ABSTRACT

DISPERSION OF GEOMETRIC TITANIUM DIOXIDE NANOMATERIALS AND THEIR BIOLOGIC EFFECT

by Trevor Tilly

Tunable chemical and physical properties of engineered nanomaterials are achievable by changing their geometry and morphology. Titanium dioxide (TiO$_2$) based nanofilaments—nanotube, nanowire, nanorod—have gained interest in industrial, energy, and as of recent, medical applications due to their superior performance over TiO$_2$ nanoparticles. Safety assessment of these nanomaterials is critical to protect workers, patients, and bystanders as these technologies become widely implemented. Additionally, TiO$_2$ based nanofilaments can easily be inhaled by humans and their high aspect ratio may make them toxic in the air passageway, like asbestos fibers. The tendency of TiO$_2$ nanofilaments to aggregate makes their nanotoxicity assessment difficult and the results controversial because incomplete dispersion results in larger particle size exposure that is no longer at the nanoscale. In this study, a microfluidic device was utilized to produce stable dosing solutions necessary to evaluate the toxicity of TiO$_2$ nanomaterials by eliminating any toxicity caused by aggregated TiO$_2$ nanomaterials. The toxicity results could then be directly correlated to the TiO$_2$ nanostructure itself. Well dispersed TiO$_2$ based nanomaterials were nontoxic to cells. Whereas, aggregated 100 μg/ml concentrations of nanowires and nanotubes reduced viability up to 27%, indicating that in vitro toxicity results may be controlled by the dispersion of dosing solutions.
DISPERSION OF GEOMETRIC TITANIUM DIOXIDE NANOMATERIALS AND THEIR BIOLOGIC EFFECT

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Chapter 1: Background

1.1 Nanosized TiO$_2$ and its Application

Nanotechnology is predicted to be a trillion dollar industry within the next decade because of the many benefits nanomaterials provide. The small size of nanomaterials, with one dimension less than 100 nm in length, gives them superior properties over larger particles. Titanium dioxide (TiO$_2$) is a naturally occurring metal oxide that is widely used in food (E171), coating, and paint industries (US$17 billion) due to its opacity, brightness, and whiteness. The highly redox active nature of TiO$_2$ also makes for applications in solar cells, environmental decontamination, and as a photocatalyst.$^{1-6}$ Furthermore, since the water splitting abilities of TiO$_2$ were discovered in 1972, geometric TiO$_2$ nanomaterials have gained even greater interest due to their electrical, optical, and photocatalytic properties.$^7$ The properties of TiO$_2$ are controlled by the material synthesis techniques, and subsequently related to the size, surface chemistry, and geometry.$^8$

Sterilization and decontamination applications for TiO$_2$ nanoparticles have already been discovered due to their photocatalytic nature. TiO$_2$ nanoparticles are useful for removing volatile organic compounds, greenhouse gases, and mercury from air and water.$^2$ In addition, TiO$_2$ was shown to be effective in killing bacteria in air, water, and food by a photocatalytic process.$^{1-3,5}$ The TiO$_2$ photocatalyst follows a two-step mechanism, which simultaneously oxidizes and reduces the starting products to complete the reaction without consumption of the TiO$_2$ catalyst. This reaction of TiO$_2$ nanoparticles has been shown to reduce bacteria population by 90%, which was an improvement from the 10% reduction observed for the UV control.$^3$ Similarly, the survival rate of S. choleraesuis, V. parahaemolyticus, and L. monocytogenes was greatly decreased when nanosized TiO$_2$ was irradiated by UV.$^2$ Likewise, the efficiency for removing the common air pollutant ozone by UV irradiation was increased from 4.5% to 55% when TiO$_2$ nanoparticles were added.$^1$ The sterilization and decontamination process of the TiO$_2$ nanoparticles are from the generation of free radicals at the surface, and improvement to the overall efficiency is possible by manipulating the morphology.$^9$

Synthesis of different nanogeometries such as: whiskers, rods, wires, spheres, and tubes have been published in literature.$^{10}$ Many of the applications of these TiO$_2$ nanomaterials are still being discovered, but foreseen applications include nanoscale reactor vessels (hollow
sphere), drug delivery systems (hollow spheres, tubes), sensing devices (tube, wire), biologic imaging devices (all), cancer treatment nanomedicine (all), and protective capsules for biologically active agents (tube, hollow sphere). The open geometry of TiO$_2$ nanotubes gives them large specific surface area allows promising applications in electronics, sensors, and pharmaceuticals.

Nanofilaments—nanotube, nanowire, nanorod—have greater specific surface area than nanoparticles, which is reason they have been discovered in even more applications. Nanofilaments are geometric structures with two aspects, length and width, at least one of which being 100 nm or smaller. TiO$_2$ based nanofilaments have become increasingly popular due to their ability to adsorb particles, photocatalytic properties, ion exchange abilities, and large specific surface area. These properties make them an exciting material for enhancing sensors, medical implants, drug delivery, lithium ion batteries, and in environmental decontamination. TiO$_2$ nanofilament potential for wide use in industrial applications and medical uses like drug delivery, implant coatings, and biomedical imaging makes imperative the assessment of their biocompatibility.

The use of geometric TiO$_2$ nanomaterials in dye-sensitized solar cells is an example of one application where they offer greater performance to nanoparticles. The large surface area and geometry of TiO$_2$ nanofilaments, makes researchers believe that the photoconversion efficiency of solar cells can be increased from 10.6% to 31%. Additionally, the morphologic change from nanoparticle to nanofilament of the TiO$_2$ caused an increase in both charge and light scattering efficiency by 25%, which resulted in improvement to the overall photocconversion efficiency.

Furthermore, TiO$_2$ nanofilaments have been used in literature as a drug delivery system. Likewise, the biocompatibility TiO$_2$ nanotubes have been shown promise as a drug delivery system because of its biocompatible and photocatalytic properties. For drug delivery, TiO$_2$ nanotubes are loaded with the drug of choice, and then stimulated by UV or x-ray to release the drug into the body. This mechanism allows for localized drug delivery to the targeted area of the body.
1.2 TiO$_2$ Nanofilament synthesis

The composition and mechanism for the formation of nanofilaments by low temperature (90-150°C) hydrothermal synthesis is complicated. Many factors, including the synthesis temperature, autoclave filling fraction, time, strong base selection, strong base concentration, and nanoparticle concentration, have been shown to directly control morphology, length, crystal structure, and surface chemistry.

