NANOPARTICLE TOXICITY ON AIRWAY EPITHELIAL CELLS

CARA L. SHERWOOD

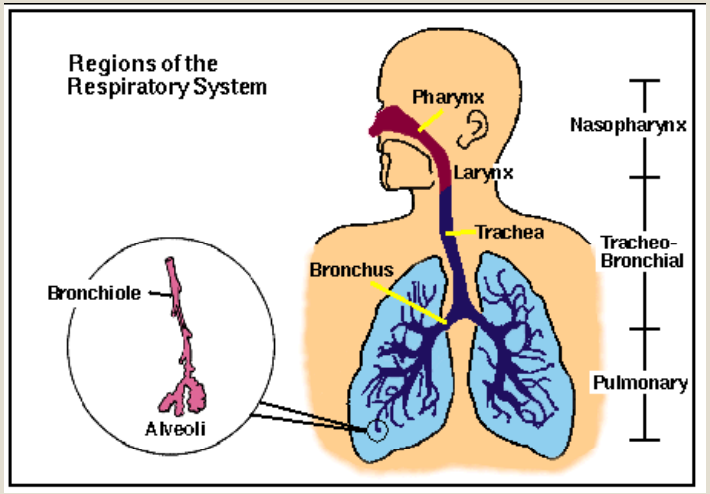
RESPIRATORY CELL PHYSIOLOGY LAB
UNIVERSITY OF ARIZONA
SEPTEMBER 13, 2011

Presentation Outline

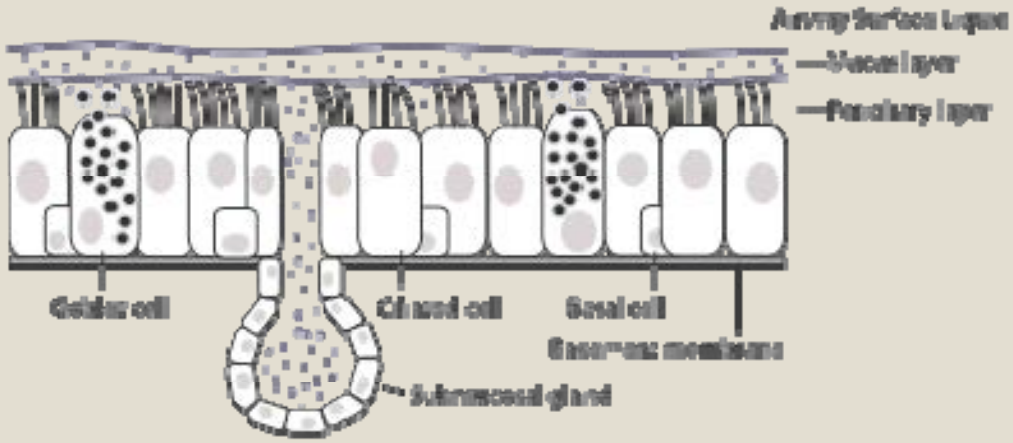
- Introduction to airway epithelial biology
- Characterization of engineered nanoparticles (ENPs) and micron-sized particles
- Cytotoxicity testing (live/dead assay vs. RTCA)
- ATP-induced cellular signaling (RTCA)
- Ca^{2+} signaling using digital imaging microscopy
- Conclusions

Conducting Airway Epithelium

- The conducting airway epithelium provides first line of defense from inhaled particulates and pathogens



http://www.mfg.mtu.edu/cyberman/environment/air/anatomy.html



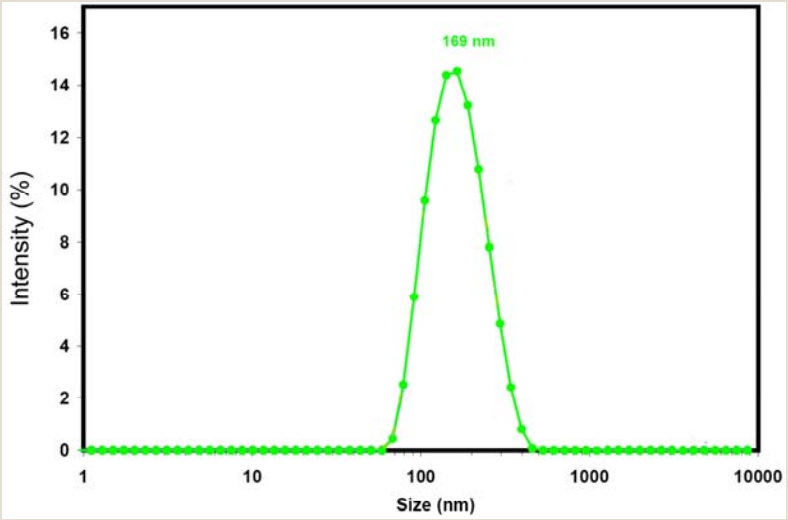
- Our cell line (16HBE14o-) models the airway epithelium

