2019 NanoEHS Webinar Series
Potential Respiratory Effects of Engineered Nanomaterials in Relation to Physicochemical Properties

September 10, 2019
Webinar will begin at 12 PM EDT
Audio will be broadcast through your computer's speakers

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MODERATOR
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Webinar viewers will be able to submit questions for the panelists to answer during the Q&A period. Submitted questions will be considered in the order received and may be posted on the NNI website. A moderator will identify relevant questions and pose them to the speakers. Due to time constraints, some questions may be grouped and some may not be addressed during the webinar.

>> Will Boyes: Hello, everyone, and welcome to today's 2019 the National Nanotechnology Initiative NanoEHS webinar series. My name is William Boyes. I'll be the moderator for today's seminar, and it's my pleasure to introduce Dr. Jim Zhang.

We'll do that in just a second. First just a little bit of housekeeping. We'll let Dr. Zhang present his webinar, and then at the end of that, there will be an opportunity for everyone to ask questions. We'll do that online, so please type your questions into the question cue box that will appear beside the Adobe Connect webinar. At the end we'll go through as many of the questions as we have time for.

Potential Respiratory Effects of Engineered Nanomaterials in Relation to Physiochemical Properties

Junfeng (Jim) Zhang, PhD
Professor of Global and Environmental Health

2019 NanoEHS Webinar Series
September 10, 2019

DUKE GLOBAL HEALTH INSTITUTE

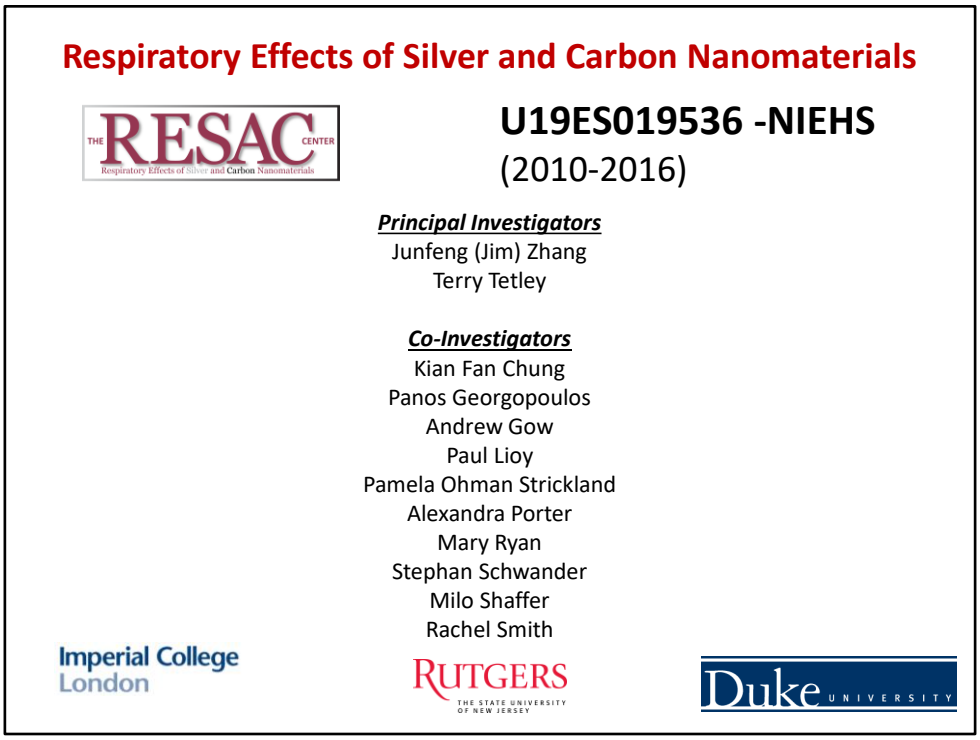
昆山杜克大学 DUKE KUNSHAN UNIVERSITY

Duke NICHOLAS SCHOOL OF THE ENVIRONMENT

>> Will Boyes: Without any further ado, Dr. Jim Zhang is today's speaker. He's a professor of global and environmental health at Duke University. Dr. Zhang's research involves exposure assessment, toxicology, epidemiology, and clinical research, all to study the health effects and underlying biological mechanisms of environmental chemical exposures. Recently he's been working on air pollutants with a focus on particulate matter and ozone. From 2010 through 2016 he led two large multidisciplinary and multi-institutional collaborations to examine potential health effects of engineered nanoparticles and nanomaterials. That's what he'll focus on for today's seminar.

Dr. Zhang is the author of more than 220 peer-reviewed publications. In 2012 he received the Jerome Wesolowski award, which is the highest award from the International Society of Exposure Science. And in 2013 he was elected to be a fellow for the American Association for the Advancement of Science.

Without further delay, it's my pleasure to introduce Dr. Zhang. The title of his talk is “Potential Respiratory Effects of Engineered Nanoparticles in Relation to Physicochemical Properties.” Dr. Zhang.



>> Jim Zhang: Thank you very much, Dr. Boyes, for that kind introduction. I appreciate everyone's attention for the next 40 minutes or so, allowing me to summarize observations or discoveries that we have done through one of the projects that Will just told you about, of the collaborative work that we call RESAC [Respiratory Effects of Silver and Carbon Nanomaterials]. You can see these are the principal investigators, from Rutgers, from the Imperial College London, and from Duke University.

These are not the only people on this effort, of course. There are many post-docs, graduate students, lab technicians, and other research staff. Their contributions are reflected in publications resulting from this effort, RESAC. This is a U19 center funded by NIEHS, National Institute of Environmental Health Sciences. The other centers---I believe there were five---three or four of them form a consortium of this larger program. I will mention some of the common work that is done by the whole consortium in addition to RESAC, my own U19 center.

Publications

1. Chen S, Goode AE, Skepper JN, Thorley AJ, Seiffert JM, Chung KF, Tetley TD, Shaffer MS, Ryan MP, Porter AE. Avoiding artefacts during electron microscopy of silver nanomaterials exposed to biological environments. J Microsc. 2015. doi: 10.1111/jmi.12215.
2. Seiffert J, Hussain F, Wiegman C, Li F, Bey L, Baker W, Porter A, Ryan MP, Chang Y, Zhang J, Zhu, J, Tetley TD, Chung KF. Pulmonary Toxicity of Instilled Silver Nanoparticles: Influence of Size, Coating and Rat Strain. PLoS ONE. 2015,10(3): e0119726. doi: 10.1371/journal.pone.0119726
3. Marchetti M, Shaffer MS, Zambianchi M, Chen S, Superti F, Schwander S, Gow A, Zhang JJ, Chung KF, Ryan MP, Porter AE, Tetley TD. Adsorption of surfactant protein D from human respiratory secretions by carbon nanotubes and polystyrene nanoparticles depends on nanomaterial surface modification and size. Philos Trans R Lond B Biol Sci. 2015, 5;370(1661):20140038. doi: 10.1098/rstb.2014.0038.
4. Botelho DJ, Bey L, Massa CB, Sarkar S, Tetley T, Chung KF, Chen S, Ryan MP, Porter A, Zhang J, Schwander S, Gow A. Low dose AgNPs reduce lung mechanical function and innate immune defense in the absence of cellular toxicity. Nanotoxicology (accepted April 2015)
5. Theodorou IG, Ryan MP, Tetley TD, Porter AE. Inhalation of silver nanomaterials--seeing the risks. Int J Mol Sci. 2014 Dec 22;15(12):23936-74 (invited review)
6. Sweeney S, Theodorou IG, Zambianchi M, Chen S, Gow A, Schwander S, Zhang J, Chung KF, Shaffer MS, Ryan MP, Porter AE, Tetley TD. Silver nanowire interactions with primary human alveolar type-II epithelial cell secretions: contrasting bioreactivity with human alveolar type-I and type-II epithelial cells. Nanoscale (Accepted April 2015)

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THE RESAC CENTER
Respiratory Effects of Silver and Carbon Nanomaterials

