# Cellular Response to Nanoparticle Exposure

G.W. Erdos*, D. Moraga*, M. Palazuelos**, K. Powers** and K.Kelley*
*Interdisciplinary Center for Biotechnology Research and **Particle Engineering Research Center,
University of Florida, Gainesville, FL USA gwe@ufl.edu

## ABSTRACT

The cellular response to exposure to nanoparticulate $TiO_2$, Al, silica and quartz has been investigated in vitro using a lung carcinoma cell line, A549. Cultures were exposed to a suspension of particles of various concentrations and for different time durations. All particles were taken into the cells by endocytosis and shuttled to lysosomal compartments. The lysosomes subsequently fused resulting in large vacuolar bodies within the cells. There was no eveidence that the cells attempted to expel the particles. Lysosomes sometimes showed eveidence of rupture and release of contents into the cytoplasm. Evidene of mitochondrial stress was also observed. Cell toxicity was measured by assay of released lactic dehydrogenase (for lysis) and caspase activity (for apoptosis). Al, silica and quartz showed toxic effects. $TiO_2$ did not, under these conditions. Tests for respiratory activity were inconclusive.

***Keywords***: nanotoxicity, titania, aluminum, silica, quartz

## 1 INTRODUCTION

With the rapid growth in research and application of nanomaterials, there is considerable concern over the possible toxic effects to both humans and the environment. Very little is known about nanosized materials, even though bulk forms of the same material might be considered safe. The area of nanotoxicology is a very new discipline and standards for the characterization of nanostructures used in toxicological studies and appropriate testing procedures for toxicity have not been established. A recent, high level workshop on nanotoxicology, held at the University of Florida, reinforced this notion (Proceedings, in preparation.). The public awareness of this situation makes all the more important that these studies are done carefully.

## 2 EXPERIMENTAL

A lung carcinoma cell line, A549 was obtained from the American Type Culture Collection and grown according to their instructions. $TiO_2$ particles of 47 nm diameter were obtained from Nanophase Technologies, Romeoville, IL http://www.nanophase.com/). Al particles of 80 nm diameter were obtained from Nanotechnologies, Austin, TX. Quartz particles, of mixed size smaller than 5 µm, were obtained from U.S. Silica Co., Berkeley Springs, WV (http://www.u-s-silica.com/) . Silica nanospheres were the kind gift of Dr. S. Santra (1). Cells were exposed to particles in growth media. Times of exposure were from 4-72 hours at concentrations of 31-500 µg/ml. Cell lysis was monitored by assaying of lactate dehydrogenase using the Cytotoxicity Detection Kit from Roche. Apoptosis was determined by caspase activity using the Apo-ONE kit from Promega.

Electron microscopy was performed on cells grown on polycarbonate membranes. They were fixed in 2.5% glutaraldehyde and 1% $OsO_4$, embedded in epoxy resin, sectioned and observed on a transmission electron microscope.

## 3 RESULTS

All particles were taken into cells by either bulk phase endocytosis .(Fig.1) or through association with coated pits indicating receptor mediated endocytosis (Fig.2).

