2012 NSF NANOSCALE SCIENCE AND ENGINEERING GRANTEES CONFERENCE. PANEL FOCUS: BIOLOGICAL INTERACTIONS: MICROBES AND IN VITRO CELL CULTURES.

THE CHALLENGES OF *IN VITRO* TOXICITY TESTING WHEN USING NANOMATERIALS.

SUSAN J. BRAUNHUT, PH.D.

DEPARTMENT OF BIOLOGICAL SCIENCES

UNIVERSITY OF MASSACHUSETTS-LOWELL

University of Massachusetts Lowell

Center for High-rate Nanomanufacturing

NSF

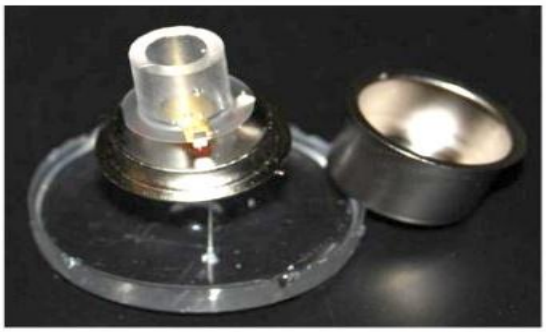
Some of the problems associated with nanotoxicity testing:

- Inconsistency of reported data is affected by the material characterization criteria used and provided.
- Evaluation of toxicity using many conventional assays is significantly underestimated due to interference of the reporter by nanomaterials.
- Methods of testing do not model the most relevant exposure scenarios.
- Few methods provide mechanistic information about how the nanomaterial proves toxic to cells, plants or animals.

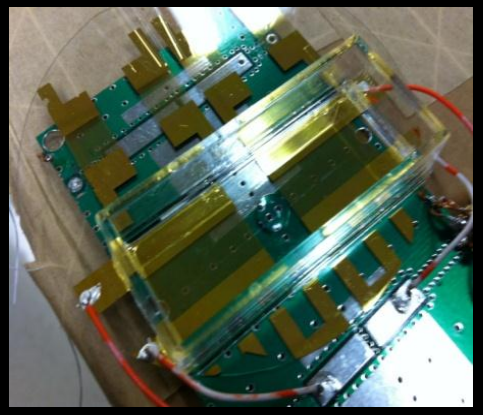
One Approach-Nanocanary ™

Our multiplex in vitro toxicity test employs acoustic wave devices to measure changes in mass, distribution of mass and viscoelastic properties of living cells.

- Cell exposures to nanomaterials:
  - result in alterations in cellular substructures.
  - a change in loading on a piezoelectric substrate to which the cells are attached.
  - a change in oscillation frequency which is read out electrically.
- Two devices are being used:
  - Quartz Crystal Microbalance: Utilizes bulk acoustic waves for sensing cellular changes.
  - Surface Acoustic Wave sensor: Utilizes surface acoustic waves for sensing.



