Task ID: 425.035

Task Title: High-Throughput Cellular-Based Toxicity Assays for Manufactured Nanoparticles and Nanostructure-Toxicity Relationships Models

Deliverable #2: Completion of data package for Set 1 MNPs and Set 2 MNPs

Note: *In the revised version of the project budget and the scope of the work, this deliverable was changed to completion of data package for Set 1 MNPs.*

Summary/Abstract:

MNPs set 1 comprised of carbon, aluminum oxide and titanium dioxide nanoparticles (NPs) was evaluated for toxic end points using cell based assays. Toxicity evaluation was carried out using A549 alveolar epithelial cells. In comparison to aluminum oxide and titanium dioxide, carbon NPs appear to be toxic based on the results from cell based MTT and ROS assay and acellular ABTS assay. Carbon NPs due to their inherent hydrophobicity probably act as a vehicle to carry iron/nickel in cells and these impurities might play a major role in eliciting the observed toxic effects.

Technical Results and Data:

MNPs set 1 consists of a small set of carbon, aluminum and titanium dioxide NPs acquired from commercial sources. The focus of study for MNPs set 1 was to compare the toxicological profiles of different composition but approximately similar sized NPs on A549 alveolar epithelial cells. MNPs were characterized for particle size by dispersing MNPs in DI water at concentration of 1mg/ml by using bath sonication for a cycle of 3 X 60 sec. Dynamic light scattering was used to measure the particle size. Zeta potential was measured using Malvern nano zetasizer.

MNP Type	Manufacturer	Particle size * range (nm)	Particle size in DI water (nm)	Zeta potential (mv)
Carbon	American Elements	55 - 100	611.7 ± 510.5	-21.1 ± 4.6
Aluminum Oxide	Alfa Aesar	40 - 50	488.8 ± 318.7	-17.7 ± 7.4
Titanium dioxide	Nano-Amor	30 - 40	511.7 ± 336.7	-25.3 ± 5.2

Table 1: Particle size and zeta potential of MNPs set 1. * Particle size provided by manufacturer.

Table 1 shows the results from characterization of MNPs set 1. In general, the average particle size measured was 10 folds higher than the size provided by the manufacturer.

The potential of MNPs to induce free radical reaction in an a-cellular in-vitro system was determined by incubating nanoparticles in a 96 well plate with 60 mM ABTS reagent (ABTS = 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid diammonium salt) for 24 hrs **.** The

formation of ABTS radical was monitored by measuring absorbance at 734 nm, characteristic for the ABTS radical. As control, NPs alone without ABTS reagent were monitored for absorbance at 734 nm. The results from this assay are shown in Fig.1 (a). The fold increase in absorbance has been corrected for any background absorbance from NPs alone.

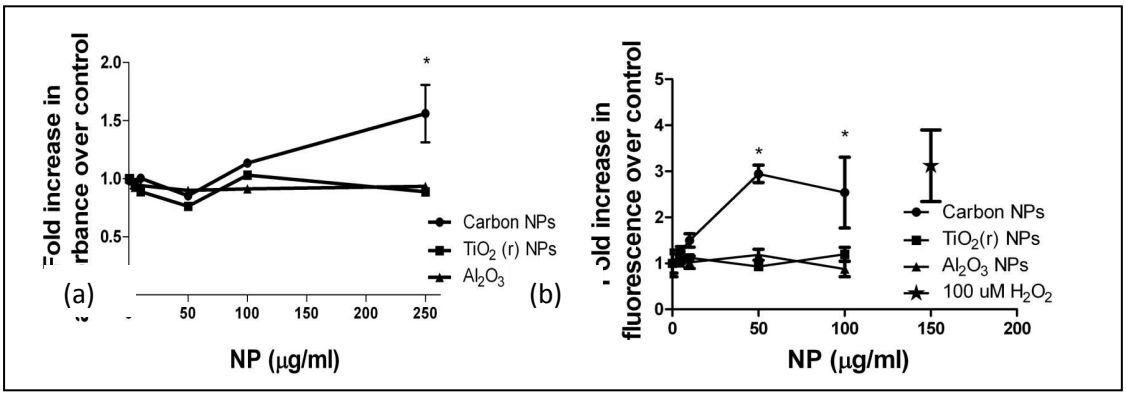

Fig. 1: (a) ABTS assay is an in-vitro a-cellular assay that gives an indication about reactivity of MNPs. (b) ROS or Reactive Oxygen Species assay gives an indication of the ability of MNPs to induce oxidative damage in cells

ROS or Reactive Oxygen Species assay was carried out to assess the ability of MNPs to induce oxidative damage in cells. A549, human alveolar epithelial cells (25,000 per well), were incubated with NPs for 4 hr. Observed fluorescence is due to cleavage of carboxy H2DCFDA to fluorescein inside cells. Data were corrected for fluorescence from NPs and fluorescence due to NP reaction with carboxy H2DCFDA in the absence of cells. 100 μM H_2O_2 was used as positive control. Fig.1(b) shows the results of ROS assay with set 1 MNPs. In both a-cellular ABTS and cell based ROS assays for assessing the reactivity of MNPs, carbon NPs appear to be most reactive whereas aluminum and titanium dioxide NPs did not show any signs of reactivity. To further evaluate the toxicity of MNPs set 1. Mitochondrial function was evaluated by using MTT assay. A549, human alveolar epithelial cells (25,000 per well), were incubated with NPs for 24 hr. Absorbance measured at 570 nm was due to reduction of MTT dye inside viable cells. Data was corrected for absorbance from blank NPs.

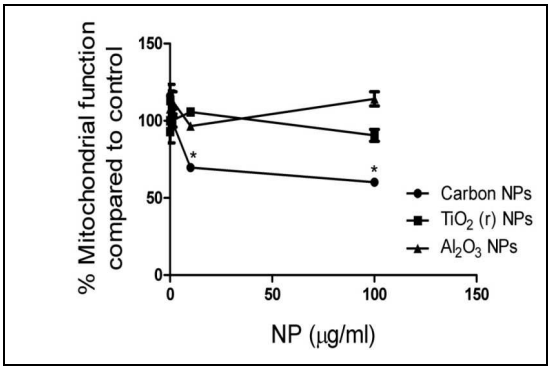

Fig. 2: MTT assay gives an indication of the ability of MNPs to alter mitochondrial function in cells.

Similar to the results from other assays, the results from MTT assay (Fig.2) also show that only carbon NPs in MNPs set 1 were able to alter the mitochondrial function. Certificate of analysis from manufacturers show the presence of transition metal impurities in only carbon NPs. A possible explanation for observing toxicity only with carbon NPs can therefore be that carbon NPs being hydrophobic can act as carriers to ferry these metal impurities across cell membranes where these metal impurities due to their inherent reactive nature are able to induce ROS and alter with mitochondrial function.

Conclusions:

- Particle size measurement by dynamic light scattering shows that NP sizes are different from those provided by the manufacturer.
- ABTS assay was successfully developed as an in-vitro acellular assay to assess the free radical forming potential of NPs. The assay is simple, adaptable to 96 well plate and cost effective.
- Carbon NPs appear to be more toxic as compared to other NPs as shown by in-vitro MTT and ROS data.
- Carbon nanoparticles probably act as a vehicle to carry iron/nickel in cells. This is a very plausible scenario as hydrophobicity provided by carbon NPs facilitates iron entry in cells.
- Results from ABTS assay correlates well with ROS and MTT cytotoxicity data