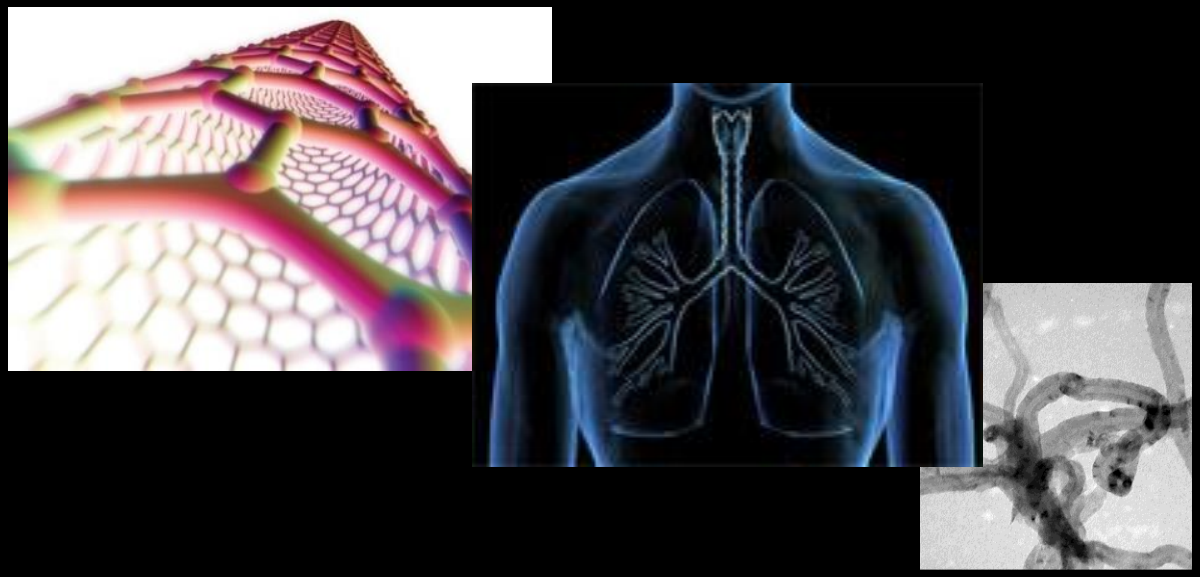
Workshop: Design of an In Vitro Testing Strategy to Assess the Inhalation Toxicity of Nanomaterials

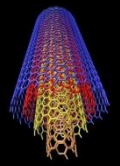


In Vitro Assays for Predicting Chronic Lung Diseases Induced by Nanomaterial Exposure

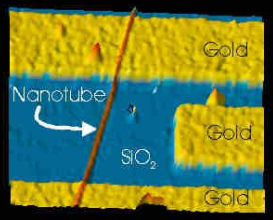
James Bonner, PhD
North Carolina State University, Raleigh, NC

February 24th -25th, 2015, Washington D.C.

The Rise of Nanotechnology

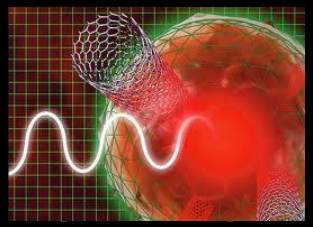


- Nanoscale electronics& Energy



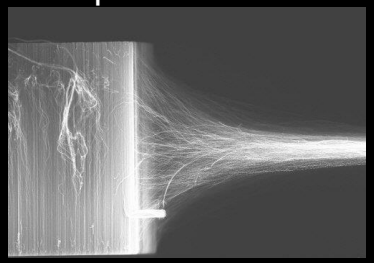
Nanotube field effect transistor

- Medicine



Cancer Therapeutics

- Tissue Engineering



Artificial muscle

- Light-weight, super-strong material

Bike Frame

Air Frame

- Future Applications

Space elevator

Artificial muscle suit

Military Apps

The Need to Refine, Reduce, Replace (3R's) *In Vivo* Testing of Engineered Nanomaterials (ENMs)