For synthesis of TiO$_2$ nanotubes, the dilute acid (0.1 M HCl) rinse step was originally believed to cause the tubular geometry, however synthesis of sodium titanate nanotubes in the absence of rinsing have disproven this theory. The sodium ions from the sodium hydroxide solution provide thermal stability to the nanotube structure, and subsequent dilute acid rinsing with HCl replaces these structural sodium ions with hydrogen. This ion exchange consequently decreases the thermal stability, and leads to the destruction of the tubular structure upon annealment at temperatures greater than 400 ºC. In addition, by altering synthesis temperature, type of strong base, and strong base concentration, the geometry of the TiO$_2$ nanomaterials can be changed from nanotube, nanowire, nanorod, and nanobelts.

1.3 Inhalation Transport and Deposition of Nanomaterials

The inhalation of nanomaterials is a complex system that includes physiologic factors, material factors, as well as fluid transport mechanisms. Primary particle size governs whether the nanoparticle transport is by diffusion or sedimentation.\textsuperscript{27} Particles with diameter less than 10 nm are controlled principally by diffusion, whereas particles larger than 200 nm rely on sedimentation.\textsuperscript{27} Particles ranging in between these values with diameter 10-200 nm are transported by both sedimentation and diffusion, which results in a slower transport of materials with this size range.\textsuperscript{27,28} Deposition and transport of nanomaterials upon inhalation is further affected by humidity, temperature, and torturous path. The geometry of the inhalation tract determines particle deposition in each localized area. The sharp geometry of the nasal cavity results in nanoparticle deposition by impaction, and the low flow in the alveolar sacs causes the nanoparticles to be deposited by diffusion.\textsuperscript{29} Changes in humidity and temperature cause nanoparticle aggregation into larger particles, thus changing their transport mechanism by diffusion to sedimentation.\textsuperscript{28} Deposition by sedimentation often governs \textit{in vitro} nanotoxicology experiments due to the agglomeration of nanomaterials in cell growth media.\textsuperscript{27}
1.4 Toxicity of TiO$_2$ nanomaterials

The wide range of materials and synthesis techniques for producing engineered nanomaterials has made it difficult to fully evaluate and understand their potential toxicity. Despite all of this, TiO$_2$ has become one of the most extensively studied metal oxides in toxicity research due to its common use in industrial applications, however its toxicity remains unclear.$^{30-38}$ Extensive *in vivo* and *in vitro* journal articles dealing with TiO$_2$ nanoparticles were recently utilized by the National Institute for Occupational Health and Safety (NIOSH) for making recommended airborne exposure limits for fine (0.1–3 µm) and ultrafine (<100 nm) particles, 2.4 and 0.3 mg/m$^3$, respectively.$^{39}$ From these values, relevant dosing concentrations were determined for this study by using an *in vivo* to *in vitro* method previously described in Braydich-Stolle et. al.$^{40}$ A concentration range was determined to accommodate for differences in deposition between the length and diameter aspects of the nanofilaments. More explanation about this dosing calculation is given in the methods section.

TiO$_2$ nanofilament toxicity studies are limited in literature. Since the morphology, geometry, chemical composition, and crystal structure of TiO$_2$ nanomaterials is controlled by the synthesis conditions, understanding current cytotoxicity data is confusing. Additionally, the differences in *in vitro* models, dosing concentration, and dispersion methods in these journal articles make it difficult to draw clear conclusions. Table 1 summarizes all known *in vitro* toxicity studies dealing with TiO$_2$ nanofilaments that have been published.
Table 1- TiO$_2$ nanofilament toxicity published in literature.

<table>
<thead>
<tr>
<th>Primary Author</th>
<th>Year</th>
<th>TiO$_2$ Nanomaterial Type</th>
<th>In Vitro Model</th>
<th>Dispersion</th>
<th>Dosage concentration</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magrez</td>
<td>2009</td>
<td>Hydrothermal: 6 g anatase particle, 15 M NaOH, 72h, 150°C</td>
<td>H596: human lung tumor cell</td>
<td>Dilute gelatin, Tween 80, sonication</td>
<td>0.02 - 2 µg/ml</td>
<td>Protonated nanotube were more toxic than nanowires and sodium nanotubes</td>
<td>[38]</td>
</tr>
<tr>
<td>Wadhwa</td>
<td>2011</td>
<td>1) P25 nanoparticle 2) 30% Nanotube 70% nanorods</td>
<td>A549: human lung epithelial cell line</td>
<td>PBS, 1 minute sonication</td>
<td>0.1 - 1.1 mg/ml</td>
<td>Nanotubes at high concentration do not reduce cell viability</td>
<td>[39]</td>
</tr>
<tr>
<td>Pierzchala</td>
<td>2011</td>
<td>1) P25 nanoparticle 2) Nanowire: 35 nm diameter, 0.5-1.0 µm length</td>
<td>1205Lx: derived from lung metastases of WM793B cells</td>
<td>PBS, bath sonication for 40 min.</td>
<td>2.5 µg/ml</td>
<td>P25 Nanoparticles were more toxic than nanowires</td>
<td>[40]</td>
</tr>
<tr>
<td>Hamilton</td>
<td>2009</td>
<td>1) Nanoparticle: 60-200 nm diameter 2) Nanobelt: 60-300 nm diameter, 0.8-30 µm length</td>
<td>murine alveolar macrophages</td>
<td>PBS/ 3.5% BSA + brief sonication and 1 min vortexing</td>
<td>100-200 µg/ml</td>
<td>Long nanobelts were most toxic</td>
<td>[41]</td>
</tr>
<tr>
<td>Schanen</td>
<td>2009</td>
<td>1) Nanoparticle: anatase 7-10 nm diameter 2) Nanoparticle: Rutile 15-20 nm diameter 3) nanotubes 10-15 nm diameter, 70-150 nm length</td>
<td>Human peripheral blood mononuclear cells, human umbilical vein endothelial</td>
<td>PBS, sonication, vortexing</td>
<td>0.004 - 8 µg/ml</td>
<td>TiO$_2$ may reduce metabolism without causing cell death</td>
<td>[42]</td>
</tr>
</tbody>
</table>