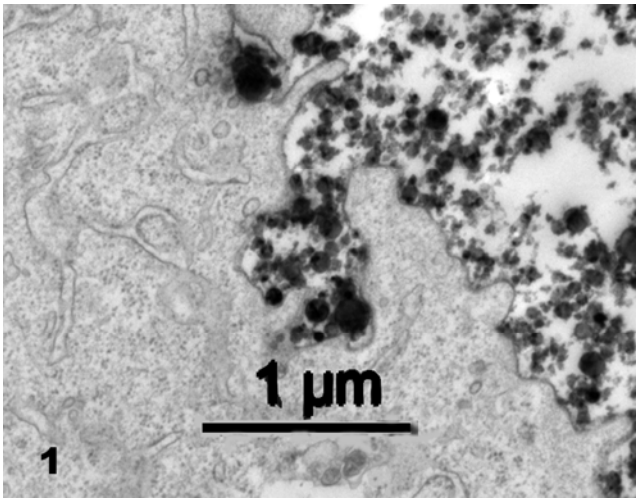



**Fig. 1.** Bulk phase endocytosis of $TiO_2$ particles from growth medium.

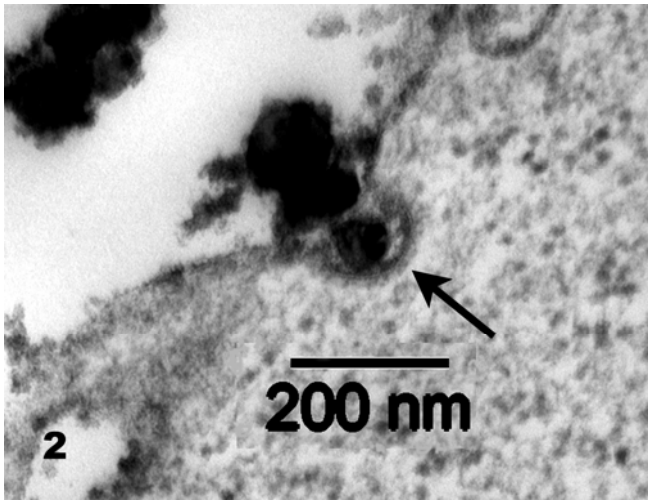



Fig. 2. Uptake of $TiO_2$ Particle via a clathrin coated pit. (Arrow).

All particle types were taken up in a similar manner and came to rest in primary endosomes.(Figs.3-5).

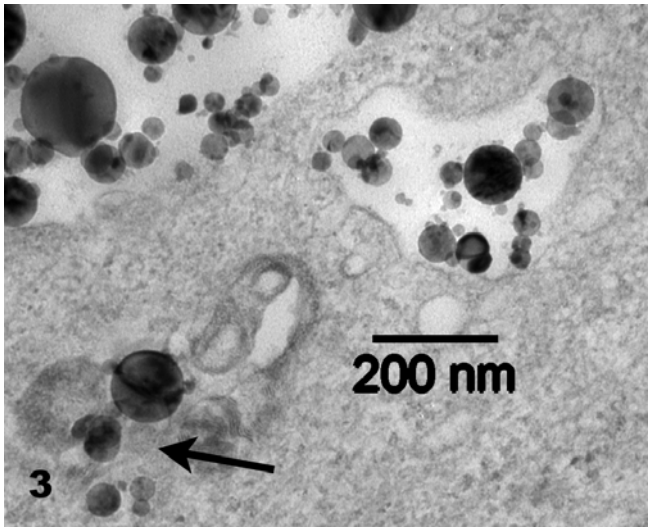



Fig.3 $TiO_2$ Particles in a primary endosome. Arrow indicates particles released from broken endosome.

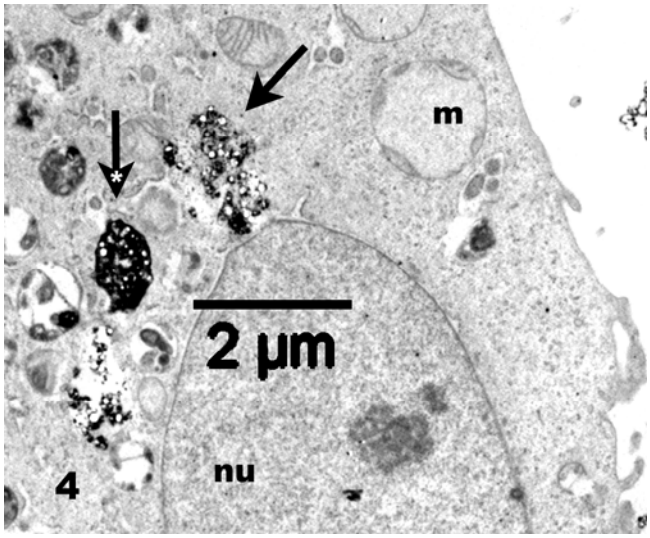



Fig. 4. Al Particle inside an endosome (arrow). Dense endosome with Al indicating chemical changes (*arrow).Dilated mitochondrion (m) indicating respiratory stress. Nucleus (nu) showing signs of apoptosis.

