Environmental Safety and Health (ESH) Impacts of Emerging Nanoparticles and Byproducts from Semiconductor Manufacturing

Tasks 425.023 and 425.024

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Buddy Ratner

University of Washington Engineered Biomaterials Center, UWEB

Project Tasks

Task 1

NP Characterization

PIs

- F. Shadman
- B. Ratner
- R. Sierra

Students

- **Jeff Rottman**
- R. Daneshvar
- L. Platt
- M. Rodriguez
- H. Wang

Other Scientists

- A Luna

Task 2

Toxicity Assessment & Prediction

PIs

- **J. Field**
- F. Shadman
- **S. Boitano**
- R. Sierra

Students

- **Lila Otero**
- I. Barbero
- A. Cuevas
- M. McCorkel
- C. Sherwood

Other Scientists

- A Luna
- **C Garcia**

Goals for the Past Year

- Validation Real Time Cell Analyzer (RTCA)
- NP oxidation of proteins
- NP impact on Sub-Lethal Cellular Effects: cell signaling (*e.g.* ATP signaling)
- NP toxicity to well-differentiated mouse airway epithelial
- Labeled NPs for Environmental Transport & Cellular Studies

Outline Presentation

- Introduction — Dr. Jim Field
- Cytoxicity (RTCA Validation) — Lila Otero, PhD Student
- Sub-Lethal Cellular Effects — Dr. Cara Sherwood, former PhD student
- Environmental Transport NPs — Jeff Rottman, PhD Student
- Summary and Conclusions — Dr. Jim Field

Application and Validation of a Real-Time Cell Analyzer to Assess Nanotoxicity

Lila Otero-González, Reyes Sierra, Jim A Field

Dept. Chemical and Environmental Engineering

University of Arizona

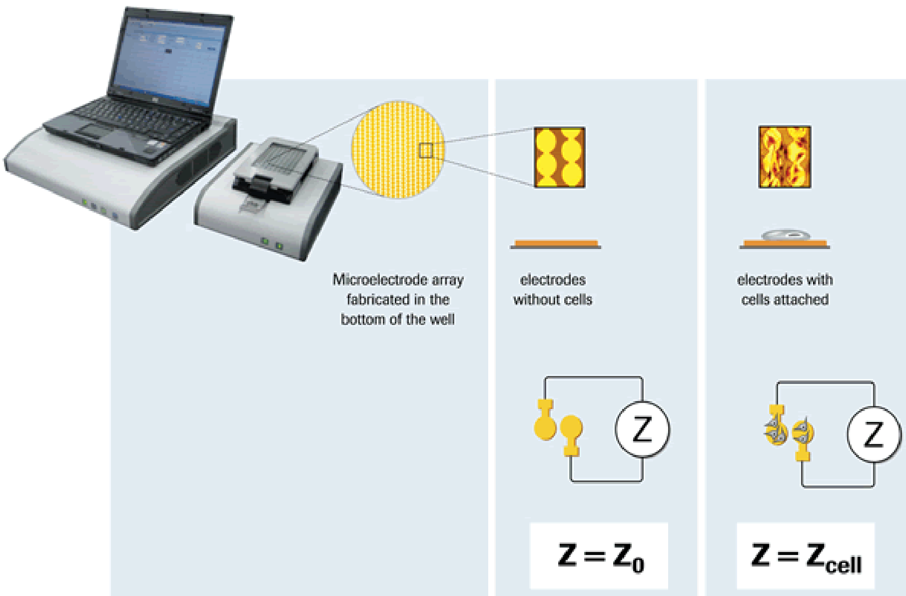
Objectives

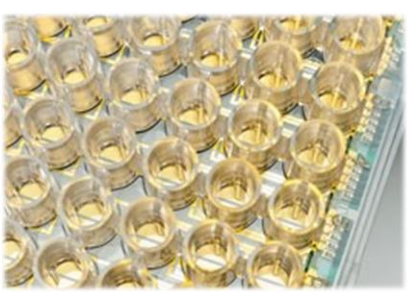
Real Time Cell Analysis

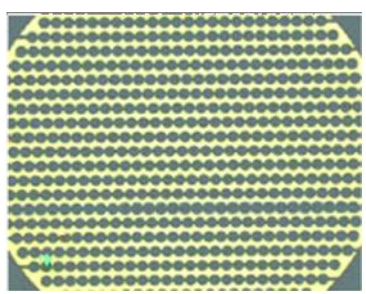
- Investigate the applicability of a new impedance-based real time cell analyzer (RTCA) system as a tool for **high-throughput** assessment of nanoparticle (NP) cytotoxicity.
- Compare the cytotoxicity results obtained using RTCA with those determined with a traditional toxicity assay, mitochondrial toxicity test (MTT).
- Characterize the aggregation of nanomaterials in the biological medium.

Real Time Cell Analyzer (RTCA)

Monitor Impedance Based Real Time Cell Assay (RTCA) with xCELLigence (Roche)

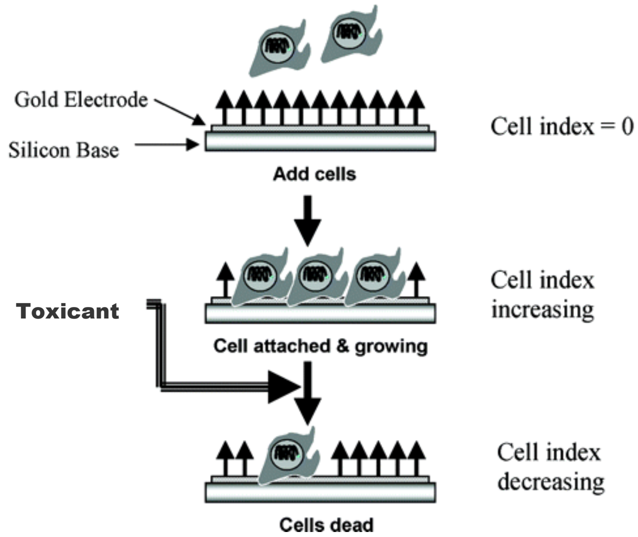






- RTCA system measures electrical impedance across interdigitated micro-electrodes integrated on the bottom of tissue culture E-Plates.
- Real time data output
- RTCA system does not need fluorescent labels.
- Does not target specific physiological process.

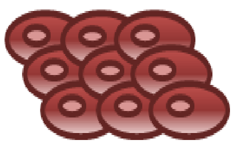
Real Time Cell Analyzer (RTCA)



Chem. Res. Toxicol. ***2005, 18, 154-161***

- The presence of the cells on top of the electrodes leads to an increase in the electrode impedance.
- The impedance also depends on the quality of the cell attachment.