Inhalation is predicted to be the common route for human exposure to TiO$_2$ nanofilaments, which can be seen in Table 1 by the in vitro respiratory models chosen for the previously reported toxicity studies. Similarly, the high aspect ratio of TiO$_2$ nanofilaments has caused some concern due to their structural similarity to asbestos, a material toxic due to geometry alone. In one study, TiO$_2$ nanofilaments were determined to be cytotoxic, as TiO$_2$ based nanotubes and nanowires reduced the viability of H596 human lung tumor cells.$^{41}$ Oppositely, TiO$_2$ nanotubes at high concentration of 1.1 mg/ml did not reduce the viability of A549 cells, and was similar to cells exposed to P25 TiO$_2$ nanoparticles despite its much greater surface area.$^{42}$ Furthermore, P25 TiO$_2$ nanoparticles had a higher phototoxic potential than anatase based TiO$_2$ nanowires, upon exposure to UVA light.$^{43}$ In murine alveolar macrophages, Long TiO$_2$ nanobelts, larger than 15 µm in length, initiated an inflammatory response and reduced cell viability compared to shorter nanobelts and nanoparticles.$^{44}$ Lastly, Schanen et. al. found that TiO$_2$ nanoparticles and nanotubes may affect the metabolism of human peripheral blood mononuclear and umbilical vein endothelial cells without causing apoptosis.$^{45}$

*In vitro* toxicology using human cell lines has become a promising and popular way to evaluate the potential harm of nanomaterials because of its rapid screening time, low cost, and use of physiologically relevant cells. However, *in vitro* nanotoxicity is often scrutinized due to
results being irreproducible and irrelevant to \textit{in vivo} studies. Some of these shortfalls, especially with high aspect ratio materials, are hypothesized to be due to the incomplete dispersion of nanofilament aggregates in dosing solutions, which causes a concentration gradient. This gradient can alter deposition and effective exposure concentration administered to the \textit{in vitro} cell culture; consequently making concentration based toxicity trends confusing and reproduction of the results difficult.

Preparing dosing solutions with TiO$_2$ nanofilaments was found to be challenging because conventional mechanical dispersion methods, by sonication and vortexing, provided polydisperse solutions containing a mixture of individual filaments, aggregates, and particle debris. This difficulty experienced in dispersing aggregated nanofilaments may explain the many discrepancies in toxicity data, famously carbon nanotubes. For example, well dispersed individual carbon nanotubes showed no pulmonary toxicity in mice, whereas granuloma-like structures were observed after treatment with carbon nanotube aggregates.$^{46}$ Interesting, the formation of granuloma-like structures by cells exposed to nanotube aggregates were similar to previously reported literature, which poses the question of whether previous studies assess the toxicity of individual carbon nanotubes or aggregates.

Mechanical dispersion by sonication and vortex are commonly used to prepare \textit{in vitro} dosing solutions for toxicity studies. These conventional mechanical methods may only temporarily suspend the nanomaterials, which make it difficult to produce uniform exposure over the entirety of the study. Additionally, mechanical dispersion methods may change the morphology of geometric nanomaterials, consequently altering the physical properties that are being assessed. The most common dispersion technique by sonication produces is understood to produce local temperatures and pressures greater than 5000 K and 20 MPa, respectively. This extreme environment has been reported to shorten the length of TiO$_2^{12}$ and carbon$^{47,48}$ nanotubes.

Chemical surfactants like dimethyl sulfoxide (DMSO), Tween 80 (T80), and bovine serum albumin (BSA) have been employed to dissipate aggregates and aid against agglomeration, however DMSO and T80 are toxic to cells$^{49}$. The surface chemistry of the nanofilaments is changed with the addition of these surfactants, and may make the biologic exposure unnatural and unrealistic.$^{50}$ Even with chemical surfactants, a mechanical force is
necessary to separate aggregates, which consequently combines the pitfalls of both chemical and mechanical dispersion methods.

Here, the toxicity of TiO$_2$ nanomaterials was assessed for four geometries. Aggregated nanofilaments were a result from the drying step of synthesis, and require a dispersion device to separate them for proper evaluation in vitro. In this study, microfluidic method is innovatively used as a chemical and ultrasonic free dispersion method to disband TiO$_2$ based nanotube, nanorod, and nanowire aggregates. The M-110Y microfluidic device utilizes shear force to separate highly aggregated nanofilaments by processing mixtures through micro-channels. Subsequently, producing well dispersed dosing solutions of TiO$_2$ nanofilaments stable for weeks.$^{51}$ This quality dispersion was employed to systematically assess the biocompatibility for four TiO$_2$ based nanomaterials upon initial human inhalation in RPMI 2650 nasal epithelium cells.
Chapter 2: TiO$_2$ material synthesis and characterization methods

2.1 TiO$_2$ nanofilament synthesis

The hydrothermal process was used to synthesize the TiO$_2$ based nanofilaments. Degussa P25 Titanium dioxide nanoparticles with ratio 3:1 anatase to rutile phases, with diameters 25nm and 85 nm\textsuperscript{52}, were used as a precursor for TiO$_2$ nanotubes, nanorods, and nanowires. Synthesis of the nanotubes, nanowires, and nanorods followed methods published previously.\textsuperscript{53–55} Figure 1 demonstrates how synthesis methods affect the TiO$_2$ nanofilament morphology.

![Figure 1- Process flow chart of TiO$_2$ nanofilament synthesis leading to the nanotube, nanowire, and nanorod geometries from P25 TiO$_2$ nanoparticles.](image)

For the synthesis of nanotubes and nanorods, 0.5 g of P25 nanoparticles were added to 30 ml of 10 M NaOH aqueous solution, and sonicated in a Branson ultrasonic bath for 1 h to homogenize the solution.\textsuperscript{56} The solution was then transferred into two 20 ml Teflon lined autoclaves and placed in a furnace at temperature ranging from 100-130 °C for 24 h. After the 24 h thermal treatment, the furnace was turned off and the autoclave was allowed to cool in the oven to room temperature. Then suspension was rinsed with deionized (DI) water till pH ~8, and left in 0.1 M
HCl aqueous solution for at least 3 h. Then the suspension was separated from the solution via centrifugation or vacuum filtration with 0.45 μm filter and rinsed with DI water until the rinse solution pH ≈ 7. The suspensions were dried into powders at 80 °C in an oven. Nanorods were synthesized by subsequently annealing the dried powder in a furnace at 400 °C for 1 h. Nanowires were synthesized the same way as nanotubes, but in an aqueous 10 M KOH solution with elevated hydrothermal temperature of 130 °C.