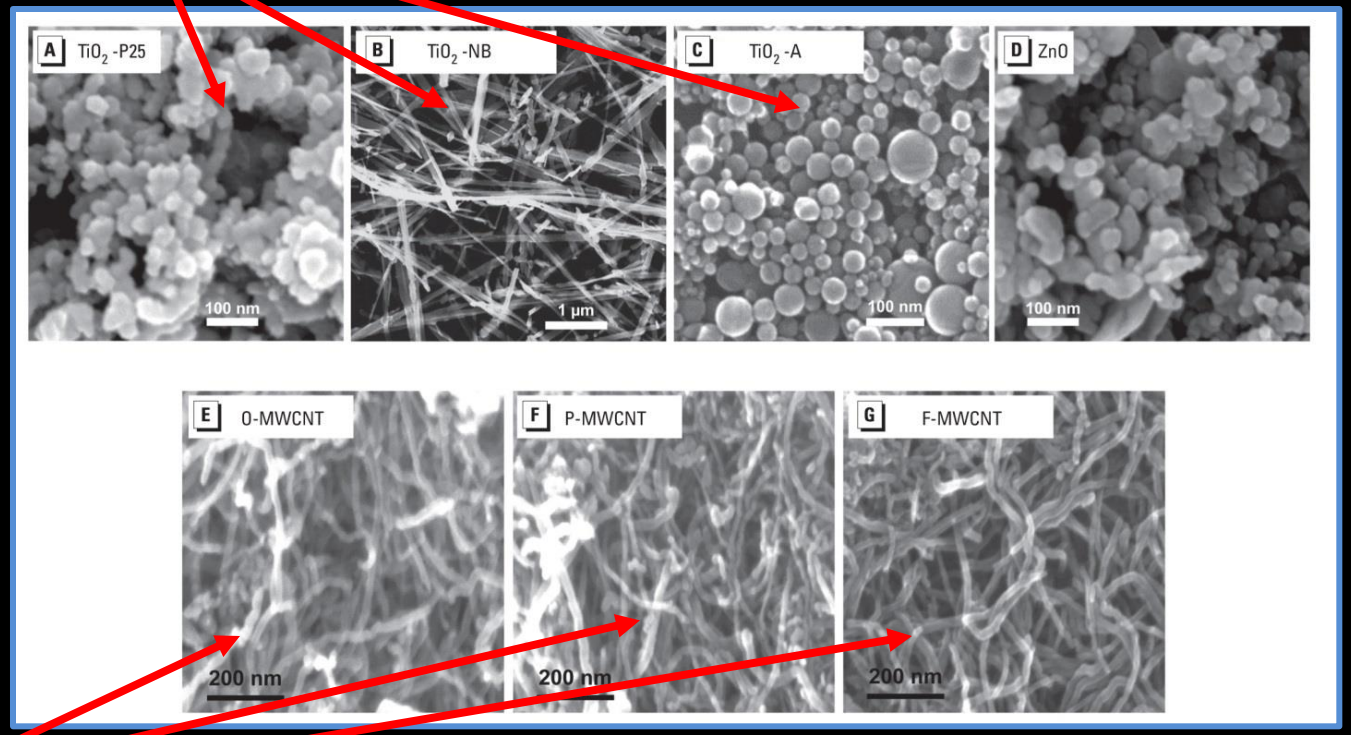
1) The number and variety of ENMs is rapidly growing with many different functionalizations of any one type of nanomaterial.

2) Ethical considerations and high costs of standard 2 year rodent bioassays limits the number of ENMs that can be tested for chronic diseases.

3) Rodent models are of limited value for some chronic diseases (e.g., mesothelioma), possibly due to relatively short life span (~2 years).