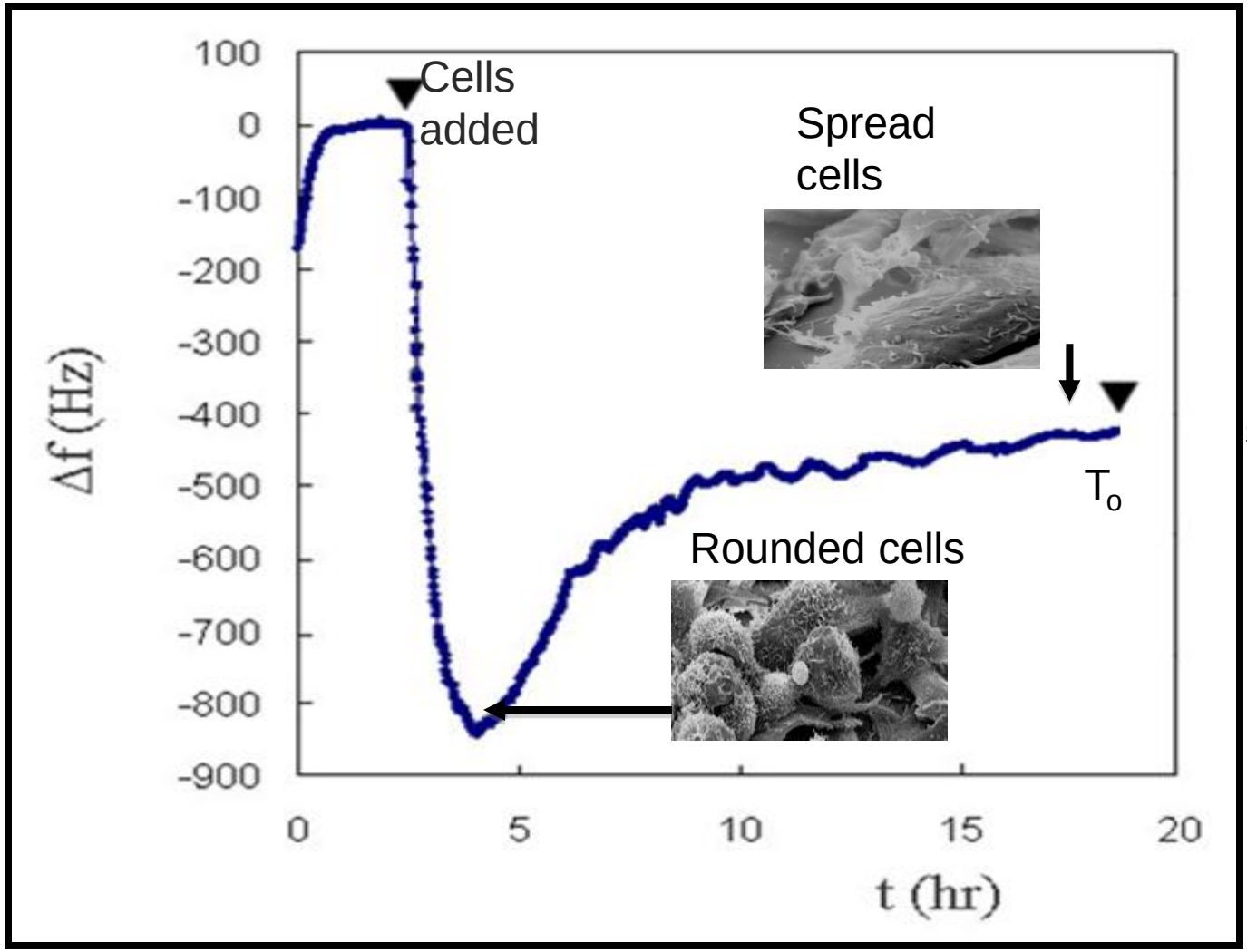
QCM sensor



SAW sensor

U.S. Patent Number 7,566,531.

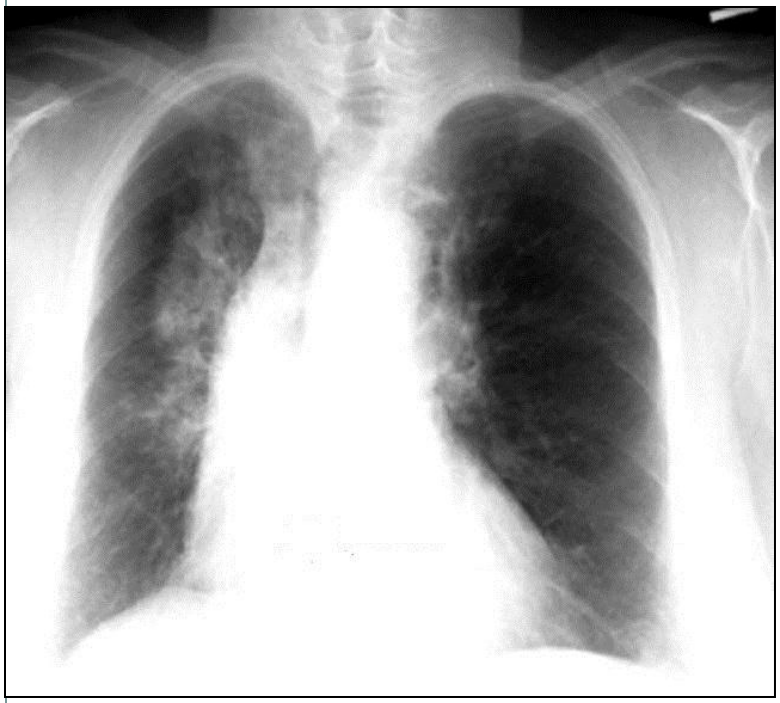
How the toxicity assay incorporates living cells- cells become the sensing element in a mass measuring device.



