Real Time Cell Electronic Sensing (RT-CES) for Nanotoxicity Evaluation

Reyes Sierra\textsuperscript{1}, Lila Otero\textsuperscript{1}, Scott Boitano\textsuperscript{2} & Jim Field\textsuperscript{1}

\textsuperscript{1}Dept Chemical & Environmental Engineering
\textsuperscript{2}Dept Cell Physiology
The University of Arizona

RSIERRA@EMAIL.ARIZONA
NANOPARTICLES

Nanoparticles (NPs) are particles sized in less than 100 nm.

Nano = Dwarf (Greek) = $10^{-9}$
INTRODUCTION

Conventional Filtration

Microfiltration

Ultrafiltration

RO

Nanoparticles

SRC/Sematech Engineering Research Center for Environmentally Benign Semiconductor Manufacturing
Unique Properties of Nanoscale Materials

- Small size
- High specific surface area (>100 m²/g)
- Quantum effects
  (dual behavior, wave- and particle-like) → unique mechanical, electronic, photonic and magnetic properties
Increase of Surface Area with Decreasing Particle Size

Nel et al. Science, 2006, 311:622-627
NPs - Applications

Increasing industrial / commercial applications

- Catalysis
- Medicine
- Environmental technology
- Cosmetics
- Semiconductors
- Microelectronics

Nanotechnology → 1 trillion US $ market by 2015.
NPs in Semiconductor Manufacturing

CMP slurries

- SiO$_2$
- Al$_2$O$_3$
- CeO$_2$

NPs for immersion lithography

Carbon nanotubes

Colloidal silica (10-130 nm)
(Source: www.bjgrish.com)
NPs in Chemo-Mechanical Planarization

Untreated CMP effluent (50-500 mg/L)
Concern about the adverse effects of NPs on biological systems

- ENM: unusual properties due to their small size
- Increasing evidence that some NP cause toxicity

Poor understanding of “nanotoxicity”
- Uncertainty about the real-life hazards of engineered NPs

Need for improved bioassays to evaluate “nanotoxicity”
Problems Assessing Nanotoxicity

- **Interference in classical methods** dependent on colorimetric or fluorimetric measurements.

- **NP characterization** → most studies do not include characterization of NPs in biological medium.
The RT-CES system measures **electrical impedance** across interdigitated micro-electrodes integrated on the bottom of culture plates.
E-plate

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

**CI**- Quantitative measure of the overall status of the cells:

- Cell number
- Cell adhesion and spreading
- Cell morphology

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

Increase in Cell Index with cell number

Cell morphology before (B) and after 3h treatment with As(III) (C)

*Chem. Res. Toxicol. 2005, 18, 154-161*
Impedance-based Real Time Cell Analysis

Advantages

- (Fluorescent) label free
- Dynamic data of the biological status of the cells
- Noninvasive
- High throughput technique

Limitations

- Requires adherent cells
- Correlation analysis between RT-CES and classical toxicity endpoints performed on a limited number of compounds
- Limited information of its applicability to NPs.
OBJECTIVES

- Assess the applicability of a real time cell electronic sensing (RT-CES) technique based on impedance measurements to evaluate the cytotoxicity of **nanosized inorganic oxides** used in semiconductor manufacturing.

- **Validation** of the RT-CES assay results: RT-CES vs. MTT.

- Characterizing the aggregation of nanomaterials in the biological medium.
RT-CES vs. MTT bioassays

Human bronchial epithelial cells (16HBE14o-)

RT-CES
- Impedance based RT-CES assay:
  - (xCELLigence, Roche)

MTT
- $O_2$ uptake
- Cell membrane integrity
**IMPEDANCE-BASED RT-CES**

(RT-CES, xCELLigence, Roche) - Lung epithelial cells: 16HBE14o-

16 HBE culture
- MEM\(^1\) (10% FBS\(^2\))
- 37°C

E-Plate 96
- MEM (3.75% FBS)
- 100,000 cells/well
- 37°C

RT-CES system
- 37°C

- Cells are transferred to the E-Plate at 100,000 cells/well.
- NPs are dosed after **16 h** of incubation.
- Cells are monitored for at least **48 h**.

