Dispersion, Bioaccumulation, and Mechanisms of Nanoparticle Toxicity

(Task Number: 425.042)

PIs:

- **Steven O. Nielsen (PI), Chemistry, UT Dallas**
- **Rockford K. Draper (co-PI), Chemistry and Mol. & Cell Biol., UT Dallas**
- **Paul Pantano (co-PI), Chemistry, UT Dallas**
- **Inga H. Musselman (co-PI), Chemistry, UT Dallas**
- **Gregg R. Dieckmann (co-PI), Chemistry, UT Dallas**

Graduate Students:

- **Blake Wilson & Elizabeth Braun, Chemistry, UT Dallas**
- **Tyler Hughes, Mol. & Cell Biol., UT Dallas**

Undergraduate Students:

- **Samee Vakil & Dakota Deutsch, Mol. & Cell Biol. UT Dallas**
- **Michael Yukica, Chemistry, UT Dallas**

Other Researchers:

- **Ruhung Wang, Senior Scientist, Chemistry and Mol. & Cell Biol., UT Dallas**

Cost Share (other than core ERC funding):

-

Objectives

- **Computational modeling and experimental validation of correlations between nanoparticle size, cellular uptake, toxicity, and dispersant effectiveness.**

- **Initial focus on multi-walled carbon nanotubes (MWNTs): Prepare MWNTs that differ only in length, dispersant, and agglomeration. Test effects of length, dispersant, and agglomeration on cytotoxicity and pro-inflammatory responses in macrophages. Provide material to other members of ERC/SRC nanotox consortium for testing.**

ESH Metrics and Impact

1. **Reduction in the use or replacement of ESH-problematic materials**
   - **Identification of problematic materials requires accurate toxicity tests. We found common sources of error in assessing toxicity of carbon nanoparticles and developed procedures to eliminate chemicals that caused false positives.**
2. **Reduction in emission of ESH-problematic material to environment**
   - **Assess inherent nanomaterial ESH properties and by-products.**
3. **Reduction in the use of natural resources (water and energy)**
4. **Reduction in the use of chemicals**

Dispersion, Bioaccumulation, and Mechanisms of Nanoparticle Toxicity

(Task Number: 425.042)

PIs:

- **Steven O. Nielsen (PI), Chemistry, UT Dallas**
- **Rockford K. Draper (co-PI), Chemistry and Mol. & Cell Biol., UT Dallas**
- **Paul Pantano (co-PI), Chemistry, UT Dallas**
- **Inga H. Musselman (co-PI), Chemistry, UT Dallas**
- **Gregg R. Dieckmann (co-PI), Chemistry, UT Dallas**

Graduate Students:

- **Blake Wilson & Elizabeth Braun, Chemistry, UT Dallas**
- **Tyler Hughes, Mol. & Cell Biol., UT Dallas**

Undergraduate Students:

- **Samee Vakil & Dakota Deutsch, Mol. & Cell Biol. UT Dallas**
- **Michael Yukica, Chemistry, UT Dallas**

Other Researchers:

- **Ruhung Wang, Senior Scientist, Chemistry and Mol. & Cell Biol., UT Dallas**

Outline of Talk

Rocky Draper:

1. Nanotoxicology
   - Is an immense field.
   - Is a challenging field.
   - Meeting the challenges.

2. A case study: Multi-walled carbon nanotubes (MWNTs)
   - What properties of MWNTs are responsible for toxicity?
   - An approach to understanding MWNT toxicity.

Tyler Hughes:

