I Round Robin Results: CeO₂

Group effort all nanotoxicity projects

II Project: ESH Emerging Nanoparticles Semiconductor Manufacturing

UA,UW project (Tasks 425.023 and 425.024)

SRC/Sematech Engineering Research Center for Environmentally Benign Semiconductor Manufacturing

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Cytotoxicity of Nanoscale CeO₂: Summary of Round Robin Findings

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- Y. Chen, Georgia Institute of Technology
- R. Mumper, A. Tropsha, Univ. of North Carolina/Chapel Hill
- R. Draper, S. Nielsen, P. Pantano, Univ. of Texas/Dallas
- B. Ratner, Univ. of Washington

Objectives

Instructions Round Robin: Compare physical chemical properties and cytoxicity of the same CeO_2 sample in different laboratories

Sigma-Aldrich, P/N 643009 10 wt. % in pH 4.6 H_2O Size = <25 nm

- 1) Measure particle size distribution (PSD) and zeta potential (ZP) of CeO_2 suspension at acidic pH.
- Measure PSD and ZP of CeO₂ suspension at pH 7 in water, phosphate buffer (PB) and biological medium
- 3) Measure change in PSD, ZP and CeO_2 concentration after 24 h
- 4) Measure cytoxicity up to a maximum concentration of 1,500 mg/L of CeO₂.

How Much CeO₂ was in Sigma 10% w/w Sample?



Sample and Analyses

Conclusion: Based on ICP, the sample contained approx. 57% of the expected CeO₂ concentration. Purity could also be rapidly estimated with a simple dry weight measurement.

Intensity Averaged Particle Size in Water and Phosphate Buffer (PB)



Conclusion: Excellent dispersion of CeO_2 NP in water at pH 4.5 and PB pH 7.4. Dispersion could be repeated from lab to lab. Unstable dispersion of CeO_2 NP in neutral water, variability within and between labs.

Zeta Potential in Water and Phosphate Buffer (PB)



Conclusion: High absolute values of ZP in water at pH 4.5 and PB pH 7.4 to keep NP dispersion stable. ZP values could be repeated from lab to lab.

Unstable and highly variable values of ZP in neutral water.

Light Intensity Averaged Particle Size in Biological Media



Organization

Conclusion: Many of the media types cause moderate to severe agglomeration of CeO_2 NPs. FBS and HEPES decrease agglomeration, both together are very effective in dispersing CeO_2 .



Change in CeO₂ Concentration in 24 h (only tested by UA)



Conclusion: In bioassay media, CeO₂ NP agglomerated and settled such that the concentration in suspension decreased by more than 90% in 24 h.

Cytotoxicity of CeO₂



Conclusion: In most assays CeO₂ is either non-toxic or displays low toxicity. A moderate toxicity was observed with HaCat cells tested with the Live/Dead assay using PBS. (Note real conc. was approx. 600 mg/L)

Conclusions

- > Manufacturer reported concentration of $CeO_2 \cong 2$ -fold off
- Acid water & 5 mM phosphate buffer (pH 7.4) provide stable CeO₂ NP dispersions
- > Neutral water and most assay media cause instability and agglomeration of CeO_2 NP.
- A few media types (e.g. those including FBS, HEPES & Dispex) provide stability of CeO_2 NP dispersions in biological media.
- CeO₂ caused no to low inhibition in most of the assays. Moderate inhibition was only observed with HaCat cells assayed in PBS.

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

Tasks 425.023 and 425.024

Research Team

PIs:

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- Reyes Sierra, Dept. Chemical and Environmental Engineering, UA
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Other Researchers:

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- Angel Cobo, Exchange MS Student, Chemical and Environmental Engineering, UA
- Jacky Yao, Research Scientist, Chemical and Environmental Engineering, UA

Cost Shares

Cost Share (other than core ERC funding):

- **\$80k** from UA Water Sustainability Program
- **\$150k** to purchase real-time toxicity monitoring system (UA Water Sustainability Program)
- **Postdoctoral fellowship** to C. Garcia (Mexican Science Foundation, CONACyT)
- Doctoral fellowship to J Gonzalez (Mexican Science Foundation, CONACyT)
- Doctoral fellowship to M McCorkel (SRC graduate fellowship program)