Methodology **Human Lung Epithelial Cell Line, 16HBE14o-**

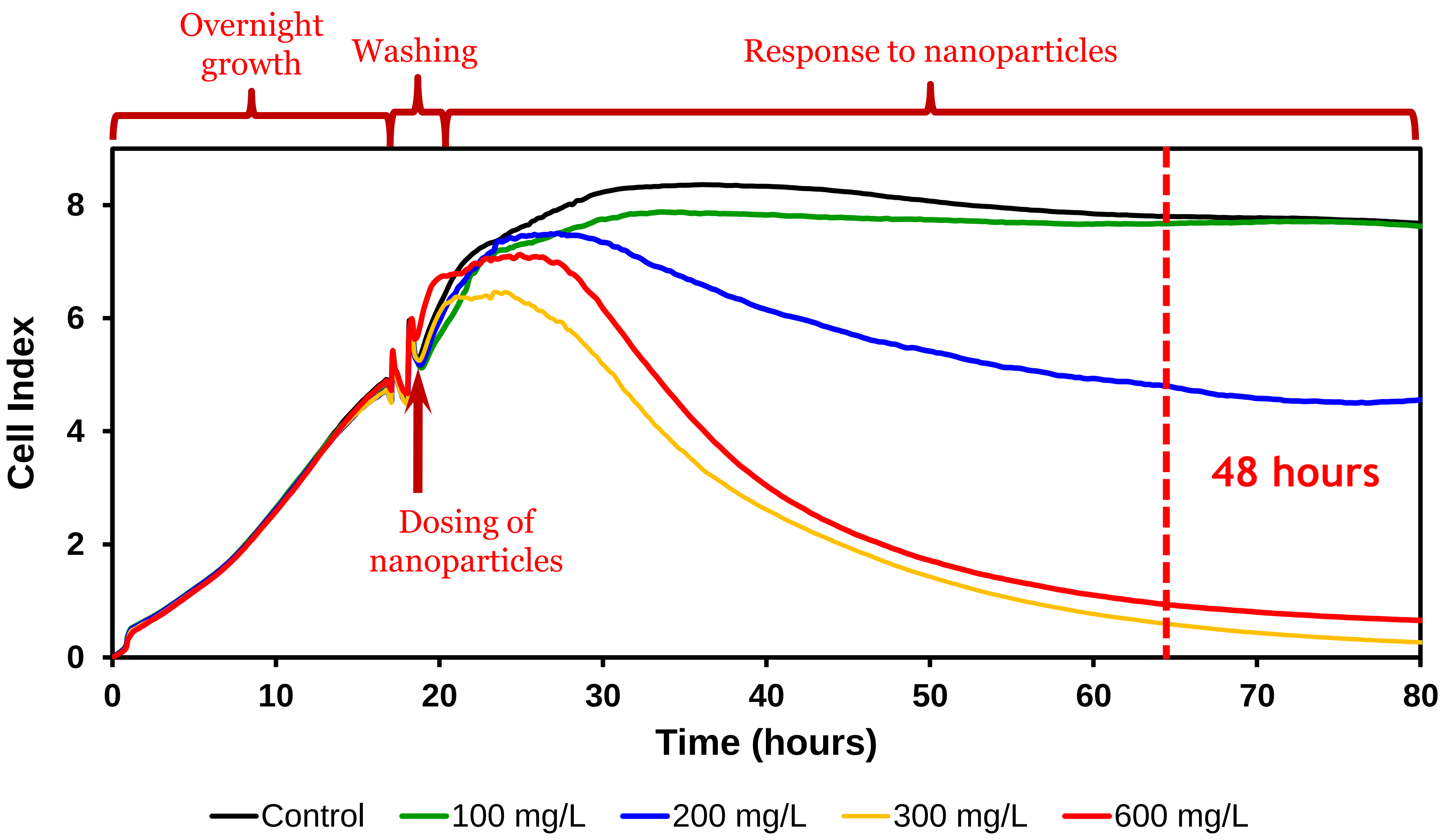


16HBE14o- culture

E-plate 96

RTCA device

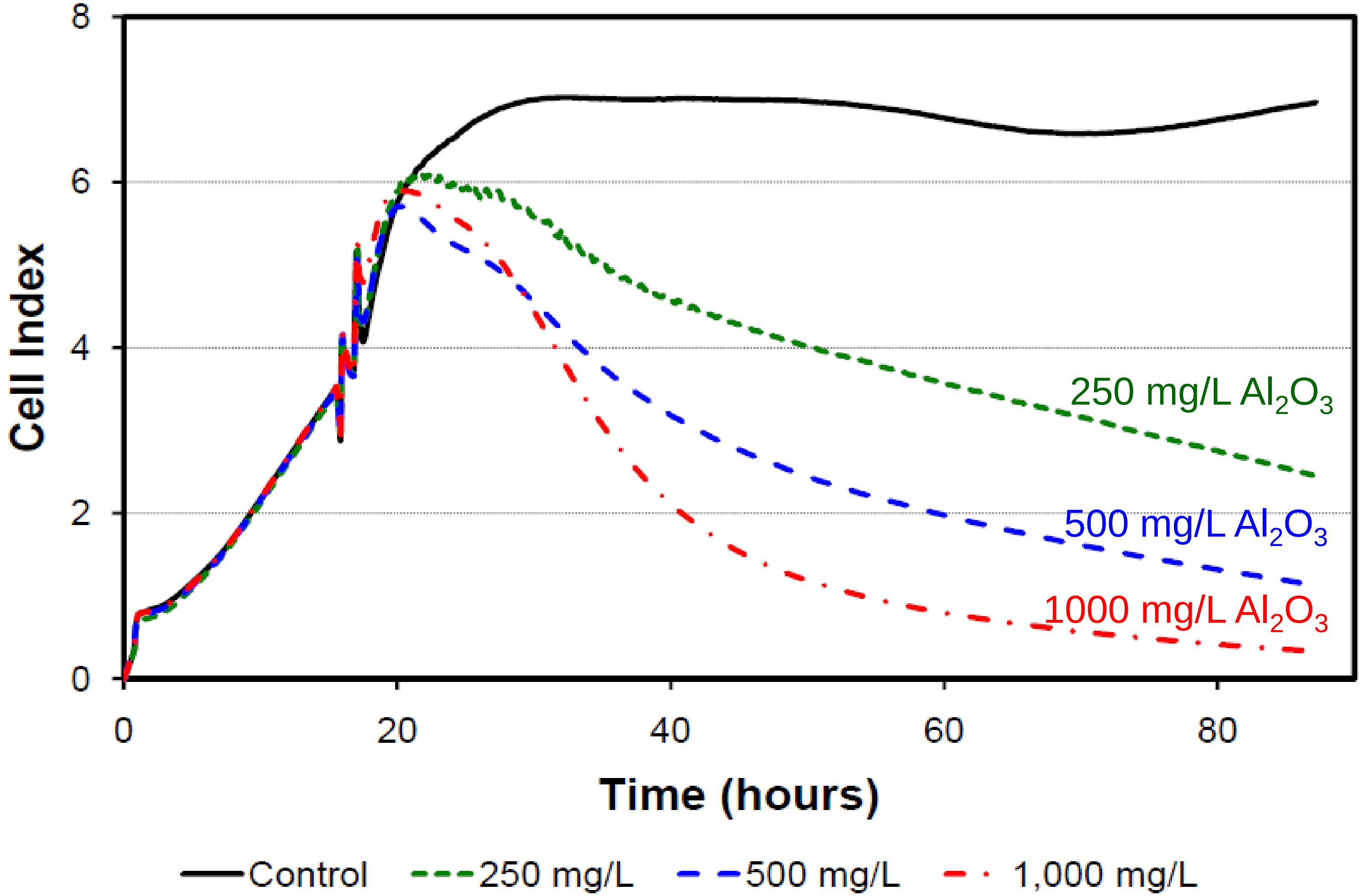
Results – Exposure to SiO_2



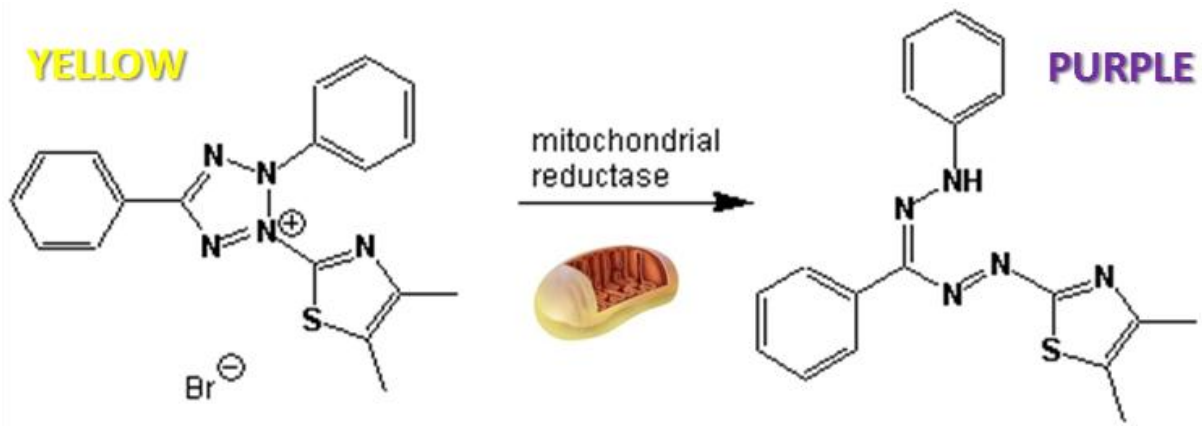
Nano-sized SiO_2 was inhibitory at concentrations above **200 mg/L**.