When frequency stabilizes at T_0 calibrate Δf to 0 for toxicology experiments

Marx KA, Zhou T, Montrone A, Schulze H, Braunhut SJ. A quartz crystal microbalance cell biosensor: detection of microtubule alterations in living cells at nM nocodazole concentrations. Biosensors and Bioelectronics 2001;16(9-12):773-782.

To simulate an inhalation route of exposure, human lung cells (type 1 and 2 pneumocytes, macrophages, endothelial cells) are used in individual AW devices and tested simultaneously using the same nanomaterial at varying doses.



Pneumonitis

Class	Material Type	Particle size
SWCNTs; Short	OD = 1-2 nm; L ≤ 5 µm	
MWCNTs; Short	OD = 10-30nm;L ≤ 5 µm	
MWCNTs; Long,	OD = 10-30 nm; L=10-30 µm	

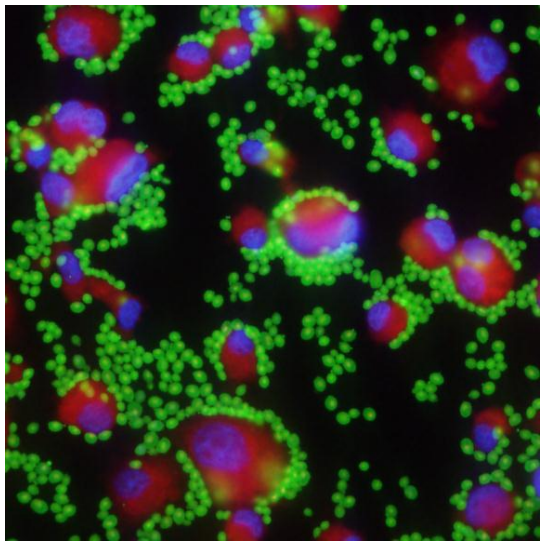
ENMs' Physicochemical Parameters

1. **Specific Surface Area** by BET
2. **Total metals** (Fe, Cr, Co, Ni, Mo, Mn, Zn, Y, etc).
3. **Morphology by TEM**
4. **Crystalinity by XRD**
5. **Surface Charge in PBS**
6. **Dispersion Efficiency & Uniformity**
7. **Reactive Oxygen Species (ROS)**

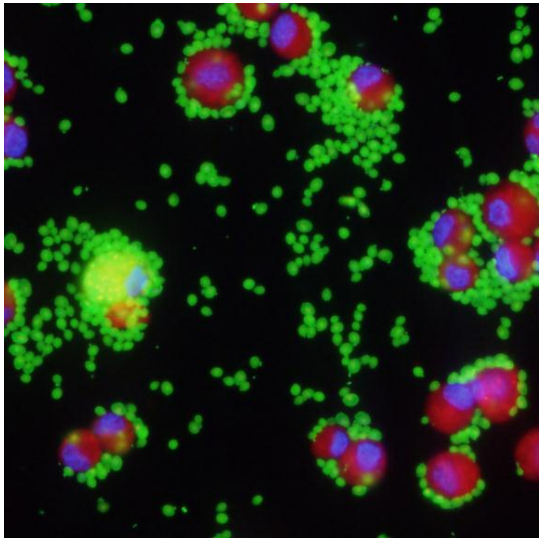
Materials and characterization provided by UML CHN faculty member: Dr. Dhimiter Bello.

Wang G, Dewilde AH, Zhang J, Pal A, Vashist M, Bello D, Marx KA, Braunhut SJ, Therrien JM. 2011.Part Fibre Toxicol. 2011 Jan 25;8:4.