Overall Objectives

- Characterize toxicity of current and emerging nanoparticles (NP) & NP byproducts
- > Physicochemical characterization NPs
- > Oxidative Stress as a Marker for Toxicity
- Develop new rapid methodologies for assessing and predicting toxicity

<u>Task 1:</u>

Physical Characterization and Preparation of Nanoparticles (NP)

Synthesis and Characterization of NP

- Characterizing and Improving NP Dispersion in Biological Medium
- Physicochemical Characterization
- Synthesis of NPs

Application for Abatement

• NP Removal in Porous Media

<u>PIs:</u>			
Reyes Sierra			
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Non-toxic Polyacrylate Surfactants Enhances Dispersion



The polyacrylate dispersant, Dispex, enhanced NP dispersion in water and biological medium, yeast extract peptone dextrose (YEPD).

Effect of Protein on the Stability of NPs in Biological Media



Fetal Bovine Serum (FBS) addition increases the stability of NP dispersions in biological medium, mineral essential medium (MEM).

Surface Characterization of Nanoparticles

Objective: Characterization of the surface sites on nanoparticles that contribute to concentration, retention, and enhanced transport of toxic chemicals.

Method approach: Surface hydroxylation (adsorption and desorption of contaminants).



Adsorbent concentration in the gas phase:

$$\frac{\partial C_g}{\partial t} = D_e \frac{\partial^2 C_g}{\partial x^2} + (1 - \varepsilon) \frac{3}{r} [k_d C_s - k_a C_g (S_0 - C_s)]$$

Adsorbent concentration on the surface:

$$\frac{\partial C_s}{\partial t} = k_a C_g (S_0 - C_s) - k_d C_s$$

- concentration in the gas phase, gmol·m⁻³
- $C_g C_s$ concentration on the surface, gmol·m⁻²
- k_a adsorption rate coefficient, m³·gmol⁻¹·s⁻¹
- desorption rate coefficient, s⁻¹ k_d
- S_o maximum capacity of the surface, gmol·m⁻²
 - packing porosity
- radius of nanoparticle, m r
- effective diffusivity, m²·s⁻¹ D

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Effect of NP Material and Size on Surface Retention



- Surface retention depend on the material as well as on the particle size.
- The affinity of nanoparticles for adsorption and retention decreases in the order: $CeO_2 > HfO_2 > SiO_2$. The surface available sites under certain challenge concentrations decreases in the order: $SiO_2 > HfO_2 > CeO_2$.

Synthesis of Fluorescent Silica Nanoparticles

Method

- Fluorescent core made with mixture of SiO₂ and NHS-Fluorescein
- Coat with pure SiO₂ shell



Advantages

- Maintains surface chemistry of pure silica NPs
- Easy to monitor
- Low detection limit
- Final size can be adjusted



Fate of Nanoparticles in Porous Media



Experimental Results

50

Model Fit

40

0.6

0.4

0.2

0

0

10

20

30

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Conclusions

- Dispex and FBS protein stabilize NP in biological media and are useful for toxicity testing.
- NP material and size affect active site density and energetics and therefore contaminant retention.
- A method was selected and tested for the synthesis of size controlled fluorescent silica NP
- A packed bed is a promising technique for the abatement of NPs in waste streams depending on bed conditions; a method was developed for selecting these parameters.

Objectives: University of Washington Project

- <u>Surface</u> chemical characterization of CeO₂ nanoparticles from the "round robin" standardization experiment
 - ESCA (XPS)
 - SIMS (static ToF-SIMS)
 - SEM
 - TEM
- Nanoparticle toxicity assessed by a human foreskin organ culture model

Hypothesis: toxicity of nanoparticles may result from interactions of surface species coating these particles with biological organisms

Sampling Depths

electron spectroscopy for chemical analysis (ESCA) secondary ion mass spectrometry (SIMS) (static mode)



Sample preparation

40 μl 10 wt.% CeO₂ in water from Sigma-Aldrich (particle size label <25 nm)



Drop of sample on Si wafer







Dried sample



Survey scan spectra shows that the elements present on the outermost 10 nm layer of nanoparticles are Ce, C, and O



- High resolution C1s peak consists of hydrocarbon and unsaturated carbon peak
- Unsaturated C1s peak with a 4 eV shift from hydrocarbon peak could be associated with either carboxylate, or, less likely, carbonate