Results – Exposure to Al_2O_3

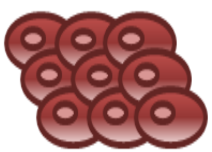
Example Output RTCA with Al_2O_3 NPs



MTT Assay

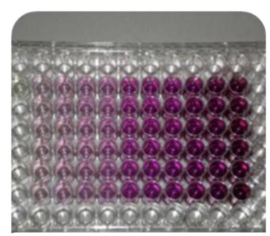


Methodology



16HBE14o-
culture

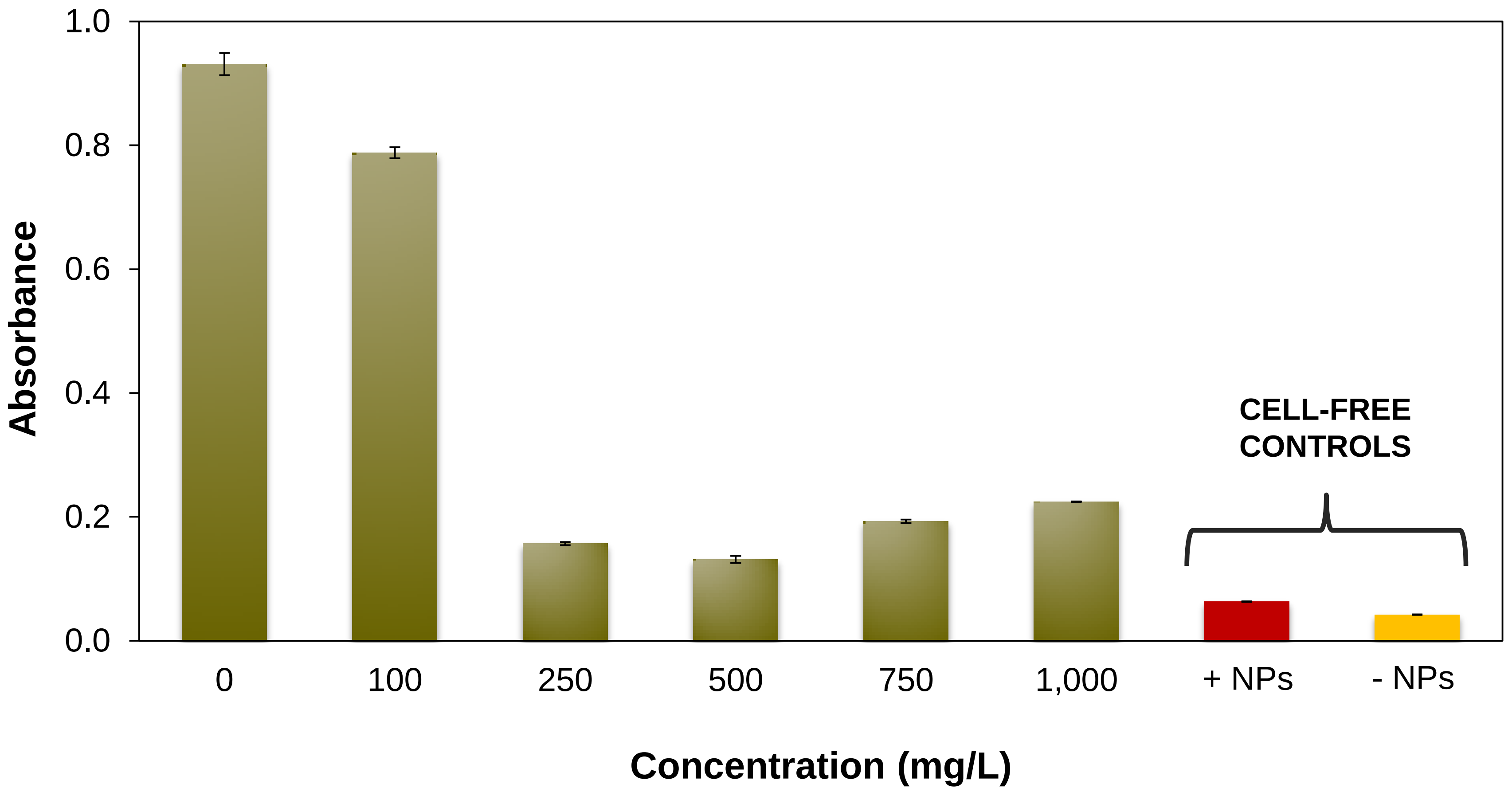
Incubation
with NPs



Addition of
MTT reagent

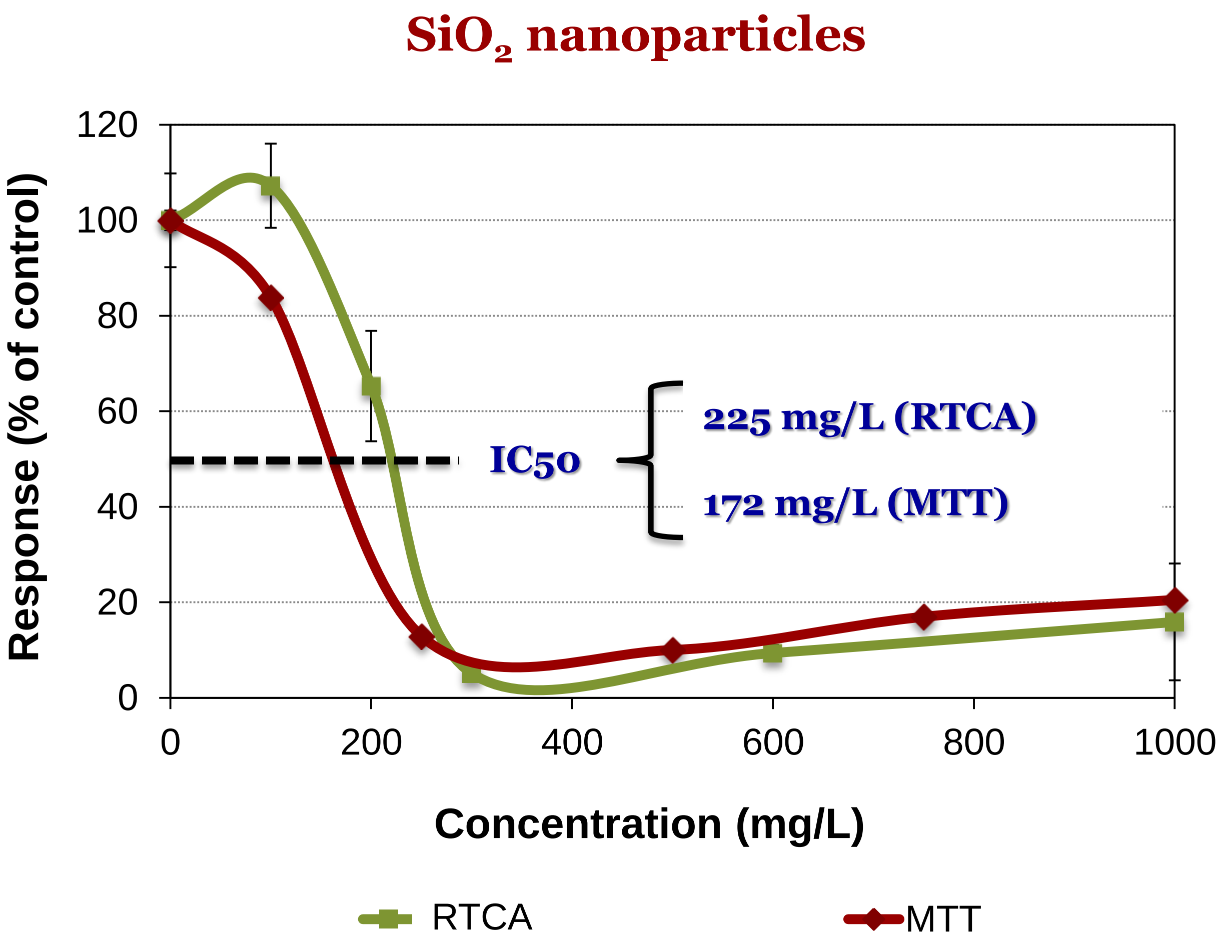
Measurement of
absorbance

Results – Exposure to SiO_2

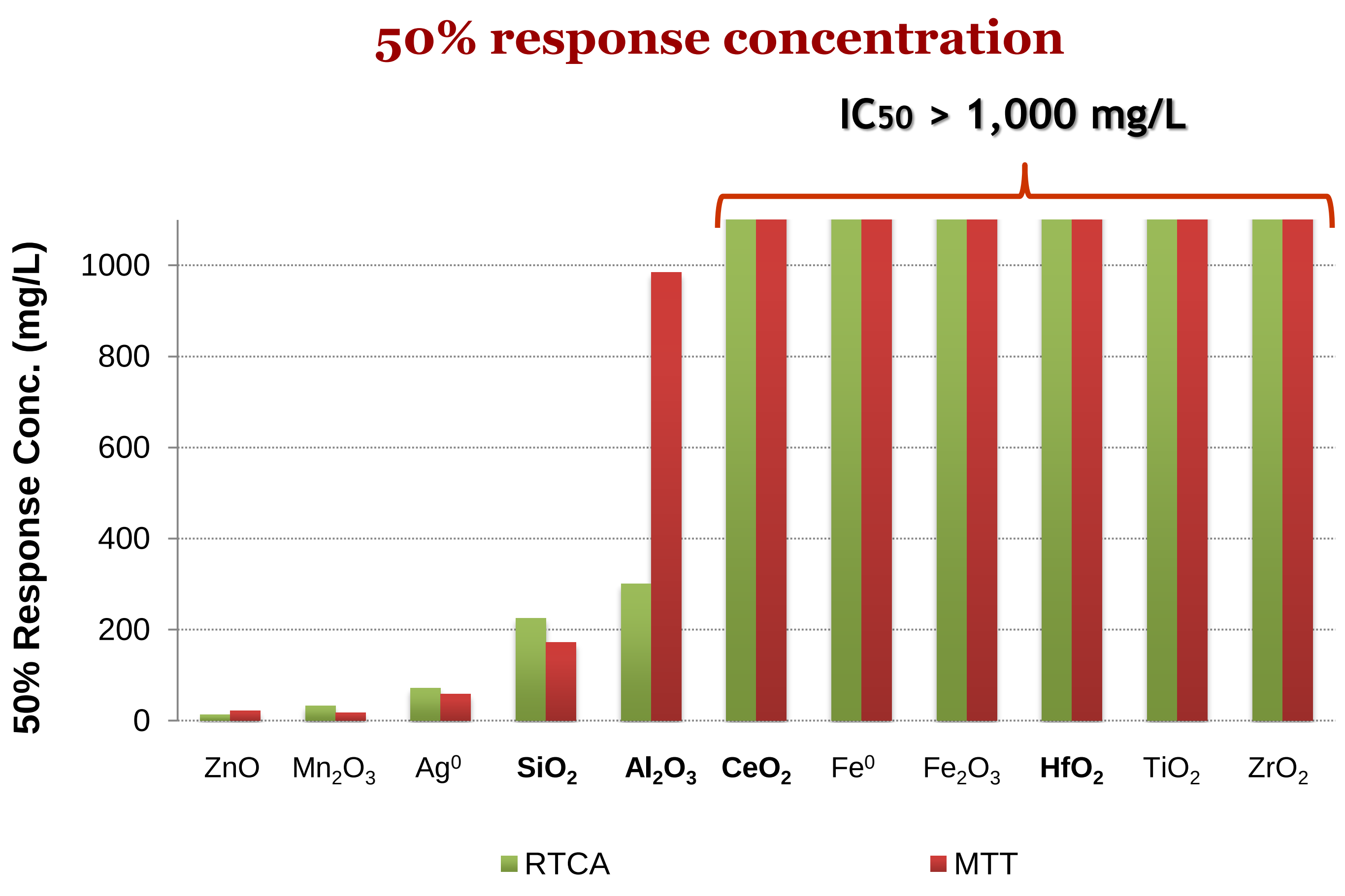


Cell-free controls with the highest NP level caused a marginal increase of the absorbance relative to the NPs-free control (2-3% of max. absorbance, depending on the NP used).

Comparison RTCA vs. MTT Results



Comparison RTCA vs. MTT Results



Good correlation between RTCA and MTT results

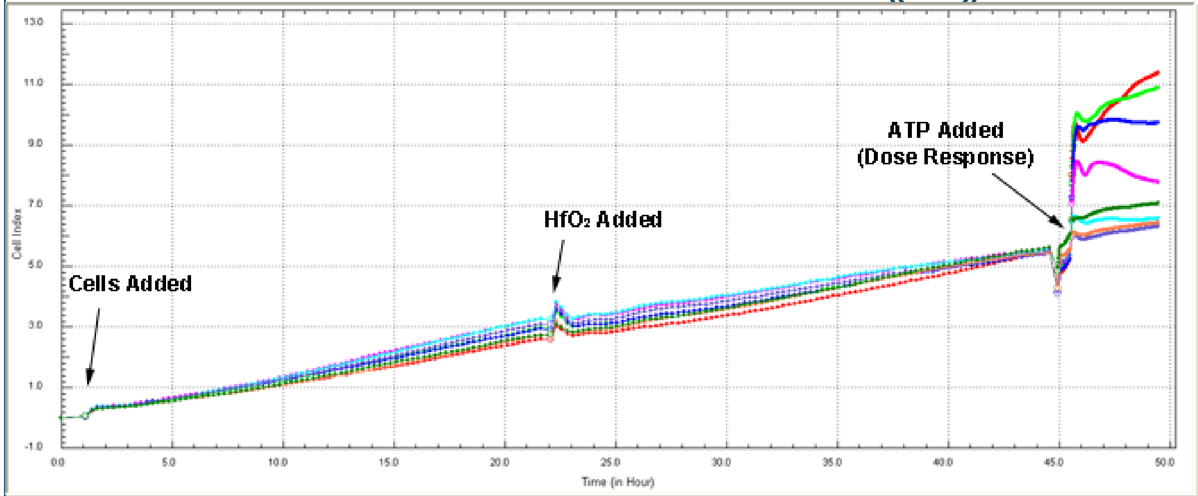
Conclusions RTCA

- The RTCA assay is a suitable technique for rapid screening of cytotoxicity of NPs.
- The inhibitory concentrations determined with RTCA technique correlated well with those obtained by a commonly used cytotoxicity assay (MTT).

