

PS 1934 **Small Airway Epithelial Cells Exposure to Printer-Emitted Engineered Nanoparticles Induces Cellular Effects on Human Microvascular Endothelial Cells in an Alveolar-Capillary Coculture Model**

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The printer is one of the most common office equipment. Recently, it was reported that toner formulations for printing equipment constitute nanoenabled products (NEPs) and contain engineered nanomaterials (ENMs) that become airborne during printing. To date, insufficient research has been performed to understand the potential toxicological properties of printer-emitted particles (PEPs) with several studies using bulk toner particles as test particles. These studies demonstrated the ability of toner particles to cause chronic inflammation and fibrosis in animal models. However, the toxicological implications of inhalation exposures to ENMs emitted from laser printing equipment remain largely unknown. The present study investigates the toxicological effects of PEPs using an *in vitro* alveolar-capillary co-culture model with Human Small Airway Epithelial Cells (SAEC) and Human Microvascular Endothelial Cells (HMVEC). Our data demonstrate that direct exposure of SAEC to low concentrations of PEPs (0.5 and 1.0 µg/mL) caused morphological changes of actin remodeling and gap formations within the endothelial monolayer. Furthermore, increased production of reactive oxygen species (ROS) and angiogenesis were observed in the HMVEC. Analysis of cytokine and chemokine levels demonstrates that interleukin (IL)-6 and MCP-1 may play a role in the cellular communication observed between SAEC and HMVEC and the resultant responses in HMVEC. These data indicate PEPs at low, non-cytotoxic exposure levels are bioactive and affect cellular responses in an alveolar-capillary co-culture model, which raises concerns for potential adverse health effects.

PS 1935 **Nanoparticle Ingestion Alters Nutrient Absorption in a Physiologically Based *In Vitro* Model of the Gastrointestinal Tract**

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The gastrointestinal (GI) tract serves as an interface between the internal circulation and external environment by absorbing nutrients and defending from outside threats. Nanoparticle (NP) ingestion from food and food packaging is nearly unavoidable, and the role of NP exposure on GI health and function is not well understood. We are testing the overall hypothesis that ingestion of some types of nanoparticles can alter mineral and nutrient absorption, change cellular gene and protein expression, and ultimately result in physiological consequences. Our *in vitro* model is composed of Caco-2 and HT29-MTX cells co-cultured on a semi-permeable membrane, and contains a mucus layer and simulated digestion. This *in vitro* model was exposed to physiologically relevant doses of 30 nm TiO₂ or SiO₂ NPs for acute (4 hours) or chronic (5 days) time periods. Following NP exposure, transport of stable isotopes (⁵⁸Fe and ⁶⁷Zn) across the cell monolayer was used to model iron or zinc transport into the bloodstream. Acute exposure to both TiO₂ and SiO₂ NP significantly increased iron transport, but decreased zinc transport. These changes in nutrient transport were not due to alterations in tight junction functionality, as transepithelial electrical resistance (TER) and occludin protein expression remained statistically the same between controls and NP-exposed cultures. Gene expression analysis showed that acute TiO₂ NP exposure decreased DMT1, which codes for the Fe²⁺ import protein, to 50% of control levels. ZIP1, which codes for a zinc import protein, was increased two-fold compared to untreated controls following acute SiO₂ NP exposure. Overall, these results suggest that intestinal epithelial cells are affected at a functional level by physiologically relevant exposure to NPs, and that the cells are working to regulate the iron and zinc transport mechanisms disturbed by NP ingestion.

PS 1936 **Evaluation of an *In Vitro* Assay for Nanoparticle-Induced Complement Activation**

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The ability to activate the serum complement cascade is a major determinant as to whether a nanoparticle will evoke immunotoxicity. Recently an *in vitro* assay for nanoparticle-induced complement activation was developed by Dr G Lanza et al