Surface elemental ratio

• Hydrocarbon and unsaturated carbon contents are calculated from the ratio of their respective area under the detailed scan peaks

	Ce %	O%	Total C%	Unsaturated Carbon%	Hydrocarbon %
Spot 2	19.3	60.2	20.5	4.7	15.8
Spot 5	17.1	54.4	28.5	9.1	19.4

SEM characterization of CeO₂ sample



SEM micrographs of CeO₂ nanoparticles. a. 10 wt.% CeO₂ (the original sample concentration) b. Surface between cracks shown in a. c,d. Diluted sample (0.5 wt. %)

Transmission Electron Microscopic Images



Static ToF-SIMS – CeO₂ 2.5wt% (+) spectrum



Static ToF-SIMS – CeO₂ 2.5wt% (-) spectrum



C = circular featureStatic ToF-SIMS – CeO₂ 2.5wt% (-) spectrum – C



Summary

- Nanoparticle surface chemical structure was characterized by XPS and surface were homogenous with respect to elemental species and oxidation state of Ce⁴⁺
- Carboxylate species probably present on nanoparticle surface
- SIMS show inhomogeneity and will permit us to identify specific species

Toxicity Experiments Past Year

Tasks 425.024

- Completed study on chemical ROS production.
- Completed study on yeast O₂ uptake assay.
- Evaluated flow-cytometry for yeast membrane integrity assay.
- Impedance-based Real Time Cell Analysis (RTCA) for high throughput screening.

Experiments: Chemical ROS - 1



Experiments: Yeast O2 uptake

Methods

20% 02

Monitor O₂ uptake by yeast *S. cerevisiae* in pH 6.5 Yeast Extract Peptone Dextrose (YEPD) medium (effect dispersant, Dispex)



NPs

02

(GC-TCD)

Experiments: Yeast Flow Cytometry - 1

Flow cytometry for assaying membrane integrity of yeast *S. cerevisiae* in pH 6.5 Yeast Extract Peptone Dextrose (YEPD) medium exposed to NP dispersed in Dispex



Experiments: Yeast Flow Cytometry - 2

Flow cytometry example plots (right plot with explanation of quadrants)



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Experiments: Yeast Flow Cytometry - 3

Flow Cytometry Summary of Results



Experiments: xCELLigence - 1

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

Human Lung Epithelial Cell Line, 16HBE14o-



- The xCELLigence System measures electrical impedance across interdigitated microelectrodes integrated on the bottom of tissue culture E-Plates.
- The impedance provides information about the biological status of the cells.
- The xCELLigence system does not need fluorescent labels.

Experiments: xCELLigence - 3



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Experiments: xCELLigence 4

50% Response Concentrations from all RTCA experiments with NPs



Experiments: Conclusions

Mos	t toxic NPs: ZnO, Ag ⁰ and Mn ₂ O ₃		
—	ZnO and Ag ⁰ NPs solubilize during the assays and are almost equivalent in toxicity to Zn^{+2} and Ag ⁺ salts.		
	Mn_2O_3 is <u>oxidative</u> (directly oxidizes ROS-dye, can also form ROS, and had a detectable impact in yeast membrane integrity test).		
Consistently moderately toxic NPs: Al ₂ O ₃ and SiO ₂			
	AI_2O_3 and SiO_2 had good NP stability in assay media utilized.		
_	AI_2O_3 and SiO_2 also have moderate to low chemical ROS production.		
Som	etimes moderately toxic NPs: CeO ₂ and Fe ⁰ CeO ₂ and Fe ⁰ strongly enhance chemical ROS production.		
Con	sistently non-toxic NPs: ZrO ₂ and HfO ₂		

New Experiments 1: Oxidation Macro Molecules

BSA protein oxidation by NPs





Protein Oxidation Carbonyl (CO) groups on side protein chain mainly from: Pro, Arg, Lys, and Thr

Protein carbonyls measurement by ELISA test

Rapid detection/quantification as an index of oxidized proteins (Kit OxiSelect)



New Experiments 2: Oxidation Macromolecules

Preliminary Result \rightarrow BSA protein oxidation by Mn₂O₃ NPs



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New Experiments – 3: Organ Cultures

