Developing a Yeast Cell Assay for Measuring the Toxicity of Inorganic Oxide Nanoparticles

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Outline

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Nanotechnology

Understanding and control of matter at dimensions of roughly 100 nm where unique physical properties make novel applications possible

Unusual Physicochemical properties

- Small size
- Chemical composition
- Surface structure
- Solubility
- Shape
- Aggregation

http://www.fda.gov/consumer/updates/nanotech072507.html
Nanotechnology has an important role to create many new devices and materials with an infinite range of applications.
Organization for Economic Cooperation and Development (OECD)
List of Nanoparticles (NPs)

- Fullerenes (C60)
- Carbon nanotubes
- Silver
- Iron
- Carbon black
- Titanium dioxide
- Aluminum oxide
- Cerium oxide
- Zinc oxide
- Silicon dioxide
- Polystyrene
- Dendrimers
- Nanoclays

http://www.nanolawreport.com/2008/07/articles/oecd-to-begin-testing-nanoparticles/
Engineered NPs in Semiconductor Industry

Chemo-Mechanical Planarization (CMP) slurries

- $\text{SiO}_2$
- $\text{Al}_2\text{O}_3$
- $\text{CeO}_2$

Immersion Photolithography

- $\text{HfO}_2$

- Nanowires
- Nanotubes
- Quantum dots
**Examples of NP Toxicity Studies**

<table>
<thead>
<tr>
<th>NPs</th>
<th>Studies</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO₂</td>
<td>In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility.</td>
<td>Brunner et al. 2006</td>
</tr>
<tr>
<td>TiO₂</td>
<td>Cytotoxicity of titanium and silicon dioxide nanoparticles</td>
<td>Wagner et al. 2009</td>
</tr>
<tr>
<td>ZnO</td>
<td>In vitro cytotoxicity assessment of selected nanoparticles using human skin fibroblasts</td>
<td>Dechsakulthorn et al. 2007</td>
</tr>
<tr>
<td>CeO₂</td>
<td>Toxicity of cerium oxide nanoparticles in human lung cancer cells</td>
<td>Weisheng et al. 2006</td>
</tr>
</tbody>
</table>
Release of Nanomaterials to the Environment

(Wiesner et al., 2009)
Methods to Evaluate

Ecotoxicity of Nanoparticles
Ecotoxicity Testing of Nanoparticles

Microorganisms
- Foundation of all known ecosystems
- Basis of food webs and the primary agents for global biogeochemical cycles
- Important components of soil health

Microbial ecotoxicology test are used to study the toxicity of nano-materials and to elucidate cytotoxicity mechanisms that could be extrapolated to eukaryotic cells
YEAST (Saccharomyces cerevisiae)

- Unicellular eukaryotic model organisms
- Short generation time
- Used in the toxicological evaluation of chemicals
Concerns About the Evaluation of Nanoparticles Cytotoxicity

- Interferences of NPs on spectrophotometric based techniques

**Mitochondrial Toxicity Test (MTT)**

- NPs agglomerate in biological medium complicating interpretation of data from toxicity studies
- Poor characterization of NPs
Objectives

- Develop a yeast-based, $O_2$-uptake test to evaluate the toxicity of NPs

- To select non-toxic dispersants to enhance the stability of NPs in biological media using in toxicity testing.

- To characterize some physicochemical properties of NPs in toxicity assay medium: particle size distribution and NP concentration.

- Apply the developed method to test the toxicity of NPS utilized in semiconductor manufacturing and in other important industries, in the presence and absence of dispersant.
Materials

CeO$_2$
(50 nm)

SiO$_2$
(10-20 nm)

Al$_2$O$_3$
(30 nm)

ZnO
(10-30 nm)

TiO$_2$
(25 nm)

Mn$_2$O$_3$
(30-60 nm)

HfO$_2$
(20 nm)

Nanoparticles (NPs)

ZrO$_2$
(20-30 nm)
Toxicity Test with Yeast

*S. cerevisiae (0.1%)*
YEPD* medium, pH: 6.5

NPs +

Dispersant (Dispex)
10:1 w/w

Sonicated
5 min. 70% amplitude
DEX® 130

20% O₂

O₂ (GC-TCD)

Incubation: 10 h at 30°C and 200 rpm

*YEPD = Yeast Extract Peptone Dextrose*
Experiment Design: Estimating $O_2$ Uptake from Theoretical Oxygen Demand (ThOD)

\[ \text{Predicted } O_2 \text{ consumption } \sim \text{Amount } O_2 \text{ in head space} \]

25 mL of YEPD medium, 135 mL of head space and 5 g ThOD/L
Characterization of NPs

• Similar conditions to the toxicity test.
• Studies without yeast were carried out.