Particulate Matter Studies

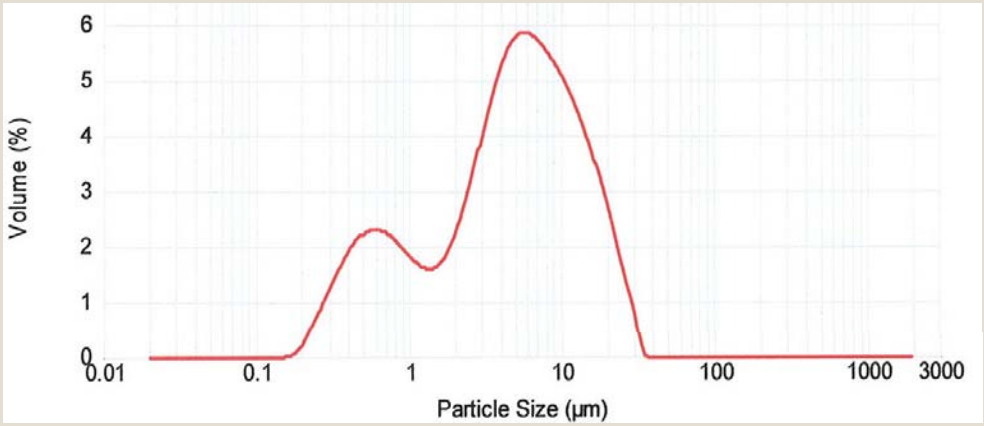
- Studies examined particulate matter in pollution (PM_{10} and $PM_{2.5}$)
  - Increased mortality and morbidity due to cardiovascular and respiratory effects
  - Increased hospital admissions in patients with chronic obstructive pulmonary disease (COPD) and asthma
- Ultrafine particles (ENP size) cause inflammation and airway epithelium injury
- ENPs are similar in size, but have different physical/chemical properties

Characterization of HfO_2 particles

- Particle characterization is important in elucidating affected cellular mechanisms
- Measured particle size distribution (PSD) for HfO_2 ENPs
- Only a fraction of HfO_2 ENPs were in the nano-range (i.e. <100 nm)



(PSD by dynamic light scattering)

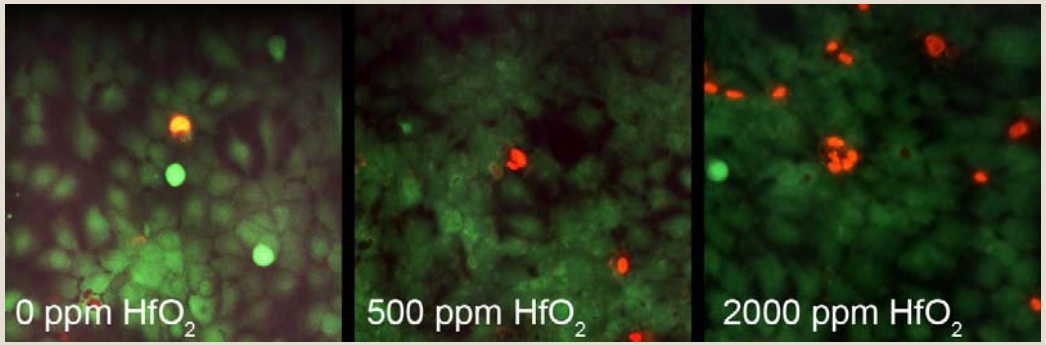


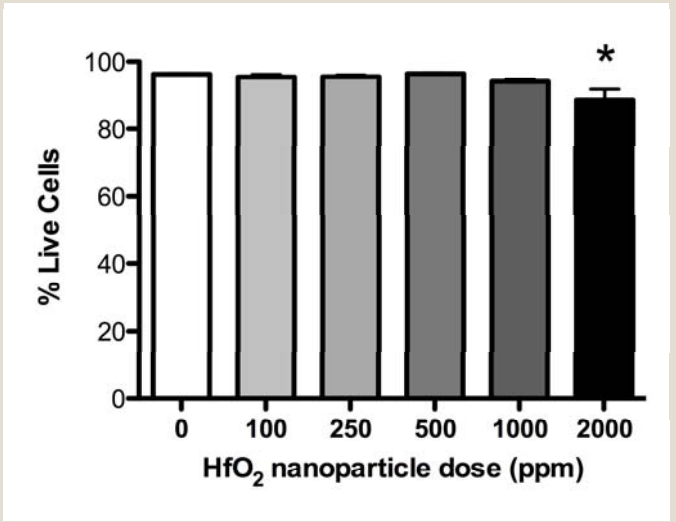
- PSD for micron-size HfO_2 was wide, with average particle size 6.768 µm

Examination of HfO_2 ENP cytotoxicity: live/dead assay

Fluorescent Assay:

- Grew 16HBE14o- cells to confluence (24 mm tissue culture wells)
- Incubated 16HBE14o- cells in culture media +/- ENPs for 2 hr
- Evaluated cytotoxicity with fluorescent dyes
  - Cell permeant green dye (Calcein-AM) = live cells
  - Cell impermeant red dye (ethidium homodimer) = dead cells