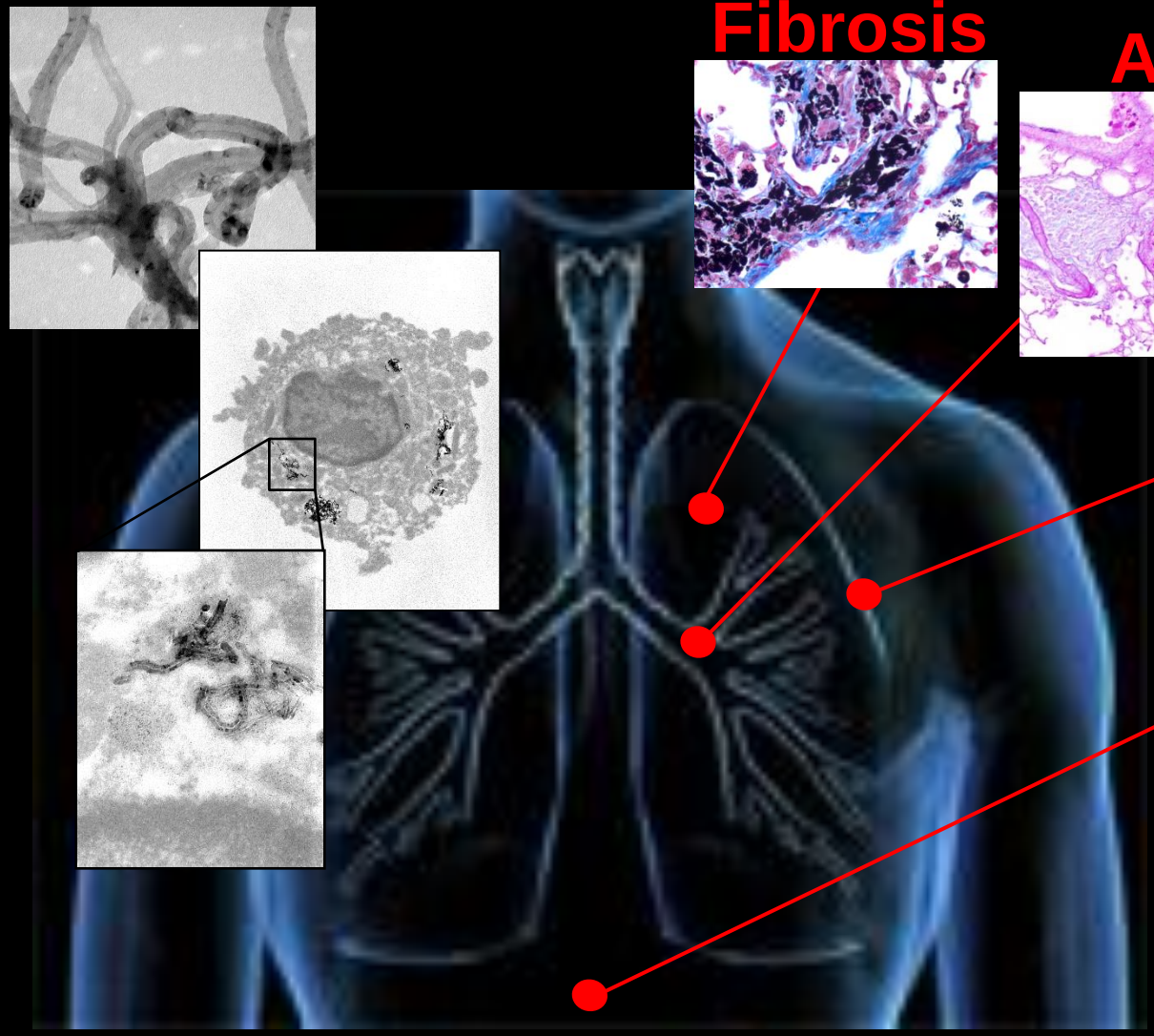
A Rapidly Increasing Number and Variety of Engineered Nanomaterials (ENMs)

ENMs of the same chemical composition can be designed with different physico-chemical characteristics: (e.g., shape, size, charge, solubility).



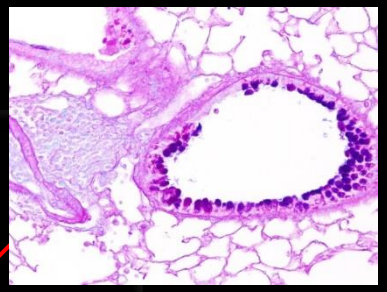
Xia et al., 2013, Environ Health Perspect, 121:683.
Bonner et al., 2013, Environ Health Perspect, 121: 676.