UA: Development of Well-Differentiated Human Airway Epithelia

Air-Liquid Interface



Apical Airway Epithelium

Filter Support

UW: Skin organ culture model



Skin punch sizes: Circles with in 8 mm diameter and about 3mm thickness





Shall the and the shall be shall be

Basally-supplied Medium

Epithelia develop transepithelial resistance (TER) > 500 Ω^* cm² Cell differentiation occurs

Differentiated Cells in Culture: Pseudostratified, ciliated epithelium

Future Plans



- NP oxidation of large biomolecules (proteins, *etc*)
- Correlation multiple parameters to measured toxicity
- NP impact on oxidative stress at cellular level
- NP impact on cell signaling (*e.g.* ATP signaling)
- NP toxicity to well-differentiated human airway epithelia, including impact on Trans-Epithelial Resistance (TER) barrier level
- Validation RTCA

UW

- Analyze SIMS spectra perform principal components analysis to clarify differences
- Begin experiments with better defined CeO₂ from the Central Florida University and gold NPs
- Test samples for cytotoxicity in the human foreskin rafted organ culture model (data analysis by light microcopy and TEM)

Journal Publications from Project

- 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. (Submitted)
- 2. Garcia-Saucedo C, Field JA, Otero L, Sierra-Alvarez R. 2011. Toxicity of HfO₂, SiO₂, Al₂O₃ and CeO₂ nanoparticles to the yeast, *Saccharomyces cerevisiae*. J. Haz. Mat. (Submitted)
- 3. 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. <u>Chemosphere</u> (*Submitted*)
- 4. Gomez-Rivera F, Field JA, Brown D, Sierra-Alvarez R. 2011. Fate of cerium dioxide (CeO₂) nanoparticles in municipal wastewater during activated sludge treatment <u>Bioresource Technol. (Submitted</u>)
- 5. Wang H, Yao J., Shadman F. 2011. Characterization of the surface properties of nanoparticles used in semiconductor manufacturing. (*Submitted*)

Conference Papers Project

- 6. Rottman J, Shadman S, Sierra-Alvarez R. 2010. Interactions of CMP nanoparticles and sewage sludge. <u>SESHA</u> <u>Journal</u>, Summer 2010. pp. 8
- Gomez-Rivera P, Brown D, Field JA, Shadman F, Sierra-Alvarez, R. 2010. Fate of CeO₂ nanoparticles during laboratory-scale activated sludge treatment. <u>SESHA Journal</u>, Summer 2010. pp. 10.

Conference Presentations

- Field JA, Luna-Velasco A, Garcia C, Otero L, Sierra-Alvarez R. 2010. Toxicity and environmental fate of nanoparticles. 2010 Nano Monterrey Forum: Nanotechnology Industrial Applications. 2010. Nov. 18-19, Monterrey, Mexico. [ORAL]
- Rottman J, Barbero I, Rodriguez M, Sierra-Alvarez R, Shadman F. 2010. ate of CMP nanoparticles in municipal wastewater treatment. TECHCON Conference: Technology and Talent for the 21st Century. Austin, TX. Sept. 13-14. <u>Prize for best</u> presentation in the Environmental session. [ORAL]
- Gomez-Rivera, P., Brown, D., Field JA, Shadman F, Sierra-Alvarez, R. 2010. Fate of CeO₂ Nanoparticles During Laboratory-Scale Activated Sludge Treatment. 32nd Semiconductor Environmental, Safety & Health Association (SESHA) Annual Int. High Technology ESH Symp. Exhibition, Scottsdale, AZ, April 26-29. Prize for 3rd best student presentation in the Environmental session. [ORAL]
- Rottman J, Barbero I, Rodriguez M, Shadman F, <u>Sierra Alvarez R</u>. 2010. Fate of CMP nanoparticles during municipal wastewater treatment. 32nd SESHA Annual Int. High Technology ESH Symp. Exhibition, Scottsdale, AZ, April 26-29, 2010. <u>Prize for 2nd best student presentation [ORAL]</u>
- Rodriguez M, Barbero I, Luna A, Shadman F, Field JA, <u>Sierra-Alvarez R</u>. 2010. Impact of Wastewater Components on the Aggregation Behavior of CMP Nanoparticles 32nd SESHA Annual Int. High Technology ESH Symp. Exhibition, Scottsdale, AZ, April 26-29, 2010. [ORAL]
- Field JA, Gomez-Rivera F, Barbero I, Rottman J, Rodríguez M, Luna A, Shadman F, Sierra-Alvarez R. 2010. Fate of Inorganic Oxide Nanoparticles in Semiconductor Manufacturing Effluents during Activated Sludge Treatment. 10th American Inst. Chemical Engineers (AIChE) Annual Meeting, Salt Lake City, UT, Nov. 7-12, 2010 [ORAL]
- Rottman J, Barbero I, Rodríguez M, Shadman F, Sierra-Alvarez R. 2010. Fate of metal oxide nanoparticles in municipal wastewater treatment. WSP Water Forum: Our Water Future. Nov. 22, 2010, Tucson, AZ. [Poster]

