

## Air Force Research Laboratory

### In Vitro Toxicity of Aluminum Nanoparticles in Rat Alveolar Macrophages

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## ***In Vitro* Toxicity of Aluminum Nanoparticles In Rat Alveolar Macrophages**

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### **Abstract:**

The purpose of this research was to investigate and characterize the *in vitro* cellular effects of exposing rat lung macrophages to aluminum oxide nanoparticles (30 and 40nm average size) compared to aluminum metal nanoparticles (50, 80, and 120nm). This study used toxicity endpoints involving cell viability, mitochondrial function, phagocytotic ability, and inflammatory response. Results indicated none to minimal toxicological effects occurred with exposure of macrophages as high as 500 µg/ml for 24 hours with aluminum oxide nanoparticles. However, there was significant delayed toxicity that occurred at 96 and 144 h post exposure. Exposure to aluminum metal nanoparticles indicated slight to moderate toxicity after 24 hours exposure at 100 and 250 µg/ml. The phagocytic ability of these cells was significantly hindered by exposure to all tested aluminum nanoparticles at 25 µg/ml for 24 hours, but not by the aluminum oxide nanoparticles. A series of cytokine and nitric oxide assays performed showed aluminum nanoparticles did not induce an inflammatory response.

### **Introduction:**

- The recent revolution in nanotechnology brought advantages in areas of our lives as diverse advancements in engineering, information technology, and diagnostics fields.
- NASA is currently investigating oxide coated Al particles ranging in size from 20 to 100 nm. These particles allow for increases in fuel density, safety, and exhaust velocity while reducing fuel slosh, leakage, and the overall size of the vehicle (Palaszewski, 2003).
- The US Army Research Lab is investigating metallic nanopowders e.g., Aluminum nanoparticles in explosives (Miziolek, 2002).
- U.S. Naval Air Warfare Center is investigating aluminum nanocomposites as "green" bullet primers (Loney, 2004).
- Currently the Navy is using a nanocomposite of alumina-titania as wear resistant coatings on propeller shafts (DoD, 2005).
- Existing minimal data suggests that nanoparticles may be able to have adverse effects at their portal of entry, for example, the lungs, as well as gain entry into deep tissue sites.
- Alveolar macrophages are the first line of capture and immunological defense from inhaled particles. They serve as a good model to understand how inhaled particles can adversely affect health (Kleinman et al., 2003).
- In view of importance application of nanoparticles, the current study was undertaken to study toxicity of Al nanoparticles in alveolar macrophages.

**Objective:**

- To assess and compare the relative toxicity of various states of commonly used aluminum nanoparticles in systems
- To investigate any functional changes in phagocytosis and inflammatory response due to exposure.

**Method:**

**Cell Culture:** Alveolar macrophage cells obtained from ATCC (CRL-2192). Cells were cultured in rat tail collagen coated flasks, with Ham's F12K medium (Sigma) containing 20% FBS (fetal bovine serum) and penicillin/streptomycin; incubated in a 5% CO<sub>2</sub> incubator at 37°C. In preparation for *in vitro* experiments, macrophages were seeded in coated 24-well plated for mitochondrial function loss (MTT) or 6-well plates for cytokine, nitric oxide (NO) assays or on 2 chambered slides for phagocytosis assay.

**Nanoparticles:** All nanoparticles were obtained from Nanotechnologies Inc. Austin Tx. (Aluminum oxide nanoparticles 30 and 40 nm average size and pure aluminum nanoparticles 50, 80, and 120 nm). Dry particles were suspended in deionized water to a concentration of 10mg/mL (stock solution). The stock solution prior to each use was sonicated for 20 seconds to reduce agglomeration of particles. Media/nanoparticle suspensions were then pipetted into 6 and 24 well plates or slides at an established concentration ranging from 5 to 500 µg/ml.

**Viability Assay:** Mitochondrial function was used to establish how viable the alveolar macrophages were after and exposure of Al nanoparticles. Results were determined spectrophotometrically by measuring the reduction of the tetrazolium salt MTT to formazan by succinate dehydrogenase (Carmichael et al., 1987).

**Phagocytosis:** Phagocytic function was measured by the uptake of 2 µm latex beads and was observed on an Olympus IX71 inverted fluorescent microscope and CytoViva. Phagocytosis index is described by Paine *et. al.*, 2004.

