluate potential nanomaterial genotoxic effects. Initially, growth and metabolic characteristics of the model were evaluated over a seven day period. The growth parameters investigated involved surface area measurements and cell viability using Trypan blue staining. Albumin and aspartate transaminase secretion, and CYP1A2 expression were the metabolic liver functions investigated. Optimal growth and metabolic characteristics were achieved after four days. Genotoxic assessments for dextran coated superparamagnetic iron oxide (Fe2O3) nanoparticles were then conducted on the fourth day. Micronucleus frequencies were investigated using a cytokinesis block micronucleus assay that showed significant ($P<0.05$) increases in micronucleus formation. Significant differences were not observed for the replicative indexes determined. Cell viabilities over 90% were retained after 48 hours of exposure. Scanning electron microscopy was used to observe the surface distribution of these nanoparticles. Additionally, synchrotron X-ray fluorescence was employed to investigate the distribution of these nanoparticles within the model. The 3D spheroid models represent an improved and more realistic test environment to conduct *in vitro* genotoxicity studies than standard 2D systems. Its versatility also enables the application of various methods involved in nanomaterial studies.

2773 Genotoxicity of Pristine, Heat-Treated, and Nitrogen-Doped Multi-Walled Carbon Nanotubes at Occupationally Relevant Doses

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The unique physiochemical properties of multi-walled carbon nanotubes (MW) make respiratory exposures likely in workers. Previously, *in vitro* exposure to MW has led to cell cycle disruption, chromosome errors, mitotic aberrations, and increased clonal growth at occupationally relevant doses. Combining the effects seen *in vitro* with the potential for lung deposition in the workplace, MW should be considered as a potential health hazard. Altering the physiochemical properties of MW has been shown to reduce toxicity. Nitrogen-doped MWCNT (NDMW) material is less inflammatory than pristine MW (PMW). PMW exposed to extremely high temperatures (HTMW) removes impurities and reduces their bioreactivity in acellular systems. To investigate genotoxicity of NDMW and HTMW compared to PMW at an occupationally relevant dose, we used two cell types, an immortalized human lung epithelial cell BEAS-2B and primary lung epithelial cell SAEC. All MW were necrotic in both cell types at the 24 µg/cm2 dose. There was no effect on the cell cycle in BEAS-2B cells. All MW induced a significant G0/G1 phase block in SAEC cells after 24 hour exposure to 24 µg/cm2 and a significant G1/S phase block after 72 hour exposure to 2.4 µg/cm2. Clonal growth in SAEC cells was inhibited by PMW at all doses, HTMW at 24 µg/cm2 and

NDMW at 2.4 and 24 µg/cm2. By contrast, number and size of colonies was increased by 0.024 and 0.24 µg/cm2 HTMW and NDMW and 2.4 µg/cm2 HTMW. Significant increases in mitotic aberrations, predominantly monopolar, were observed by exposure to all MW for 24 hours. All MW were found to penetrate the nucleus and associate with the DNA, mitotic spindle and centrosomes. Nuclear penetrations were greatest for the PMW followed by HTMW and NDMW, respectively. Significantly increased fragmentation of the centrosome and centromere were found in response to all MW exposure at all doses indicating a possible mechanism of genotoxicity, however a lower limit was not apparent. A dose-dependent increase in aneuploidy was observed from exposure to all MWs. These data indicate that altering the physiochemical properties of MWs may not reduce their genotoxic effect.

2774 Size and Crystal Structure Dependent Inhibition of Human Mesenchymal Stem Cell Adipogenic Differentiation by Nanoscale Titanium Dioxide

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Titanium dioxide (TiO2) has been used in a broad spectrum of consumer products, including food, cosmetics and various medical products. Increased use of nano-scale TiO2 in recent years has raised concern regarding their safety. In the current study, we characterized TiO2 nanoparticles with different crystal structures and particle sizes through electro microscopy, diffraction and scattering techniques. . Subsequently, we determined the impact of TiO2 nanoparticles on cell viability using LDH, ATP assays, and the adipogenic differentiation capacity using Oil red O Staining assay in human mesenchymal stem cells (hMSCs). We further investigated whether the impact of TiO2 nanoparticles was associated with specific particle size and/or crystal structure. Data revealed that TiO2 nanoparticles exhibited minimal acute (up to 72 hours exposure) cytotoxicity in hMSCs. There was a size- and crystal structure- dependent inhibition of hMSC adipogenic differentiation (21 days) by TiO2 nanoparticles. Cellular uptake and media "stripping" studies indicated that the inhibition of hMSC adipogenesis was likely due to direct cellular response to TiO2 nanoparticles instead of a "charcoal-stripping" effect of TiO2 leading to depleted growth factors in the culture media. Additional exploratory gene expression array analyses suggested that TiO2 nanoparticles inhibit hMSC adipogenesis by down-regulating key genes involved in adipogenesis promotion, including FGF2, IRS1, CEBPA, CEBPB, and ACACB, etc. Findings from this study indicate that TiO2 nanoparticles, while exhibiting minimal acute cytotoxicity, may impost long-term impact on hMSC adipogenic differentiation. Future planned studies will reveal the mechanism of TiO2 nanoparticle interaction in stem cell models. Disclaimer: The views presented in this article do not necessarily reflect those of the Food and Drug Administration.