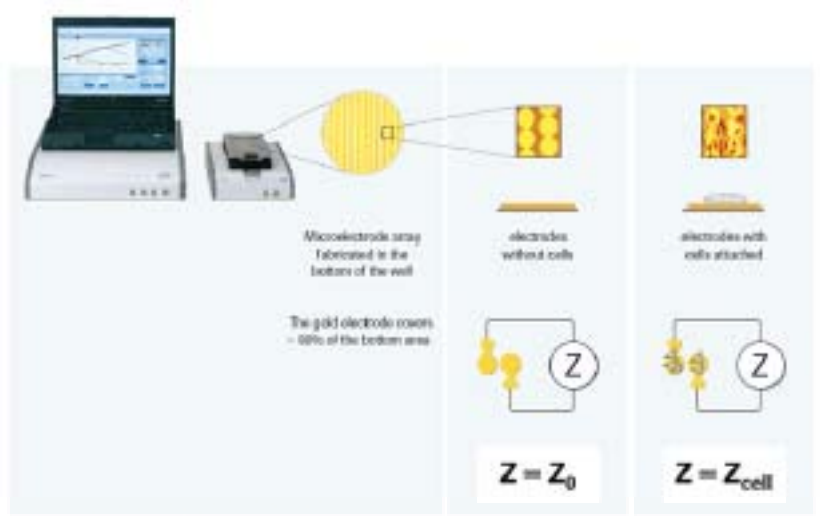


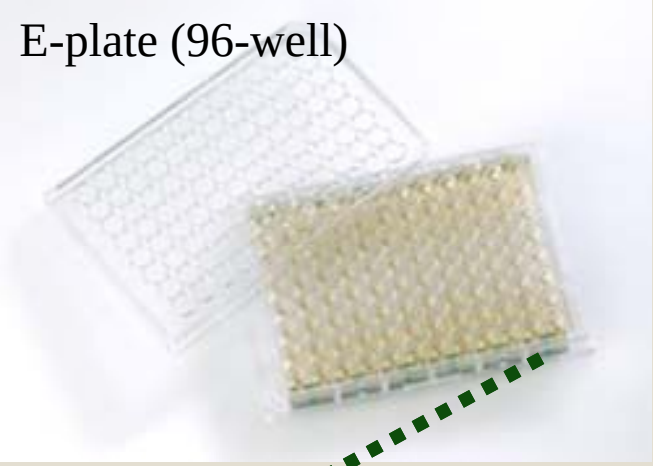


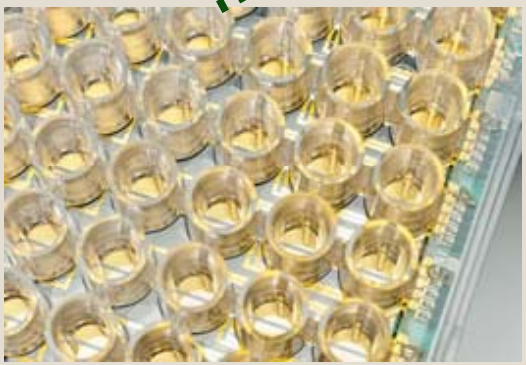
Limitations:

- Single time point response
- Time-intensive analysis (not high-throughput)

Examination of HfO_2 ENP cytotoxicity: Real Time Cell Analysis (RTCA)