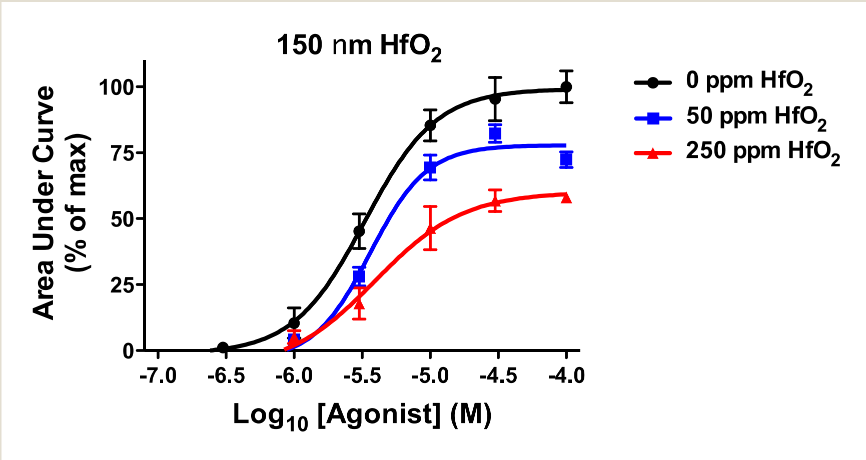
Beyond cytotoxicity: cellular effects of HfO_2

- Cell death is the end point for toxicity testing
- Detrimental cellular effects can occur in the absence of cell death
  - Cell transformation – e.g., Cancer
  - Loss of ability to respond to cellular signals or stress
- Are there adverse effects in lung epithelial cells from ENPs (HfO_2) exposure in the absence of cell death?
- We used a high-throughput physiological assay to evaluate low-dose ENP exposure on cellular signaling mechanisms in airway epithelial cells
  - We initiated signaling with ATP
  - We measured signaling with RTCA

Quantification of physiologic response to ATP following ENP and micron-sized HfO_2 exposure



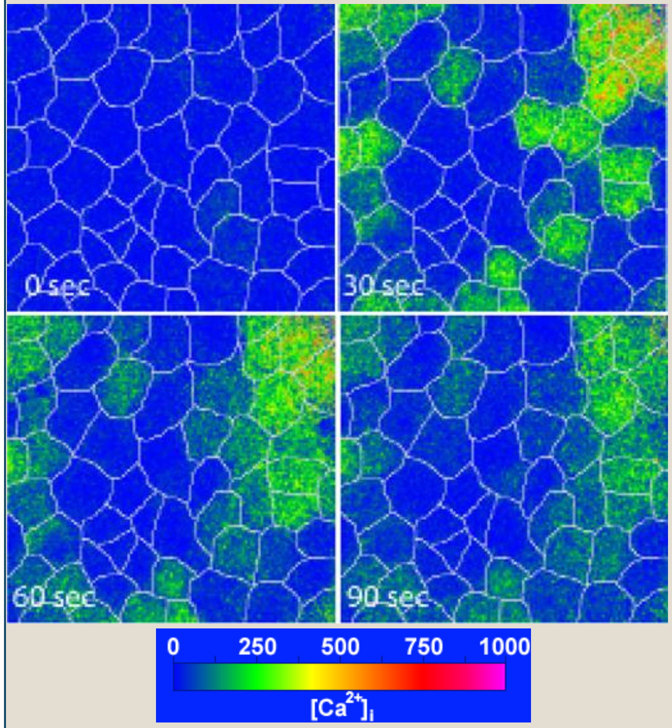
- Cells Seeded at time "0"
- HfO_2 Added at 24 hours
- ATP added at 48 hours
- 4 hours of signaling analyzed