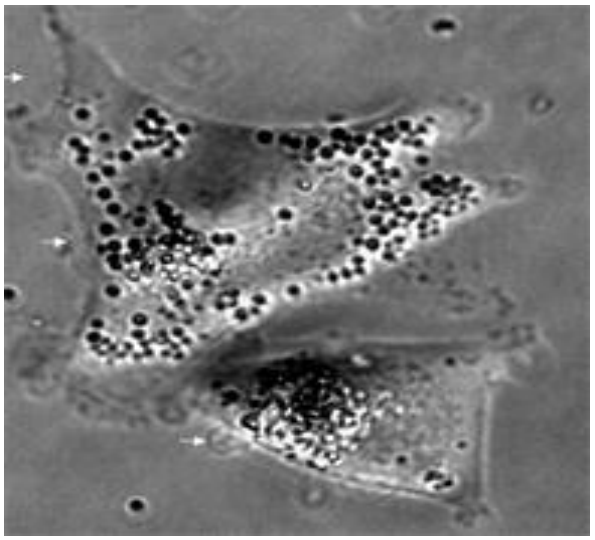
Normal phagocytosis by macrophages: using yeast zymosan and polymer beads.



2 hrs

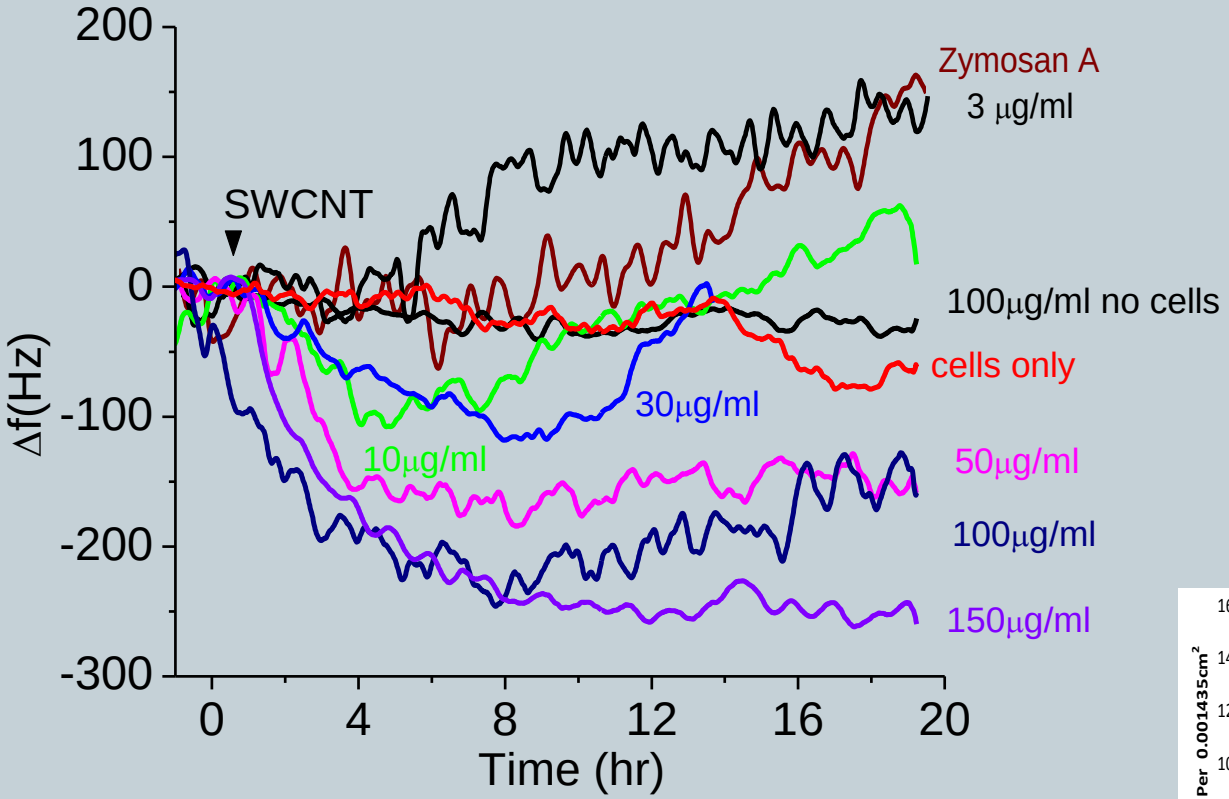


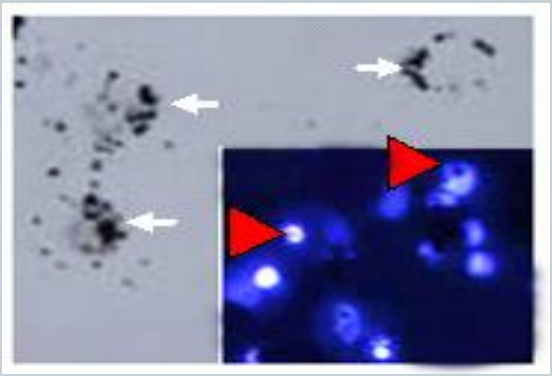