2.2 Characterization of TiO$_2$ nanomaterials

Size and morphology of the hydrothermally grown nanofilaments before and after dispersion was examined using transmission electron microscopy (TEM). 10-1000 μg/ml solutions were prepared in DI water and a droplet from the solution was placed on formvar stabilized carbon 200 mesh Cu TEM grid purchased from GloEMT. A hair dryer on low power setting was used to rapidly evaporate the droplet from the grid to minimize agglomeration of the nanomaterial in the droplet. The prepared grids were imaged at 120 kV on a JEOL JEM-1200 EX II TEM 120 kV.

The length and diameter of the nanomaterials was determined by measuring TEM images using Image J software. Aspect ratio was determined by dividing the length by the diameter of obtained from the TEM images. Crystalline diffraction patterns of each nanomaterial was measured using powder x-ray diffraction (XRD) using the XI Advanced Diffraction System by Scintag Incorporated, and analyzed using MDI Jade 7 program.

BET specific surface area was determined with a Micrometritics TriStar II. Samples were degased at 190 °C for 1h in order to evaporate water and remove gas adsorbed on the surface of the nanomaterials, and then analyzed.
Chapter 3: In vitro cell culture, toxicity, and statistical analysis methods

3.1 In vitro cell culture

Sterile laboratory practices were followed, and all cell culture and dosing solution preparation was done in a biological hood. RPMI 2650, a human nasal epithelial cell line was used to model the first point of exposure to TiO$_2$ based nanomaterials. RPMI 2650 cell line is extensively studied for use in nasal drug delivery studies. It has a similar diploid number and karyotype as human male epithelial cells, which is not commonly found in carcinomas.

There has been debate over RPMI 2650 cells ability to form a monolayer in classic submerged in vitro culture, and its success in modeling the nasal epithelium for nasal permeation experiments. In submerged cell culture conditions, cells are dispersed in cell growth media in a collagen coated flask, and they settle and attach to the collagen surface. Under these conditions, the nasal epithelial cells do not form a monolayer, but instead grow in clusters. More recently, monolayer RPMI 2650 have been produced by culturing at the air-liquid interface. Basic culture procedures for the RPMI 2650 cell line are shown in Table 2.

Table 2 Breakdown of RPMI 2650 nasal epithelial cell line culture procedures.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>RPMI 2650 Human Nasal Epithelial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>Minimum essential medium Eagle (10% FBS, 1% Pen/Strep)</td>
</tr>
<tr>
<td>Media Renewal</td>
<td>Twice a weekly</td>
</tr>
<tr>
<td>Cleaning</td>
<td>Three times rinse 10 ml media (Without FBS)</td>
</tr>
<tr>
<td>Splitting</td>
<td>1-2 times biweekly</td>
</tr>
<tr>
<td>Detachment</td>
<td>Add 2-4ml of 0.25% trypsin let sit for 10 min in incubator.</td>
</tr>
<tr>
<td>Plating</td>
<td>150,000 cells/ml, 100μl in each well of 96-plate</td>
</tr>
</tbody>
</table>

3.1.1 Cell growth

Cells were grown in cell culture flasks in an incubator at 37°C, and a controlled 5% CO$_2$ atmosphere. Human nasal epithelial (NE) cell line (RPMI 2650) was grown in Minimum essential medium Eagle (Earle’s BSS), which consists of 2.0 mM glutamine, 0.1 mM non-essential amino acids, and 1.0 mM sodium pyruvate. The addition of 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin were added to promote a protein rich environment for cells to grow without infection by bacteria. Penicillin, streptomycin, and FBS are commonly
used in cell growth media. The cells were split from a previously cultured flask, which its standard procedure is explained by the cell growth process, pictured in Figure 2.

![Diagram of cell culture process]

Figure 2- The experimental approach for cell culture of RPMI 2650 cells.

RPMI-2650 cells and most other cell type rely on attachment to the cell culture flask to grow. When plating or splitting however, detachment from the flask is necessary. The cleaning step must be performed to remove any residual FBS in the flask because it neutralizes trypsin (the detachment agent). First, the used growth media was aspirated from the flask, and 5 mL of media without FBS was added. The flask was gently rocked back and forth to rinse the cell surface before the media is aspirated from the flask. This process was repeated three times.

### 3.1.2 Detachment and splitting

For detachment of the cells from the culture flask, 2-4 ml of 0.25% trypsin was added and removed from the flasks three times to neutralize the FBS. On the third rinse, the 0.25% trypsin was not removed, and the flask was returned to the incubator for 10 min while the cells were allowed to detach. 2-4 ml of growth media was added to the flasks (equal to the volume of
trypsin) to neutralize the trypsin. The resulting solution was then transported to a conical tube to prevent reattachment during splitting or plating.

A total volume of 10 ml was added to the cell culture flasks during splitting, which consists of the cell solution and growth media. The amount of growth media added to cell culture flasks was dependent on the concentration of the cell solution and foresight of upcoming experiments. For normal growth, 3-5 ml of cell solution was added with the difference growth media. When experiments were closely approaching and cells were needed, a higher concentration of cells was added to the flasks.

3.1.3 Cell density optimization

Standardization and optimization for the RPMI 2650 cell line to prevent cell death caused by overcrowding the seeding density was performed using light microscope. Cells were cultured for 24 h at concentrations ranging from 200,000 to 700,000 cells/ml in a 6 well plate. Light microscope images used to visualize the attachment and growth of the RPMI 2650 cells are shown in Figure 3.

![Figure 3- RPMI 2650 cells seeded at a) 200k cells/ml b) 500k cells/ml and c) 700k cells/ml for 24 h in a 6 well plate to determine seeding concentration for in vitro experiments.](image)

As can be seen in Figure 3, RPMI 2650 cells grow in clusters as described in literature. Figure 3 shows that as the seeding density went up, more clusters of cells were observed. The RPMI 2650 cells will have an uneven exposure to the TiO$_2$ nanomaterials when they are grown in
clusters because cells on top will be exposed to the nanomaterials while underlying cells will be unexposed. This will potentially add error to the system.