Chronic Diseases and Nanomaterials

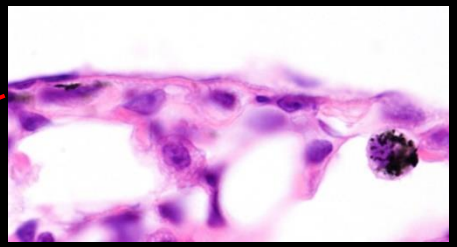


Fibrosis

Asthma



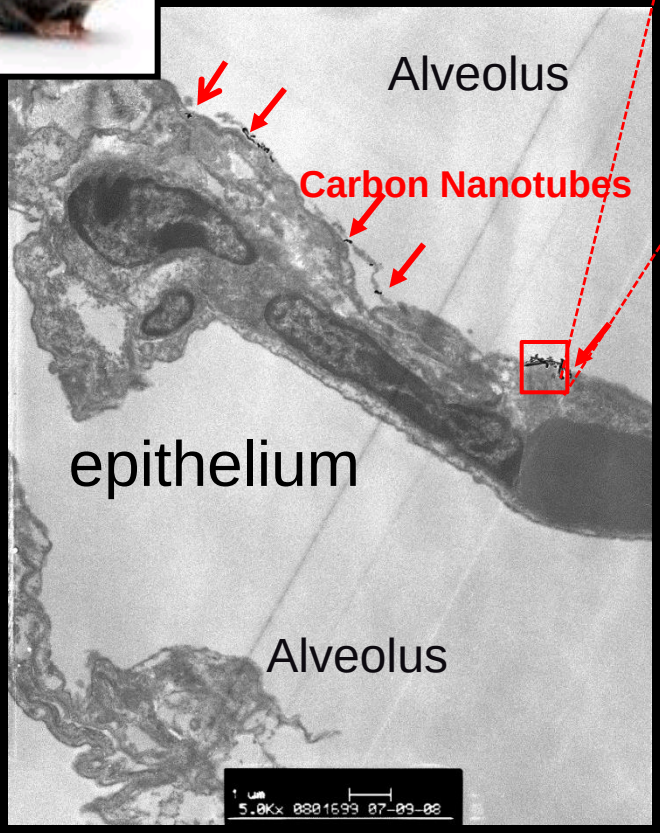
Cancer

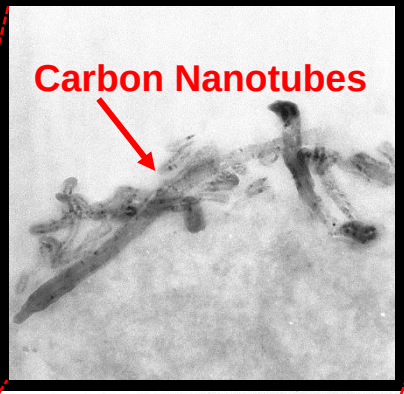


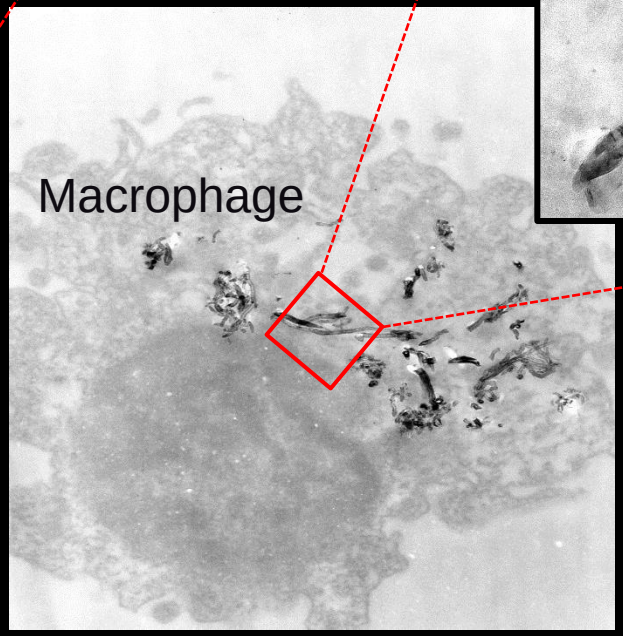
Systemic Effects
-Heart
-Spleen
-Liver
-Lymphatics

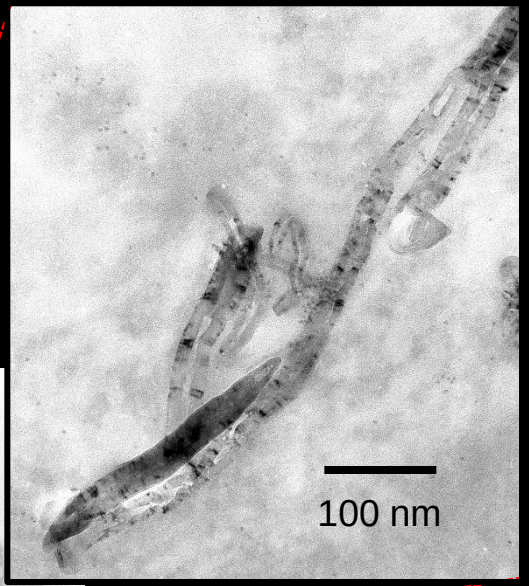
Particle Deposition Site Determines Local Injury…