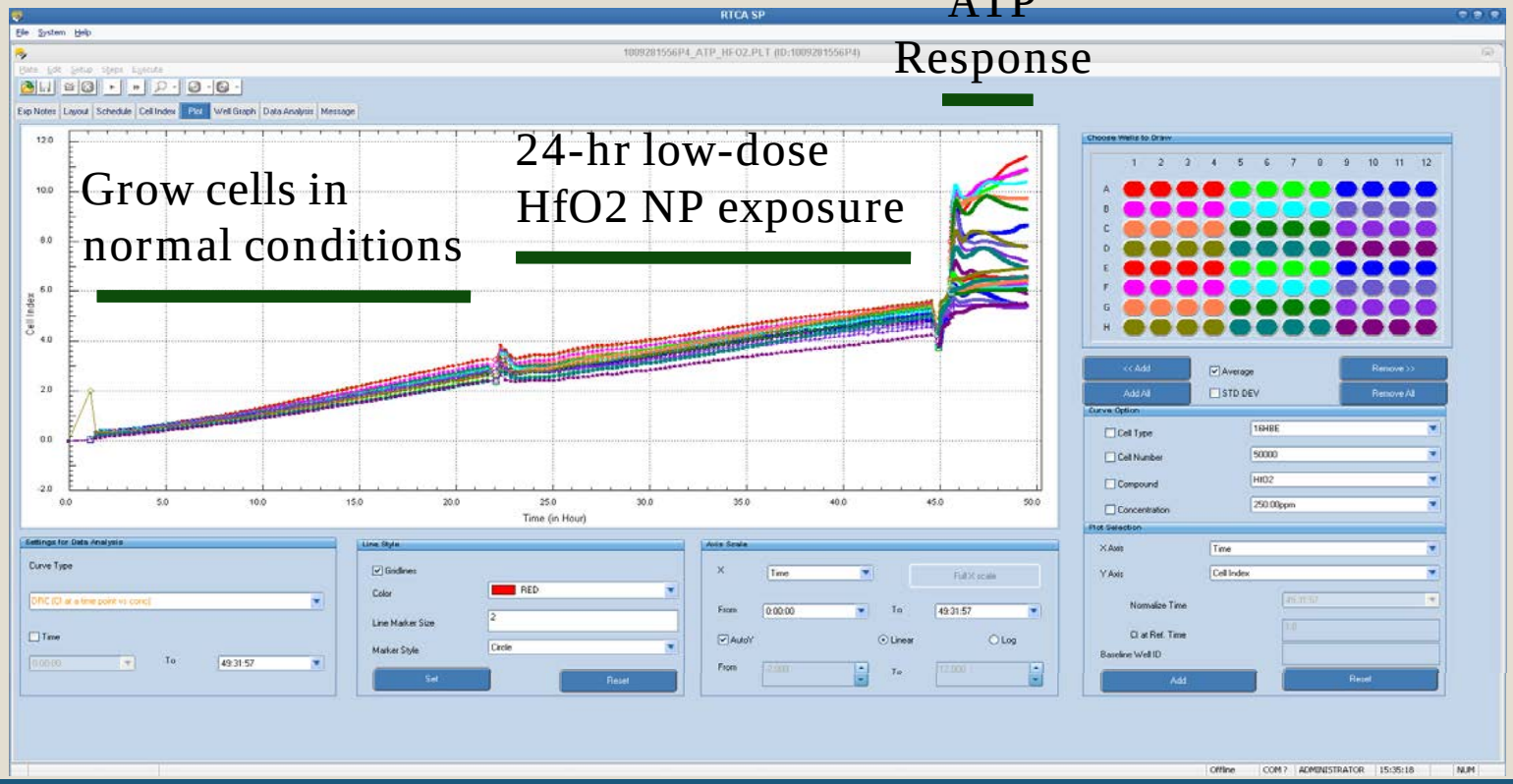




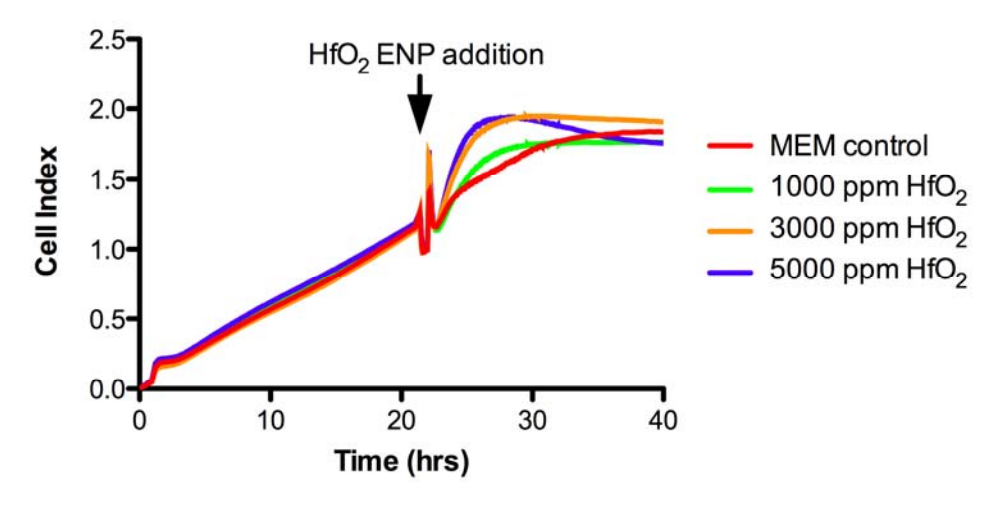
- RTCA measures cellular lipid contact with E-plate surface
- Quantified by “cell index”
  - Change in impedance divided by background value
- Cytotoxicity is indicated by a dramatic loss in cell index

RTCA Assay

- Raw data obtained from RTCA

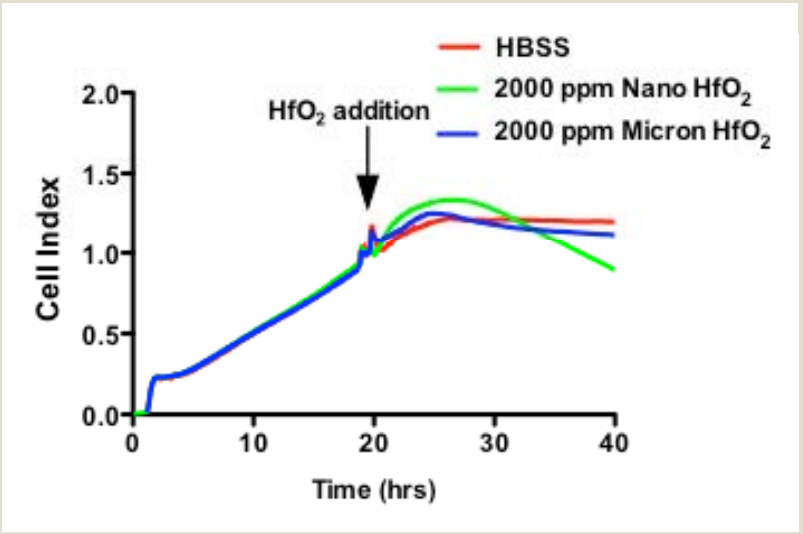


RTCA results with 16HBE14o- cells



- HfO_2 ENPs do not result in significant cytotoxicity
- HfO_2 ENPs do show cellular response

- Compared ENP HfO_2 with micron-sized HfO_2 and untreated controls
- No significant difference in HfO_2 cytotoxicity between different sized particles