3.1.4 Plating

The cellular concentration of the cell solution from detachment was determined before plating cells for the experiments. A Nexcelcom Cellometer® automated cell counter was used to count the cells in the solution. Each cell count slide has two wells capable of holding 15 μl of solution. The solution was pipetted into both wells until completely full and the cells on the slide were counted. The average of the two readings was used to determine the amount of media to be added to have a concentration of 250,000 cells/ml for plating. Equations 1 and 2 are the equations used to calculate the necessary amount of cell solution and media to create the desired concentration.

\[
\frac{\text{Cells needed/ml}}{\text{Cells counted/ml}} \times \text{Plating Volume} = \text{volume of cell solution to add} \quad (1)
\]

Where cells needed/ml refers to the optimized concentration of RPMI 2650 cells, the cells counted/ml refers to the concentration of cells determined by the automated cell counter, and the plating volume is the volume of cell suspension for the experiments (10 ml for a 96 well plate).

\[
\text{Plating Volume} - \text{Volume of the cell solution} = \text{volume of cell solution to add} \quad (2)
\]

Cell Splitting

Human nasal septum carcinoma RPMI 2650 cell line (American Type Culture Collection) was used as a nasal epithelial model in this experiment. Eagle’s Minimum Essential Medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin was used as the cell growth media and will be denoted as EMEM. Cells were grown according to instructions given by the supplier in a culture flask, and maintained in a humidified incubator at 37 °C and 5% CO₂.

Once at least 70% confluent, cells were rinsed three times with phosphate buffer solution (PBS), and detached from culture flask by adding 4 ml of 0.5% trypsin for 10min in a humidified incubator. After detachment, cells were counted by a Vision Cellometer by Nexcelom.
Bioscience following the manufacturer’s protocols, and a calculated volume of EMEM was added to the flasks to neutralize the trypsin and produce the necessary concentration to be seeded into 6 well plates for uptake, 96 well plates for cell viability, and 2 chamber slides for localization studies.

3.2 Dosing solution concentration calculation

The dosing concentrations for experimentation were determined based on a previously mentioned method, and in vivo to in vitro correlation of NIOSH’s Recommended Exposure Limit of TiO$_2$ nanoparticles. The theoretical work day exposure for an employee handling TiO$_2$ nanomaterials was determined by equation 3, which was used to determine the in vitro dosing concentration from eqn. 4.

\[
\text{Workday exposure (mg/day)} = (\text{ventilation}) \times (\text{A-L volume conversion}) \times (\text{TLV-TWA}) \times (\text{exposure time})
\]  
(3)

Where workday exposure is measured in mg, ventilation is 20,000 ml/min, the air to liquid volume conversion is $10^{-6}$ m$^3$/ml, TLV-TWA is the threshold limit value time weighted average recommended by NIOSH, and exposure time was based on an 8 h work day.

\[
\text{Daily in vitro dose concentration (µg/ml)} = \frac{\text{(workday exposure)}/(\text{surface area target organ}) \times (\text{surface area of tissue culture dish})}{(\text{volume tissue culture dish})}
\]
(4)

Where the daily in vitro dosing concentration is measured in µg/ml, the workday exposure is the value obtained from eqn. 3, the volume of the tissue culture dish is 0.2 cm$^3$, the surface area of the human nasal epithelium (target organ) is 203 cm$^2$, and surface area of the culture dish is 0.3 cm$^2$.

3.3 Cytotoxicity methods—cell viability, inflammation, morphology, uptake, and localization

3.3.1 Cell viability- Health of Cells

Cell viability determined by the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay was performed in triplicate in a 96 well plate. This assay uses fluorescence to measure the adenosine triphosphate (ATP) production of the mitochondria to determine cellular survival. TiO$_2$ nanotubes, nanowires, nanorods, nanoparticles, and copper nanoparticle (30 nm) control were exposed to cells at concentration 10, 50, and 100 µg/ml. 96 well plates were seeded with RPMI 2650 cells at
concentration of 250,000 cells/ml, and allowed to attach for 24 h in an incubator. The cells were rinsed 3X with PBS, and then exposed to TiO$_2$ based nanomaterials for 24 h. The cells were rinsed 3X with PBS, and 100 µl of cell media without FBS and 20 µl of MTS was added and placed in the incubator for 1 h. The 96 well plate absorbance was then read by the automated plate reader at wavelength 480 nm.

3.3.2 Inflammation and cellular morphology- Cellular Response to Nanomaterials

Inflammation in the RPMI 2650 cell line was determined by ELISA purchased from Invitrogen for detecting the cytokine tumor necrosis factor alpha (TNF-α) after 24 h exposure to each nanomaterial. The procedures provided by the manufacture were followed.

The cell morphology after nanomaterial exposure was evaluated by light microscope. RPMI 2650 cells were platted in a 6 well plate, and placed in an incubator for 24 h. After attachment, the cells were exposed to 100 µg/ml of nanotubes, nanowires, nanorods, and P25 nanoparticles for 24 h. An Olympus IX71 Microscope was modified with a light condenser, and used to image the cells post exposure at 20X magnification in bright-field microscopy. QCapture Pro software was used to correct the images. A lense magnification of 20X was used to image the cells with QCapture Pro imaging software.

3.3.3 Localization and Uptake- Nanomaterial Transport in Cells

Localization of the nanomaterials in the RPMI 2650 cells was performed by cell staining. Cells were cultured in a 2 chamber slide, and exposed to nanotubes, nanorods, nanowires, and nanoparticles. The cells were fixed with 2% paraformaldehyde, permeabilized with 0.1% Triton-X-100. The cells were rinsed two times with PBS and incubated in 1% Bovine Serum Albumin (BSA Calbiochem, #12659) for 30 min. The BSA was removed and the membrane was stained, at room temperature for 20 min, with 1 ml of Alexa Fluor 555 Phalloidin (1:40 Alexa Fluor: 1%BSA, Life Technologies, # A34055). The cells were rinsed two times with PBS, and the chambers were removed. Samples were then air dried for 5 min, and two drops/chamber of Prolong Gold Antifade agent with DAPI counterstain (Life Technologies, #P36931) were added. A coverslip was placed over the slide, and sealed in place with nail polish. The slide was allowed to cure overnight, and imaged in QCapture Pro Imaging Software from images obtained by the Olympus IX71 Microscope platform coupled with a URI system.
Cellular uptake of TiO$_2$ based nanomaterials was visualized by TEM. RPMI 2650 cells were seeded in a 6 well tissue culture dish, and placed in an incubator at 37 °C, 5% CO$_2$. After cells were approximately 85% confluent, 2 wells were dosed for each TiO$_2$ nanomaterials at concentration of 100μg/ml concentration. Cells were washed 3X with PBS and then detached from culture dish with 1ml of 0.25% trypsin. Trypsin was neutralized by EMEM and then solution was centrifuged. The cell precipitate was transferred to a specimen embedding capsule. Fixation of cells was achieved by adding 2% paraformaldehyde/2.5% gluteraldehyde for 2 h. Once fixed, cells were rinsed three times with PBS, and then stained with 1% osmium tetroxide. Samples were rinsed three times with PBS, and dehydrated by serial dilutions of ethanol from 50-100%. Then samples were placed in 100% resin overnight at 60 °C in EM capsules. These capsules were sectioned on a Leica ultramicrotome at thickness 70 nm and collected on a TEM grid. Cells were imaged on a JEOL JEM-1200 EX II TEM at 100 kV.