Publications -continued

7. Chen S, Hu S, Smith EF, Ruenraroengsak P, Thorley AJ, Menzel R, Goode AE, Ryan MP, Tetley TD, Porter AE, Shaffer MS. Aqueous cationic, anionic and non-ionic multi-walled carbon nanotubes, functionalised with minimal framework damage, for biomedical application. Biomaterials. 2014, 35(17):4729-38.
8. Hu S, Chen S, Menzel R, Goode AD, Ryan MP, Porter AE, Shaffer MS. Aqueous dispersions of oligomer-grafted carbon nanomaterials with controlled surface charge and minimal framework damage. Faraday Discuss. 2014.
9. Mukherjee D, Leo B, Royce S, Porter AE, Ryan MP, Schwander S, Chung KF, Tetley TD, Zhang J, Georgopoulos P. Modeling physicochemical interactions affecting in vitro cellular dosimetry of engineered nanomaterials: application to nanosilver. Journal of Nanoparticle Research. 2014, 16(10):1-16.
10. Mukherjee D, Royce SG, Sarkar S, Thorley A, Schwander S, Ryan MP, Porter AE, Chung KF, Tetley TD, Zhang J, Georgopoulos P. Modeling in vitro cellular responses to silver nanoparticles. Journal of Toxicology. 2014, 2014:13.
11. Royce S, Mukherjee D, Cai T, Xu S, Alexander J, Mi Z, Calderon L, Mainelis G, Lee KB, Lioy P, Tetley TD, Chung KF, Zhang J, Georgopoulos P. Modeling population exposures to silver nanoparticles present in consumer products. Journal of Nanoparticle Research. 2014, 16(11):1-25.
12. Marchetti M, Shaffer MS, Zambianchi M, Chen S, Superti F, Schwander S, Gow A, Zhang J, Chung KF, Ryan MP, Porter AE, Tetley TD. Adsorption of surfactant protein D from human respiratory secretions by carbon nanotubes and polystyrene nanoparticles depends on nanomaterial surface modification and size. Philosophical Transactions B. online 12/22/2014

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Publications -continued

13. Thorley AJ, Ruenraroengsak P, Potter TE, Tetley TD. Critical determinants of uptake and Translocation of Nanoparticles by the Human Pulmonary Alveolar Epithelium. ACS nano. 2014.
14. Sarkar S, Song Y, Sarkar S, Kipen HM, Laumbach RJ, Zhang J, Strickland PA, Gardner CR, Schwander S. Suppression of the NF-κB pathway by diesel exhaust particles impairs human antimycobacterial immunity. J Immunol. 2012, 188(6):2778-93.
15. Chen S, Goode AE, Sweeney S, Theodorou IG, Thorley AJ, Ruenraroengsak P, Chang Y, Gow A, Schwander S, Skepper J, Zhang J, Shaffer MS, Chung KF, Tetley TD, Ryan MP and Porter AE. Sulfidation of silver nanowires inside human alveolar epithelial cells: a potential detoxification mechanism. Nanoscale. 2013, 5(20):9839-47.

16. Leo BF, Chen S, Kyo Y, Herpoldt KL, Terrill NJ, Dunlop IE, McPhail DS, Shaffer MS, Schwander S, Gow A, Zhang J, Chung KF, Tetley TD, Porter AE and Ryan MP. The stability of silver nanoparticles in a model of pulmonary surfactant. Environ Sci Technol. 2013, 47(19):11232-40.

17. Chen S, Theodorou IG, Goode A, Gow A, Schwander S, Zhang J, Chung KF, Tetley T, Shaffer MS, Ryan MP and Porter AE. High resolution analytical electron microscopy reveals cell culture media induced changes to the chemistry of silver nanowires. Environ Sci Technol. 2013, 47(23):13813–21.

18. Mukherjee D, Botelho D, Gow A, Zhang J and Georgopoulos PG. Computational multiscale toxicodynamic modeling of silver and carbon nanoparticle effects on mouse lung function. 2013, PLOS One 8(12):e80917.DOI: 10.1371/journal.pone.0080917

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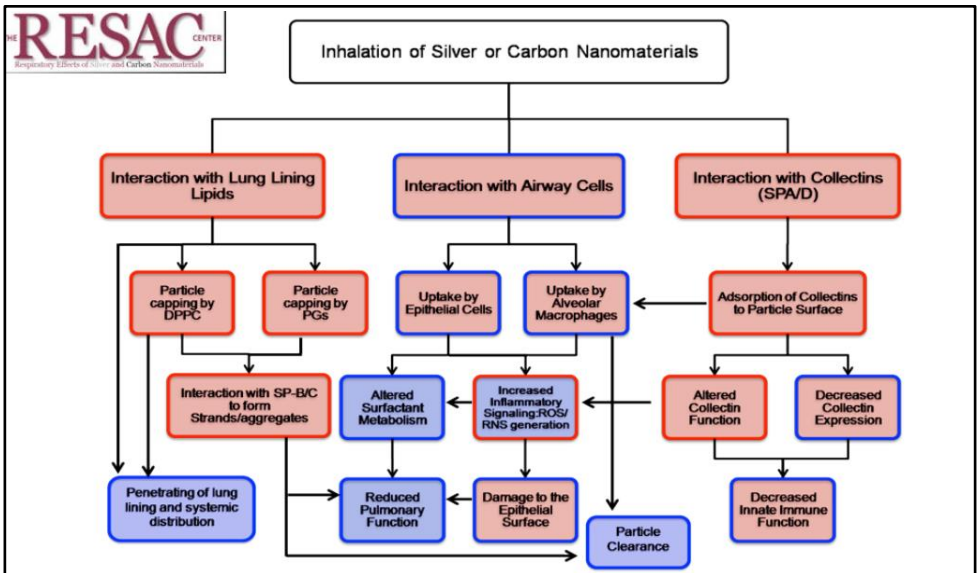
Overarching Hypotheses

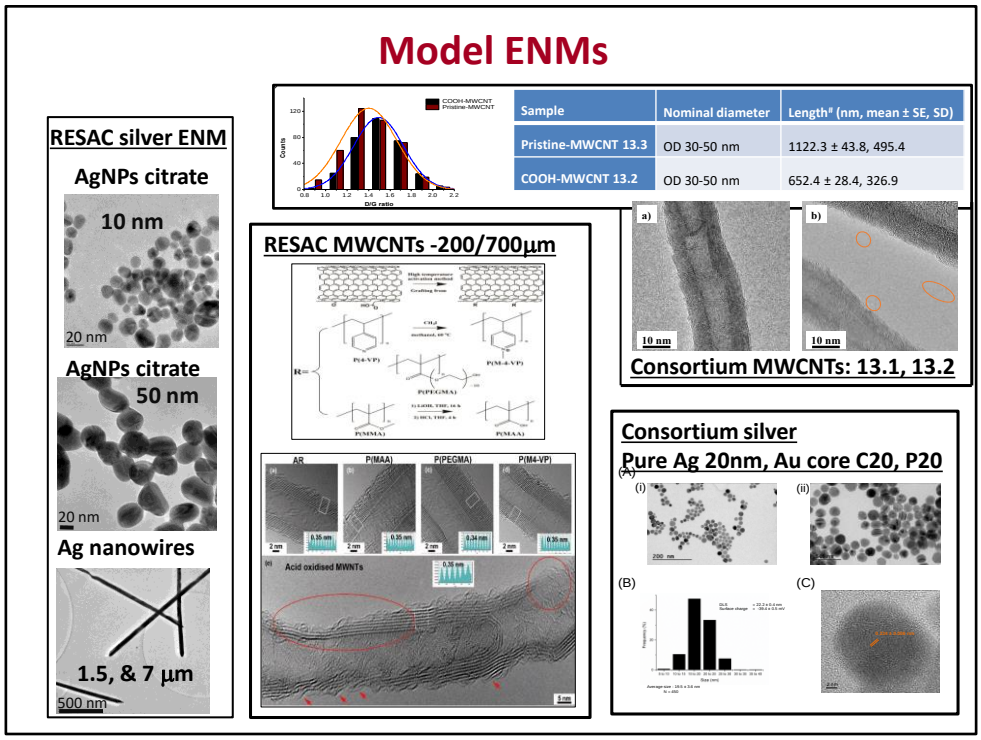
- The interaction between ENMs and the components of the lung lining fluid and cells is essential in determining lung tissue and lung function effects.
- Physicochemical properties of ENMs determine this interaction which, in turn, modifies physicochemical properties of ENMs (two-way interactions).