**Cytokine and Nitric Oxide Assays:** Nitric Oxide, MIP-2, and TNF- $\alpha$  assays were used to characterize what effects Al nanoparticles might have on an inflammatory response. Performed as directed by the manufacturer (Promega) and (Biosource). Lipopolysaccharide (LPS) was used as a positive control.

## Results:

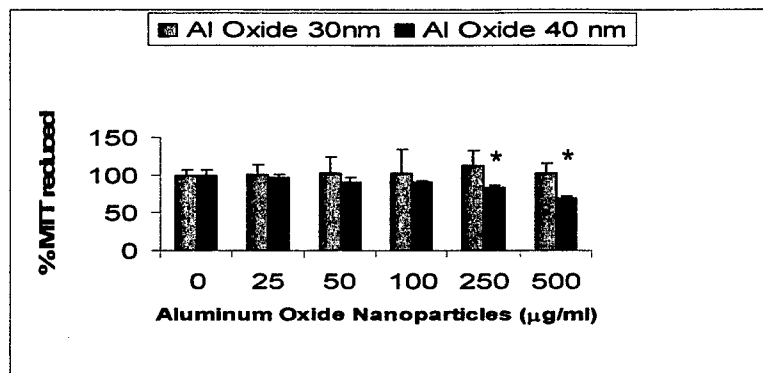


Figure 1: Percent MTT reduced by Alveolar Macrophages after 24 hours of exposure to aluminum oxide nanoparticles. MTT values observed in triplicate and data reproduced in 3 separate experiments. Results confirm that  $\text{Al}_2\text{O}_3$  particles do not have a large impact on the viability of these cells even at concentrations as high as 500  $\mu\text{g/ml}$ .  $\text{Al}_2\text{O}_3$  40 nm at doses between 250 and 500  $\mu\text{g/ml}$  were the only data points that indicate a statistical significant difference between the cells not exposed to any aluminum particles (control) with a  $p$  value  $< .05$ . (\* asterisk indicates doses that are significantly different than the zero control)

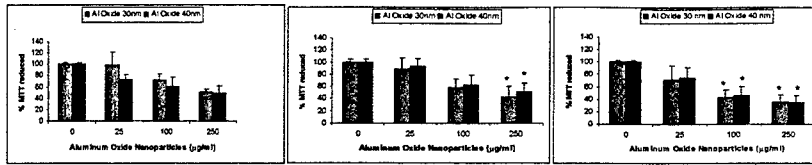


Figure 2: Percent MTT reduced by Alveolar Macrophages after A) 48 hours, B) 96 hours, and C) 144 hours of exposure to  $Al_2O_3$  nanoparticles. MTT values observed in triplicate and data reproduced in 3 separate experiments. Results illustrate the amount of delayed toxicity of  $Al_2O_3$  nanoparticles on these cells. MTT reduction after 96 hrs in both  $Al_2O_3$  30 and 40 nm were significant at a dose of 250  $\mu g/ml$ . MTT reduction after 144 hrs in both  $Al_2O_3$  30 and 40 nm were significant at 250  $\mu g/ml$  with a decrease in reduction to approximately 35% for both and at 100  $\mu g/ml$  where it was reduced 43% and 45% respectively. (\* asterisk indicates doses that are significantly different than the zero control)

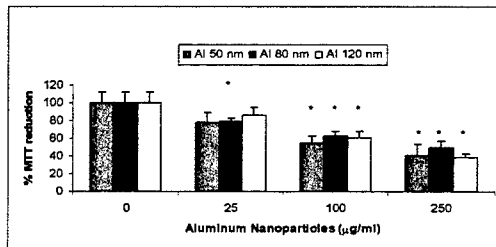


Figure 3: Percent MTT reduced by Alveolar Macrophages after 24 hours of exposure to aluminum nanoparticles. MTT values observed in triplicate and data reproduced in 3 separate experiments. Results indicate that aluminum nanoparticles have a more drastic effect on cell viability. Al 50 and 120 nm created a significant reduction in MTT production at 100 and 250  $\mu g/ml$ . Al 50 nm reduced MTT reduction to 54 and 40% respectively and Al 120 nm reduced it to 61 and 39% respectively. Al 80 created a significant reduction of MTT at all three dosing points at 25 mg/ml MTT reduction was reduced to 79%, at 100 mg/ml to 63%, and at 250 mg/ml to 49%. (\* asterisk indicates doses that are significantly different than the zero control)