Conferences Presentations (continued)

- Gomez-Rivera F, Barbero I, Rodríguez M, Luna-Velasco A, Shadman F, Field JA, Sierra-Alvarez R. 2010. Interactions Between Inorganic Oxide Nanoparticles and Municipal Wastewater Constituents: Implications for Nanoparticle Removal during Biological Wastewater Treatment. Proc. Leading Edge Technology 2010, Phoenix (AZ), June 2-4. p. 55 [Poster]
- Sierra-Alvarez R, I. Barbero, J. Rottman, M. Rodríguez, A. Luna-Velasco, F. Shadman, JA Field. Removal of CeO₂ and Al₂O₃ Nanoparticles in Semiconductor Manufacturing Effluents during Activated Sludge Treatment. Proc. Leading Edge Technology 2010, Phoenix (AZ), USA, 2-4 June 2010. p. 48. [Poster]
- Boitano S, M. McCorkel, I. Barbero, JA Field, R. Sierra-Alvarez. 2010. Nanoparticle Toxicity: Chemical Adsorbents Increase Cytotoxicity. TECHCON Conf.: Technology & Talent for the 21st Century. Austin, TX. Sept. 13-14. Pub. ID: P056590. [Poster]
- Sierra-Alvarez R, Gomez F, Barbero I, Rottman J, Rodríguez M, Shadman F, Field JA. 2011. Removal of Inorganic Oxide Nanoparticles in Semiconductor Manufacturing Effluents during Activated Sludge Treatment. 2nd International Congress on Sustainability Science & Engineering (ICOSSE '11). Jan. 9-14, Tucson, AZ. [Poster]
- Boitano S, Sherwood CL, Flynn AN, McCorkel M, Field JA, Sierra-Alvarez R. 2010. Use of xCELLigence RTCA to assay cellular signaling and nanocytoxicity responses in an adherent human bronchial epithelial cell line. Mountain 28th Annual Regional Chapter Meeting of the Mountain West Society of Toxicology, Tucson, AZ, Sept. 9 –10, 2010. [Poster].
- McCorkel M, Boitano S. 2011. Nanoparticle toxicity on airway epithelial cells. Society of Toxicology, Washington, D.C. March 6-10. [Poster].

Seminars

- Field JA, Sierra-Alvarez R. 2010. Brownbag presentation: Nanoparticle Interaction with Biological Wastewater Treatment Processes, Water Sustainability Program, Phoenix, Arizona. Jan . 2010 at Arizona Cooperative Extension.
- Garcia-Saucedo C, Otero L, Field JA, Sierra-Alvarez R. 2010. Developing a Yeast Cell Assay for Measuring the Toxicity of Inorganic Oxide Nanoparticles. Teleseminar series of Semiconductor Research Corporation/Sematech Eng. Research Center for Environ. Benign Semiconductor Manuf. May 6th, 2010, Tucson, AZ.
- Otero, L., Garcia-Saucedo, C., Field JA, Sierra-Alvarez, R. 2010. Comparison of Nanoparticle Toxicity to Yeast Cells and Human Lung Epithelial Cells. ERC Teleseminar series. Dec. 2, 2010, Tucson, AZ>
- Boitano, S. 2009. Measuring cytotoxicity of nanoparticles in human cells. ERC Teleseminar Series. Sept. 17, Tucson, AZ.
- Sierra-Alvarez, R. 2009. Toxicity characterization of HfO₂ nanoparticles. ERC Teleseminar Series, Aug. 6, 2009. Tucson, AZ.