>> Jim Zhang: The overarching hypotheses for our particular U19 RESAC center were: (1) The interaction between engineered nanomaterials (ENMs) and the components of the lung lining fluid at the cells is essential in determining lung tissue and lung function effects; and (2) Physicochemical properties of ENMs determine this interaction which, in turn, modifies physicochemical properties of the ENMs---this is a two-way interaction between the biological components in our lungs and the engineered nanomaterials.



The conceptual model showing studying approaches. The boxes with red border and pink fill represent components of **in vitro studies**; the boxes with blue border and pink fill represent the components of **both in vitro and in vivo studies**; and the boxes outlined and filled with blue represent the components of **in vivo studies**. These processes are **mathematically modeled**.

>> Jim Zhang: This hypothesis can be seen. (I'm not sure how well you can see on the screen…) This is the conceptual model. We focused on three components. Here, one is the lung lining lipids, the other part is cells in the airways, and the third component was collectins. We focused on surfactants, proteins (especially SP-A and SP-D). Then each of the interactions going downstream determine cell uptakes, clearing mechanisms, that sort of thing: oxidative stress, inflammatory responses, all the way down to how they affect function, lung function, for example.



>> Jim Zhang: We used several selected ENMs as our model materials for testing these hypotheses. We called “RESAC” the materials that were used by our center. Some of the materials were produced by our scientific core; we do have materials scientists on our team from the Materials Science Department of Imperial College.

We used silver nanoparticles, those are spheres with different capping agents---citrate, also PVP (polyvinylpyrrolidone, I think, should be shown here---then with different diameters: 10-nanometer, 50-nanometer---and also we used short nanowires, only 1.5 micron in length or 7 micron, that's the long nanowires. Then we also used carbon-based nanomaterials to test this hypothesis because we wanted to compare different materials, silver versus carbon.

In carbon, we used carbon nanotubes with different aspect ratios. And we also tested our hypothesis (*animation*) using materials we called “consortium materials”; those are the ones that all the centers participating in this nano program are using; this was provided by the consortium. This included multiwall carbon nanotubes (MWCNTs) and silver nanoparticles, one with pure 20-nanometer spheres and the other one with a gold core material. You can see there are contrasting chemical-physical properties---they have a different surface modification, they have different aspect ratios, they have different shapes---to test the hypothesis.

Main Study Findings -1

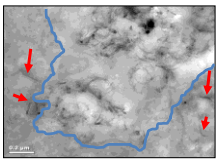
- **Physicochemical properties of ENMs markedly affect cellular uptake, bioreactivity, and tissue/organ function.**

>> Jim Zhang: I'm going to summarize several main findings, several features.

The first one is directly related to our hypothesis, which is physicochemical properties of the ENMs affect cellular uptake, bioreactivity, and tissue/organ function. In this case we focus on lung function of rodents, as you will see later.

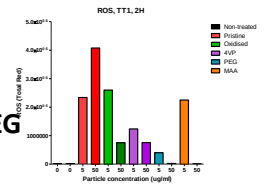
Effects of MWCNTs on Alveolar Epithelial Cells and Microvascular Pulmonary Endothelial Cells

Surface modification altered cellular bioreactivity



AT1 Cell Uptake:

Endosomal and mechanical piercing processes;
PEGMA > PMAA > AR > AO > M(4-PV)

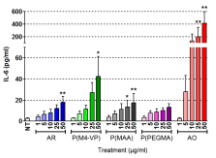


Induction of ROS in AT1-like cells:

As received > Acid oxidized = MAA > P(M4-VP) > PEG

***IL-8 and IL-6 release* (cell type dependent)**

Inhibited in AT1-like and AT2; stimulated in HPMEC.
Acid oxidized MWCNTs had the most effect.

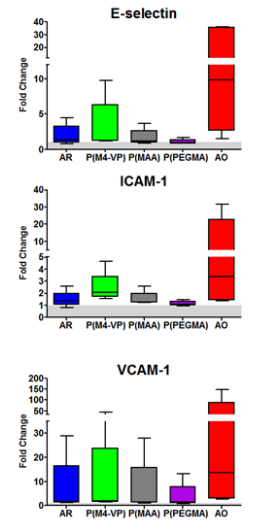


>> Jim Zhang: This slide demonstrates just how important surface modification is in terms of determining cellular bioreactivity. In AT1 cells, we look at uptake, how much material is put into the cell. This is *in vitro* work. We can see that this is a different surface modification (materials scientists would know those things). As we see, these are the ones we got from AR, from the consortium. We also get them washed with acid to get the surface a little more smooth, or purified. We find that for uptake, you can see that order of bioreactivity.

And then down here, this is the reactive oxygen species induction in AT1-like cells. You can see there, the order of bioreactivity is different from the ones above, in which the outcome is the cell uptake.

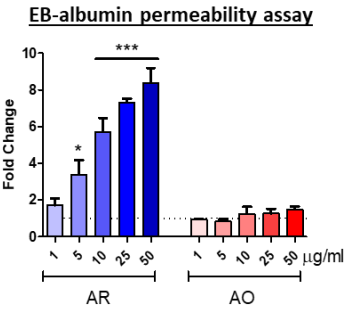
Down here, when we look at the proinflammatory cytokine release, I wanted to show that different cells have a different response. In AT1-like and AT2, cells, we see inhibitory effects, but in the HPMEC (human pulmonary microvascular endothelial cell) cells, we saw a stimulatory effect. Different surface modification had a different level of effect: acid-oxidized MWCNTs showed the strongest effect.

Surfaces of MWCNTs affect human pulmonary microvascular endothelial cells (HPMEC) cell adhesion, monolayer permeability



- **Acid-oxidized MWCNT has the strongest effect in upregulation of E-selectin, ICAM-1 and VCAM-1 gene expression.**
- **As received MWCNT had the strongest effect in disruption of HPMEC monolayer integrity.**

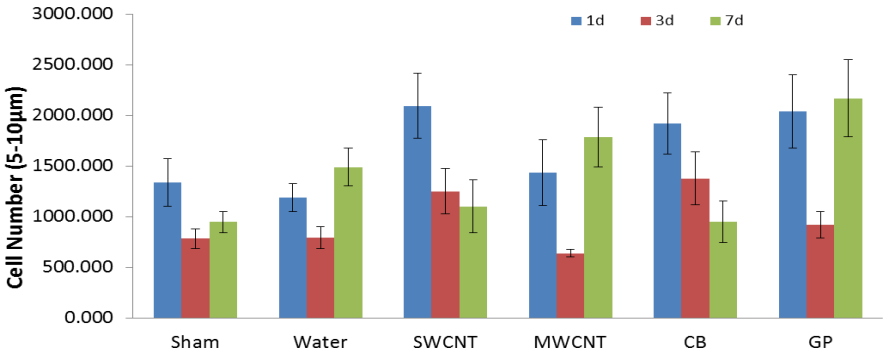
Acid oxidised



>> Jim Zhang: Here is where we really tried to compare the acid-washed carbon nanotubes with, as we see, those that were not treated, just as we got them from a commercial source. And you can see that acid-oxidized materials have the strongest effect in upregulation of the E-selectin and gene expression of the ICAM-1 and the VCAM-1. But as we see, carbon nanotubes had the strongest effect in disruption of the monolayer integrity permeability measure in this assay. So clearly, different surfaces will effect different outcomes. That's the message from this slide.

Installation of CNTs and Nanoplates into Sprague-Dawley Rats

- **Shape is important**
- **Graphene nanoplates produce a delayed 2nd inflammation (7 days)**



Airway inflammation
(mainly BAL neutrophils)

>> Jim Zhang: Here I wanted to show the shape of the material is also important. Here we have the nanoplate, this is a graphene nanoplate, another carbon material. We compared to the carbon nanotubes, single-walled nanotubes and multiwalled carbon nanotubes.