…And Nanoparticle Deposition Site Determines Local Lung Injury and Disease



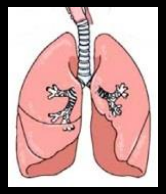


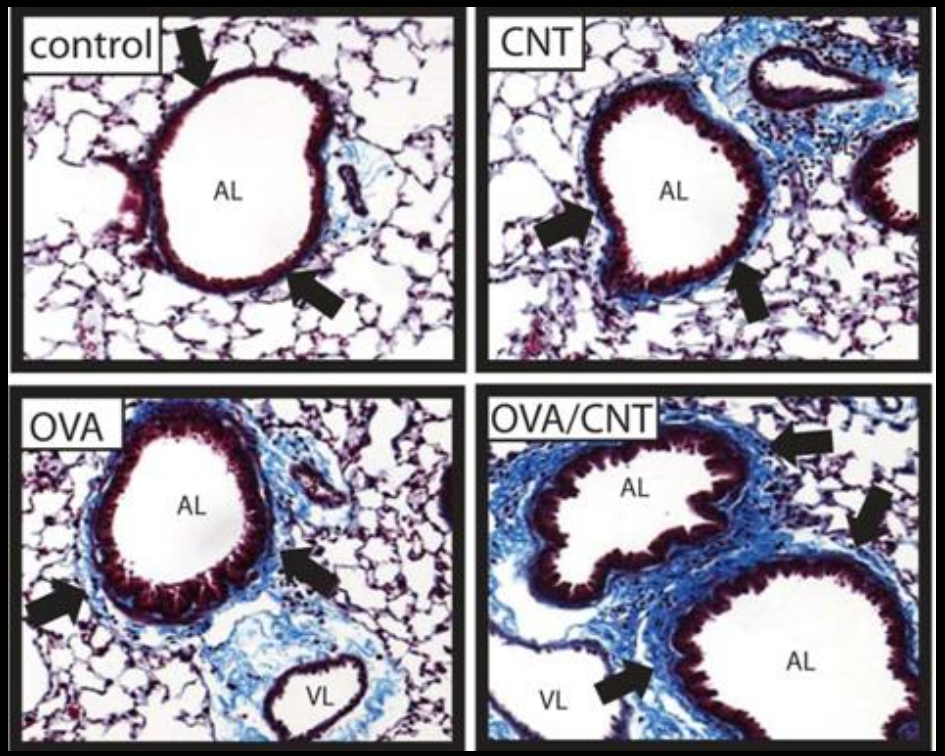


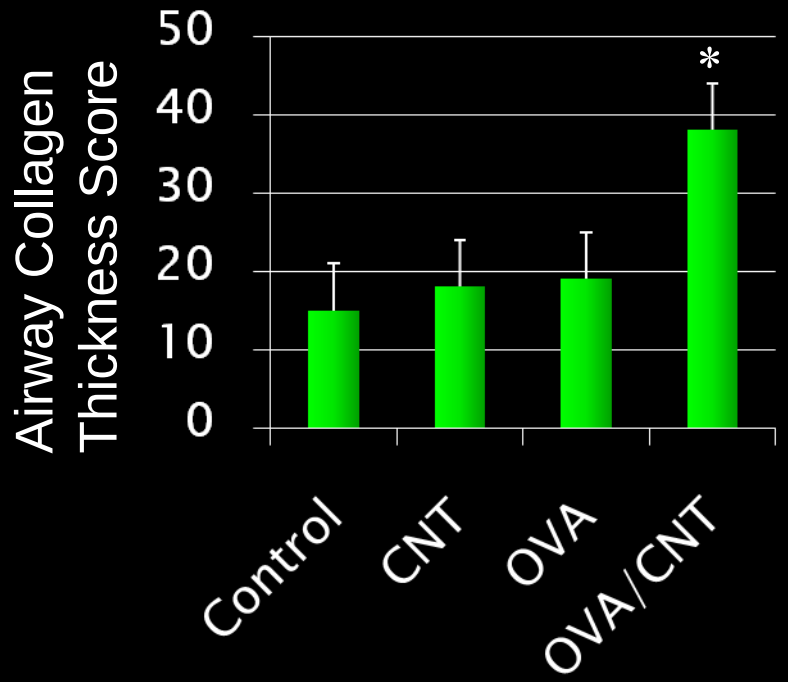


Ryman-Rasmussen et al. *AJRCMB 40:349-358, 2009*

Susceptibility is a Major Determinant of Disease