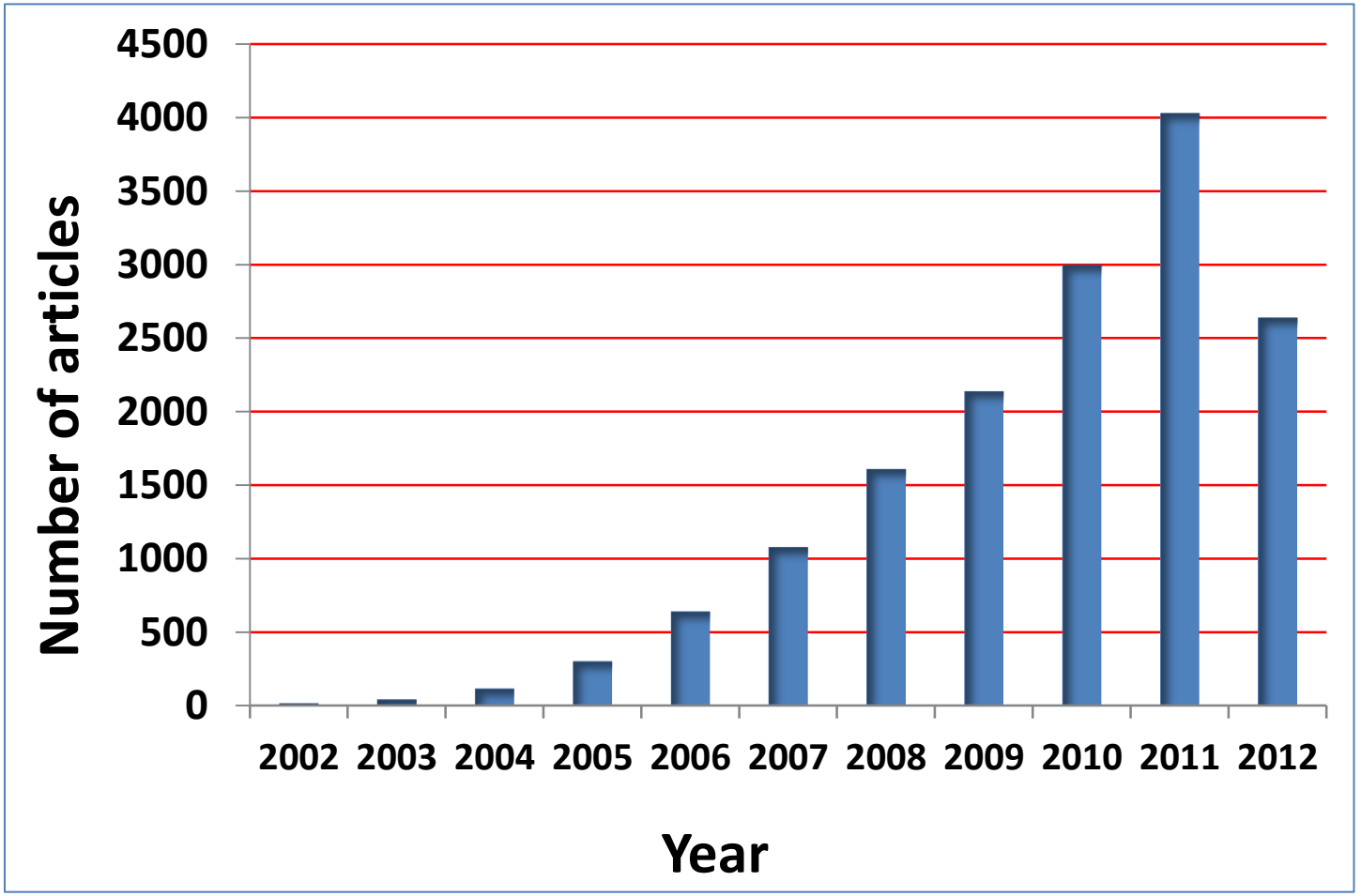
1. Preparation of matched MWNTs that differ only in length, dispersant, or state of agglomeration.
   - Sonication, centrifugation, and dialysis.

2. Characterization
   - Collaborative effort among SRC consortium laboratories.
   - Length by TEM.

3. Future Work

Number of Articles from Google Scholar Containing the Term "Nanotoxicology" 2002-2012

(excluding patents and citations)



Nanotoxicology is Challenging

1. There are potentially many thousands of chemically distinct nanoparticles. Even the same nanoparticle in a different nanoscale size range may be different.

2. Many nanoparticles have unknown contaminants in them. It is difficult to understand or predict toxicity of impure samples.

3. Nanoparticles often aggregate, and/or interact with many different types of biological molecules, which can change their properties and potential toxicity.

4. How to best assay nanotoxocity: With *in vitro* cell lines? Animal models? Computational predictions? How to translate model studies to human dosimetry?

The literature on Silica Nanoparticles

1. **"Focusing the research efforts"**
   F. Schrurs F. & D. Lison (2012) *Nature Nanotechnology* 7:546-548.

2. **Compared results of 38 papers with *in vitro* assays regarding basic questions of the toxicity of silica nanoparticles (SNPs), a common metal oxide. Questions on which there was no clear agreement:**

- **Are SNPs more cytotoxic than their larger counterparts?**
- **Do SNPs penetrate into cells?**
- **Does the cytotoxic activity of SNPs vary with cell type?**
- **Does SNP aggregation/agglomeration influence the cytotoxic activity?**
- **Which properties of SNPs drive their cytotoxic activity?**
- **Technical interferences and positive controls.**

General Recommendations

1. **Laboratories should work together in a consortium approach.**

2. **Use a well characterized reference material for inter-laboratory comparisons.**

3. **Develop standard operating procedures for tests that all labs can use.**

4. **Use a common set of cell lines for *in vitro* work.**

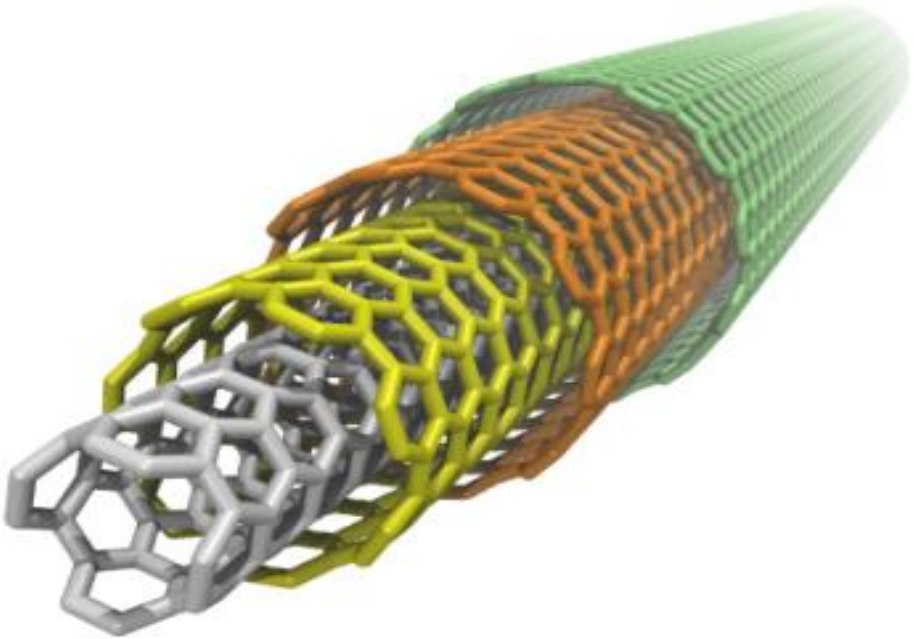
5. **Assess interactions of nanoparticles with cells. Do they enter the cells and if so, how much? Is entry related to biological consequences?**