One point I wanted to really highlight from this slide is that the nanoplate seems to have a delayed inflammatory effect than other materials at Day 7---this is a single instillation study given to the SD (Sprague Dawley®) rats. Then we measure these responses, in this case, the neutrophils---mainly neutrophils, but inflammatory cells---in the lung lavage. We see one day, after three days, and seven days after the exposure, the nanoplate seems to have prolonged inflammatory effects on some of this while the others sort of decayed over time.

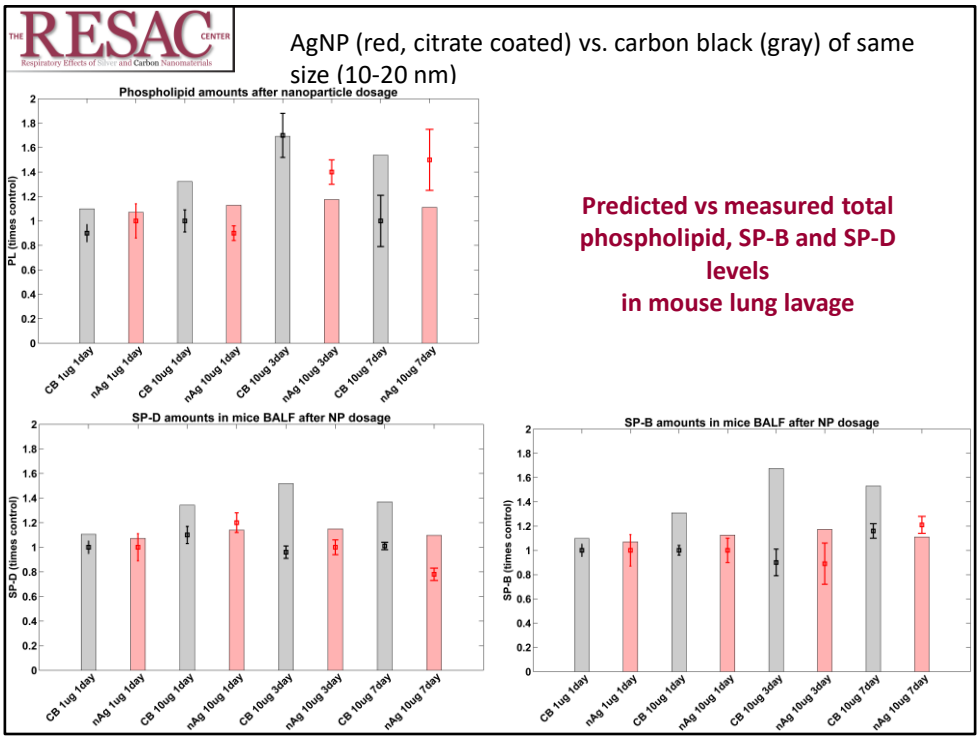
Installation of AgNP into C57-Bl6 Mice

- At doses when carbon black causes toxicity, AgNPs do not result in acute lung injury and inflammation but **acutely increase tissue stiffness** (loss of surfactant function) with delayed inflammation responses.

- AgNP functionalization and size alters interaction with lung lining components -
  - PVP coating (vs. citrate-coating) reduces self-aggregation but promotes surfactant disruption.
  - Larger size (110 vs 20 nm) favors surfactant disruption.

>> Jim Zhang: Here, this is the silver nanoparticles. We use a different model in this case; in this case, it's not rats, it's mice. And we focus on comparing silver nanospheres with the carbon black spheres. They're all, when we compare the two materials, we use the same diameter: 20-nanometer carbon black compared with 20-nanometer silver nanospheres.

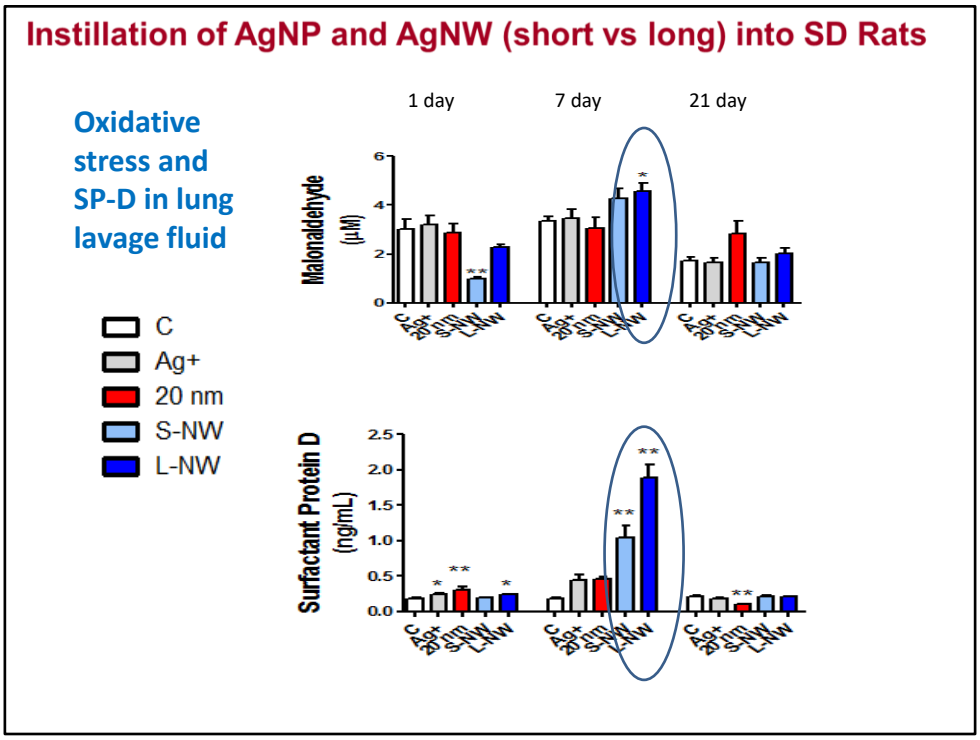
We saw that silver nanospheres do not result in acute lung injury and inflammation but acutely increase tissue stiffness. This is reflected as loss of surfactant function, and there is a delayed inflammatory response. Later as we look into the size of the silver nanoparticle effect, we find that larger size has a stronger surfactant disruption effect. And we look at two different coatings, PVP coating versus citrate coating, and PVP has a stronger effect on aggregation of the silver nanoparticles in the surfactant when interacting with the surfactant. That promotes more surfactant disruption.



>> Jim Zhang: This one is showing the effects on phospholipids and surfactant protein B and D in the mouse lung lavage, comparing the silver nanoparticles with the carbon black. (*I'm sorry, the slide is probably not very clear. The gray is the carbon black; the red is the silver particles.*) We have a different time after the exposure---this is showing that.

I have two points to show. First, the effects are different between the carbon black and silver materials. Secondly, this is showing that we have, as I mentioned, we have a computational component in the whole study. We have *in vitro* studies, we have *in vivo* studies, and we also have mathematical computational studies within the center. This mathematical model actually can predict those effects quite well.