4 hrs

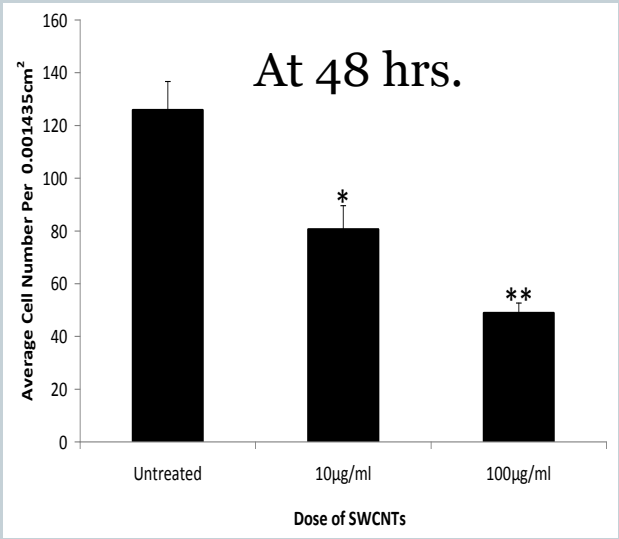


4 hrs

Macrophages of the lung were the cell most sensitive to single walled carbon nanotubes (SWCNT), which caused their apoptosis.