---

\(^1\) Minimum Essential Medium  
\(^2\) Fetal Bovine Serum
**RT-CES Bioassay**

- **Experiment stages**

  - **nano-Fe**
  - Washing
  - Overnight growth
  - Exposure to nanoparticles
  - Response to nanoparticles

  ![Graph showing experiment stages](image)

  - Control
  - 10 mg/L
  - 100 mg/L
  - 1,000 mg/L

**SRC/Sematech Engineering Research Center for Environmentally Benign Semiconductor Manufacturing**
RT-CES Bioassay

Cell Index

\[ \text{CI} = \max_{i=1 \ldots N} \left[ \frac{R_{\text{cell}}}{R_b} - 1 \right] \]

- \( R_{\text{cell}} \) = Electrode impedance with cells present
- \( R_b \) = Electrode impedance without cells
- \( N \) = Number of points measured = 3
Example Output RT-CES with As(III)

Dosing of As(III)

Control

0.5 mg/L

30 mg/L

100 mg/L

10 mg/L

2.5 mg/L

Time (hours)

Control
As 500 ppb
As 2,500 ppb
As 10,000 ppb
As 30,000 ppb
As 100,000 ppb

Cell Index

SRC/SEMATECH Engineering Research Center for Environmentally Benign Semiconductor Manufacturing
RT-CES: $\text{Al}_2\text{O}_3$ Nanoparticles

$\text{Al}_2\text{O}_3$ Nanoparticles (50 nm) - $\text{IC}_{50} = 300 \text{ mg/L}$

![Graph showing the effect of $\text{Al}_2\text{O}_3$ Nanoparticles on cell index over time for different concentrations: control, 250 ppm $\text{Al}_2\text{O}_3$, 500 ppm $\text{Al}_2\text{O}_3$, and 1000 ppm $\text{Al}_2\text{O}_3$.}
RT-CES: SiO$_2$ nanoparticles

SiO$_2$ Nanoparticles (10-20 nm) - IC$_{50}$ = 225 mg/L
RT-CES: CeO$_2$ nanoparticles

CeO$_2$ Nanoparticles (50 nm) - IC$_{50}$ > 1,000 mg/L
**MTT Bioassay**

- Assay relies on the reduction by live cells of the water-soluble tetrazolium MTT salt to a colored formazan dye.

- Indicator of cell redox activity and viability
MTT BIOASSAY

- Cells are transferred to a 24-well plate (5 $10^5$ cells/well)
- NPs dosed after 24 h of incubation
- After 48 h, cells are washed and stained with MTT reagent
**MTT BIOASSAY: NANO- Al$_2$O$_3$**

Al$_2$O$_3$ NP (IC$_{50}$ = 980 mg/L)

- NP did not interfere with the MTT analysis. Cell-free controls with the highest NP level caused a marginal increase of the absorbance relative to the DMSO control (2-3% of max. absorbance, depending on the NP used).
SiO$_2$ NPs - $IC_{50} = 225$ mg/L (RT-CES), 172 mg/L (MTT)
**Comparaison RT-CES vs MTT Results**

Conclusions: Good correlation between RT-CES and MTT results. 
Al₂O₃ and SiO₂ moderate toxicity, CeO₂ not toxic.
Aggregation of NPs in Biological Medium

- Particle size distribution (PSD) & chemical analysis
  - Same conditions as in toxicity assays

Sampling ➔ PSD & Zeta Potential (DLS) ➔ Concentration (ICP-OES)
AGGREGATION OF NPs IN BIOLOGICAL MEDIUM

Dispersion | Aggregation | Sedimentation

- Supernatant
- Total
In bioassay media, CeO$_2$ NP agglomerated and the concentration that is effectively dispersed decreased many-fold.
PROTEIN ADDITION (FBS)-
Effect on Stability of Al₂O₃ NPs

FBS in the growth medium (MEM) stabilizes the Al₂O₃ NP dispersions
CONCLUSIONS

- RT-CES is a useful, high throughput technique for dynamic monitoring of NP cytotoxicity. The test relies on impedance measurements, avoiding interference problems often associated with colorimetric/fluorimetric tests.

- The inhibitory concentrations determined for NPs using the RT-CES technique correlated well with those generated by a commonly used cytotoxicity assay (MTT).

- Al₂O₃ and SiO₂ NPs showed moderate toxicity in the MTT and RT-CES assays, CeO₂ was not toxic at very high concentrations (1,000 mg/L).

- Most of the nanoscale inorganic oxides tested showed a high tendency to aggregate in the RT-CES & MTT medium resulting in micron-size aggregates that settled out of the dispersion.
ONGOING RESEARCH:
CYTOXICITY MECHANISMS OF INORGANIC OXIDE NPs

- Release of toxic products
- Disruption of cell membrane (flow cytometry)
- ROS formation (intra- and extracellular generation)
- Protein damage (ELISA bioassay)
ONGOING RESEARCH:

Reducing Aggregation of NPs in Biological medium

![Graph showing particle size distribution](image)

**CeO2 + Displex pH7**

**CeO2 pH7**

Particle Size Distribution (d nm)

Intensity (%)

[src/sematech Engineering Research Center for Environmentally Benign Semiconductor Manufacturing](src/sematech)
ACKNOWLEDGEMENTS

- SEMATECH / SRC

- UA Nanotoxicity group