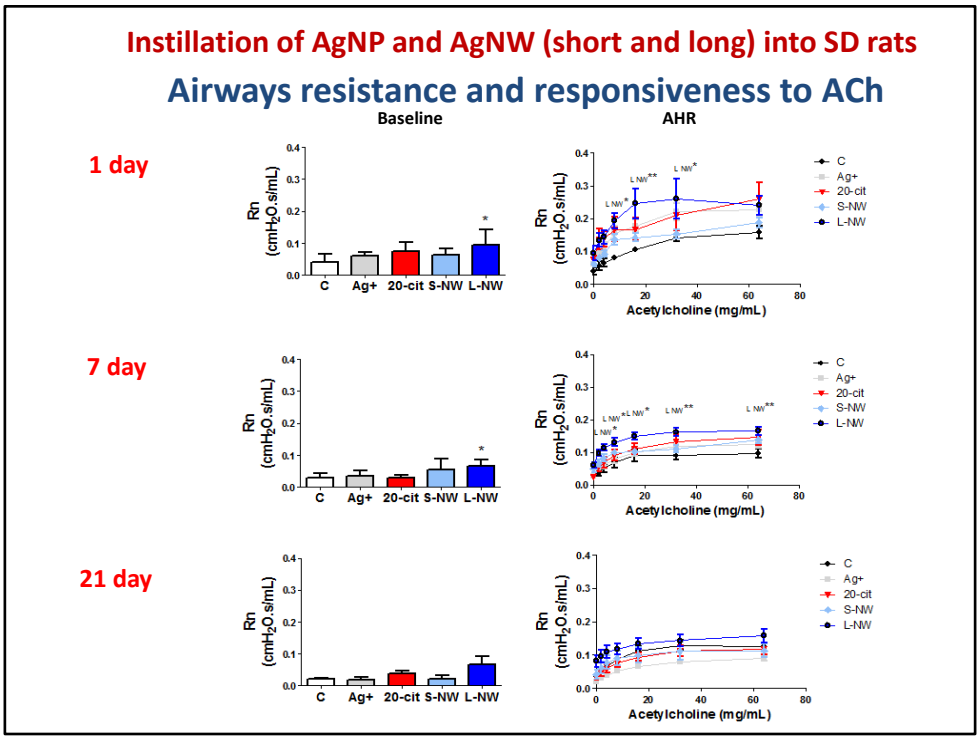
Here, the bar is showing the measured effects; if you can see the points, they are showing the predicted effects. So we are doing quite well with model predictions.



>> Jim Zhang: Here I want to show, this is all silver nanomaterials. We have a silver control, which you see here; that's just the vehicle we used in those *in vitro* studies. (*I am sorry, I tried to use the pointer, just it doesn't work very well with my computer*.)

So we have a silver ion, we have a 20-nanometer silver sphere, and we have nanowires with a short---which is about a 1.5-micron in length and a longer one, which is a 7-micron in length. So here, showing those different materials, the top one has the oxidative stress marker a lot higher in the lung lavage fluid and down here is the surfactant protein D, and at different days. One day after the instillation, this is instillation through intratracheal injection of materials into the rats.

You can see the nanowires showing effects on the 7th day, and the effects persist longer than the spheres and the ion.



>> Jim Zhang: This is the same study but a different outcome. In this case, we measured lung function and with the same timeline--one day after exposure, 7 days after exposure, 21 days after exposure--the left panel is showing the airway resistance at baseline, that means there's no acetylcholine challenge.

Just look at the airway resistance: you can see that the very right bar, the blue bar--that is the longer nanowire--appears to have the largest effect. And then the right panel--that's the airway resistance after the rats were challenged with acetylcholine--we're looking at the responses.

It's hard to see this line, but the message is that the longer nanowire had the strongest effect in terms of increasing airway resistance, which means the airflow limitation was worsened, which is not good for breathing.

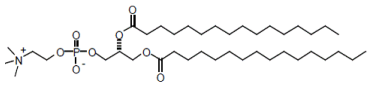
Study Findings -2

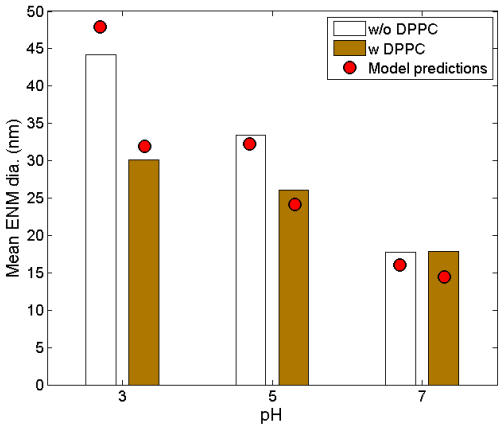
- **Pulmonary surfactants affects dissolution, internalization, and physiochemical properties of ENMs**

>> Jim Zhang: So now, the second part is the finding that this interaction between pulmonary surfactant and engineered nanomaterials, that surfactant is going to affect dissolution, internalization, and physicochemical properties of nanomaterials.

Extension of Agglomeration, Diffusion, Sedimentation, Reaction Model (ADSRM) to biologically relevant systems

Change in mean ENM agglomerate size with and without phospholipids

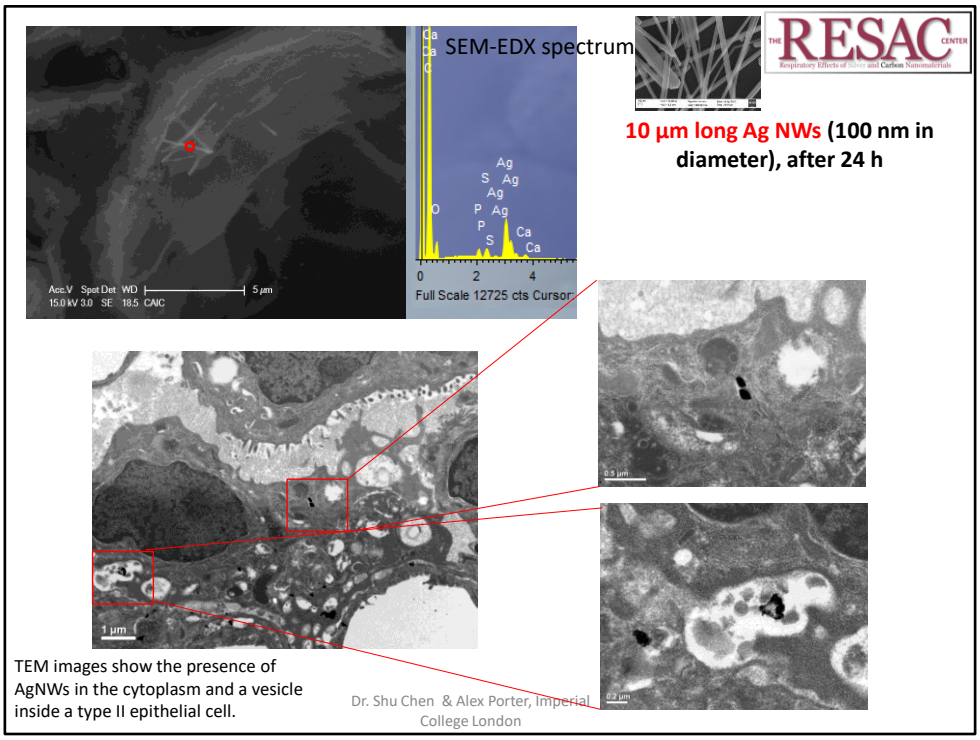




In vitro measurements for citrate-stabilized AgNPs from Leo *et al.*, *ES&T* 2013

>> Jim Zhang: Start with the effects, here, showing on the phospholipids of airways--this is simulating airways in an *in vitro* assay.

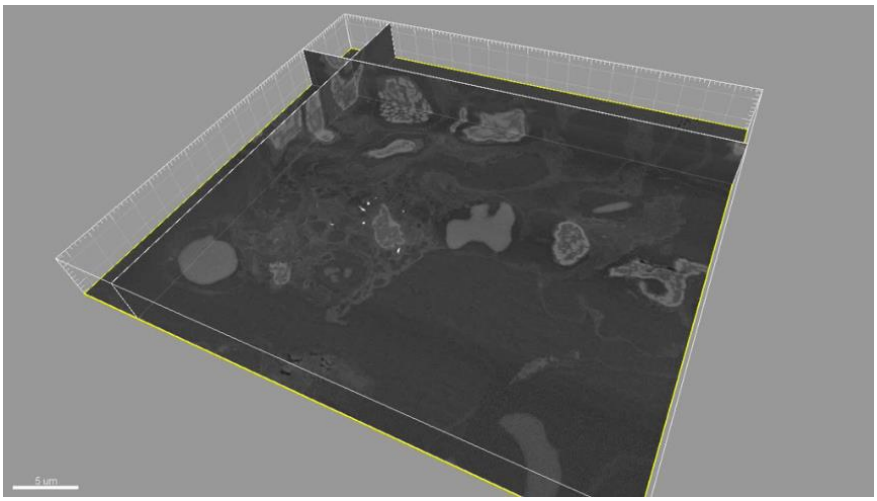
We put, in this case, silver nanoparticles in DPPC (dipalmitoylphosphatidylcholine) with different pH levels. You can see that with the different pH of this DPPC, the diameters of the nanomaterials are different; so the lower the pH, the more agglomeration occurred with the materials that we have put into the system. So the surfactant in the lung, the airway surfactant, the fluid, is going to affect what happens to the materials that get into the airways.



