Detection of Silver (Ag) and Titanium Dioxide (TiO2) Nanoparticles using Light Scatter by Flow Cytometry and Darkfield Microscopy.

R.M. Zucker, L. Degn, J. Ortenzio and W. K. Boyes. USEPA, ORD, NHEERL, TAD, Research Triangle Park, NC

Titanium Dioxide (TiO_2) and Silver (Ag) nanoparticles are used in many domestic applications, including sunscreens and paints. Evaluation of the potential hazard of manmade nanomaterials has been hampered by a limited ability to detect and measure nanoparticles in cells. In the present study, six different types of silver nanoparticles (AgNP) and six types of titanium dioxide nanoparticles (TiO_2NP) were incubated with cells from a human-derived retinal pigment epithelium cell line (ARPE-19).

Following 24 hour incubation of different sized AgNPs coated with polyvinylpyrrolidone (PVP) or citrate, uptake by ARPE-19 cells was detected by flow cytometry (Stratedigm 1000 and FACSCalibur). A dose dependent increase of side scatter was observed following cell exposure to both PVP- and citrate-coated 50 nm and 75 nm AgNP. Citrate-coated AgNP revealed slightly more side scatter than did PVP-coated AgNP. Side scatter of 75 nm AgNP was greater than that of 50 nm and 10 nm AgNP. Results demonstrate that the uptake of AgNP can be detected by standard flow cytometers in an apparent dose dependent manner using side scatter. A dose dependent inhibition in the S phase of the cell cycle was observed after 24 hour exposure using a flow cytometry procedure that involved nuclear isolation followed by RNase incubation and PI staining. After 24 hours incubation with AgNP, the S phase and G2/M phases increased while the G1 phase decreased.

Cells grown on BD glass chamber slides were used for microscopic analysis of cellular nanoparticles. The TiO_2NP and AgNP showed unique patterns of agglomeration within the cells as observed by darkfield microscopy. Morphological evaluation by dark field microscopy showed that increased uptake of AgNP (50 nm and 75 nm) correlated with the flow cytometry measurements, and showed more clumped nanoparticles concentrated around the nucleus. This observation was supported using the PARRIS spectral imaging system which showed larger nanoparticles surrounding the nucleus and smaller particles located closer to the edge of the cell. Fluorescent and dark field microscopy observations suggested that nanoparticles entered cells by endocytosis and accumulated into clumps and single particles in the endoplasmic reticulum near the Golgi complex and surrounding the nucleus. At concentrations of 10 and 30 μg/ml, the entire cytoplasm appeared to be loaded with nano particles. TiO_2NP showed more clumping at the same dose than AgNP.

These data suggest that the uptake of nanoparticles into cells can be monitored by both flow cytometry and dark field microscopy. They also establish an efficient measurement of the intracellular dose of nanoparticles during *in vitro* experiments that should enhance our ability to link cellular dose to potential nanomaterial toxicity.

1. Zucker RM, Massaro EJ, Sanders KM, Degn LL, Boyes WK. Detection of TiO2 nanoparticles in cells by flow cytometry. Cytometry A. 77:677-85 2010.