3.4 Statistical Analysis Methods

To determine the statistical significance of the cell viability data, a two-tailed t-test in Microsoft Excel was used. Additionally, all biological experiments were performed in triplicate. The two-tailed t-test uses Student’s t-test, which assumes a normal probability distribution. Additionally, since nanomaterials can increase or decrease cellular viability, as measured by the MTS assay, the two-tailed method was chosen. The Excel two tailed t-test function used eqns. (5-8) to determine if treated samples were statistically significant at p level P=0.05.

The pooled standard deviation ($s_{X1X2}$), was calculated by eqn. (5), below.

\[
s_{X1X2} = \sqrt{\frac{1}{2}(s_{X1}^2 + s_{X2}^2)}
\]

The value obtained from eqn. (5) represents the estimated variance of the control cells and tested samples, where $s_{X1}^2$ represents the unbiased estimator of the variance. The t statistic of the data was calculated by eqn. (6).

\[
t = \frac{\bar{X}_1 - \bar{X}_2}{s_{X1X2} \cdot \sqrt{2/n}}
\]
Where \( n \) is the number of wells in the 96-well plate dosed at a certain concentration (10, 50, 100 \( \mu \text{g/ml} \)) or with a certain material (\( \text{TiO}_2 \) NT, NW, NR, NP, untreated, Cu NP control), and \( \bar{X}_t \) is the mean of the wells, and \( s_{x_1 x_2} \) was determined by eqn. (5).

Critical value (CV) and degrees of freedom (DF) were calculated to determine the \( t \) value from Table 3.

The CV was determined at significance level \( \alpha=0.05 \) and used eqn. (7).

\[
CV = 1 - \frac{\alpha}{2}
\]  

(7)

The DF were determined by eqn. (8)

\[
DF = 2n - 2
\]  

(8)

Where \( n \) represents the number of wells as in it did in eqn. (6).

The CV and DF were calculated by eqns. 7-8 used to be used with Table 1 to determine the significance of the data. Absolute values of the test statistic greater than the critical value were determined to be significant.

Table 3- Critical values of Student's \( t \) distribution with degrees of freedom (DF) \(^{17} \). Bold value is the critical value used for significance testing.

<table>
<thead>
<tr>
<th>CV</th>
<th>0.9</th>
<th>0.95</th>
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<th>0.995</th>
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<tr>
<td>1</td>
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Chapter 4: Characterization, dispersion, and in vitro exposure of TiO$_2$ based nanomaterials

4.1 TiO$_2$ nanomaterial characterization

Hydrothermal synthesis conditions were controlled to produce nanotube, nanowire, and nanorod geometries of TiO$_2$ from P25 TiO$_2$ nanoparticles. The TEM images used in determining the size and morphology of the four TiO$_2$ nanostructures are shown in Figure 3.

![TEM images of TiO$_2$ based a) P25 nanoparticle b) nanotube c) nanorod and d) nanowire dispersed in DI water.]

Figure 4 shows the morphologic differences between the geometric TiO$_2$ nanomaterials. A summary of the size analysis and calculated aspect ratio for the TiO$_2$ nanostructures is in Table 4.

<table>
<thead>
<tr>
<th>TiO$_2$ geometry</th>
<th>Mean Length (nm)</th>
<th>Mean Diameter (nm)</th>
<th>Aspect Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanoparticle</td>
<td>25.9±11.2</td>
<td>25.9±11.2</td>
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<tr>
<td>Nanorod</td>
<td>48.8±10.8</td>
<td>9.0±1.6</td>
<td>5.4</td>
</tr>
<tr>
<td>Nanowire</td>
<td>102.1±22.1</td>
<td>8.6±2.0</td>
<td>11.9</td>
</tr>
<tr>
<td>Nanotube</td>
<td>127.0±51.2</td>
<td>8.5±1.3</td>
<td>14.9</td>
</tr>
</tbody>
</table>
The diameter of the nanofilaments was consistent for all of the geometries at ~9 nm, however the lengths of the nanofilaments ranged from 48.8 to 127 nm, and are believed to be a function of the material processing and stability. The TiO$_2$ nanorods were subject to the most processing, as they were the only geometry that was annealed before dispersion. During synthesis of the nanotube geometry, acid washing is used to replace structural sodium ions with protons, which has been previously reported to reduce structural stability upon heating. Since the nanorod geometry was the only sample annealed, thermal instability caused by the acid treatment was predicted to be the reason that nanorods had the shortest filament length of 48.8 nm and aspect ratio of 5.4. Oppositely, TiO$_2$ nanotubes and nanowire length and aspect ratio were more than double that of the nanorods. Nanotubes had length of 127.0 nm and aspect ratio of 14.9. Furthermore, nanowires were 102.1 nm in length and had aspect ratio of 11.9. Finally, P25 TiO$_2$ nanoparticles had spherical geometry, and an aspect ratio of 1.

The crystal structure of the TiO$_2$ nanomaterials was investigated using XRD, Figure 5A.

**Figure 5-**(A)XRD and (B)BET surface area patterns of TiO$_2$ based (blue) nanowire, (green) nanotube, (black) nanorod, (red) nanoparticle.

P25 TiO$_2$ nanoparticles had the sharpest, most intense XRD pattern of the TiO$_2$ materials tested, and were predicted to be the most crystalline sample tested. Oppositely, the nanotubes and
nanowires were less crystalline, which could be visualized by the less intense peaks of their XRD pattern. The XRD pattern of the TiO$_2$ nanorods revealed greater crystallinity and a mixture of anatase and rutile phases. These results indicate that the change in XRD patterns can be used for determining the morphology of the TiO$_2$ nanomaterials.