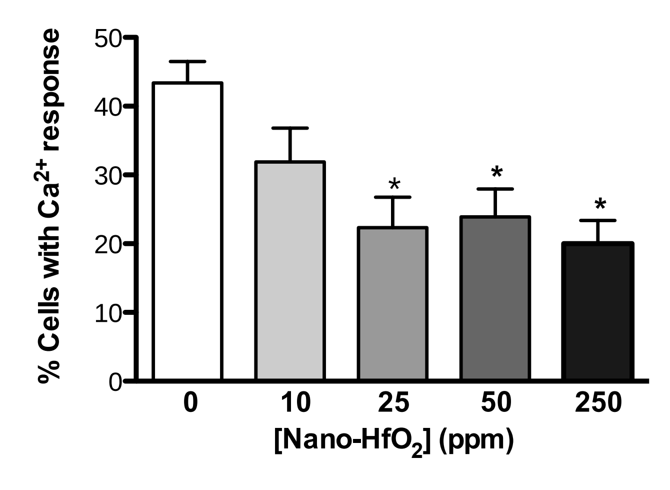


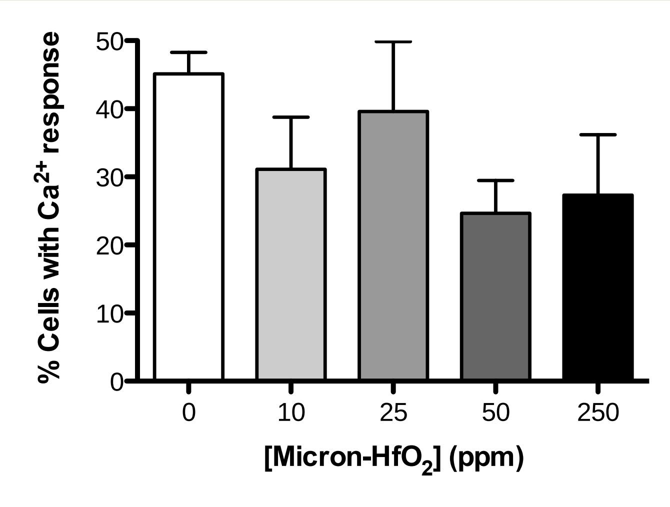
- 24-hr incubation with low-dose ENP HfO_2 reduces physiologic response to ATP:
  - $P < 0.05$ at 100 µM ATP (0 vs. 50 and 250 ppm)
  - $P < 0.05$ at 30,10, and 3 µM ATP (0 vs. 250 ppm)

Mechanistic Studies: Quantification of Ca^{2+} signaling

- ATP-induced Ca^{2+} responses in individual cells

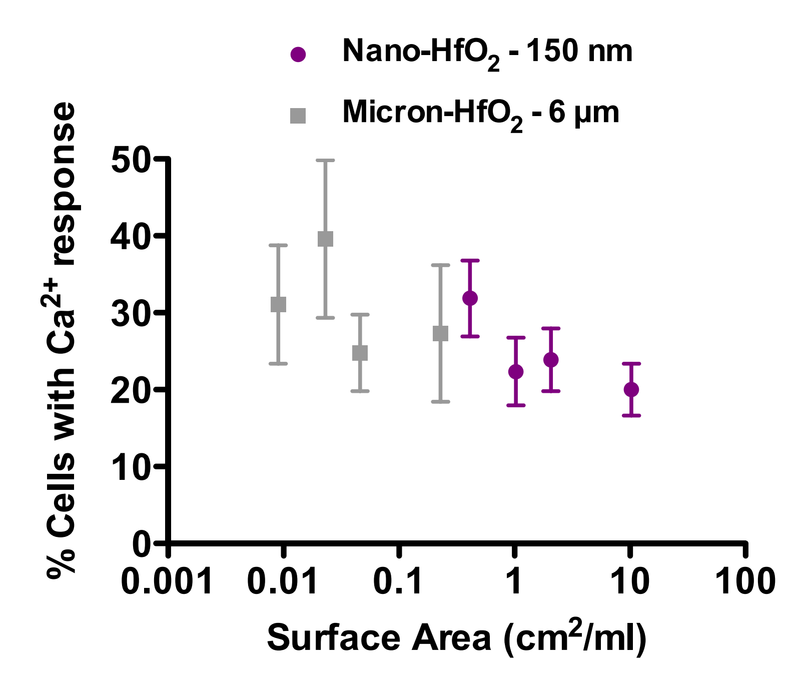






- ENP-induced "signaling toxicity" occurs at ~1/10 of cytotoxicity levels
- Micron-sized HfO_2 did cause significant signaling toxicity

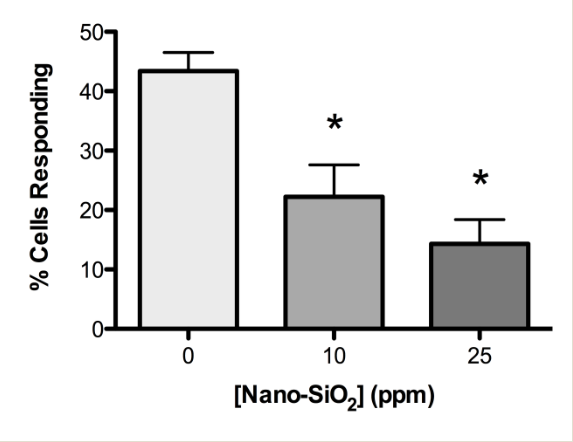
Effects of HfO_2 on Ca^{2+} signaling: surface area vs. signaling

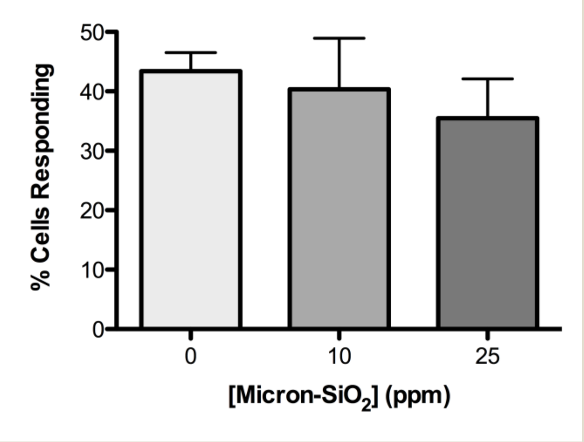


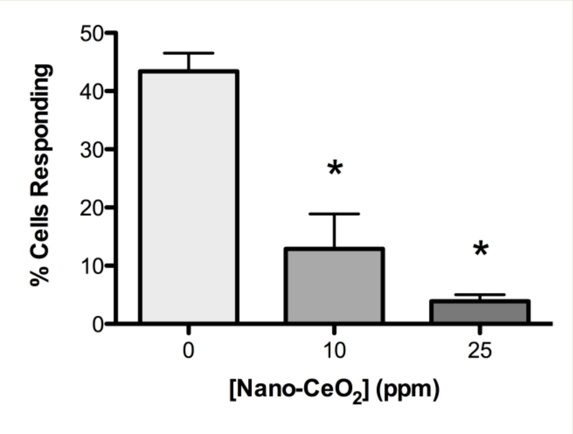
- HfO_2 Ca^{2+} signaling reductions display a logarithmic function with the surface area of particles presented
- HfO_2 Ca^{2+} signaling reductions are due to metals toxicity more than particle size

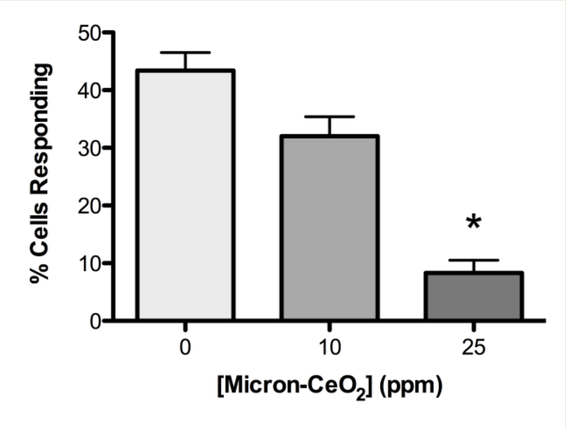
Ca^{2+} Imaging to compare other ENPs: SiO2 and CeO2

- Nano-SiO2 reduced Ca^{2+} signal; micron-sized was not reduced
- CeO2 reduced signaling was more prevalent in ENP compared to micron-sized