Initiation and Progression after Nanoparticle Exposure

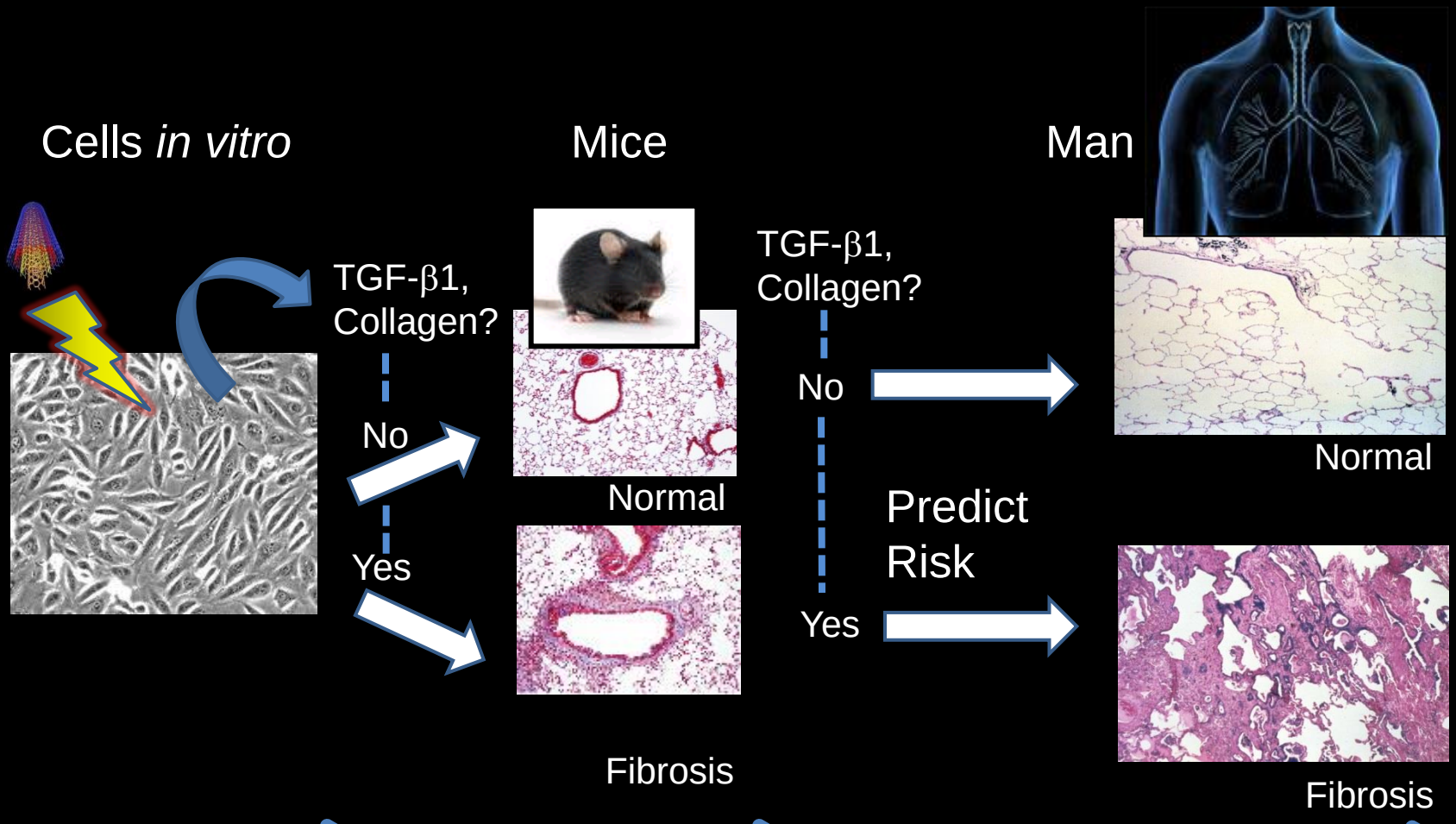
OVA (21 days) →  →  Nanotube Inhalation (6 hours) →  Collect Lung Tissues (14 days)





Ryman-Rasmussen et al. *AJRCMB 40:349-358, 2009*

In Vitro to Mouse to Man: Can We Eliminate the Middle-Man (-Mouse)?