Analytical methods:
• Particle size
• Zeta potential
• Concentration

Samples:
10 hrs shaking → 30 min. statics → Supernatant Total suspension
Particle Size Distribution

Dynamic Light Scattering

- Analyzes the velocity distribution of particle movement by measuring dynamic fluctuations of light scattering intensity caused by Brownian motion of the particle.

Zetasizer®Nano ZS
Malvern Instruments
Zeta potential

- The electrical potential that exists across the interface of all solids and liquids

<table>
<thead>
<tr>
<th>Zeta Potential [mV]</th>
<th>Stability behavior of the colloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>from 0 to ±5,</td>
<td>Rapid coagulation or flocculation</td>
</tr>
<tr>
<td>from ±10 to ±30</td>
<td>Incipient instability</td>
</tr>
<tr>
<td>from ±30 to ±40</td>
<td>Moderate stability</td>
</tr>
<tr>
<td>from ±40 to ±60</td>
<td>Good stability</td>
</tr>
<tr>
<td>more than ±61</td>
<td>Excellent stability</td>
</tr>
</tbody>
</table>
Concentration of Nanoparticles

ICP- OES

To determine the elemental composition of samples

Microwave-Assisted Digestions

To reduce interference by organic matter and to convert metals associated with particulates to a form (usually free metal) that can be determined with ICP
Results...
Toxicity of dispersants Polyethylenimine (PEI)

PEI dispersant was toxic to *S. cerevisiae*. 

![Graph showing the toxicity of PEI dispersants](image-url)
Toxicity of dispersants
Dispex (Ammonium polyacrylate)

Dispex is not toxic to yeast
Dispex can be used in toxicity test to disperse NPs.
Dispex:NPs ratio: 1:10 (w/w)
Stability of $\text{Mn}_2\text{O}_3$ NP Dispersions in Demineralized Water

pH= 6

Dispex stabilizes the NP dispersion in water (pH=6)

$\text{Mn}_2\text{O}_3$ (30-60 nm)
Stability of \( \text{Mn}_2\text{O}_3 \) NPs in Yeast Medium

\( \text{pH} = 6 \)

Dispex increased the stability of NP dispersions in biological medium

\( \text{Mn}_2\text{O}_3 \) (30-60 nm)
Mn$_2$O$_3$ Nanoparticle with Dispex

Saccharomyces Cerevisiae with Mn$_2$O$_3$ + Dispex

Calculate slope

Mn$_2$O$_3$ nanoparticle are toxic to yeast at concn. > 500 ppm
Activity of Yeast Respiration

\( \text{Mn}_2\text{O}_3 \) Nanoparticles with Dispex

IC\(_{50} = 300\) ppm
ZnO Nanoparticle with Dispex

Sacharomyces cerevisiae with ZnO + Dispex

ZnO NPs toxic to yeast at > 50 ppm
Activity of Yeast respiration

ZnO Nanoparticle with Dispex

IC$_{50}$ = 75 ppm
Possible Mechanism of ZnO NP Toxicity

Toxicity observed with ZnO NPs could be associated to the Zn(II) ion.

Kasements et al. (2009) reported that ZnO toxicity was explained by soluble Zn(II) ion...
# Nanoparticles Toxicity

<table>
<thead>
<tr>
<th>NPs</th>
<th>50% inhibition* (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnO</td>
<td>75</td>
</tr>
<tr>
<td>Mn$_2$O$_3$</td>
<td>300</td>
</tr>
<tr>
<td>CeO$_2$</td>
<td>1000</td>
</tr>
<tr>
<td>SiO$_2$</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>HfO$_2$</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Al$_2$O$_3$</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>ZrO$_2$</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

*with Dispersant
Conclusions

- Monitoring of $O_2$ uptake by yeast cells is a reliable method to study the toxicity of NPs.

- The addition of the dispersant Dispex improved the stability of the NPs in yeast bioassay medium.

- Most NPs were not toxic to yeast at 1,000 mg/L. Only CeO$_2$, Mn$_2$O$_3$, and ZnO displayed toxicity. ZnO was the most toxic compound tested.
Current and Future Work

- Complete the characterization of NPs and evaluation of their toxicity to yeast.

- Investigate the mechanisms of toxicity
  - Membrane damage
    (Live/Dead assay, flow cytometry, microscopy)
  - Production of reactive oxygen species (ROS)
    (Commercial kits)
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Thank you!
Questions?