The porous structure of TiO$_2$ made it an ideal candidate for surface area analysis by BET, shown in Figure 5B. Aspect ratio and specific surface area are correlated for TiO$_2$ nanomaterials; high aspect ratio materials have higher specific surface area. Surface area and aspect ratio of TiO$_2$ based nanomaterials followed the trend: nanotube > nanowire > nanorods > nanoparticle. Where the specific surface area of TiO$_2$ nanotubes was the largest at 389.38 m$^2$/g and nanoparticles the smallest at 55.08 m$^2$/g. Interestingly, the morphologic change of TiO$_2$ nanotubes to nanorods substantially decreased the BET surface area from 389.38 to 150.02 m$^2$/g. The hollow structure and increased length of the nanotube gives them more surface area per unit mass.

4.2 TiO$_2$ nanomaterial dispersion

TiO$_2$ nanofilaments in this study were aggregated after synthesis, and difficult to disperse individually by traditional means. Figure 6 shows TiO$_2$ nanofilaments after processing by ultrasonic probe.
A mixture of aggregates, particle debris, and a size distribution of nanofilaments are observed in Figure 6. Unlike the TiO$_2$ nanofilaments, P25 TiO$_2$ nanoparticles could be dispersed by sonication in DI water due to their spherical geometry. Similarly, low aspect ratio TiO$_2$ geometries, like the nanorods, were easier to disperse in water. Figure 6, shows the result of TiO$_2$ nanofilament processing by powder dispersion in air 6(a) and sonication in water 6(b).
Aggregates greater than 1 μm in diameter were seen in TEM images shown in Figure 6 after simple powder dispersion and sonication. These aggregates were difficult to disperse, which was potentially due to the intermolecular forces between the TiO$_2$ nanofilaments during drying at the air-liquid interface. Nanofilaments were observed with lengths greater than 1 μm, however after drying and dispersion their length was greatly reduced. To our knowledge, successful dispersion of TiO$_2$ based nanofilaments has not been achieved without reduction in length. To preserve the high aspect ratio of TiO$_2$ based nanofilaments special care must be taken. Creative dispersion and processing methods may make it possible to produce nanofilaments with aspect ratios >100.

In order to produce homogeneous dosing solutions for \textit{in vitro} toxicity testing a microfluidic dispersion device was employed, which provided solution stability for weeks. Time point images immediately after dispersion of the nanomaterials and after 24 h can be seen in Figure 8.
Figure 8- Time point images at 0 h and 24 h post dispersion by (A) microfluidic and (B) sonication in DI water. (a) DI water (b) nanotubes, (c) nanoparticles, and (d) nanowire.

As can be seen in Figure 8, the microfluidic device provides more stable, homogeneous TiO$_2$ solutions than ultrasonic probe. When probe sonication was used, polydispersion was observed in nanotube and nanowire solutions after 24h after dispersion. This result poses questions previous nanofilament in vitro toxicity studies because of the frequency sonication was used in literature.

Zeta potential was used to investigate solution stability and agglomeration tendencies of the nanomaterials as a means for quantifying the dispersion methods. Improvement in solution stability was observed with the microfluidic dispersion by the zeta potentials in Table 5. Zeta potential close to 0 mV indicated the tendency for the material to agglomerate, and deviation from this value represents increased stability of the solution.

<table>
<thead>
<tr>
<th>TiO$_2$ geometry</th>
<th>Micro (mV)</th>
<th>Sonic (mV)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanoparticle</td>
<td>-26.6</td>
<td>15.2</td>
<td>7.35</td>
</tr>
<tr>
<td>Nanorod</td>
<td>-25.3</td>
<td>-23.0</td>
<td>7.03</td>
</tr>
<tr>
<td>Nanowire</td>
<td>-36.9</td>
<td>-24.3</td>
<td>7.27</td>
</tr>
<tr>
<td>Nanotube</td>
<td>-36.2</td>
<td>-30.8</td>
<td>7.17</td>
</tr>
</tbody>
</table>

For all of the nanogeometries tested, TiO$_2$ nanowires were found to be the most difficult to disperse by either of the methods due to its high aspect ratio. Sonication dispersions were
unstable and contained a mixture of dispersed nanofilaments and aggregates. Figure 9 shows an aggregate of TiO$_2$ nanowires with dispersed nanowires.

![Figure 9 - TEM photograph of TiO$_2$ based nanowires after dispersion by sonication probe. Image shows individually dispersed nanowires and long nanowire aggregate.](image)

The TEM of the TiO$_2$ nanowires seen in Figure 9, show an aggregate greater than 700 nm in length coexisting with 100 nm well dispersed nanowires. This mixture of short and aggregated long nanowires may be the cause of the instability and subsequent agglomeration observed in the time point images seen in Figure 8.

Preservation of the TiO$_2$ nanomaterial structure after dispersion was important in correctly assessing their toxicity. The previously reported length and width reduction of TiO$_2$ based nanotubes after sonication gave us motivation to investigate their morphology before and after dispersion. The high aspect ratio of the nanofilaments makes them subject to morphologic change when aggregates are processed. The hollow structure of the TiO$_2$ nanotube make them subject to morphologic change during dispersion, which is why this geometry was used to investigate material deformation by the two dispersion methods. TEM images, Figure 10, showed that both dispersion methods preserved the TiO$_2$ nanotube structure.
Additionally, the microfluidic device provided uniform length reduction, which is shown in Figure 10. In contrast, many aggregates were still common after probe sonication, which is hypothesized to be the reason for the instability in these solutions.

The microfluidic device is considered to be a superior device for dispersion of nanofilaments because of its ability to produce uniform dispersions that are stable for extended periods of time. The microfluidic device is a continuous flow process, where the fluid and nanomaterials to be tested are dispersed by shear flow in an interaction chamber, provides the solutions uniformity. Whereas sonication is a batch process in which nanofilaments are dispersed unevenly based on their accessibility to the ultrasonic stimulation. This batch process is nonuniform, and consequently does not disperse some nanofilament aggregates and over processes others, leading to destruction of their morphology. The continuous flow process and
uniform dispersion provided by the microfluidic dispersion device shows potential for future upscale in engineered nanomaterial processing.