A Missing Gap in Information…

….is the ability to reliably predict *in vivo* outcomes with *in vitro* tests.

Alternative *In vitro* tests are not yet available to reliably predict some adverse health effects

Repeated dose toxicity (problems related to long term, repeated exposure).

Toxicokinetics (the penetration into, fate within and elimination from the body of a toxic substance, including its absorption, distribution, metabolism and excretion).

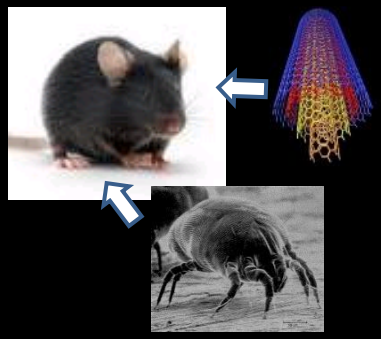
Carcinogenicity (the ability of substances to cause cancer).

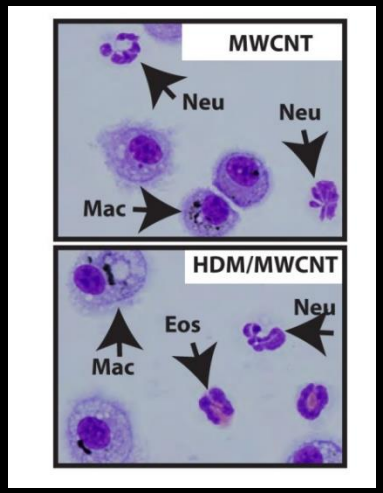
Immune sensitization (the toxicological impact associated with chemicals that have the intrinsic ability to cause allergy).

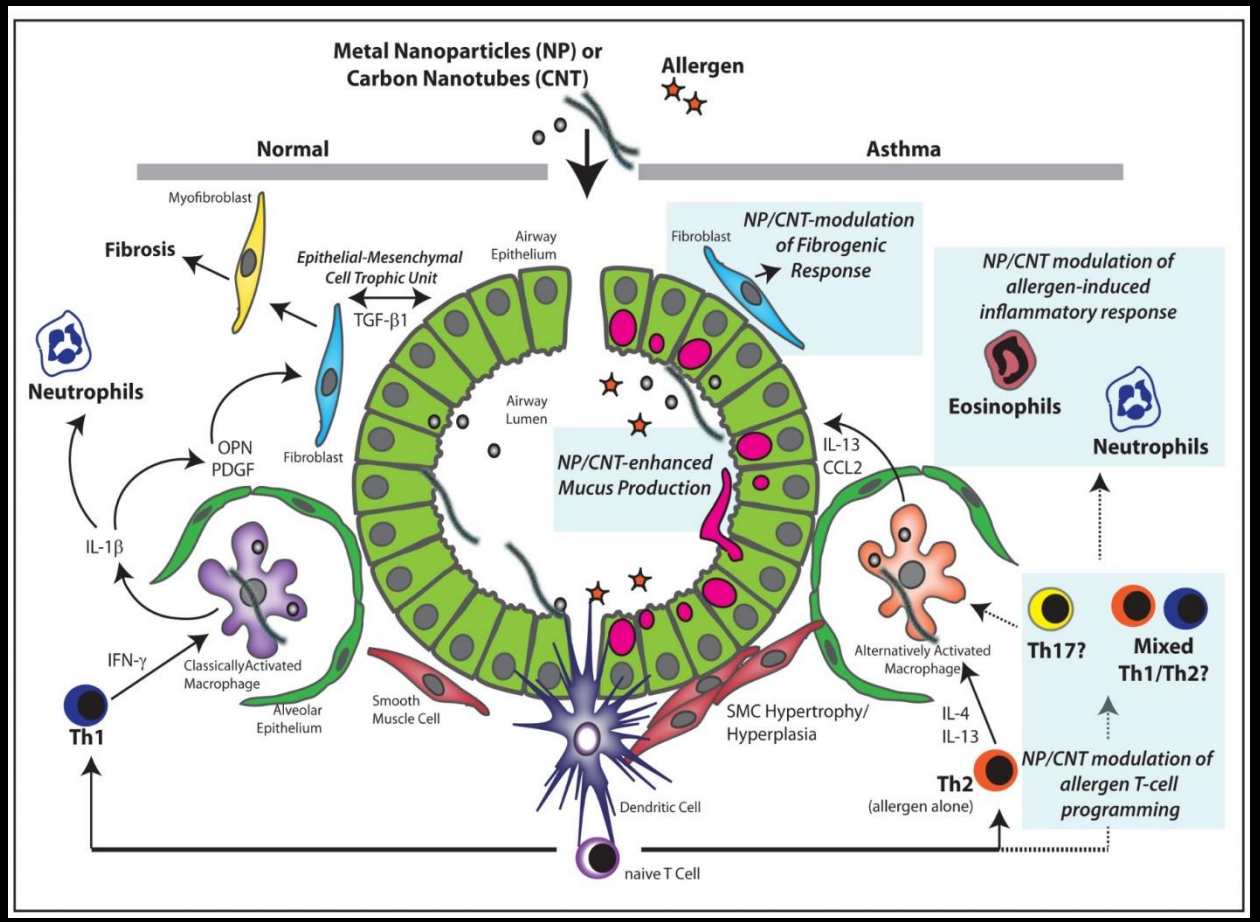
Adapted from: European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)