6. **Use *in vitro* assays for mechanistic studies and *in vivo* assays for hazard assessment. Work to make *in vitro* assays predictive of *in vivo* hazards.**

Multi-walled Carbon Nanotubes

1. **As a sample case, consider multi-walled carbon nanotubes (MWNTs).**

- **Carbon nanotubes nested within one another**
- **Many useful properties and potential applications**
- **On the ITRS roadmap**
- **Evidence of pulmonary toxicity *in vitro* and *in vivo***



What properties of MWNTs cause toxicity?

1. There are opposing data in the literature on what properties of MWNTs cause toxicity and proinflammatory responses:

   - MWNT length.
   - Different dispersants used to prepare MWNT suspensions.
   - MWNT agglomeration state.
   - Different amounts of MWNTs taken up by cells.

2. Our objectives:

   - Develop well-characterized reference MWNT material that differs only in length, dispersant type, agglomeration state.
   - Share this material with consortia members.
   - Assess toxicity and proinflammatory responses with fibroblast and macrophage cell lines as models for pulmonary toxicity.
   - Quantify the amount of MWNTs taken up by cells.
   - Correlate toxic responses with amount of material in cells.

Minimizing Physical Differences between MWNTs

1. Start with one type of MWNT from a single source.

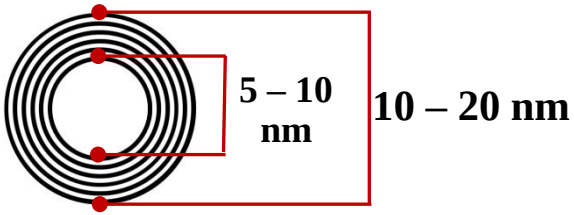
2. Carefully characterize the MWNT powder.
   - Check for contaminants from synthetic method: metal catalysts, support components, oxidation fragments.
   - Do not rely on manufacturers analysis results.

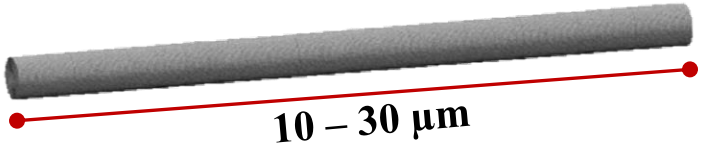
3. Prepare suspensions with consistent methods using characterized non-toxic dispersant.
   - Beware of sonication artifacts.

4. Characterize physical and chemical properties of suspensions.
   - Make sure any toxic synthesis catalysts are removed.
   - Characterize size and Zeta potential in suspensions and in biological media that will be used on cells.

Preparing Reference MWNTs that Differ in Length, Dispersant, and Agglomeration Part 1

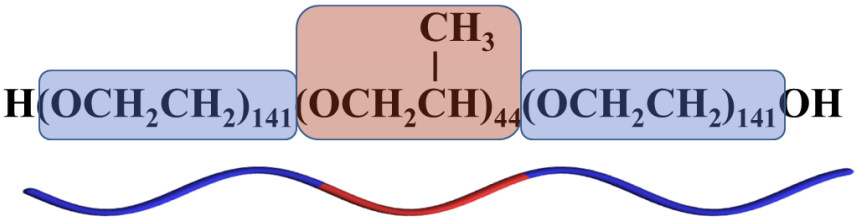
1. Starting pristine MWNT powder purchased from NanoAmor, Inc.