(Pham et al, Nanomed. 10, 651-60, 2014). As part of an FDA program to beta-test this assay for general use in nanotoxicology research, we have evaluated the assay using a spectrum of gold nanoparticles and compared the response of human serum with that of cynomolgus monkey, beagle dog and Sprague-Dawley rat serum. The assay measures residual complement activity in a serum sample by titrating the serum with hemolysin-sensitized sheep erythrocytes and determining the level of hemolysis. The serum samples are pre-exposed to nanoparticles so that the degree of nanoparticle-dependent complement depletion can be determined. The assay was evaluated using coated perfluorooctylbromide (PFOB) nanoemulsions provided by Dr Lanza's group. The assay worked well, providing quantitative and reproducible results with commercially available cryopreserved human and animal serum and with both commercially available and freshly prepared sensitized erythrocytes. Results with PFOB emulsions were correspondent to published data. Species differences were observed in complement activating potential of gold nanoparticles. Human and monkey serum was more sensitive to complement activation than that of dog and rat. E.G. a comparison of human and dog serum showed that 50 nm BioPure gold particles (nanoComposix bPEI, zeta Potential 79.9 mV) caused a 90.2% complement depletion in human serum, but only a 7.1% depletion in dog serum. Larger, 100 nm gold particles (nanoComposix bPEI, zeta Potential 41.6 mV) caused a 97.1 and 95.2% depletion in human and dog serum respectively. The data suggests that *in vitro* species comparisons should be performed prior to designing animal studies to evaluate nanoparticle immunotoxicity.

PS 1937 **Effect of Nanoscale Surface Features of TiO₂ Implant Coatings on Biological Activity of Human Bone Marrow-Derived Stromal Cells**

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Biomaterials with nanoscale topography have been increasingly investigated for medical device applications to improve tissue-material interactions. Nanoscale surface modifications of dental and orthopaedic implants have shown promise *in vitro* and *in vivo*, though a comprehensive understanding of cellular response to nanostructured surfaces is needed. The aim of this study was to evaluate *in vitro* cellular response to titanium dioxide (TiO₂) coatings and to determine the effect of nanoscale surface topography on functional changes in human bone marrow-derived stromal cell (hBMSC). TiO₂ coatings of 50 nm and 250 nm thicknesses were deposited using RF sputtering and subsequently annealed (700°C and 1100°C) to fabricate coatings with varying nanoscale surface topography. Physico-chemical characterization was performed using scanning electron microscopy, atomic force microscopy, X-ray diffraction, energy dispersive spectroscopy, and contact angle measurements. *In vitro* cell viability, proliferation, morphology, and osteogenic differentiation of hBMSCs on TiO₂ coatings were evaluated. Osteogenic differentiation of hBMSCs on TiO₂ coatings was examined using evaluation of alkaline phosphatase (ALP) activity, osteopontin expression, and hydroxyapatite (HA) deposition. Flow cytometry was used to correlate progression of osteogenic differentiation with changes hBMSC surface marker expression (CD90, CD105, CD44, and CD73). Results demonstrate varying cellular activity on nanostructured TiO₂ implant coatings, including differences in proliferation, ALP activity, and amount of HA deposition. Understanding cell response to nanoscale topography is critical in evaluating safety and efficacy of medical devices with nanosurfaces.

PS 1938 **Cytotoxicity of Titanium and Cerium Dioxide Nanoparticles in HaCaT Cells**

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Metal oxide nanoparticles have the potential to contact skin due in part to their use in commercial products and potential release into the environment. The objective of this study was to assess the cytotoxicity of six titanium dioxide and three cerium dioxide nanoparticles using the human-derived keratinocyte cell line, HaCaT cells. Titanium dioxide particle sizes were 22, 25, 31, 59, 142 and 214 nm. Cerium dioxide particle sizes were 8, 40 and 58 nm. Approximately 1 x 10⁴ cells were plated in 96 well plates and placed in an incubator (37 °C, 5% CO₂, 95% relative humidity). Twenty-four h later, the cells were exposed to the particles in media (Dubelco's minimum essential medium with %10 fetal bovine serum) at doses ranging from 1 to 500 µg/ml. The particles were probe sonicated before exposing them to the cells. The exposed cells were incubated for 24 h and then assessed for cytotoxicity using the lactate dehydrogenase assay. Additional experiments were conducted to assess formation of reactive oxygen species by quantitating the fluorescent oxidant marker 2',7'-dichlorofluorescein. All of the titanium dioxide particles showed significant (p < 0.0001) dose-response relationships with respect to cytotoxicity. The cytotoxicity of the titanium dioxide particles at 500 µg/ml ranged from 22.2 to 58.2%, with the

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