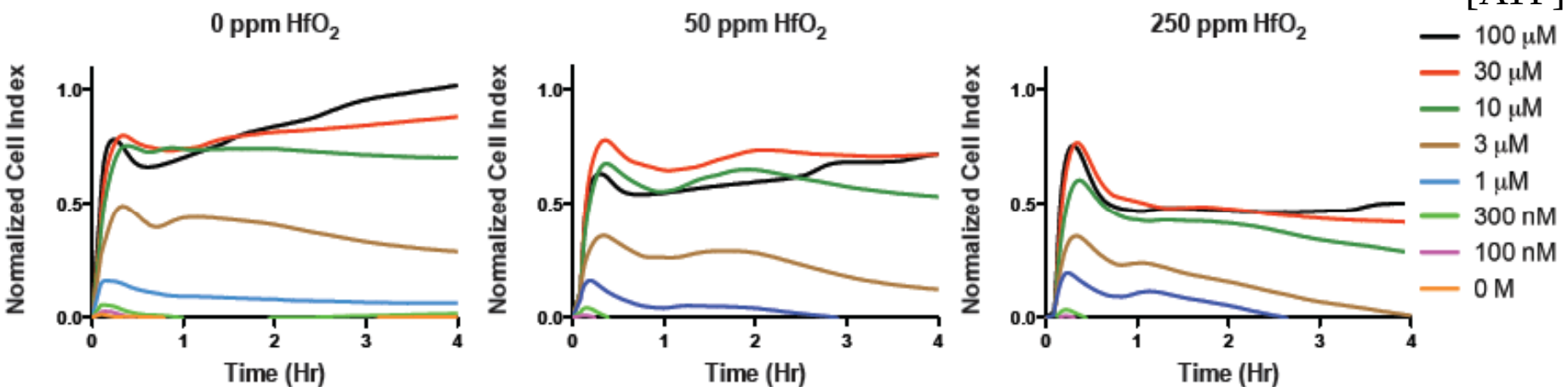


Beyond cytotoxicity: cellular effects of HfO_2

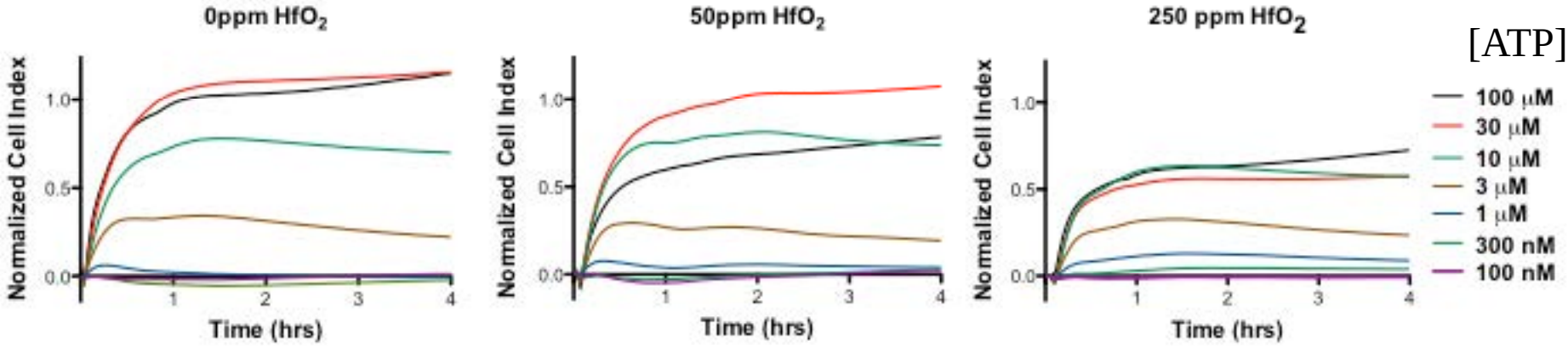
- Cell death is the end point for toxicity testing
- Detrimental cellular effects can occur in the absence of cell death
  - Cell transformation – e.g., Cancer
  - Loss of ability to respond to cellular signals or stress
- Are there adverse effects in lung epithelial cells from HfO_2 ENPs exposure in the absence of cell death?
- We used the RTCA to evaluate low-dose ENP exposure on ATP-induced airway epithelial cell signaling
  - Alterations in ATP signaling is associated with innate immune impairment and chronic lung diseases
  - ATP initiates an immediate physiological response that translates to an increase in cell index when measured by RTCA

Physiologic response to ATP following ENP and micron-sized HfO_2 exposure in 16HBE14o- cells