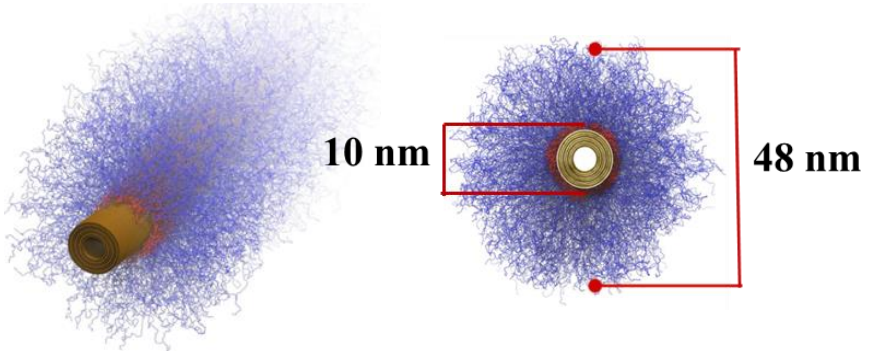




2. Dispersant: triblock copolymer Pluronic® F-108 (PF108).

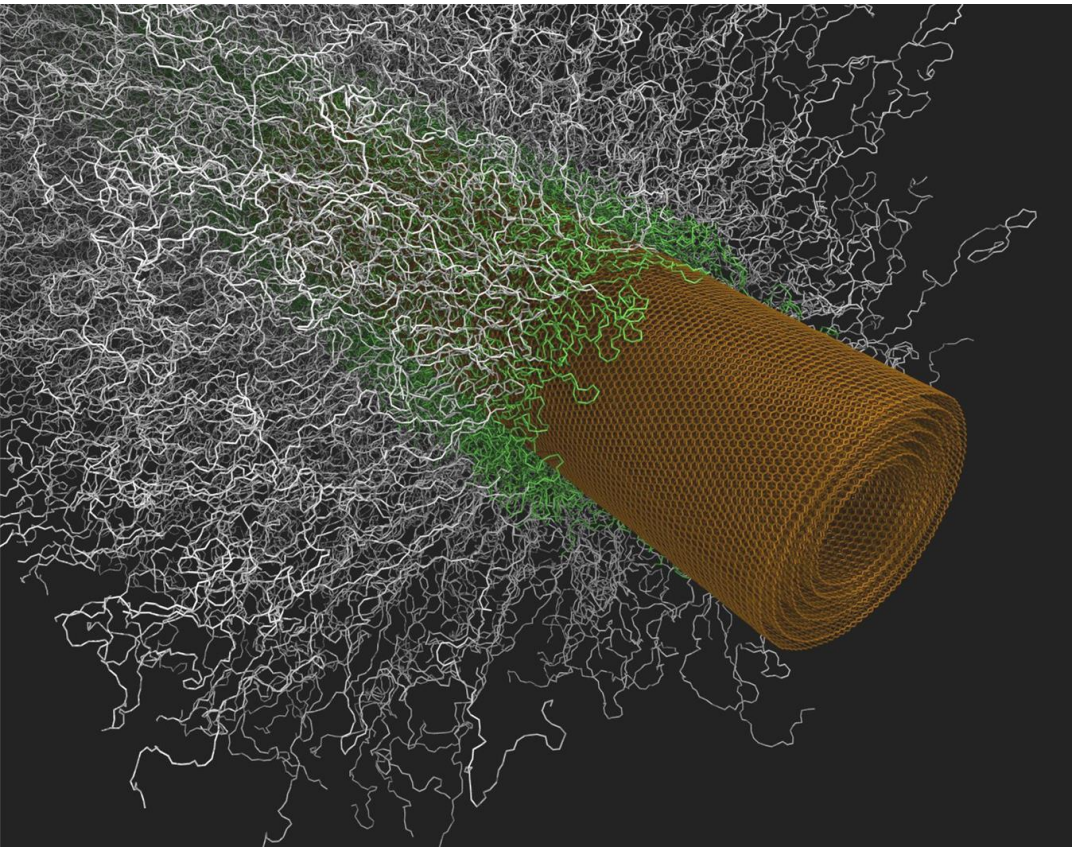
MW 14600

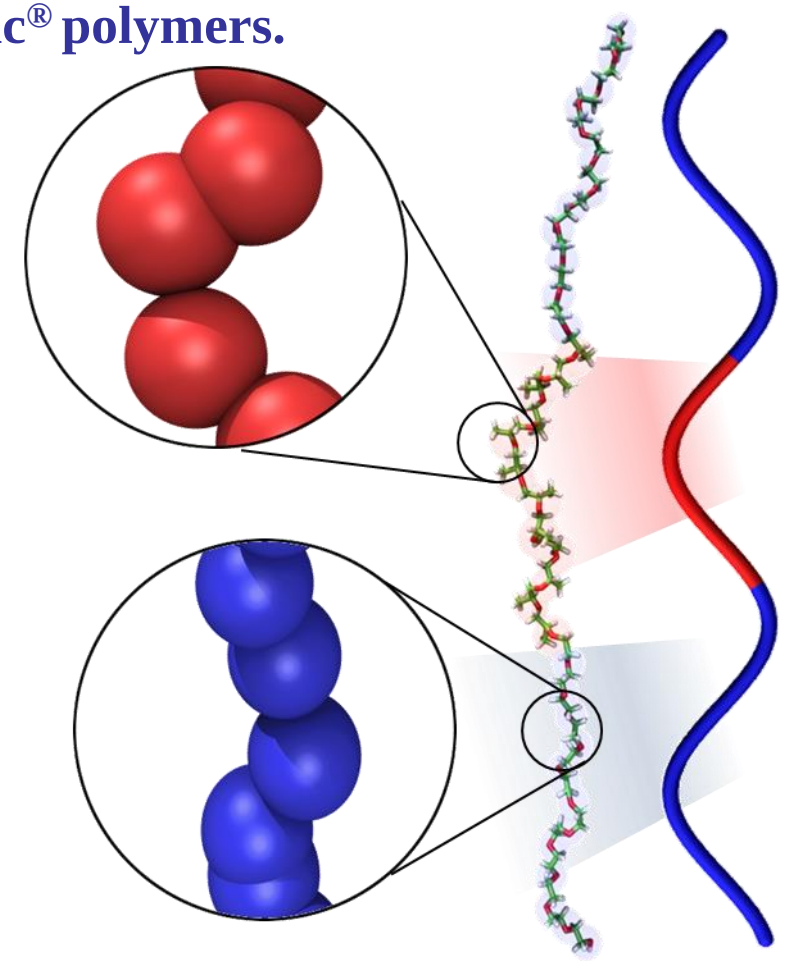




Computational Modeling

- Model molecular interactions of Pluronic® polymers with MWNTs to study dispersion and agglomeration.
- Coarse grain representation of Pluronic® polymers.