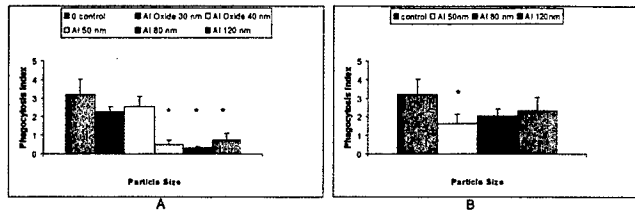


Figure 4: A) Phagocytosis Index of Alveolar Macrophages exposed to various Al nanoparticles at 25 µg/ml for 24 hours (100 cells counted for each exposure on 3 separate experiments, 300 total cells counted for each exposure). B) Phagocytosis Index of Alveolar Macrophages exposed to various Al nanoparticles at 5 µg/ml for 24 hours (100 cells counted for each exposure on 4 separate experiments, 400 total cells counted for each exposure). Results indicate that cells exposed to 25 µg/ml of various aluminum nanoparticles will have varied results. Al<sub>2</sub>O<sub>3</sub> 30 and 40 nm show a slight, but no significant decrease in phagocytosis ability (*p* value > .05 when comparing to the control). Al 50, 80, and 120nm all show a significant reduction in phagocytosis compared to the control (*p* value < .05). Cells exposed to 5 µg/ml of Al 50, 80, and 120 nm will again have a slightly reduced phagocytosis index, but only Al 50 nm is significant (*p* value < .05). (\* asterisk indicates doses that are significantly different than the zero control)

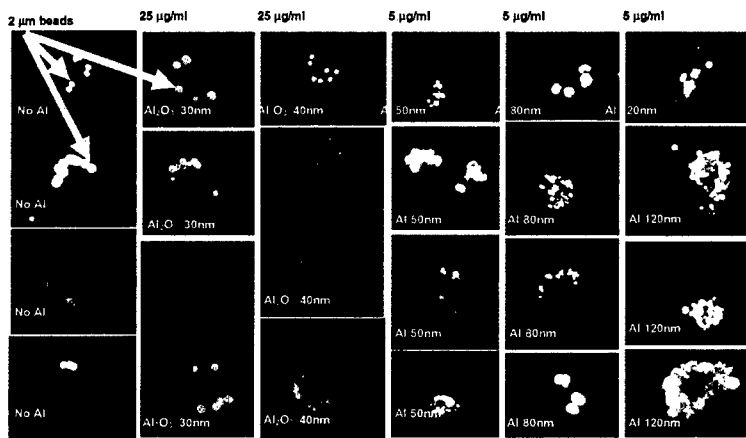


Figure 5: Various images of rat alveolar macrophages taken during phagocytosis assay with the Olympus IX71 inverted fluorescent microscope and CytoViva. 2  $\mu\text{m}$  latex beads appear as bright globular areas in the cells. Cells and beads phagocytosed by cells were counted to obtain a phagocytosis Index.