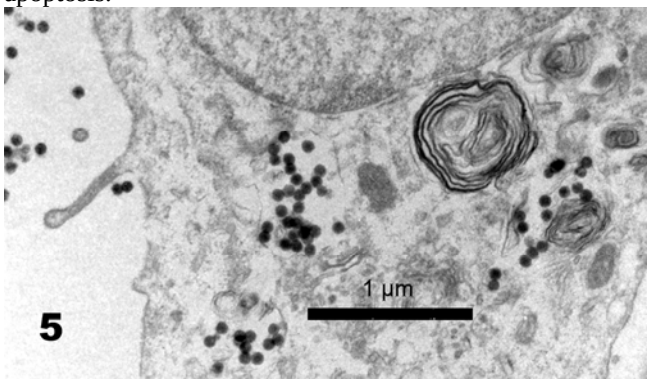



Fig. 5. Silica microspheres in endosomes.

The endosomes fuse with primary lysosomes which are some times observed to break and release their contents which includes (Fig. 3) certain proteases. In the case of Al particles the lysosomal content becomes very dense indicating that the Al is reacting with the acidic contents of the lysosome. Dilated mitochondria with few cristae are also seen indicating respiratory stress (Fig. 4) Lysosomes continue to fuse until the cells contain large membrane bound sacs filled with particles (Fig. 6) or dense content in the case of ingested Al.

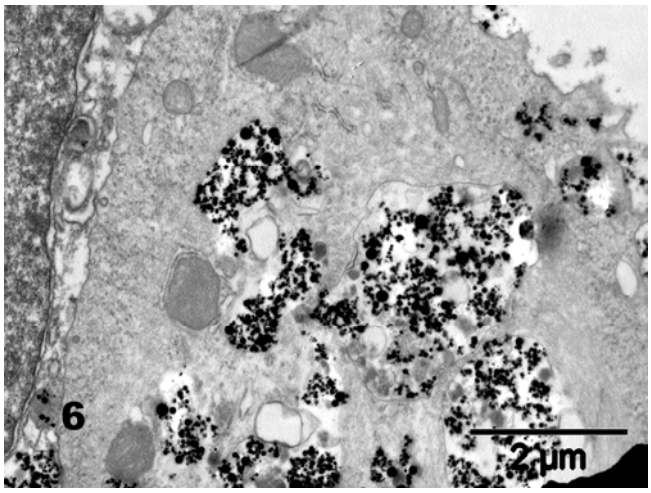



Fig. 6. Fusion lysosomes filled with $TiO_2$ particles after 48 hours.

Some nuclei show clear signs of apoptosis (programmed cell death) (figs.4 and 7)