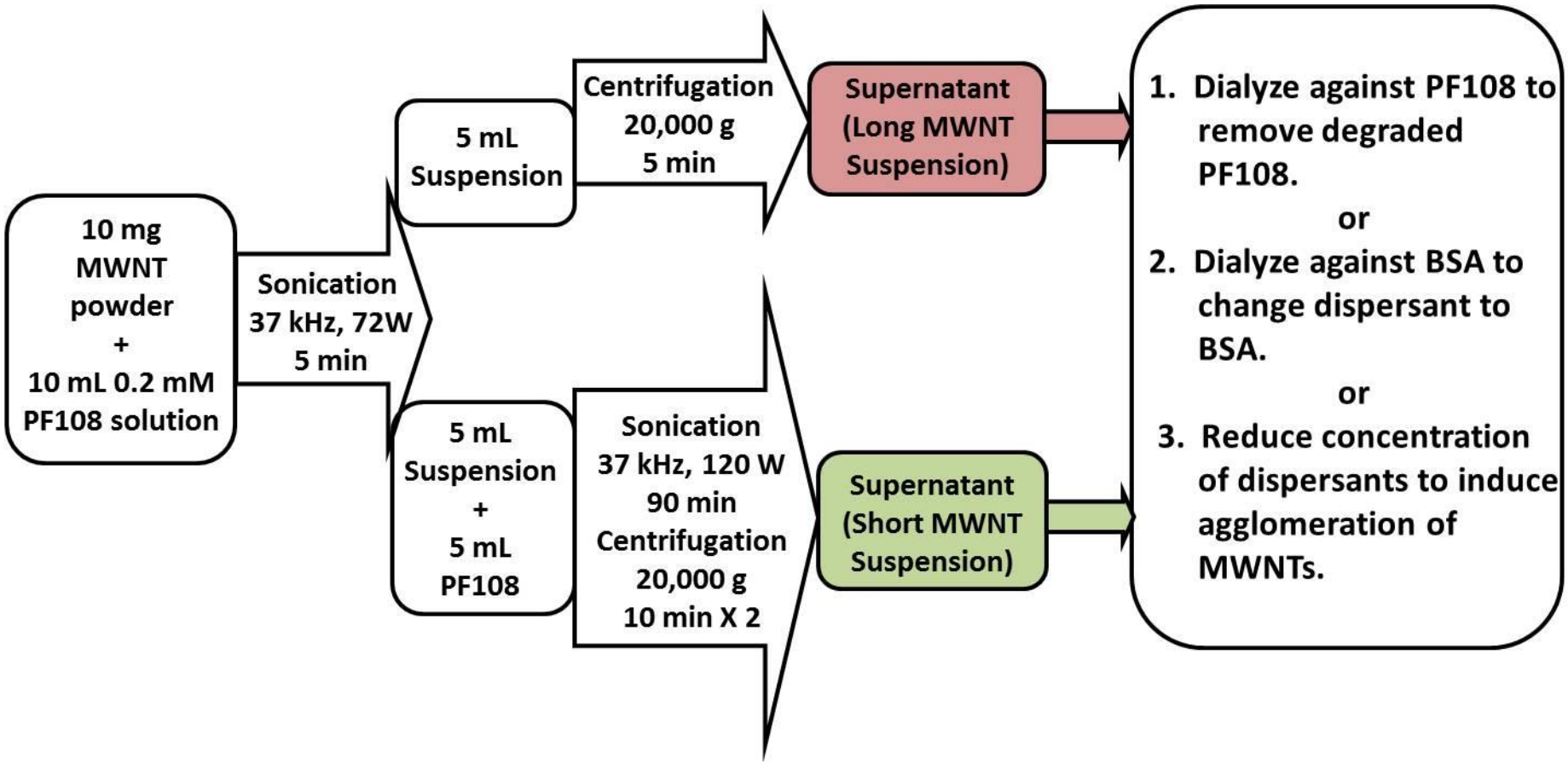
Preparing Reference MWNTs that Differ in Length, Dispersant, and Agglomeration Part 2

3. Varying sonication time and power to make long and short PF108-coated MWNTs that differ in length by 10-fold or more.

4. Centrifugation to remove aggregates and impurities.

5. Dialyze against fresh PF-108 to remove toxic sonolysis products, and/or exchange to a different dispersant, or reduce dispersant concentration to induce agglomeration.