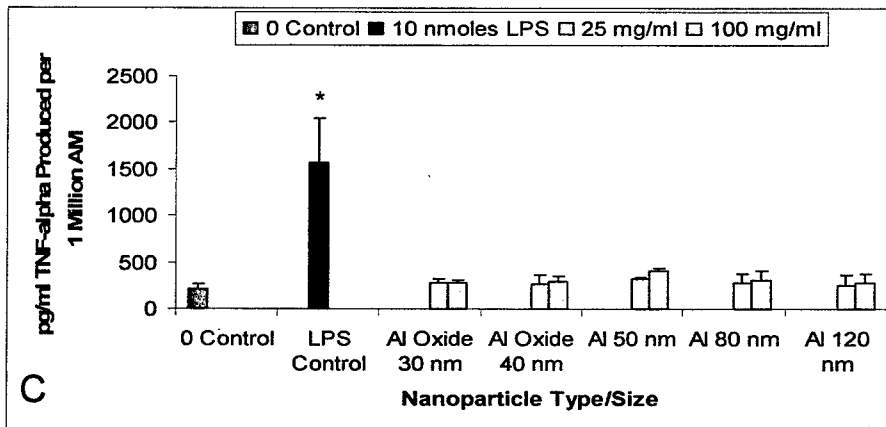
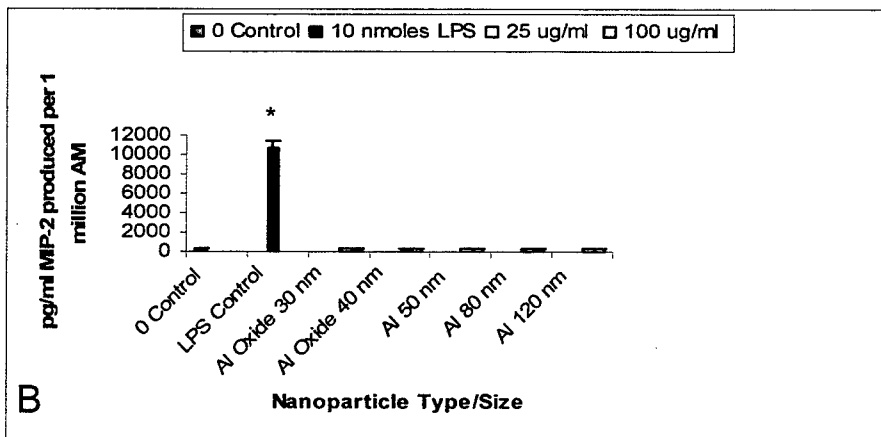
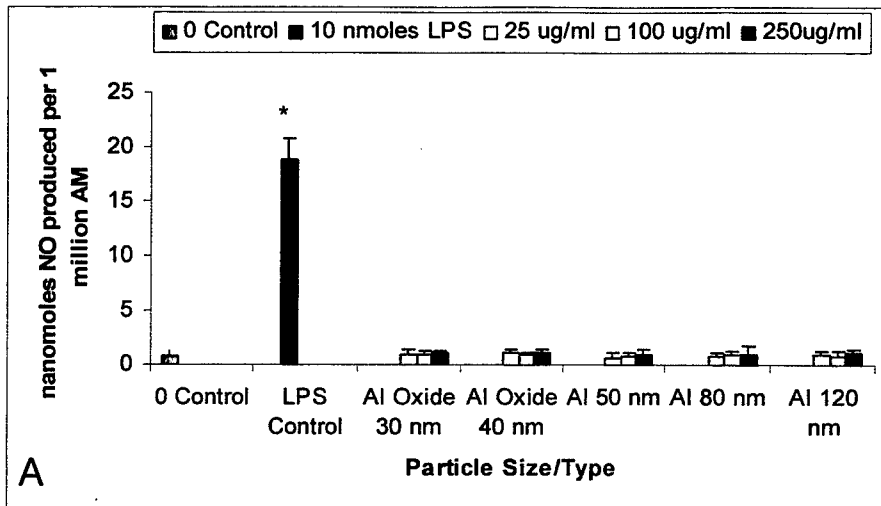


Figure 6: Inflammatory Response by Alveolar Macrophages 24 h Post Exposure to Al Nanoparticles. A) Nitric Oxide production (nanomoles/produced per 1 million macrophages, B) MIP-2 production pg/ml, C) TNF-alpha production pg/ml. Lipopolysaccharide (LPS) was used as a positive control. It is shown that inflammatory products (NO, TNF-alpha and MIP-2) were not being produced by AM (\* asterisk indicates doses that are significantly different than the zero control)

### **Conclusion:**

Aluminum oxide nanoparticles displayed significant toxicity after 96 and 144 hours post exposure at high doses (100 and 250  $\mu\text{g/ml}$ ). Aluminum nanoparticles also showed slight toxicity after 24 hours at high doses (100 and 250  $\mu\text{g/ml}$ ). When these cells were dosed at lower non toxic levels (25  $\mu\text{g/ml}$ ) Al 50, 80, 120 nm caused a significant reduction in phagocytosis. Even at a dose as low as 5  $\mu\text{g/ml}$  Al 50 nm still caused a significant reduction. None of these nanoparticles caused the induction of nitric oxide, TNF-alpha, or MIP-2, important components in inflammatory responses. In summary, based on viability, aluminum nanoparticles appear to be slightly toxic to rat alveolar macrophages. However, there was a significant reduction in phagocytic function of macrophages

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