PS 298 Impact and Implications of Gold Nanoparticle Interactions with Human Serum Proteins and Biocorona Formation

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The role of nanoparticle (NP) interaction with biomolecules to form a biocorona is the determining aspect of NP behavior and its consequences in the physiological environment, and must be considered when assessing potential toxicity. The adsorbed biomolecular corona decides the fate of a nanomaterial *in vivo* and thus its successful application in the biomedical arena, a comprehensive understanding of the dynamic interactions of the proteins with the NP is imperative. A systematic investigation on the size, surface chemistry and surface charge on time dependent adsorption kinetics and individual protein corona formation was conducted with BPEI and lipoic acid coated 40nm and 80nm gold NP (AuNP). NP were exposed to four human hard corona proteins, human serum albumin (HSA; 40mg/ml), fibrinogen (2mg/ml), immunoglobulin (IgG; 12mg/ml) and transferrin (2.5mg/ml) at physiological concentrations, for 24h. Time evolution data over 0, 6, 12 and 24h revealed that irrespective of surface chemistry, rapid and prominent binding of HSA and IgG coronas occurred over both BPEI and lipoic acid coated AuNP marking an increase in size, without agglomeration up to 24h, at 37°C. Interestingly, IgG exhibited size dependent binding, wherein IgG treated 80nm NP had a higher surface charge compared to the 40nm NP. In contrast, fibrinogen triggered agglomeration instantaneously upon contact with both BPEI and lipoic acid coated NP, while transferrin induced aggregation in BPEI coated NP. These findings suggest that protein coronas at their physiological concentrations interact variably; wherein HSA and IgG coronas adsorbed strongly onto the NP surface keeping the NP well-dispersed, while fibrinogen caused rapid, strong and irreversible agglomeration. Importantly, individual protein coronas exhibited diverse cellular uptake patterns in human umbilical vascular endothelial cells, HSA and IgG coronas showed a high cellular uptake and fibrinogen coronas reduced cellular uptake.

PS 299 Toxicological Profiling of Differently Coated Silver Nanoparticles Using a Suite of *Saccharomyces cerevisiae* Single-Gene Deletion Mutants

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Silver nanoparticles (Ag NPs), are most widely used nanomaterials in various consumer applications, mostly due to their antimicrobial properties. Although the release of Ag-ions, cell membrane damage and generation of reactive oxygen species (ROS) are thought to play the key role in toxic action of silver, the exact mechanisms behind the silver toxicity are still unclear. It has also been shown that the coating of silver NPs strongly modulates its toxicity (Bondarenko et al., *Arch Toxicol* (2013) 87:1181–1200). The yeast *Saccharomyces cerevisiae* is a promising model organism for studies of toxicity mechanisms as it is well characterized with a lot of mutants available. In this study, we used a *S. cerevisiae* wild-type and its single-gene deletion mutants (from EUROSCARF) to elucidate the mode of action of Ag NPs. The mutants sensitive to oxidative stress (OS), cell wall/membrane stress and deficient in endocytosis were used. Uncoated (nAg), polyvinylpyrrolidone-coated (nAg-PVP), casein-coated (collargol, nAg-col) Ag NPs and AgNO3 were compared for their toxicity. Toxicity was evaluated in 48-h growth inhibition test in YPD medium and 24-h cell viability test in deionized water (DI). The toxicity order was as follows: Ag-ions>nAg-col>nAg-PVP>nAg. All Ag compounds were about 40-fold more toxic in DI than in YPD, probably due to different bioavailability of silver. Quantifying solubilised Ag-ions (AAS and Ag-sensor-bacteria) suggests that Ag NPs toxicity was largely caused by Ag-ions release. Toxicity of Ag compounds to OS- and cell wall/membrane-mutants was similar to wild-type, indicating that Ag NPs were not toxic due to OS or membrane disruption. However, the mutant end3Δ, deficient in endocytosis, was more sensitive to Ag compounds than wild-type yeast, particularly to nAg-col and nAg-PVP. This study was supported by the IUT 23-5 and ETF9001.