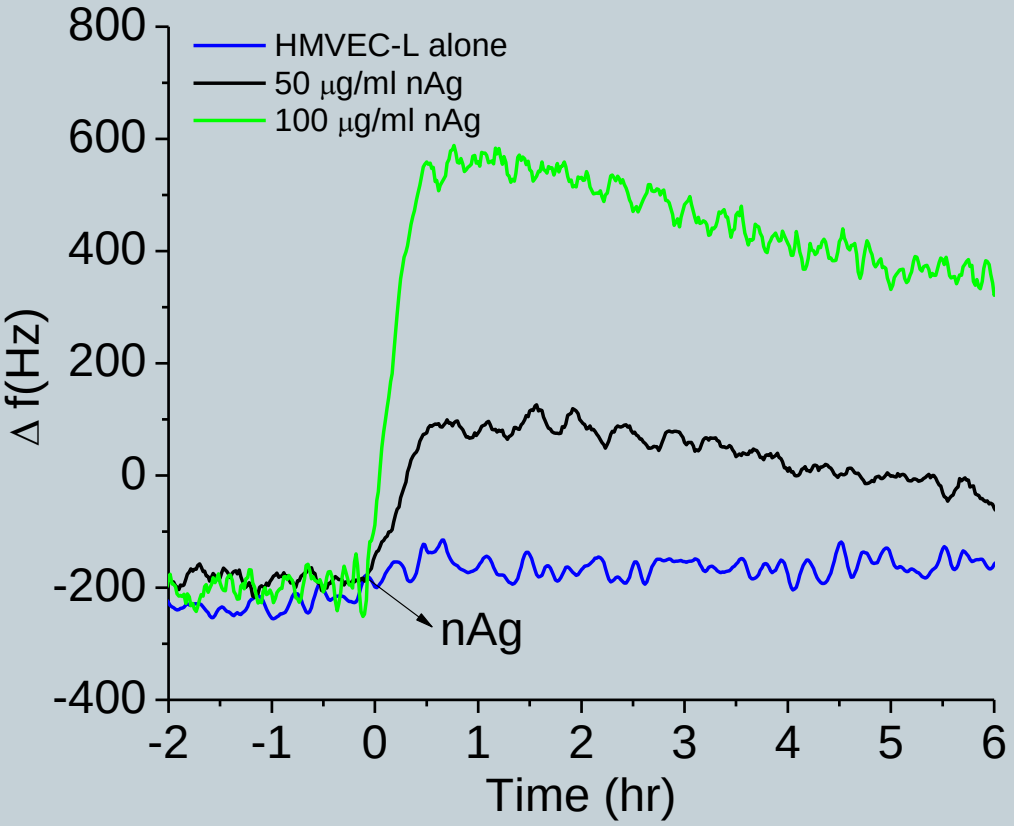


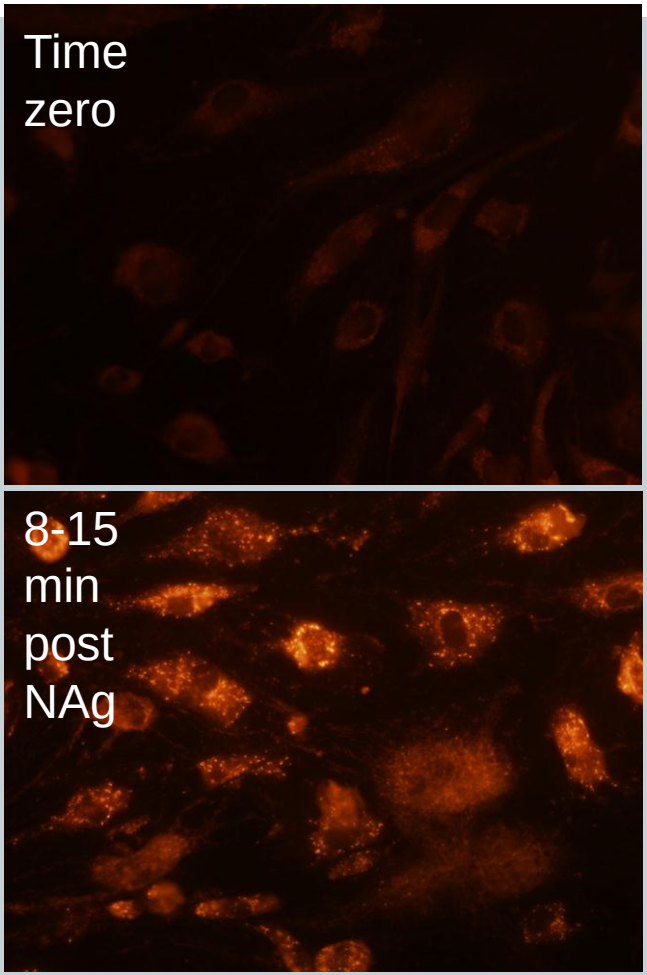




Wang G, Dewilde AH, Zhang J, Pal A, Vashist M, Bello D, Marx KA, Braunhut SJ, Therrien JM. 2011.Part Fibre Toxicol. 2011 Jan 25;8:4.

Death by mitochondrial membrane depolarization: human endothelial cells of the lung were most sensitive to apoptosis induced by nanosilver.