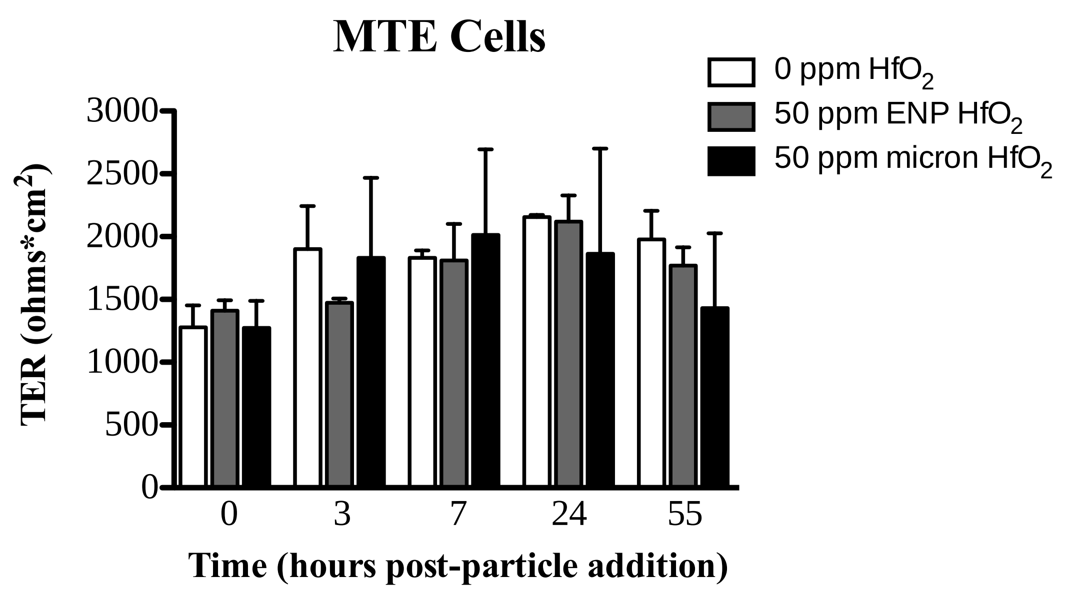


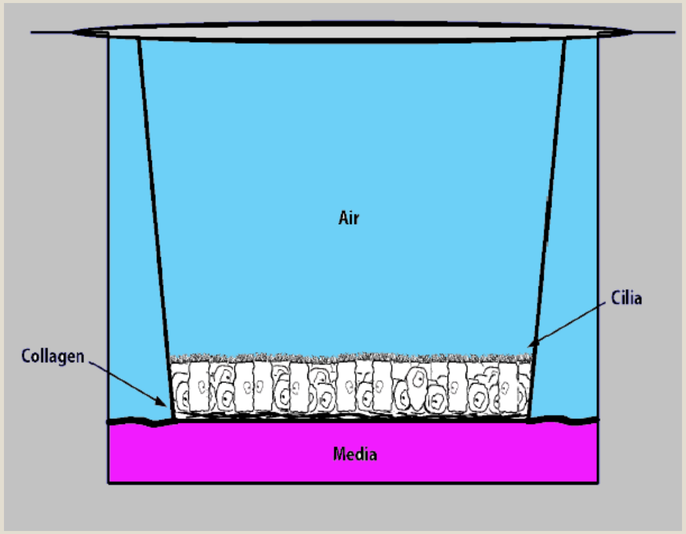




ENP exposure and barrier function

- Cultured primary mouse tracheal epithelial cells
- Exposed stable monolayers to ENP and micron-sized HfO2 particles





- HfO2 does not appear to alter transepithelial resistance significantly

Nanoparticle Retention in Porous Media

Objective

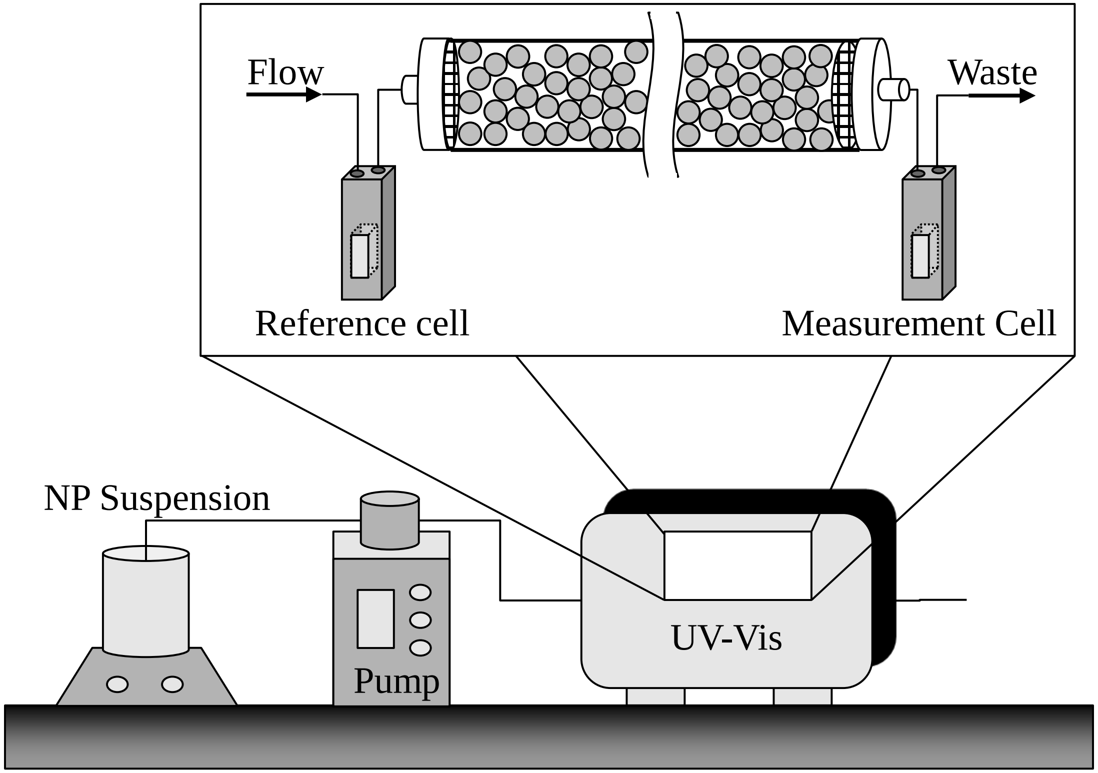
- Investigate the role of porous media in the treatment of wastewater containing nanoparticles

Method of Approach

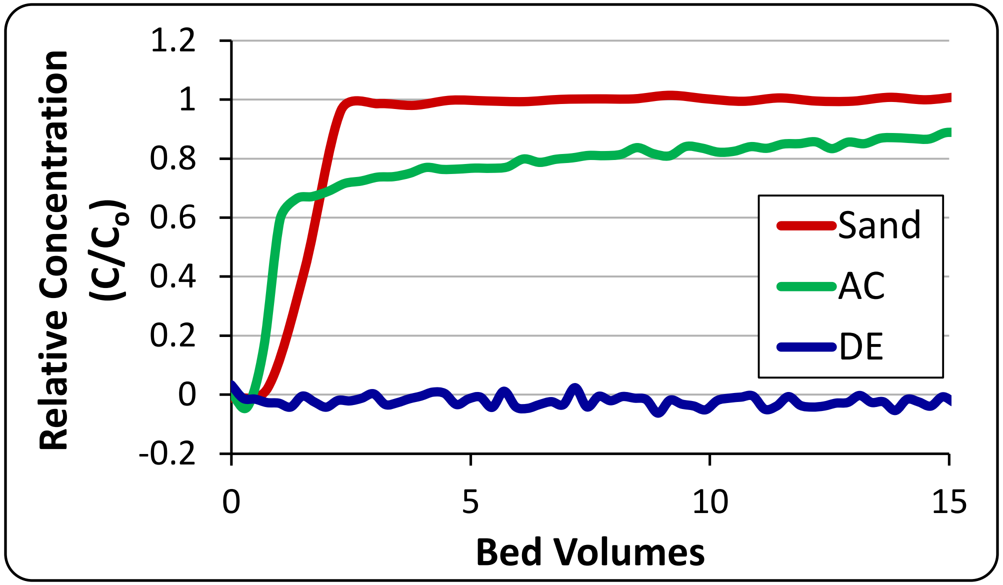
- Develop a technique to rapidly determine NP behavior in porous media.
- Test and select filtration materials that would be suitable for effective removal of NP from water and wastewater
- Develop a process model for data analysis, scale up, and process design.

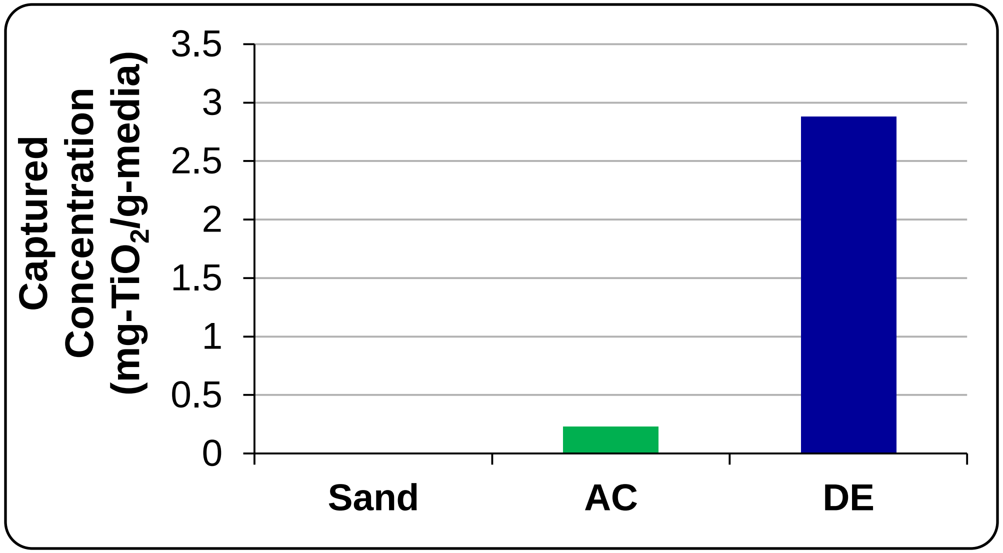
Online Retention Measurement

- Novel apparatus allows for fully online measurement of nanoparticle retention.
- The system is flexible in that it can also be configured to determine retention for other systems/techniques.