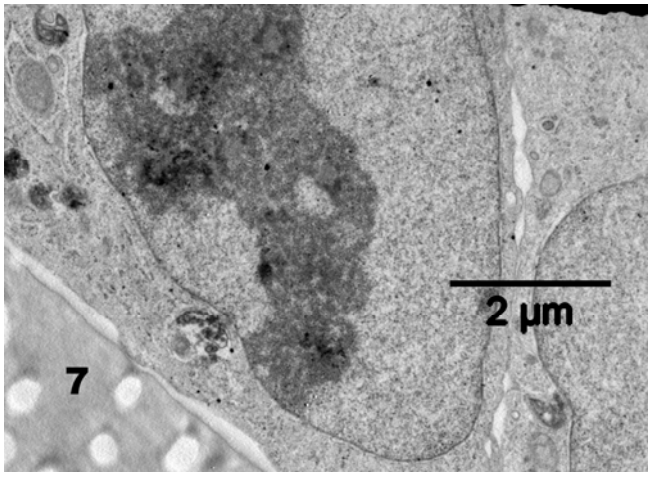
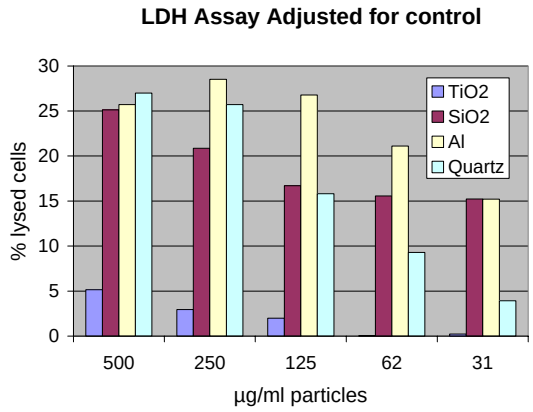


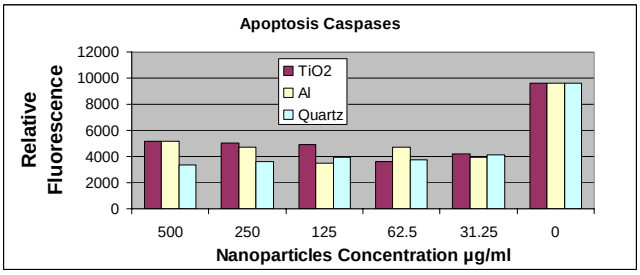

Fig.7. Apopototic nucleus of a cell exposed to Al for 72 hours

As a correlate to the morphological studies, biochemical assays typical of toxicity testing were performed. They were LDH activity to measure the percentage of cells lysed; caspase activity to measure the degree of apoptosis: and alamar blue assay to mesure cell proliferation or respiratory activity. Experiments involving alamar blue were

inconclusive and those data are not shown. Data typical of the LDH and apoptosis tests are shown below.




LDH assay show a clear concentration dependent toxic effect by AL, quartz and silica. Titania however appears to have little toxic effect when measured by cell integrity as an indicator of toxicity. It must be remembered that cell lysis is a catastrophic event and that other changes may be occurring in the cells that would show a greater toxic effect, short of cell death.




Particles had no toxic effect in regard to the induction of apoptosis. In fact the presence of the particles seemed to have an inhibitory effect on apopotosis for the three particle types tested. At least using the presence of the two caspases as an indicator of apoptosis shows this unexpected effect. However since apoptotic nuclei were observed it may be that the process is atypical in the presence of nanoparticles. The silica spheres could not be tested since they were autofluorescent.

## 4 DISCUSSION

This study is the beginning phase of a larger study to determine the appropriate testing procedures for toxicity testing of nanoparticles. It may be that those we have chosen are not the correct ones and that testing of solid particulates need different methods than those used for soluble toxins. Our data indicate that titania has relatively little toxic effect on this cell line. However the oth three do show a significant level of toxicity. The cell line, A549 has a habit of being very active in regard to endocytosis. A pertinent questions is whether we would find the same results in another cell line. Although we can do considerable characterization of the particles prior to placing them with the cell, we do not really know what the cell "sees" after interaction of the particles with the growth medium.

It is clear that there is chemistry occurring inside the lysosomes, particularly in regard to Al, which is more reactive than the other substances. Whether similar reactivity is taking place with other particles is less clear.

Before we can make any conclusions about the specific toxicity of the materials tested here or make any general conclusions about appropriate testing procedures for nanostructural materials, we must conduct other testing protocols for comparison. This work is ongoing in our laboratories.

## 5 REFERENCES

1. **Santra, S**; Wang, KM; Tapec, R; **Tan, WH 2001.** Development of novel dye-doped silica nanoparticles for biomarker application. JOURNAL OF BIOMEDICAL OPTICS, 6 (2): 160-166