>> Jim Zhang: This is a TEM [transmission electron microscopy] image showing 10-micron (this is very long; we use 7 to 10, and it's hard to make it precisely, but it's between 7 to 10 microns) silver nanowires after instillation to an SD rat. These are the type-II epithelial cells harvested from the lung of the rat. Then we put this under a microscope and try to see if we can find this material.

We did see--it's hard to see the images--but we did see the wire inside the cytoplasm. But probably you can see a little bit better, I hope, with this movie. Let me see if this is going to work.

10 µm silver nanowires inside the deep lung inside alveolar type 2 cells at 24 hrs. 3D-reconstruction movie.



Shu Chen, Alex Porter, Jeremy Skepper

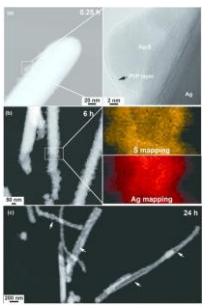
>> Jim Zhang: This is a 3D reconstruction of the image that shows the long nanowires ended up in the type-II cells. This is 24 hours after exposure in the rat lung. You can see the wires inside of the cells and also in between the cell spaces.

So this is showing that this kind of material with this shape is able to get internalized within the cells. So that has implications for the downstream cytotoxicity and bioreactivity.

Ag nanowires (AgNWs) Sulfidation

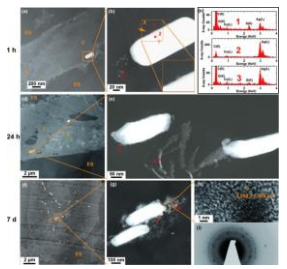
Extracellular

AgNWs became sulfidized over time in DCCM1 medium, due to solutes (not protein) in the medium



Intracellular

AgNWs within AT1 like cells generated Ag daughter particles during dissolution; both AgNWs and daughter particles became sulfidized.



>> Jim Zhang: We have thought about, as I said, what happens to these materials, the human body, our biological system? We've got to find a way to detoxify or get rid of these materials. We know that silver, if it gets into silver ion (form), that is water-soluble; whatever is going through the system probably gets excreted some way.

But this wire in the lung, the solubility or dissolution is not that great; we do see that even a day after exposure, they are still stuck in the cells. So is there any way that we can try to deal with this material? I think one thing we did is extracellular (using the left panel figure), using the a DCCM-1 medium (a serum-free medium with a high protein formulation for maximizing cell growth) that mimics some fluid in the airways. When we put the nanowires in the medium, *in vitro*, and incubated for a day, 24 hours, we do see that at the end we can measure silver sulfide. So the nanowires became sulfidized.

Then the right panel, those are the studies that put it, also *in vitro*--this is in AT1-like cells---which are the cell lines of Dr. Tetley from Imperial College, who led this work---showing that these nanowires generated what we call “silver daughters”. They are different from the beginning shape and aspect ratio and got shorter and also stickier, into one bundle, that sort of thing. Then they all became sulfidized after several days.

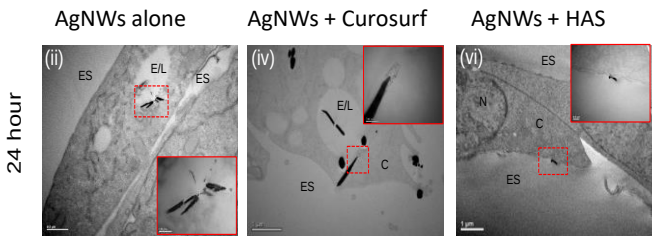
- **Ag nanowires are internalized in the cells and sulfidation may be a detoxification mechanism.**

>> Jim Zhang: We feel that sulfidation is a detoxification mechanism for materials like wires; they're probably not initially readily dissolved. But this way they become silver sulfide, and maybe that's a way to deal with this in terms of detoxifying, potentially.

Treatment of AgNWs with Human AT2 Cell Secretions (HAS) and Curosurf: Differential Effects on AT1-like Cells

AT 1 like cells:

AgNW alone	AgNWs in endosomes + cytoplasm
AgNW+Curosurf	AgNWs in endosomes only
AgNWs+HAS	AgNWs not internalized



>> Jim Zhang: Here, I want to show further the importance of surfactants in determining internalization of nanomaterials. We used AT1-like cells, and if just nanowires alone are put into cells, we find that those nanowires internalized in endosomes and the cytoplasm of the cells. But if we added Curosurf, which is a surfactant derived from the pig lung, used as a model surfactant, then we don't even see the nanowires, internalized in endosomes but cannot get into the cytoplasm. If we use human AT2 (alveolar type-II) cell secretions (HAS), we cannot see nanowires internalized at all in AT1-like cells. So that means it's a very important surfactant, as we expect, to protect us from foreign materials getting into the lung.

Instillation of MWCNTs into C57-Bl6 Mice

- MWCNTs result in acute loss of surfactant phospholipid and increases in lung tissue stiffness.
- MWCNTs increase SP-D production in a delayed fashion (3 days).
- Changes in lung function in response to MWCNTs are accentuated by lack of SP-D (SP-$D^{-/-}$ mice)
- ***In vivo*, the presence of SP-D is needed for clearance of MWCNTs.**

>> Jim Zhang: Further, we used multiwalled carbon nanotubes in a mouse model; this way, we're looking into the interaction with the lung surfactants. The nanomaterials, carbon nanotubes, result in acute loss of surfactant phospholipids and increase the lung tissue stiffness, and increase SP-D (surfactant protein-D) production in a delayed fashion compared to the effect we saw in the first bullet.

Then we used an SP-D knock-out mouse, so they don't have SP-D. Then we saw a stronger effect on the lung tissue stiffness and loss of the phospholipids. So, we believe that the SP-D is very important in carbon nanotube clearance from the lung.

- **Pulmonary surfactants subdue bioreactivity of Ag ENMs and MWCNTs, possibly reflecting binding of surfactant components.**

- **Surfactants used in our studies:**
  - **Curosurf: a sterile, non-pyrogenic pulmonary surfactant**
  - **Human alveolar secretions**
  - **SP-D knock-out mice**

>> Jim Zhang: In summary, pulmonary surfactants really subdue bioreactivity of silver nanomaterials and the multiwalled carbon nanotubes. We believe that's probably reflecting binding of the surfactant, where the materials will bind to the surfactant; that is one way to reduce the effects of the nanoparticles. The other way is that these materials will deplete the surfactant, so that's what we see the earlier slides are showing, the materials can cause loss of SP-D.

Summary on AgNPs

Silver nanospheres bind to surfactant and taken up by Type I cells and macrophages

- Larger size, more dispersion, and hydrophobic functionalization →
  - increase surfactant association
  - Increase lung tissue stiffness
  - Increase loss of SP-D
- Association (binding) with surfactants, not inflammation, mediates loss of lung function.

>> Jim Zhang: The final several slides I want to present in a different way. This is just a summary of some of the materials we tested, two groups.

Of the silver nanoparticles, *silver nanospheres* bind to surfactant taken up by type-I cells and macrophages. We find that larger size, more dispersion, and more hydrophobic surface functionalization are associated with increased surfactant association, increased lung tissue stiffness, increased disruption or loss of SP-D---an association which we believe is largely binding with the surfactants and not inflammation---and mediates loss of lung function. Because we did measure lung function of rats and lung function of mice, we believe the lung airway resistance got increased. That's mainly due to the binding with the surfactants because, especially at lower dose, we don't see a whole lot of inflammatory response in the lung. But we do see loss of lung function.