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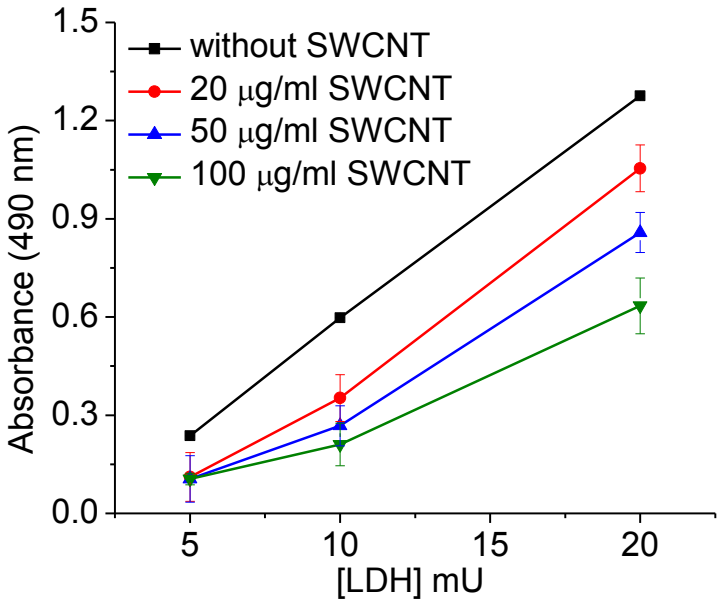
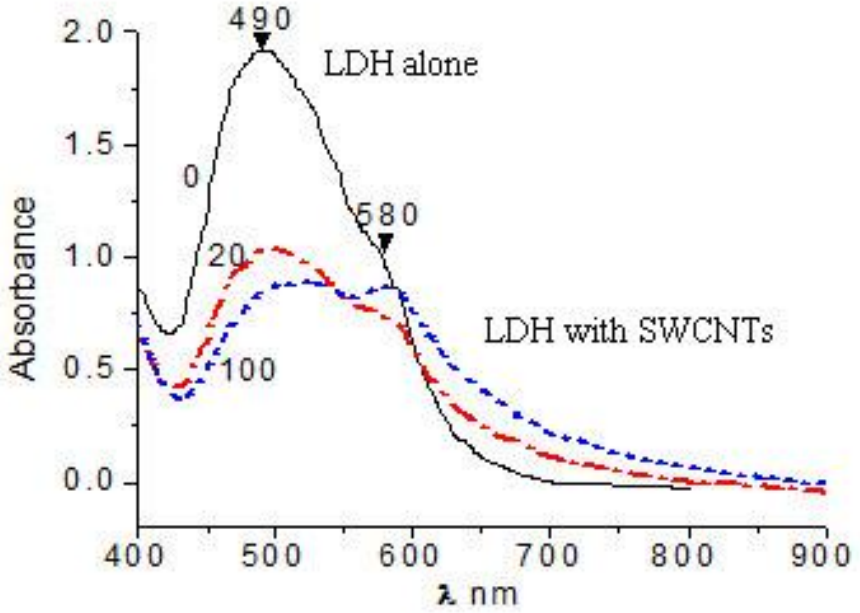
Manuscript in preparation.

Nanomaterials interfere with most conventional toxicity assays.





Using the same pure LDH standard dose curve(black), we show a significant and dose dependent decrease in the readout with SWCNTs in the assay when reading at the recommended wavelength of 490.

When we examined the entire spectra, a shift in the absorbance occurs and by using the 490 and 580 wavelength you can correct the assay when using nanomaterials.

Wang G, Zhang J, Dewilde AH, Pal AK, Bello D, Therrien JM, Braunhut SJ, Marx KA. 2012. Understanding and correcting for carbon nanotube interferences with a commercial LDH cytotoxicity assay. Toxicology. 299:99-111.

Concluding Remarks-
We need standards of practice in the following areas:

1] **Characterization :** Size distribution, surface activity (free radical generation), surface hydrophobicity, and aspect ratio, transition metals content, coatings and aggregation behavior all effect toxicity. **What attributes should be reported?**

2] **Quality control of assay outcomes**: Methods must be scrutinized for their integrity when performed with different nanomaterials. **Which methods have been validated when used with specific nanomaterials?**

3] **Relevant testing designs:** Testing with living cells in biologically relevant environments; using assays that can examine re-dosing and cumulative dose exposures. **What experimental designs simulate living cell exposures?**

4] **Mechanistic data:** Classify how nanomaterials kill specific cell types: as a DNA mutagen, mitochondrial poison, pro-apoptotic signaling, or membrane disruptor. What are the most sensitive cells in each tissue. **How do nanomaterials prove toxic for the organism, plant, or cell?**

Acknowledgements

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