AN INVESTIGATION OF TOXICITY AND IMMUNE EFFECTS OF COMMON METAL OXIDE NANOPARTICLES

By

Zhicheng Wei

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Examining Committee:

Lupita D. Montoya, Thesis Adviser

Deanna M. Thompson, Member

Linda S. Schadler, Member

Marianne C. Nyman, Member

Rensselaer Polytechnic Institute
Troy, New York

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ABSTRACT

Nanotechnology is presently considered one of the greatest engineering innovations since the Industrial Revolution and is estimated to become a $1 trillion market by 2015. A major component of nanotechnology is engineered nanoparticles (<100 nm), defined as nanometer size particles specifically synthesized to display particular properties. To date, titanium dioxide (TiO_2), aluminum oxide (Al_2O_3) nanoparticles have been widely used in a number of commercial applications. Expected increases in their commercial production worldwide could place consumers and workers at risk of exposure to those nanoparticles with still unknown consequences. Currently, the U.S. Environmental Protection Agency and the National Institute of Occupational Safety and Health are leading scientific efforts to understand the potential risks of nanomaterials to humans. There is a great need for methods, tools, and efforts to collect, characterize, and test nanoparticles to assess their health effects.

Midget impingers have been used to collect a variety of biological particles, and they appear to be good candidates for the collection of nanoparticles as well. Research presented here determined the efficiency of a 25 ml midget impinger for the collection of ultrafine TiO_2 and SiO_2 particles (from 3 to 100 nm). This research studied the effect of different parameters (collection fluid volume and type, flow rate, and particle type) on the collection efficiency of the impinger. Results showed that the smallest particles (3 nm) are collected with around 80 % efficiency. Increasing the volume of the collection liquid improved the collection efficiency of the impinger. This research represents the first published data on collection efficiency of impingers for particles below 15 nm.

The toxicity effects of TiO_2 and Al_2O_3 nanoparticles were investigated with *in vitro* models using human lung epithelial cells (A549). Careful particle characterization was performed by various methods to provide accurate physicochemical properties of the nanoparticles used in these studies. Clonogenic assay results showed that the highest concentration level of nanoparticles (10 mg/ml) inhibited cell viability and proliferation. Both clonogenic assay and alamarBlue® confirmed that the small nanoparticles (5 nm TiO_2 and 10 nm Al_2O_3, in primary size) included in this study had greater cytotoxicity effects than the large particles (200 nm TiO_2 and 50 nm Al_2O_3, in primary size). The correlation between cytotoxicity effects and particle size is only limited to primary size

not hydrodynamic size. These *in vitro* studies revealed that particle primary size plays a key role in determining the cytotoxicity effect of nanoparticles, but TiO_2 and Al_2O_3 nanoparticles are materials of relatively low toxicity.

The immune effects of TiO_2 and Al_2O_3 nanoparticles at low concentration levels were also studied using an *in vitro* model of the antigen presentation process using murine macrophages. Results showed that nanoparticles suppressed the T-cell cytokine production during antigen presentation. It was hypothesized that this response could occur because: 1) nanoparticles are able to interrupt the T cell's normal function, 2) nanoparticles have a direct effect on macrophages altering the way they present the antigen and subsequently altering the T-cell cytokine production or 3) nanoparticles cause macrophages to release a soluble factor that alters T-cell cytokine production. The results showed that nanoparticles are capable of altering the antigen presentation process by impacting antigen presentation cells (macrophages) via two possible routes: 1) upregulation of the surface signal molecules and 2) change of soluble factors/cytokines' concentration levels. The domain of these two mechanisms depends on the concentration of nanoparticles to which macrophages are exposed.

This research endeavors to deepen our understanding of the possible risks of using nanomaterials as the field of nanotechnology continues to emerge. These discoveries will provide valuable information to researchers, policy-makers and the public to make more educated decisions about the development, use, storage and disposal of nanomaterials.