2775 *In Vitro* Toxicity of Tio2 Nanoparticles Immobilized on Clay to Human Hepatic Cells

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Introduction: Nanotechnology is growing in rapid pace, discovering innovative and attractive nanomaterials for several applications. The fact that nanoparticles induce more damage and are more biologically active when compared to larger micro-sized particles encouraged the emergence of nanoarchitectonics. The immobilization of nanoparticles on the surface of inorganic or organic supports results in the creation of nanocomposites that combine the best properties of both components. Although it has been stated that micro-sized particles induce less toxicity than the nano-size ones, few studies have been made regarding the *in vitro* characterization of cellular responses to this type of materials. Objective: Evaluate *in vitro* toxicity of TiO2 nanoparticles immobilized on clay (C-TiO2) in a hepatocellular carcinoma human cell line (HepG2) as well as of its single elements. Materials and Methods: Materials were supplied by the Ceramic for Smart System Group of the Electroceramic Department, Instituto de Ceramica y Vidrio, Madrid, Spain and characterized by scanning electron microscopy for particle morphology and dynamic light scattering for average hydrodynamic size and potential zeta. After characterization, different concentrations and time periods were tested in regards of HepG2 viability, by employing MTT and Alamar Blue assays, and DNA integrity, by using comet assay. Results and Discussion: Results showed that all studied materials were capable to induce hepatocyte cell death in a dose dependent way and for the majority of the studied periods of exposure. Besides that, the HepG2 DNA was also affected after longer periods of exposure to TiO2 NPs, kaolinite and C-TiO2 nanocomposites. Conclusions: Nanocomposites are promising materials for different nanotechnological applications. Notwithstanding, it is of paramount importance to evaluate their potential toxicity. Data obtained suggests that other subtracts must be tested to immobilize TiO2 NPs as kaolinite mineral was found to be both cytotoxic and genotoxic for the studied cell line. Acknowledgements: Financial support from TD1204 MODENA COST Action.

2776 Effects of Zinc Oxide Nanomaterials on the Cellular Responses in THP-1 Cells

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Purpose: The biological effects of nanomaterials are related to the physicochemical properties such as composition, shape, particle size, aggregation state, surface area and surface charge. An *in vitro* cellular toxicological study using well-characterized nanomaterials is conducted for evaluation of the biological effects of nanomaterials. In this study, we examined the effects of zinc oxide (ZnO) nanomaterials on the cytotoxicity of THP-1 cells and the expression of CD54 and CD86. Methods: The size distribution and the zeta potential of ZnO nanomaterials were measured by dynamic light scattering. The cellular cytotoxicity and the expression of CD54 and CD86, skin sensitization marker, were measured by cellular ATP method and flow cytometry, respectively. Results and Discussion: The primary particle size and hydrodynamic diameter of ZnO nanomaterials in distilled water suspension were <35 nm and 66 nm (Sigma-Aldrich), and 40 nm and 165 nm (NanoTeK Alfa Aesar). The zeta potentials of ZnO nanomaterials in distilled water suspension were positive (44.9 mV, Sigma) and negative (-7.5 mV, Alfa). The cytotoxicity by ZnO (Sigma) was stronger than that by ZnO (Alfa). ZnO nanomaterials increased the CD54 in a dose-dependent manner, but did not affect the expression of CD86. The CD54 relative fluorescence intensity (RFI) after treatment with 50 µg/mL of ZnO for 24h were 2007 % (Sigma) and 1207 % (Alfa). In conclusion, ZnO nanomaterials showed the cellular cytotoxicity and increased the expression of CD54. The extent of these effects caused by ZnO (Sigma) was higher than that by ZnO (Alfa). The differences of the cellular responses may due to the differences of physicochemical properties between ZnO (Sigma) and ZnO (Alfa). Further analysis at the molecular-level would help the better understanding of the relation between biological effects and the physicochemical properties of nanomaterials.

2777 Induction of the Epithelial-Mesenchymal Transition by Zinc Oxide Nanoparticles

J. J. Kim. *Sungkyunkwan University, Seoul, Korea, Democratic People's Republic of.* Sponsor: D. Y. Shin.

Zinc oxide (ZnO) nanoparticles are one of the promising materials applied in the various kinds of the commercial products such as sunscreens. At the same time, there are growing concerns about unintended toxic effects of ZnO. Many researches on the toxicity of ZnO have been studied, except for the epithelial-to-mesenchymal transition (EMT). EMT is one of the significant multistep processes. Epithelial cells reduce intercellular adhesion and increase cell mobility which is crucial for the cancer metastasis. The aim of our study is to investigate the ZnO-induced EMT in the human alveolar epithelial A549 cells. At first, size distribution of ZnO nanoparticles was observed by dynamic light scattering (DLS). The cytotoxicity test was performed to find the appropriate exposure concentration. Real-time PCR was used for the mRNA expression, related with EMT. In addition, the EMT-associated protein level was measured by western blot. The morphology changes were obtained by the microscopy. The average size of ZnO showed 208 ± 16.10 nm measured by DLS. Cell viability as the cytotoxic effects was decreased in a dose-dependent manner. When A549 cells are exposed to ZnO nanoparticles, mRNA level of EMT-related transcription factor was increased including Snail. Furthermore, the protein levels related with EMT markers were found. In microscopy, ZnO induced morphology changes of A549 to spindle-like elongated shapes. Our data demonstrated that ZnO nanoparticles induced the EMT in A549 cells.

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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.

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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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- CE Continuing Education Courses
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