Sonication, Centrifugation, and Dialysis

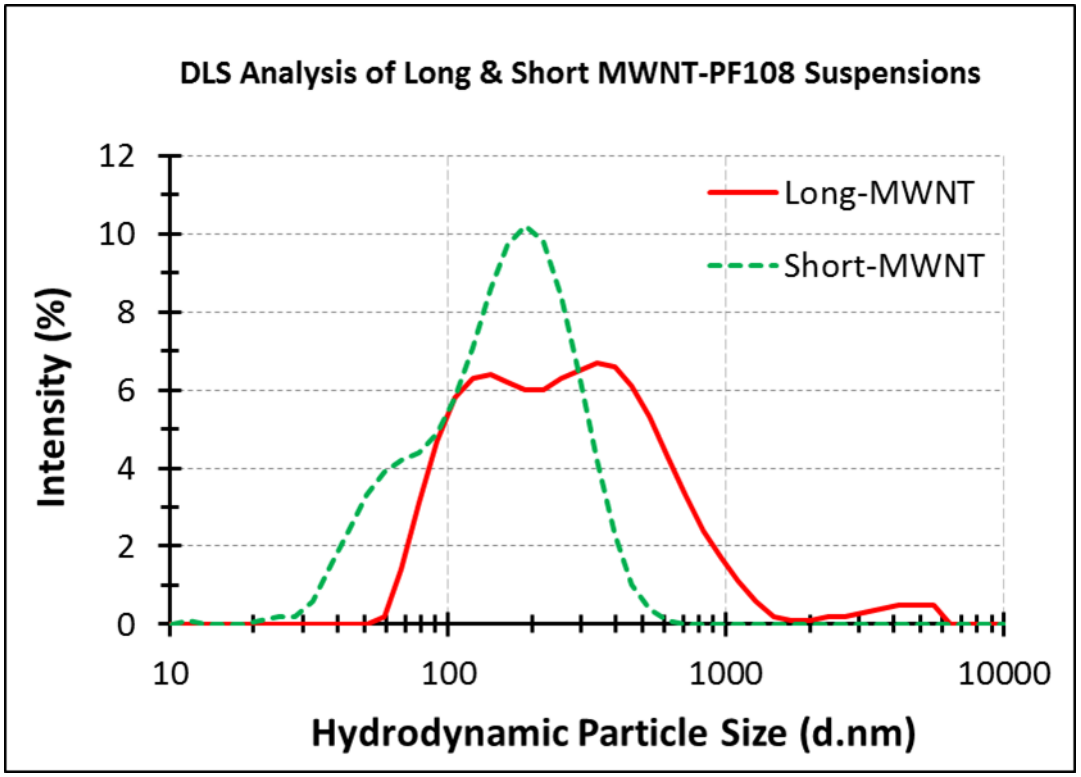


The entire procedure is done under sterile conditions so final products can be used with in vitro cell culture assays.

Length Characterization by Dynamic Light Scattering (DLS)

1. **DLS done routinely during the procedure to rapidly monitor estimated size.**

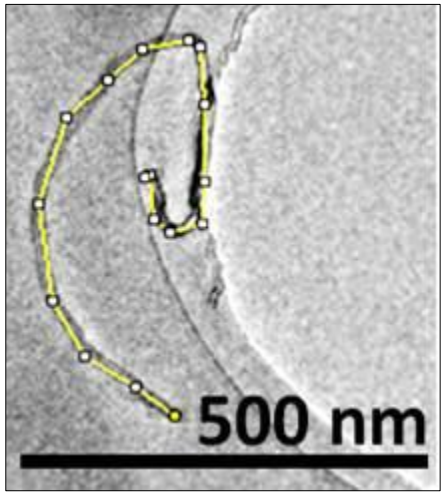
2. **Particle size distributions of final products:**

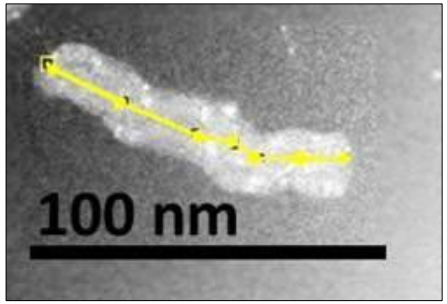


MWNT-PF108 Suspension	Average Particle Size (d. nm)
Long	215 ± 40
Short	120 ± 8

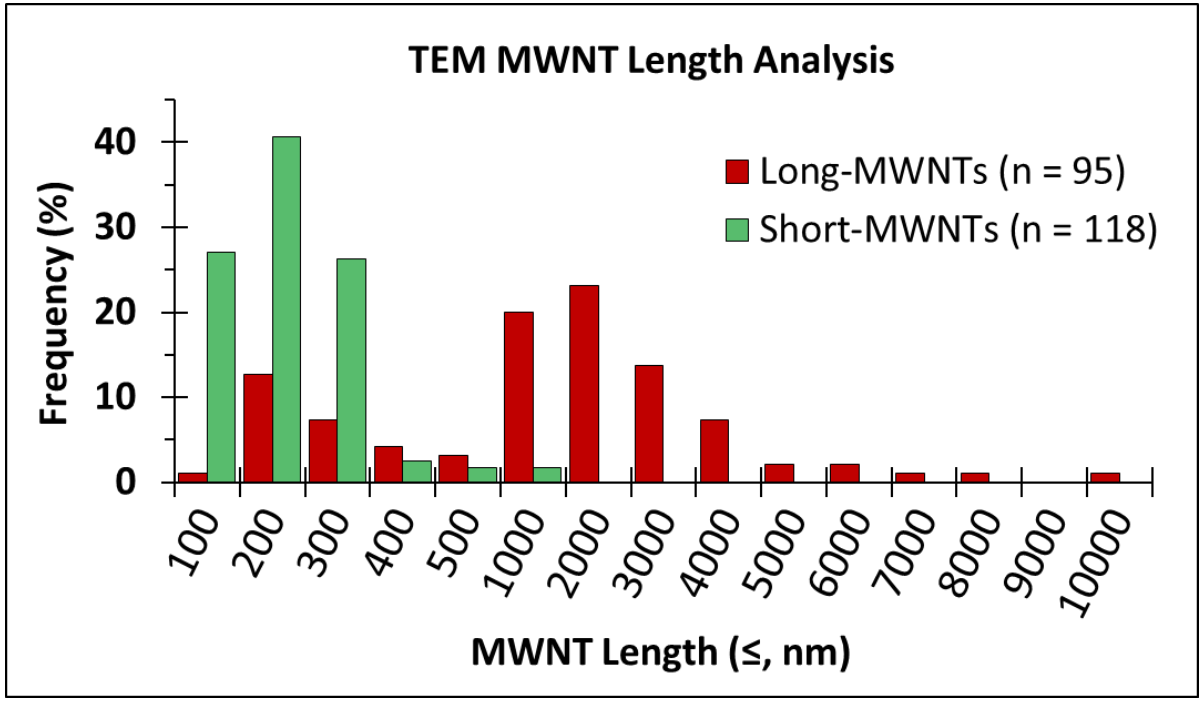
Length Characterization by TEM

Done in collaboration with Drs. Yu Yang and Paul Westerhoff of Arizona State University.