PS 300 Nickel Nanoparticles Induce Sustained Activation of the STAT6 Transcription Factor in Lung Fibroblasts to Promote Exacerbation of Asthma

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Introduction: Nickel nanoparticles (NiNPs) are used as a catalyst in the production of multiwalled carbon nanotubes and pose a risk for respiratory diseases such as asthma and fibrosis. Asthma is characterized by a Th2 lymphocytic immune response, mucous cell metaplasia, and airway fibrosis. IL-4 and IL-13 are Th2 cytokines that bind specific cell-surface receptors to induce phosphorylation of STAT6 in lung fibroblasts, which in turn leads to the transcription of profibrogenic cytokines (e.g., TGF-β1, PDGF-AA, CCL2) that promote chronic airway remodeling in asthma. We postulated that NiNPs would alter IL-4 or IL-13-induced STAT6 activation to enhance the production of profibrogenic cytokines and thereby exacerbate asthma. Methods: Human lung fibroblasts (HLF) were dosed with IL-4 or IL-13 (10 ng/ml) in the absence or presence of NiNPs (10 μg/ml). Cell lysates were collected at 0.5, 1, 4, 24, and 48 hours post-exposure and phosphorylated STAT6 (p-STAT6) or total STAT6 protein were measured by western blot analysis. Densitometry was performed to quantify p-STAT-6 signal normalized to total STAT6 protein. Results: IL-4 and IL-13 caused transient phosphorylation of STAT6 in HLF as early as 0.5 hr post-exposure that returned near control levels by 4 hr. NiNPs alone did not activate STAT6. NiNP exposure significantly enhanced IL-4- and IL-13-induced STAT6 phosphorylation at 24 and 48 hours post-exposure. No significant changes in total STAT6 protein were observed in any of the treatment groups. Conclusions: NiNPs prolong activation of IL-4- or IL-13-induced STAT6 signaling in lung fibroblasts *in vitro* for days post-exposure. Further study will elucidate the mechanism through which NiNPs sustain STAT6 phosphorylation and determine if this chronic signaling event could exacerbate airway fibrosis in asthma through increased production of profibrogenic cytokines. Funding: Supported by NIEHS grants R01-ES020897 and T32-ES007046

PS 301 Effects of the Physicochemical Properties of Nano-Scaled Cerium Oxide on Fibrogenesis

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Nano-scaled cerium oxide ($nCeO_2$) is used in a variety of applications, including use as a fuel additive, catalyst, and polishing agent, yet potential adverse health effects associated with $nCeO_2$ exposure remain incompletely understood. *In vivo* studies have shown in a rat model that inhaled $nCeO_2$ can deposit in deep lung tissues and induce fibrosis; however, little is known about how the physicochemical properties of $nCeO_2$, such as size or surface chemistry (e.g. amorphous silica coating), may affect the bio-activity of these particles. Thus, we hypothesized that the physicochemical properties of $nCeO_2$ influence its fibrogenicity. Plasma samples collected from rats 28 days after intratracheal instillation of 3.5 mg/kg $nCeO_2$ showed increased levels of the fibrotic mediator TGFβ, which is released from macrophages and platelets upon stimulation. This was not observed in response to amorphous silica-coated $nCeO_2$ ($amsCeO_2$), which induced levels consistent with saline-treated animals. Interestingly, platelets isolated from $nCeO_2$-treated rats released significantly more TGFβ than those isolated from control animals, suggesting that platelets may be contributing to $nCeO_2$-induced fibrosis. This was found to be an indirect effect since treatment of control platelets *ex vivo* did not stimulate release of TGFβ, regardless of the size or coating of $nCeO_2$. Primary alveolar macrophages demonstrated increased cell death when treated with $nCeO_2$, but not $amsCeO_2$, at doses consistent with those used *in vivo*, while neither particle directly induced TGFβ release from these cells. Furthermore, $nCeO_2$ directly induced the production of collagen I and increased cell proliferation, hallmarks of fibrogenesis, in primary lung fibroblasts *in vitro*, while $amsCeO_2$ failed to do so to the same extent. Collectively, these results indicate that differences in the physicochemical properties of $nCeO_2$ may affect the fibrogenicity of this compound, and highlight the utility of "safe-by-design" strategies for preparing engineered nanomaterials.

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