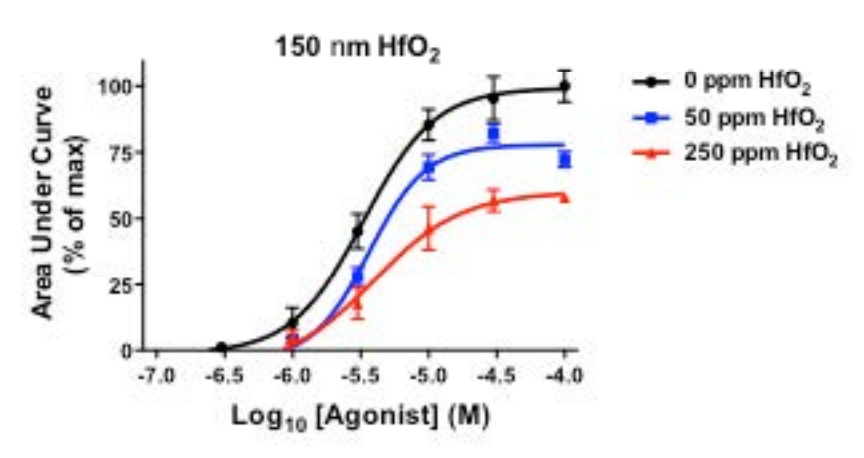
HfO_2 ENPs:

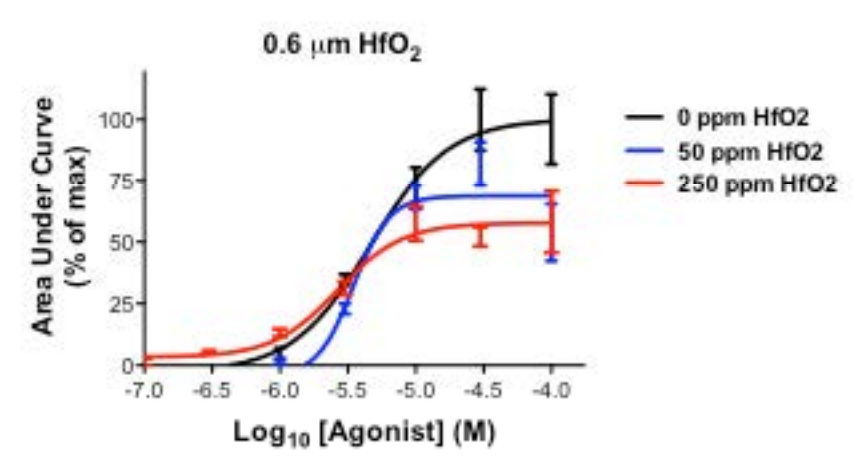


HfO_2 Micron-sized:



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

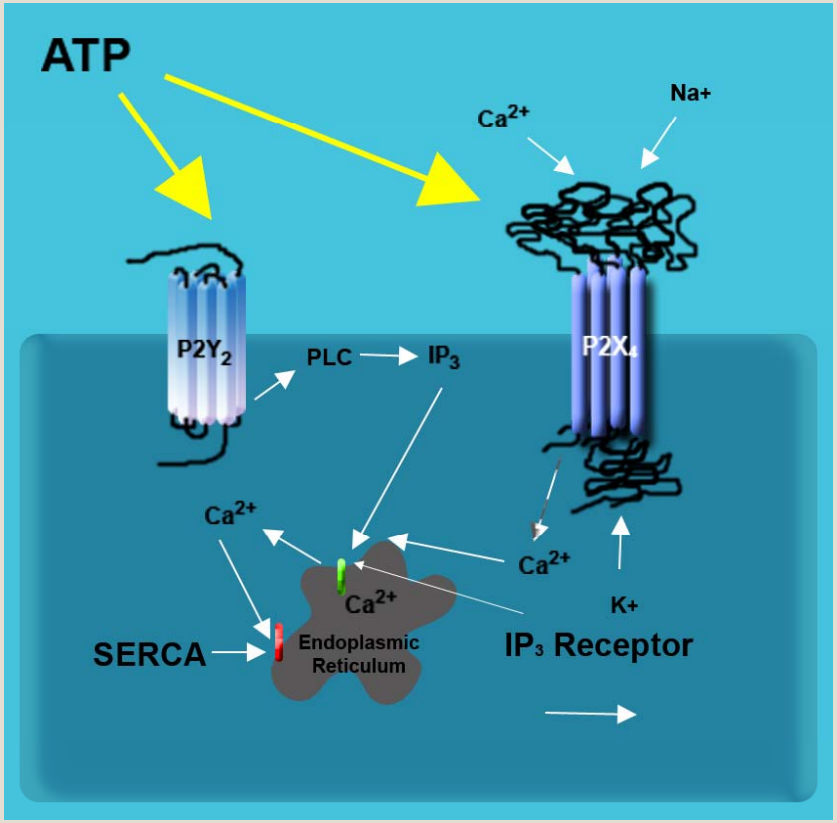




- 24-hr incubation with low-dose ENP HfO_2 reduces physiologic response to ATP:
  - P <0.05 at 100 μM ATP (0 vs. 50 and 250 ppm)
  - P <0.05 at 30,10, and 3 μM ATP (0 vs. 250 ppm)
- 24-hr incubation with low-dose micron-sized HfO_2 reduces physiologic response to ATP:
  - P <0.05 at 100 μM ATP (0 vs.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

