Task ID: 425.035

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

Deliverable #3: Begin to test set 3 MNPs from ESH and SRC and member companies

Note: *In the revised version of the budget and the project scope of the work, this deliverable was changed to begin to test set 2 MNPs from ESH and SRC and member companies. MNP set 2 has been modified since the revised proposal was submitted and now consists of matched carbon coated and corresponding bare metal nickel and copper NPs (Ni, C-Ni, Cu and C-Cu NPs), all four acquired from a single commercial source.*

Summary/Abstract:

A lot of discrepancies exist in the nano-toxicity literature related to the evaluation of toxicity of commercially available NPs, therefore there exists a need to systematically evaluate the toxicity of MNPs such that one characteristic can be studied at a time and some level of correlation can be made between the physicochemical characteristics and observed toxicity end points. Keeping in mind this aim, it was decided to elucidate the effect of carbon coating / hydrophobicity on the toxicity of matched carbon coated and corresponding bare metal nickel and copper NPs (Ni, C-Ni, Cu and C-Cu NPs) when tested in cell based assays using A549 alveolar epithelial cells. Physicochemical characterization, membrane integrity assay, MTT assay, qualitative and quantitative cell uptake studies have been performed in this direction.

Technical Results and Data:

The focus of study for MNPs set 2 was to elucidate the differences in toxicological profiles of carbon coated and uncoated metal NPs on A549 alveolar epithelial cells. MNPs were characterized for particle size by dispersing MNPs in DI water at concentration of 1mg/ml 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.

NP Type	Manufacturer	Particle Size* Range (nm)	Particle Size in DI water (nm)	Zeta Potential (mV)
Nickel	NanoAmor	20	834.6 ± 495.1	2.76 ± 0.7
Carbon coated Nickel	NanoAmor	20	466.6 ± 179.6	-16.4 ± 1.8
Copper	NanoAmor	25	662.2 ± 139.3	-9.0 ± 2.4
Carbon coated Copper	NanoAmor	25	412.1 ± 210.9	-6.21 ± 0.7

Table 1 shows the results from characterization of MNPs set 2. In general, the average particle size measured was 20 folds higher than the size provided by the manufacturer.

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

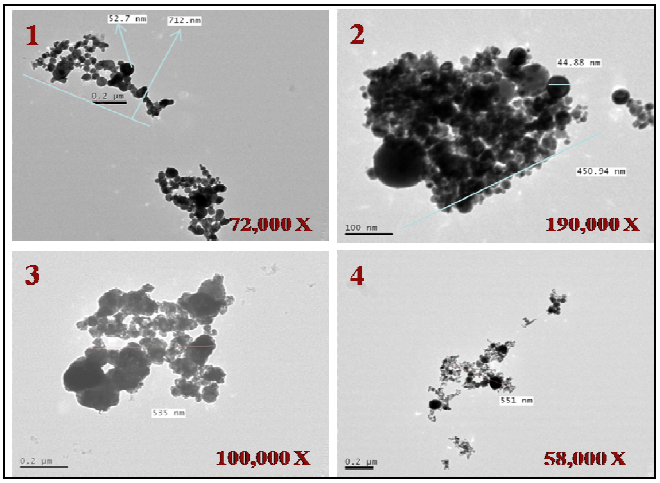

Fig 1 shows MNP morphology and size as observed under TEM. Particles were suspended at concentration of 10µg/ml in DI water using bath sonication for a cycle of 3 X 60 sec. The average particle sizes measured by TEM correlates with the dynamic light scattering data.

Fig. 1: TEM images of MNPs. 1. Ni , 2. C-Ni, 3. Cu and 4. C-Cu MNPs.