Summary on AgNW

- **Silver nanowires** appear to be internalized in type II cells
- Increased oxidative stress (MDA in BAL)
- Increased SP-D production
- Sulfidation in lung lining fluid may be a detoxification mechanism.
- Functional responses yet to be examined.

>> Jim Zhang: For the *silver nanowires*, I want to summarize, nanowires appear to be internalized in type-II cells, and they increase oxidative stress, they increase SP-D production, and the effects of nanowires compared to the nanospheres seems to be more persistent. Sulfidation in lung lining fluid may be a way to clear those materials. Functional responses we had yet to do before the project ended. We still have lots of hypotheses, lots of questions to answer. Of course, that happens to all the studies; we finish something, there will be more questions to come up.

Summary on MWCNTs

- **MWCNTs** that associate less well with surfactant phospholipids bind to SP-D
  - SP-D association results in inflammatory activation that can persist.
  - Lung function changes are consistent with acute inflammatory response.
- **Graphene plates** produce lung collapse and delayed inflammation.

>> Jim Zhang: For the multiwalled carbon nanotubes, we find that these materials, if they associate less well with the surfactant phospholipids, they will bind to SP-D more strongly. SP-D association is the association that results in inflammatory activation that can persist days after the exposure. Lung function changes are consistent with acute inflammatory responses. Graphene plates produce lung collapse and delayed inflammation. The effects are in some ways quite different from the carbon nanotubes because the shapes are very different.

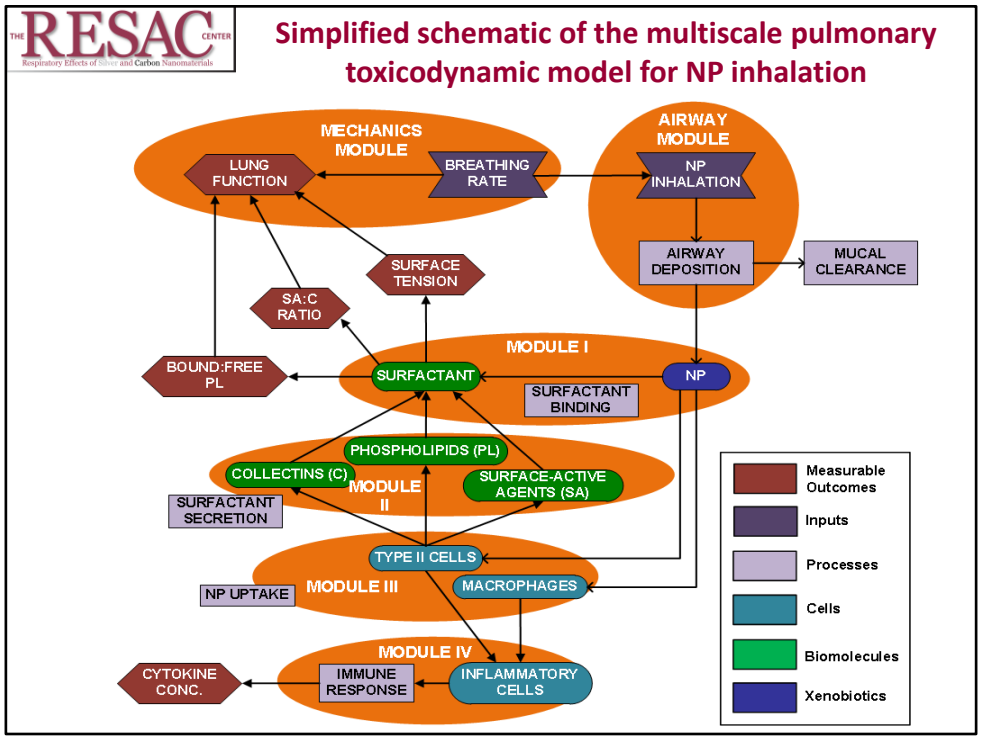
Conclusions

- The interaction between ENMs and the lung lining fluid and cells alters ENM physicochemical properties (dissolution, sulfidation, etc), consequently affecting cellular uptake, cytotoxicity and bioreactivity.
- ENM cytotoxicity, bioreactivity, and lung function effects depend on size, shape, and surface modification. This can be predicted using mathematical models.
- The magnitude and profile of biological response depend on cell types and animal models. Physiological parameters for different animal models explains the response difference well using mathematical models.

>> Jim Zhang: So, overall conclusions. The interaction between engineered nanomaterials and the lung lining fluid and cells alters original ENM physicochemical properties, including dissolution, sulfidation--that's chemistry, chemical reactions we would say--consequently affecting cellular uptake, cytotoxicity, and bioreactivity (bioreactivity meaning non-ROS [reactive oxygen species]-generation inflammatory response).

The engineered nanomaterials' cytotoxicity, bioreactivity, and lung function in rats and mice---the effects depend on size, shape, and surface modification. This can be predicted using mathematical models.

The magnitude, the profile of the biological response, depends on cell types, animal models. Physiological parameters with different animal models probably explain the response difference while using mathematical models.

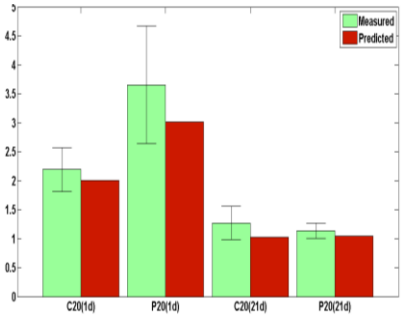


>> Jim Zhang: I didn't get a chance to really show a lot of results about the differences or comparisons between results from different strains of rats, but I would just make a point here in the context of this toxicodynamic model that we used to model what happened with this material inside the lung, all the way from surfactant disruption, inflammatory responses, to lung function being measured as airway resistance and elastance.

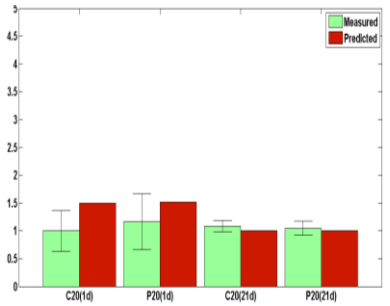
THE RESAC CENTER
Respiratory Effects of Silver and Carbon Nanomaterials

Comparison of measured and predicted lung function (Elastance at PEEP=3) *fold* changes following AgNP installation

Brown Norway rats



Sprague-Dawley rats



C20= citrate AgNP 20 nm, P20 = PVP AgNP, 20 nm

>> Jim Zhang: Here you can see that the two types of rats, Brown Norway, which has more hyperreactive lung compared to the Sprague-Dawley rats. In this case, we use citrate to cap silver nanoparticles, 20-nanometer, and also PVP. This point is already made: if you have different capping agent the responses are different. But here you can see, overall, the changes of the lung elastance; that's what's showing here. This reflects how flexible the airways are in the lung, and you can see that the effects are larger in the Brown Norway rats than in the S-D rats (the scales of the two chart graphs are the same). The point is that the models developed by Dr. Panos Georgopoulos really predict quite well what we measured in our studies.

>> Jim Zhang: Thank you very much for the attention, and I hope we have some time to answer questions.

Q&A Session

Moderator: Will Boyes

>> Will Boyes: This is Will Boyes again. Thank you, Jim. That was a really fascinating seminar. I am very impressed by what you've been doing. So for listeners on the webinar, now is question time. There should be a field on your screen where you can type in questions and we'll try to field them as they come in. In order to get things started, I have a couple of questions to just get us going…

Q/A

What effect does the surfactant have on the type-II alveolar cells?

>> Will Boyes: Maybe I'll ask about the surfactant. I found that really interesting. Is that an effect on the type-II alveolar cells? Do you think it's suppressing the generation of the lung lining fluid surfactants? In the movie it looked like the silver bars were going into the type-II cells. Is that what you're thinking?