Ca^{2+} signaling is downstream of ATP

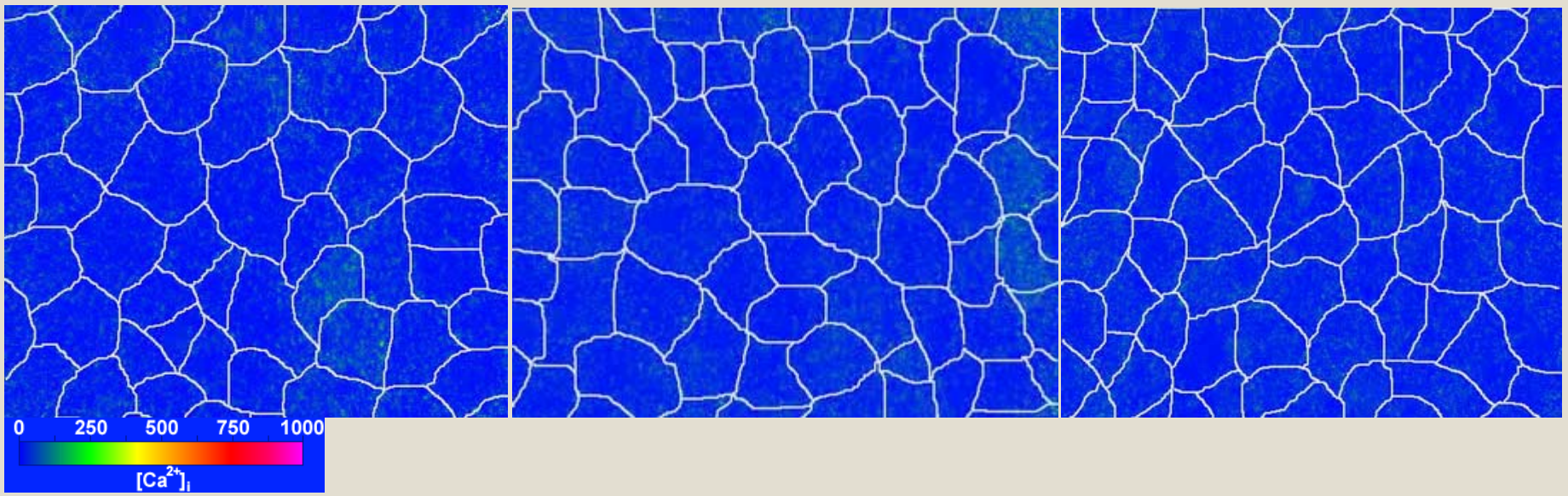
- ATP mediates an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$)
- ATP can activate P2 receptors and increase $[Ca^{2+}]_i$
  - P2Y receptors are G protein-coupled receptors
  - P2X receptors are cation-selective ion channels activated by ATP
- Hypothesis
  - Exposure of airway epithelial cells to low-dose HfO_2 will decrease their $[Ca^{2+}]_i$ response to ATP



HfO_2 ENPs reduce ATP-mediated Ca^{2+} signaling

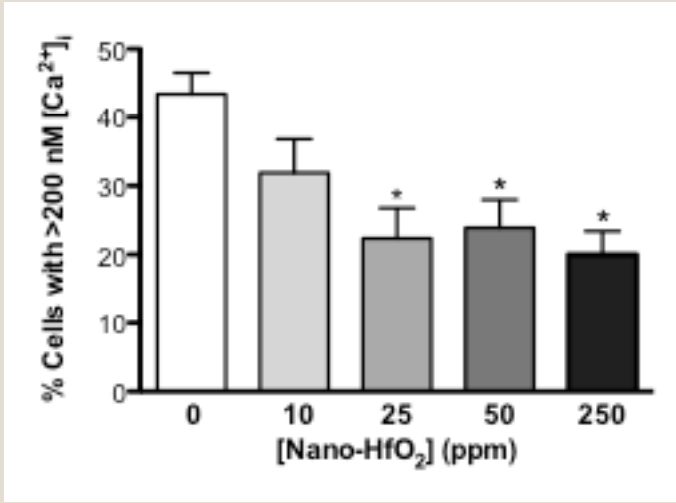
- Confluent monolayers of 16HBE14o- cells were incubated with low-dose HfO_2 ENPs for 24 hr
- 1 μM ATP was applied exogenously and intracellular Ca^{2+} $[Ca^{2+}]_i$ monitored for 3 minutes