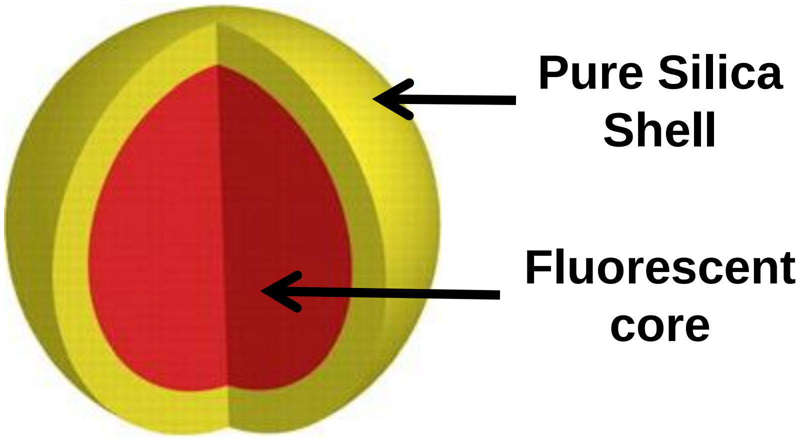
Media Comparison





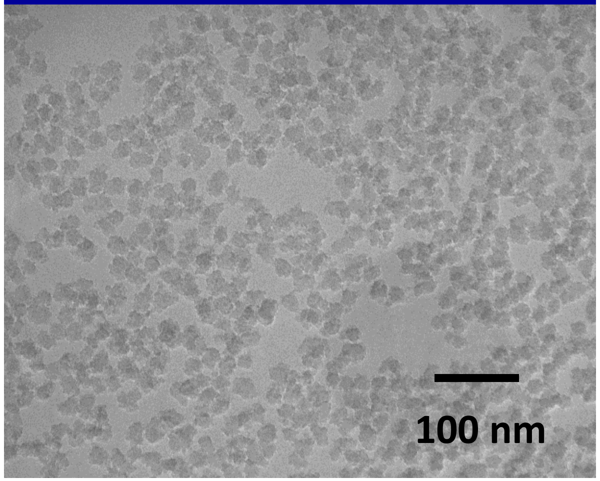
- Activated carbon (AC) shows marked improvement over sand regarding NP retention.
- Diatomaceous earth (DE) displays real promise as an adsorbent bed media, showing significant improvement in NP capture capacity.

Fluorescent Nanoparticles

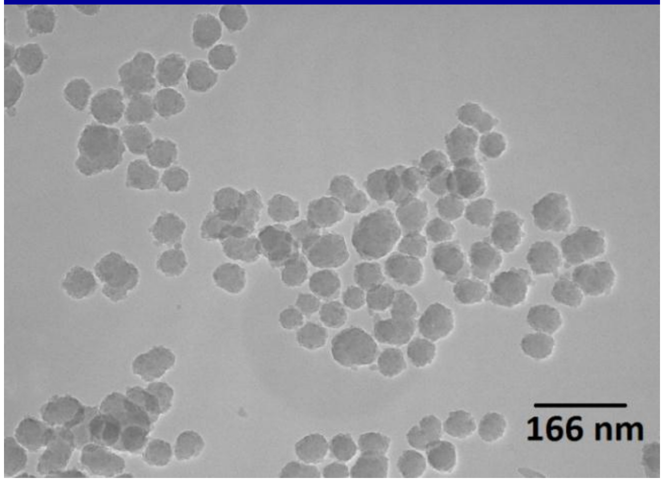


- Able to synthesize fluorescent nanoparticles of varying sizes
- Enables determination of "nano" effect in treatment techniques as well as toxicity.

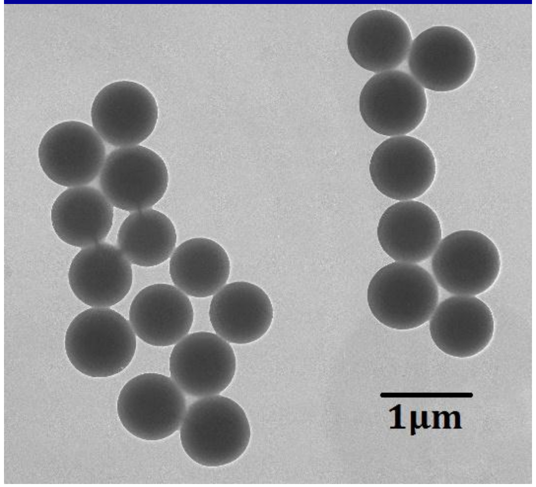
25 nm Particles



80 nm Particles



800 nm Particles

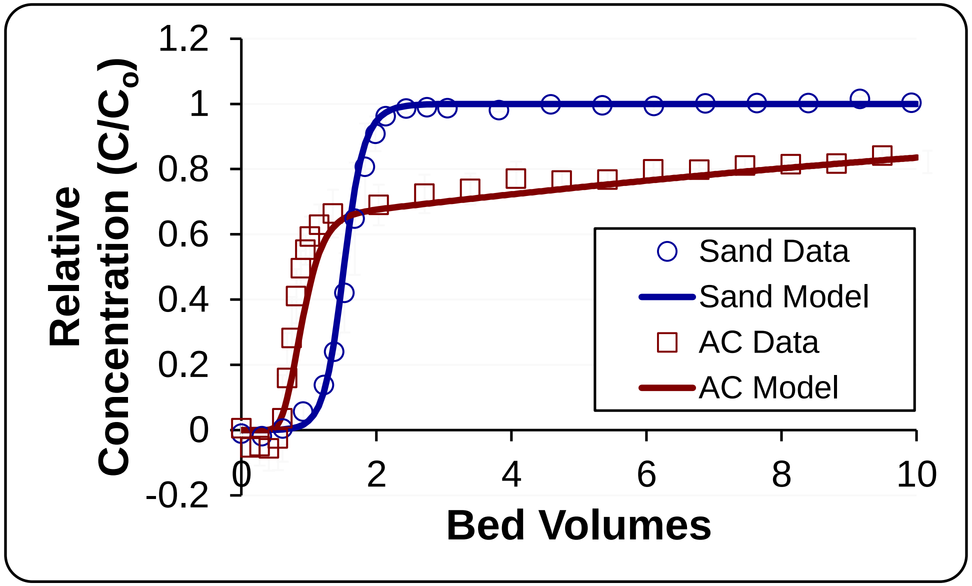


Process Simulation

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial z^2} - U\frac{\partial C}{\partial z} - \frac{3(1-\varepsilon)}{\varepsilon\, r_c}\frac{\partial C_s}{\partial t}$$

$$\frac{\partial C_s}{\partial t} = \left[\alpha_{pc}(1-\Theta) + \alpha_{pp}\Theta\right]k_a C - k_d C_s$$

Continued refinements to the process model allows more accurate predictions of NP behavior in porous media.