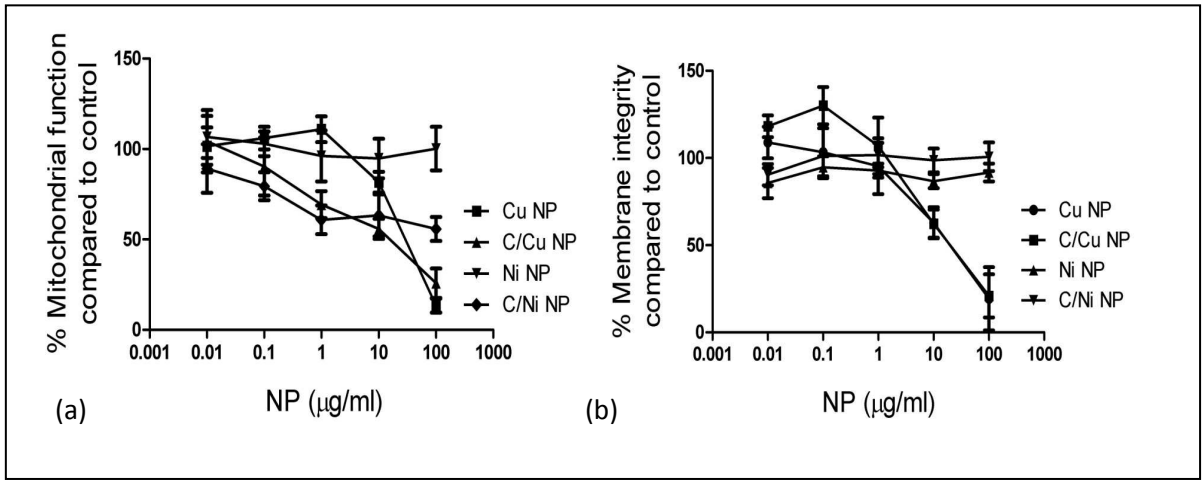

Fig. 2: (a) MTT assay with MNPs set 2. (b) Membrane Integrity assay with MNPs set2

Mitochondrial Function Assay and Membrane Integrity Assay: MTT was used to evaluate the mitochondrial function of A549 cells (25,000 cells/well in a 96 well plate) after 24 hr. of treatment with different MNP doses. Results as shown in Fig.2(a) suggest Ni NPs were most non toxic in observed concentration ranges. C-Ni NPs were moderately toxic. Cu NPs showed toxicity at higher doses and C-Cu NPs showed consistently increasing toxicity with increasing dose. For assessment of membrane integrity, neutral red dye was used. Membrane integrity was analyzed after 24 hr. of treatment with different MNP doses with A549 cells (25,000cells/well in a 96 well plate). The results of membrane integrity are shown in Fig. 2(b). Ni and C-Ni NPs did not alter the membrane integrity whereas Cu and C-Cu NPs were almost equally toxic in altering the membrane integrity.

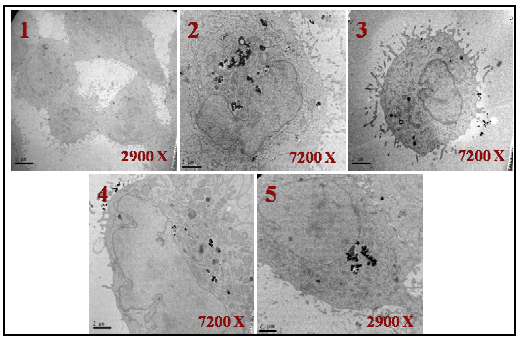

Fig.3 . Qualitative analysis of MNP uptake in A549 cells after treatment for 8hrs. 10ug/ml dose of MNP was used.1. Control A549 cells , 2. Cu treated , 3. C-Cu treated , 4. Ni treated and 5. C-Ni treated A549 cells.

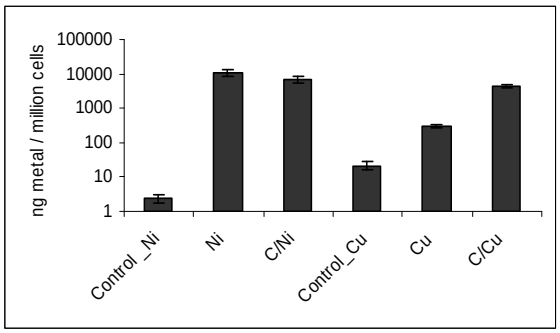

Fig. 4 Preliminary data on Cell uptake analysis of Ni and Cu from Ni, C-Ni and Cu and C-Cu NPs respectively. Control_Ni and Control_Cu signify the amount of respective metal content in untreated control A549 cells.

MNP uptake: For analysis of MNP uptake qualitatively (Fig.3), A549 cells were treated with 10µg/ml of MNPs for 8 hrs. The cells were then fixed and stained for TEM analysis. All types of MNPs are taken up by cells. They are mostly centered around nuclear membrane and in vicinity of mitochondria. For quantitative uptake analysis (Fig.4), NPs were tested at concentrations of 10µg/ml for 8 hr in A549 cells. Ni uptake from Ni and C-Ni NPs is comparable but Cu uptake from C-Cu NPs is an order of magnitude higher than uptake from Cu NPs.

Conclusions:

- Average particle size measured by DLS of all MNPs in Set 2 are on an average 20-fold higher than provided by the manufacturers.
- Ni NPs do not alter mitochondrial function and membrane integrity although their uptake by cells is comparable to C/Ni NPs.
- C-Ni NPs alter the mitochondrial function but not membrane integrity.
- Cu NPs alter mitochondrial function at100 µg/ml but can alter membrane integrity even at 10 µg/ml dose.
- Rounded morphology of Cu NP treated cells and results from membrane integrity and ICP-MS suggest that Cu NPs might act on cell surface at lower dose, possibility of alterations with cell adhesion.
- C-Cu NPs alter mitochondrial function and membrane integrity to the same extent.
- Cu and C-Cu NPs appear to be more toxic than Ni and C/Ni NPs.
- Correlation of uptake data with observed toxicity in cell based assays awaits data on detailed physico-chemical characterization.