0 ppm HfO_2 | 50 ppm HfO_2 | 250 ppm HfO_2

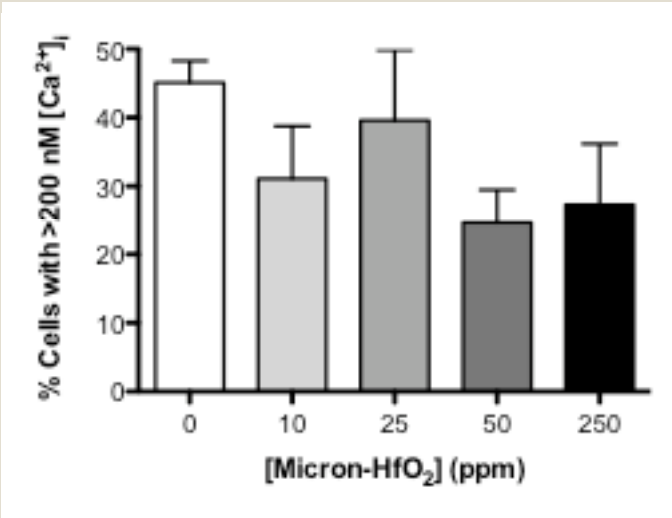


Quantification of Ca^{2+} signaling

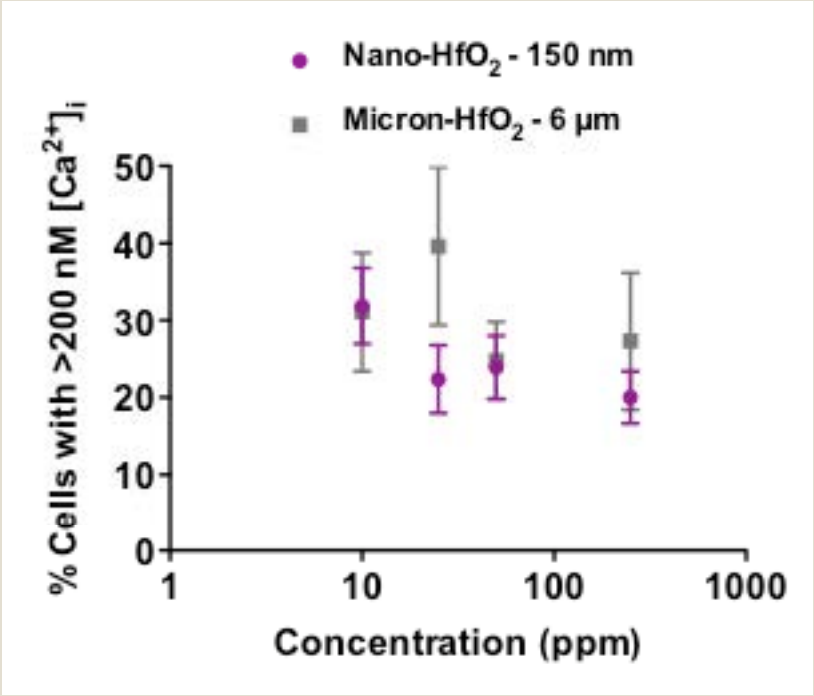
- Cells that increase $[Ca^{2+}]_i$ to 200 nM or more are considered positive
- Low-level ENP concentrations below cytotoxic levels have reduced ATP-mediated Ca^{2+} signaling

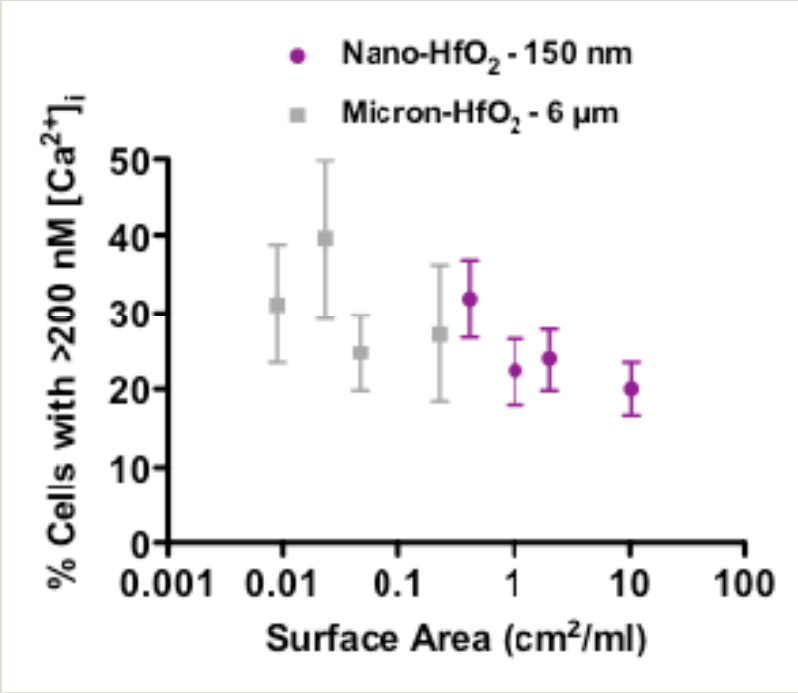


- Micron-sized HfO_2 showed some variation in ATP-mediated Ca^{2+} signaling that was not significantly reduced



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





- These graphs demonstrate HfO_2 Ca^{2+} signaling reductions are due to metals toxicity more than particle size

Conclusions

- Size of ENPs
  - Size reported by manufacturer should be verified
- HfO_2 ENPs did not cause significant cell death
- Sub-cytotoxic exposures to HfO_2 can alter mechanisms of innate immune function in lung epithelial cells
  - Cellular response to ATP is altered by ENP exposure (RTCA)
  - ATP-mediated $[Ca^{2+}]_i$ response reduced by ENP exposure (Ca^{2+} imaging)
  - ENP altered ATP-mediated $[Ca^{2+}]_i$ response appears to be a metals toxicity

Acknowledgements

- Work in this study
  - Mia McCorkle, M.S.
  - Scott Boitano, Ph.D.
- Funding
  - SRC/Sematech Engineering Research Center for Environmentally Benign Semiconductor Manufacturing (James A. Field (P.I.), Scott Boitano, Farhang Shadman, Buddy D. Ratner, Chris Barnes, and Reyes Sierra-Alvarez)
  - Intel Foundation fellowship (Mia McCorkle)