4.3 Biologic Interaction

The toxicity of geometric TiO$_2$ based nanomaterials was evaluated by the MTS cell viability assay. RPMI 2650 cells were exposed in triplicate to TiO$_2$ based nanomaterials and a Cu nanoparticle control at concentrations of 10, 50, 100 µg/ml after dispersion by either the microfluidic device or sonication. These concentrations were determined by the method previously described, and represent daily, weekly, and monthly in vitro exposure concentrations.

![MTS cell viability of RPMI 2650 cells exposed to TiO$_2$ based nanotubes, nanorods, nanowires, nanoparticles, and 30 nm Cu nanoparticle positive control dispersed by a) microfluidic device and b) sonication at concentrations of 10, 50, and 100 µg/ml.](image)

Figure 11- MTS cell viability of RPMI 2650 cells exposed to TiO$_2$ based nanotubes, nanorods, nanowires, nanoparticles, and 30 nm Cu nanoparticle positive control dispersed by a) microfluidic device and b) sonication at concentrations of 10, 50, and 100 µg/ml.

MTS cell viability results are shown in Figure 11, which suggest that TiO$_2$ nanomaterials are nontoxic to RPMI 2650 cells. TiO$_2$ nanotubes and nanowires dispersed by ultrasonication at the highest concentration of 100 µg/ml were the only samples to have statistical significance (p = 0.05) in reducing cellular viability. These ultrasonic samples of TiO$_2$ nanotubes reduced viability by 27% and the nanowires 21%, respectively. Despite the reduction in cell viability, no toxicity was interpreted from these results due to the high concentration used and the polydispersion of the samples. Interestingly, there was no reduction in viability of RPMI 2650
cells exposed to nanoparticles or nanorods at all concentrations and by either method. This result was not surprising due to the low aspect ratios of nanorods, which made them easier to disperse. Similarly, the difference in zeta potential of the nanotubes and nanowires was 5.4 and 12.8 mV, respectively, and suggests that aggregates are more common in sonicated samples. Aggregated nanotubes and nanowires have more mass than individual filaments, which make them settle in culture and interact with the cells as a larger particle.

To further evaluate the biocompatibility of TiO$_2$ based nanogeometries, microfluidic dispersion solutions were used in the cellular morphology, inflammation, uptake, and localization studies. Since there was no decrease in cell viability at concentrations of 10 and 50 μg/ml; only the highest concentration of 100 μg/ml was used for the remainder of the biologic studies. No cellular inflammation was observed by the RPMI 2650 cells after 24 h exposure to the nanomaterials, as a measure of cytokine tumor necrosis factor alpha marker TNF-α (data not published). Cellular morphology of the RPMI 2650 cells after exposure to TiO$_2$ based nanomaterials is shown in Figure 12.

![Cellular morphology of RPMI 2650 cells after exposure to TiO$_2$ based nanomaterials. a) growth media control, b) nanoparticles c) nanotubes d) nanowire e) nanorod. Scale bar is 20 μm](image)
No morphological changes in the RPMI 2650 cells after exposure to all TiO$_2$ based nanogeometries was observed by light microscope. To further see where the nanomaterials were localized in the cells, the actin and nuclei were fluorescently stained to be observed by the CytoViva microscope.

The route of the TiO$_2$ based nanotubes, nanowires, nanorods, and nanoparticles in RPMI 2650 cells were visualized by localization and uptake studies. The localization fluorescent images can be seen in Figure 13.

![Image of localization](image_url)

**Figure 13- Imaging of the localization of TiO$_2$ nanomaterials in RPMI 2650 cells by fluorescence microscope.** a) growth media control, b) nanoparticles c) nanotubes d) nanowire e) nanorod.

The nanomaterials appear to localize in the actin of the cells and not the nucleus, which may be the reason for the nontoxic response after exposure to TiO$_2$. Light scattering and agglomeration of the TiO$_2$ nanomaterials made them difficult to see individually by fluorescence microscope. Therefore, TEM was utilized to further investigate the nanomaterial cellular interaction and uptake in the RPMI 2650 cells, Figure 14.
Figure 14- TEM imaging of TiO$_2$ based nanomaterials taken up by RPMI 2650 cells. a) growth media control, b) nanoparticles c) nanotubes d) nanowire e) nanorod.
TiO$_2$ based nanotubes, nanorods, and nanoparticles were observed individualized in the vesicles of RPMI 2650 cells. In Figure 14c, TiO$_2$ based nanotubes were observed perinuclear in cellular compartments, which suggests that they may be in late endosomes or lysosomes. Interestingly, the TiO$_2$ nanowire geometry was observed as aggregates in RPMI 2650 cells, Figure 14d. Further, endocytosis was predicted as the pathway for TiO$_2$ nanomaterial cellular uptake pathway uptake by RPMI 2650 cells. Biologic imaging devices or selective drug delivery devices may be developed by selectively tuning the geometries of TiO$_2$ nanomaterials.
Conclusion

In summary, the results demonstrate that microfluidic dispersion influences the \textit{in vitro} toxicity of TiO$_2$ nanofilaments. Well dispersed TiO$_2$ nanomaterials processed by the microfluidic device at dosing concentrations relevant to NIOSH’s recommended exposure limits for TiO$_2$ were nontoxic to nasal cells, as they did not cause inflammation, alter cellular morphology, or reduce the cellular viability. Monodispersed TiO$_2$ nanofilaments were taken up by cells without any biologic coating. This result suggests that TiO$_2$ nanomaterials may be tuned for localized drug delivery and bioimaging. Lastly, it is recommended that characterization of nanomaterial morphology be performed before and after dispersion so that accurate and reproducible toxicological assessment can be made.

Future Work

In this study RPMI 2650 cells were used to model the nasal epithelium. Enhancement to the model can be made by culturing the RPMI 2650 cells at the air to liquid interface, where they will grow a monolayer, and TiO$_2$ nanopowders can be directly deposited without the need for dispersion in an aqueous solution. In addition, the TiO$_2$ nanomaterials could be evaluated in a lung cell model to further understand the effect of nanomaterial transport and deposition upon inhalation. Lastly, the cellular uptake biologically functionalized TiO$_2$ nanomaterials can be evaluated to visualize how these materials could be used in bioimaging and drug delivery applications.
References


57. NIST; SEMATACH Critical Values of the Student’s t Distribution.