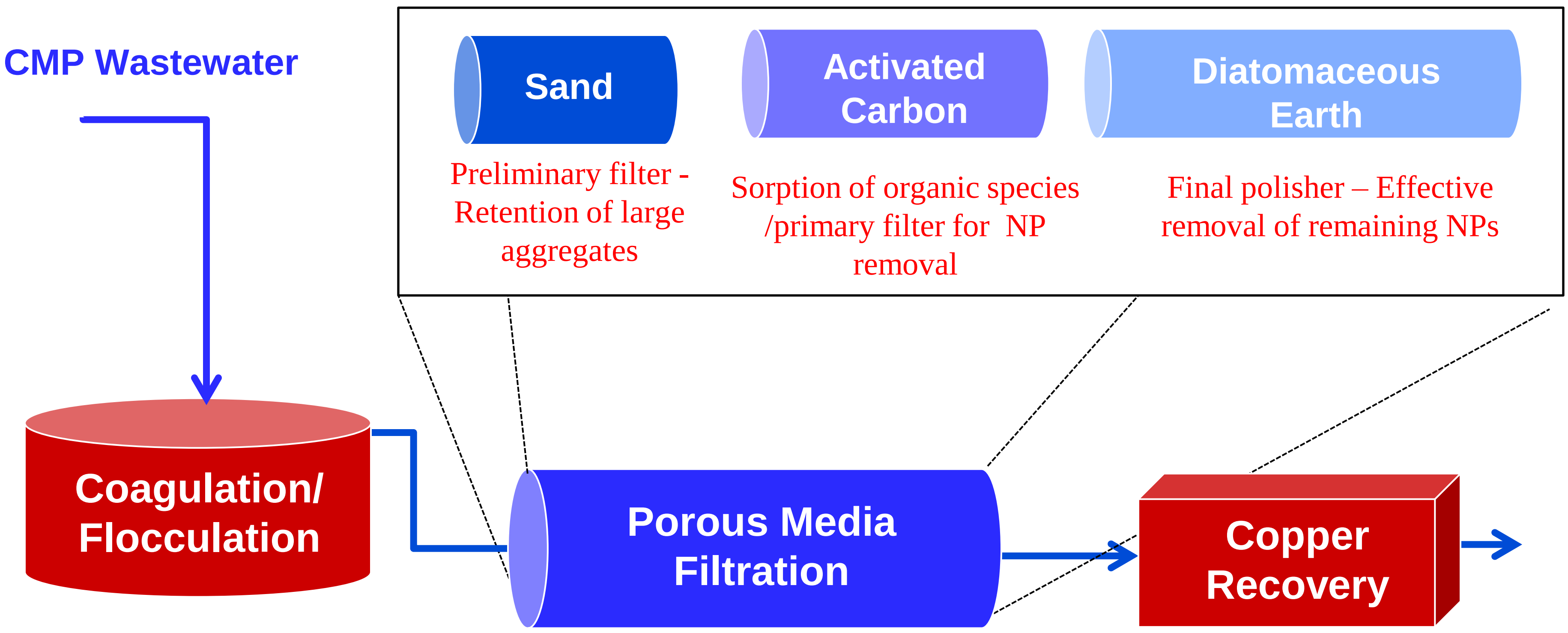
Conclusions

- Novel online measurement allows for rapid determination of media suitability as well as sensitivity to bed and solution characteristics.
- Fluorescent silica NPs enable true size comparison and act as accurate tracers.
- The continued modeling work will provide the basis for scale-up of developed treatment strategies.

Bed Integration

Proposed Design

A hybrid bed design provides the strengths of each sorbent in a simple, easily implemented design.



Main Achievements: Physical Characterization

University Arizona

- Aggregation inorganic NPs (except SiO_2) in culture media
- Protocols for dispersing NPs in medium (protein, surfactant)
- NPs adsorb other contaminants
- Porous media suited for filtering NP (diatomaceous earth)
- Transport model
- Mastered synthesis of fluorescent dye labeled NPs

Main Achievements: Physical Characterization

University of Washington

- Surface characterization HfO_2 and CeO_2 with XPS and ToF-SIMS
- Principal Component Analyses of Surface Contamination
- SEM characterization NPs in biological medium
- Methods of sterilizing NPs (without altering physical properties)

Main Achievements: Toxicity

University Arizona

- Developed Chemical ROS and Protein oxidation assays for NP
- Adapted RTCA for evaluating NP toxicity
- Validated RTCA for NP toxicity measurements
- Developed methods for evaluating sub-lethal cellular effects (cell signaling effects)
- Advanced tissue culture for lung epithelial barrier function
- Demonstrated that NPs used in CMP (SiO_2, Al_2O_3, CeO_2) or proposed for photolithography (HfO_2) have low to moderate toxicity

Main Achievements: Toxicity (*continued*)

University Arizona

- Media matters. Medium composition impacts NP cytotoxicity (CeO_2, Al_2O_3)

- Soluble metal release to medium (dissolution, corrosion) is an important mechanism for the most toxic NPs (Cu^0, CuO, Mn_2O_3, Ag^0, ZnO)

- NPs that are positive in ROS assay or protein oxidation assay tend to be NPs with high to moderate toxicity (Cu^0, CuO, Mn_2O_3, Fe^0, CeO_2)

Main Achievements: Journal Publications

Five Publications already published

1. Luna-Velasco A, Field JA, Cobo-Curiel A, Sierra-Alvarez R. 2011. Inorganic nanoparticles enhance the production of reactive oxygen species (ROS) during the autoxidation of L-3,4-dihydroxyphenylalanine (L-Dopa). J. Haz. Mat. 82:19-25.
2. Garcia-Saucedo C, Field JA, Otero L, Sierra-Alvarez R. 2011. Toxicity of HfO_2, SiO_2, Al_2O_3 and CeO_2 nanoparticles to the yeast, *Saccharomyces cerevisiae*. J. Haz. Mat. 192:1572– 1579.
3. Wang H, Yao J., Shadman F. 2011. Characterization of the surface properties of nanoparticles used in semiconductor manufacturing. *Chemical Eng, Sci*. 55, 2545.
4. Field JA, Luna-Velasco A, Boitano SA, Shadman F, Ratner BD, Barnes C, Sierra-Alvarez R. 2011. Cytotoxicity and physicochemical properties of hafnium oxide nanoparticles. *Chemosphere*. 84(10):1401-1407.
5. Gomez-Rivera F, Field JA, Brown D, Sierra-Alvarez R. 2011. Fate of cerium dioxide (CeO_2) nanoparticles in municipal wastewater during activated sludge treatment. *Bioresource Technol*. (In press). http://dx.doi.org/10.1016/j.biortech.2011.12.113

Journal Publications (*continued*)

Three publications pending review

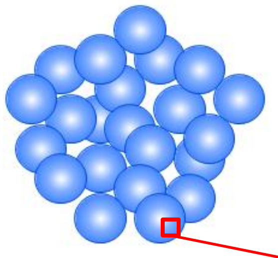
6. Rottman, J., Shadman, F., Sierra-Alvarez, R. 2012. Interactions of Inorganic Oxide Nanoparticles with Sewage Biosolids. *Water Sci. Technol.* (*Under review*).
7. Rodríguez, M., Sierra-Alvarez, R., Field, J. A., Shadman, F. 2012. Impact of Wastewater Components on the Aggregation Behavior of Nanoparticles in Chemomechanical Planarization (CMP) Slurries. *(Under Review)*
8. Otero-Gonzalez, L., Field JA, Sierra-Alvarez, R. 2012. Application and validation of an impedance based real time cell analyzer to measure the toxicity of nanoparticles impacting 16HBE14o- lung epithelial cells. Environ. Sci. Technol. (*Under review*)

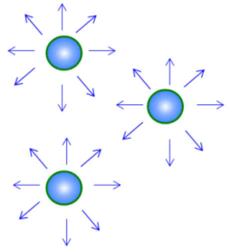
Three publications un preparation

9. Luna-Velasco, A., Cobo, A., Field, JA, Sierra-Alvarez, R. 2012. Inhibitory effect of inorganic oxide nanoparticles towards the bioluminescent bacterium *V. fisheri*. *(In preparation*).
10. Luna-Velasco, A., Sun, A., Sierra-Alvarez, R., Field, JA. 2012. Direct oxidation of proteins by inorganic nanoparticles. (*In preparation*).
11. Garcia-Saucedo, C., Otero-Garcia, L., Field JA, Sierra-Alvarez, R. 2012. Cytotoxicity of inorganic oxide nanoparticles to the yeast, *Saccharomyces cerevisiae*. *(in preparation)*

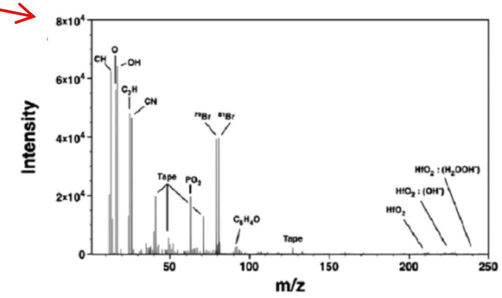
Main Achievements: Overview

Control/characterization of dispersion and aggregation

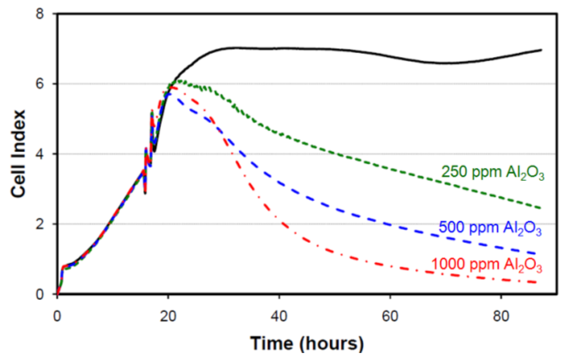




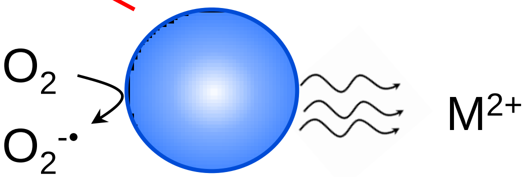
Surface characterization



Cytotoxicity, high through-put assays (RTCA, ROS)



Mechanisms: ROS and metal dissolution



Labeled NP for environmental and cellular studies