>> Jim Zhang: The first question is the surfactants. I want to make it clear to say that we're looking at two things: One is, we got into *in vitro* studies or *in vivo* studies with different cell types; we tried to see what material is going to affect the concentration of the surfactant. At the same time, we want to look at how the surfactant is going to affect the size and dissolution of the materials. So your question was, first, to clarify that?

>> Will Boyes: Are they suppressing the generation of the surfactant, or reacting with it after it gets out into the lung?

>> Jim Zhang: They sometimes actually stimulate the production of the surfactant. So we find, I think it's in AT2 cells, the concentration actually is increased after adding the materials. But also, we look at surfactant function. Dr. Andrew Gow is really the expert on surfactant biology. The function disruption, the loss of function, was another measure that, clearly, the materials will affect that. In terms of a little bit downstream, bioreactivity---normally in the addition of surfactants, the presence of the surfactants---subdued those effects.

Q/A

How was the model parameterized, what data went into it; what are the inputs and outputs; what predictions can you can make? What is the status of the model now?

>> Will Boyes: Interesting. I don't see any other questions coming in yet, so listeners, please feel free to join in. Jim, let me ask you about the model; that also was very interesting to me. Can you tell us a little bit more about how it was parameterized, what data went into it, and what sort of inputs and outputs, what predictions can you make?

>> Jim Zhang: Yes. This is work led by Dr. Panos Georgopoulos at Rutgers. He has been working on a system he calls a modeling system. He is working with different models. I did not present them here, but he also worked on an exposure pathway model, that sort of thing. Once inside of the biological system, he also has different models. One model is about cell uptake, very detailed, how the diffusion, how the sedimentation, all these processes, the physical process. They have this fundamental chemistry-physics-biophysics-biochemistry theory in developing the models.

(*continued…)*

Q/A

How was the model parameterized, what data went into it; what are the inputs and outputs; what predictions can you can make? What is the status of the model now?

(*continued*)

>> Jim Zhang: [*continued*] Then the parameterization is very important. What our approach was, use lots of experiments from our own work and use the experimental data to try some parameters, coefficients, that kind of thing; whether theoretically driven or from other previous work, we have something, and we use this to use our own data coming in to see whether those coefficients are still appropriate for the system that we're working with.

So lots of that kind of coefficients or constants, you modify it, use new data coming in; the goal is to make the parameters as generalizable as possible, reflecting/considering different biological systems and different cell types, different levels of surfactants, that sort of thing. I can't speak too much because this is really a lifetime work of Dr. Georgopoulos. But they are getting very sophisticated about the model systems. They use this for nanomaterials, they use this for other chemical compounds that are gases or have different chemical, physical properties.

Q/A

When they're functionalized, you're adding a lot of hydroxy fluids to the structure of the outside of the nanotube. Do you think that makes it more water-soluble, or do you think they're more active because they're more reactive?

>> Will Boyes: I wanted to ask you about the functionalization of carbon nanotubes too. I thought it was very interesting that in some cases the functionalization causes them to be more active and in other cases less so. So when they're functionalized, you're adding a lot of hydroxy fluids to the structure of the outside of the nanotube. Do you think that makes it more water-soluble, or do you think they're more active because they're more reactive? Do you have any ideas about that?

>> Jim Zhang: Yes, both. I think there's functionalization, it sometimes makes the material more water-soluble, polymer added, oxygen molecules, and so chemistry is very important. Sometimes it’s really affecting the solubility, sometimes it's more about redox potential---some of the chemical substances are more redox-active---we only got to the point that we set those experiments and observed the findings. But I think having an in-depth understanding of exactly what happened, that requires additional work and we haven't got to that yet.

Q/A

What are the dimensions of the silver nanospheres studied, and what are the diameters of both the silver NWs and the multiwall carbon nanotubes?

(*Question continues on next slide*.)

>> Will Boyes: We have a couple of questions coming in from the viewers: “Thank you for your presentation. What are the dimensions of the silver nanospheres studied and what are the diameters of both silver, the NWs and the multiwall carbon nanotubes?”---I guess those are the silver nanowires in the multiwall carbon nanotube---”Can your study exposing these nanomaterials to rats be extrapolated to occupational settings?”

>> Jim Zhang: Okay. (*Let me try to see this very small font*.) First of all, what are the dimensions of the nanospheres studied? The spheres, which really measure the diameter: 20 nanometer, 50 nanometer, some types we studied were a little bit larger. I think one set of studies did 110, that was extreme, to see how the size can affect the effects. What are the diameters of both the nanowires? The diameters of the nanowires, if I recall correctly, was about five nanometers, in that range, so very fine. The length is about one to ten, 1.5 to ten microns. But I recall it was very hard to make them very precise; at the time, materials scientists spent quite a bit of time getting us the best materials to study.

Q/A

Can your study exposing these nanomaterials to rats be extrapolated to some occupational settings, and why?

>> Jim Zhang: (*Reading from screen question*) Can your study exposing these nanomaterials to rats be extrapolated to some occupational settings, and why?

That's a very good question. I think there are a few things to think about. For one, the exposure relevance. Another one is the biological response relevance. Biological response relevance: rats and humans have a different physiology, that sort of thing. It's always been a challenge to directly translate the findings from rodents to humans. I think most of the studies really help us to understand the mechanisms, although the degree of effect probably will be quite different between humans and rodents, but some fundamental biochemical molecular mechanism probably will be similar, for one thing.

The other thing, the exposure relevance, I would say that the silver nanoparticles, they are through inhalation exposure. We know that they are in lots of consumer products, spray products; but we do see that carbon nanotubes are probably more of an occupation exposure issue.

Q/A

Does sulfidation occur only with the silver fibers or also with the silver nanoparticles?

>> Will Boyes: We have a question about sulfidation. Does it occur only with the silver fibers or also with the silver nanoparticles?

>> Jim Zhang: The silver nanoparticles, we actually didn't do much, but we did compare the silver nanospheres with the wires. The sphere ones (when we were putting them in the medium), much less sulfidation occurred. We guess one of the reasons was the spheres easily get dissolved (or dissociated, we don't know the detailed mechanisms), and probably didn't really need to form sulfide. So when we compared the silver sulfide between the wires and spheres, there was much less in the spheres.

Q/A

How might your findings in the animal models extrapolate to humans?

>> Will Boyes: We have about two more minutes. I don't see new questions, but I think there was a question that got grouped into another question in your response on the different animal models and how might that might influence your ability to extrapolate to humans (assuming that you're talking about the rats)…

>> Jim Zhang: Right. Just speaking to the Brown Norway rats and the Sprague Dawley rats---this work was led by Dr. Fan Chung. He is really a pulmonary-asthma clinician scientist, so he's very much interested in this because Brown Norway rats are more hyper-responsive to any stresses to the pulmonary tract. He considers that Brown Norway rats have lungs more like people with asthma. We did try to see if this nanomaterial is going there, if the lung is more responsive, especially to oxidative stress-generating materials, that sort of thing. So we do see they are responsive in different ways. Sometimes the Brown Norway rats are less responsive to materials with certain features than to others. It's not clear cut that Brown Norway rats always show greater effects.

Concluding Remarks

See https://www.nano.gov/node/1601/ for information about the final two NanoEHS webinars.

>> Will Boyes: OK, we're out of time today. I want to thank all of the viewers and listeners who tuned in to our webinar. I especially want to thank Dr. Zhang for a really stimulating webinar and for all of this excellent research that he has described for us today.

For our listeners out there, I remind you that on October 8 there will be the next of the webinars in this same NanoEHS series. The topic is "Evaluating Worker and Consumer Exposures to Engineered Nanomaterials." Paul Schulte of NIOSH and Joanna Matheson of the Consumer Products Safety Commission will be the presenters and the event will be moderated by John Howard. On November 12, "Global Harmonization of Nanoinformatics: A Case Study for Convergence and Team Science" will be presented by Christine Hendren of Duke University and Fred Klaessig of Pennsylvania Bio Nano Systems.

So thank you, everyone! I wish you a nice rest of your day.