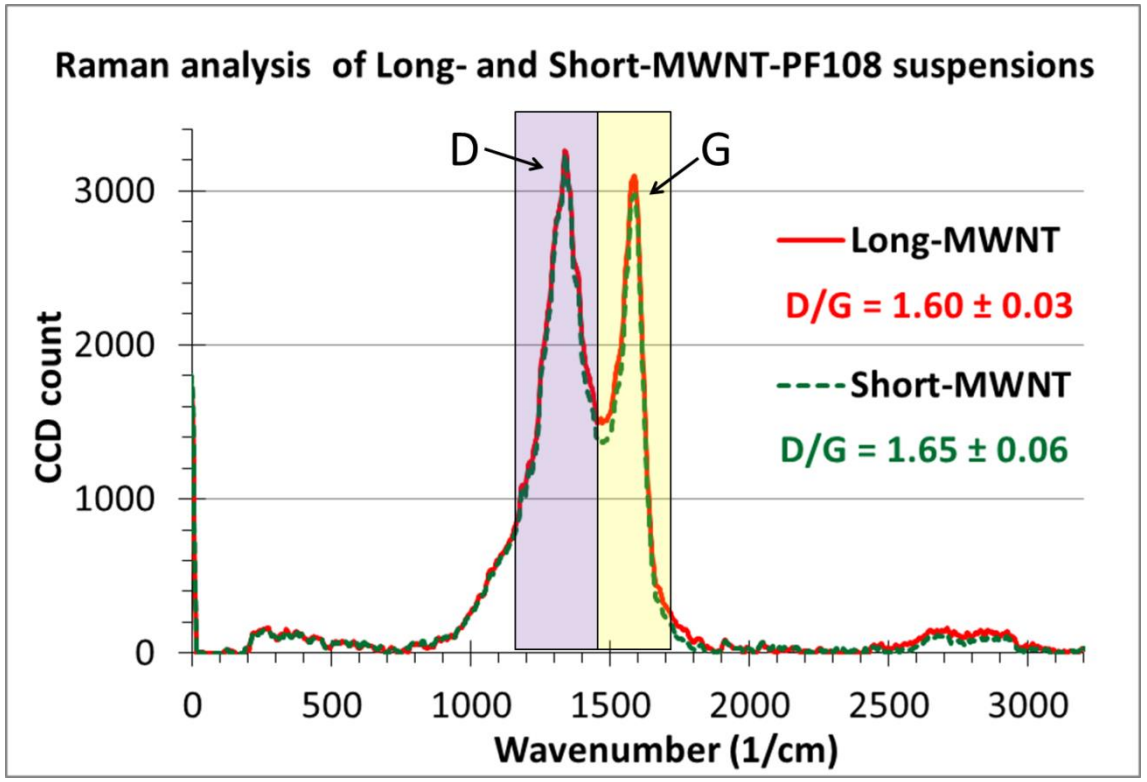


TEM MWNT Length Analysis				
MWNT-PF108 Suspension	MWNT Count	Min -Max (nm)	Average (nm)	Mode (nm)
Long	95	45 - 9512	1593 ± 1689	1001-2000
Short	118	44 - 552	167 ± 91	101-200



Characterization by Raman Scattering

Sonication of MWNTs has the potential to covalently alter the sidewall structure of MWNTs such that long and short MWNTs may differ in sidewall structure. One assay to detect differences is Raman scattering which reports on covalent sidewall modification by increasing the Raman disorder or "D" band. We found no differences in the "D" band between long and short MWNTs. More sensitive assays to detect potential structural differences are in progress.



Additional Physical Characterizations Planned by SRC Nanotox Consortium Members

1. **MWNT lengths, Arizona State University, Transmission Electron Microscopy (TEM).**

2. **MWNT crystallinity, North Carolina State A&T, X-ray Diffraction (XRD).**

3. **Validation of size, University of Arizona, Dynamic Light Scattering (DLS).**

4. **Metal impurities in MWNT samples, Colorado School of Mines, Inductively Coupled Plasma - Mass Spectroscopy (ICP-MS).**

5. **MWNT binding to lipid membranes, Johns Hopkins University, Quartz Crystal Microbalance (QCM).**

In addition, consortia members at the University of Arizona and North Carolina State A&T plan cytotoxicity assessments and the University of North Carolina at Chapel Hill will do data analysis and model building for nanotoxicity prediction.

Work in Progress and Future Work

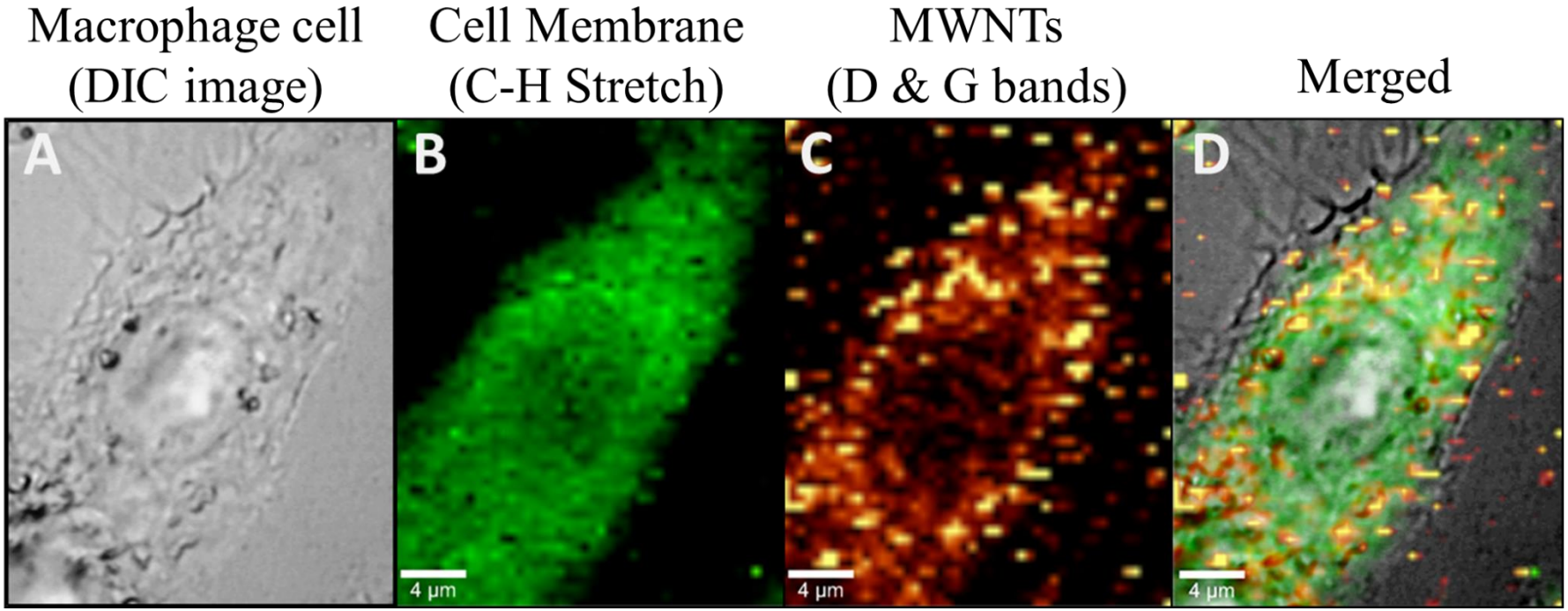
1. **Cytotoxicity with epithelial and macrophage cell models.**
   - **A549 human lung epithelial cells.**
   - **RAW264.7 mouse macrophages.**
   - **THP-1 human macrophages.**

2. **Proinflammatory cytokine secretion by macrophages.**
   - **TNFα and IL-1β.**

3. **Quantify MWNTs in cells.**
   - **Use SDS PAGE method we previously developed (Wang R, et al. 2009. Anal. Chem. 81(8):2944-2952).**
   - **Compare cytotoxicity and cytokine release with cells that have same amount of internal MWNTs.**

4. **Locate MWNTs inside cells by label-free laser scanning confocal Raman microscopy.**

MWNTs Accumulation Inside Macrophages



Industrial Interactions and Technology Transfer

- **Participated in 10 teleconferences in 2012 with consortia members and industrial liaisons.**
- **Presented two ERC/SRC teleseminars (26 July 2012 and 7 Feb 2013)**
- **Hosted two ERC/SRC teleseminars (31 May and 15 Nov 2012)**
- **Continue to interact with TI World Wide Environmental & Safety**

Future Plans

Next Year Plans

- Cytotoxicity studies with reference MWNTs.
- Proinflammatory cytokine secretion by macrophages.
- Computational and experimental studies of link between MWNT size/length, dispersant effectiveness, and cellular uptake.
- Correlate quantity of MWNTs in cells with biological assay endpoints.

Long-Term Plans

- Study uptake, bioaccumulation, and subcellular distribution of MWNTs inside cells with laser scanning confocal Raman microscopy.
- Migrate approaches to other engineered nanoparticles of interest to semiconductor manufacturers.

Publications, Presentations, and Recognitions/Awards

- **Wang, R., T. Hughes, S. Beck, S. Vakil, S. Li, P. Pantano, and R.K. Draper. 2012. Generation of toxic degradation products by sonication of Pluronic(R) dispersants: implications for nanotoxicity testing. Nanotoxicology: DOI: 10.3109/17435390.17432012.17736547.**

- **Pantano, P., R.K. Draper, C. Mikoryak, and R. Wang. 2012. Electrophoretic methods to quantify carbon nanotubes in biological cells. In Handbook of carbon nanomaterials. Vol. 3. F. D'Souza and K.M. Kadish, editors. World Scientific Publishers, Singapore. 84-107.**

- **Presented seminar in Japan at request of SRC (Draper, Jan 2012)**

- **Presented ERC/SRC teleseminar (Ranatunga and Wang, July 2012)**

- **Presented ERC/SRC teleseminar (Nielsen, February 2013)**

- **Posters at Society of Toxicology meeting (March 2012) and Sustainable Nanotechnology Conference (Nov. 2012)**

- **Students Tyler Hughes and Samee Vakil earned Undergraduate Research Awards at UT Dallas.**