Challenges to Overcome in Complex Scenarios

While *in vitro* modeling of some diseases (e.g., lung fibrosis) is within reach, modeling of more complex immune diseases (e.g., asthma) or interaction of other factors (e.g. allergens) represents major challenges.







Thompson *et al*., *J. Environ. Immunol. Toxicol.,* 2013 1:3, 150-156.

The Challenge of Predicting Nanomaterial Toxicity using Alternative Testing Strategies (ATS)

"For ethical and many other reasons, it is also not practical to test all [nanomaterials] to which human beings might be exposed in whole animal studies."

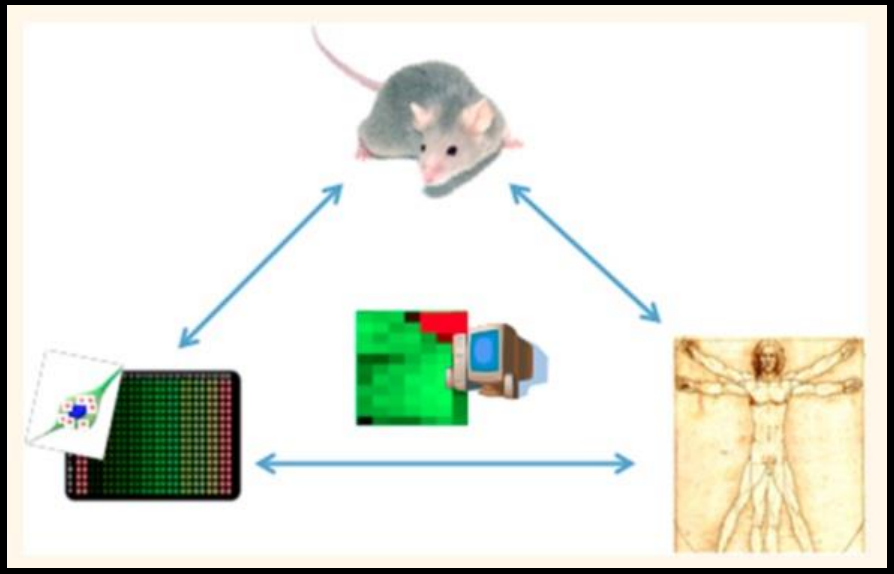
Epithelium

"The use of ATS to investigate engineered nanomaterial (ENM) hazard and prioritize ENMs for additional toxicity testing, risk assessment and product development are generally accepted goals. However, the use of ATS in lieu of *in vivo* testing for regulatory risk assessment or management purposes is not yet at the level of general acceptance."[1]

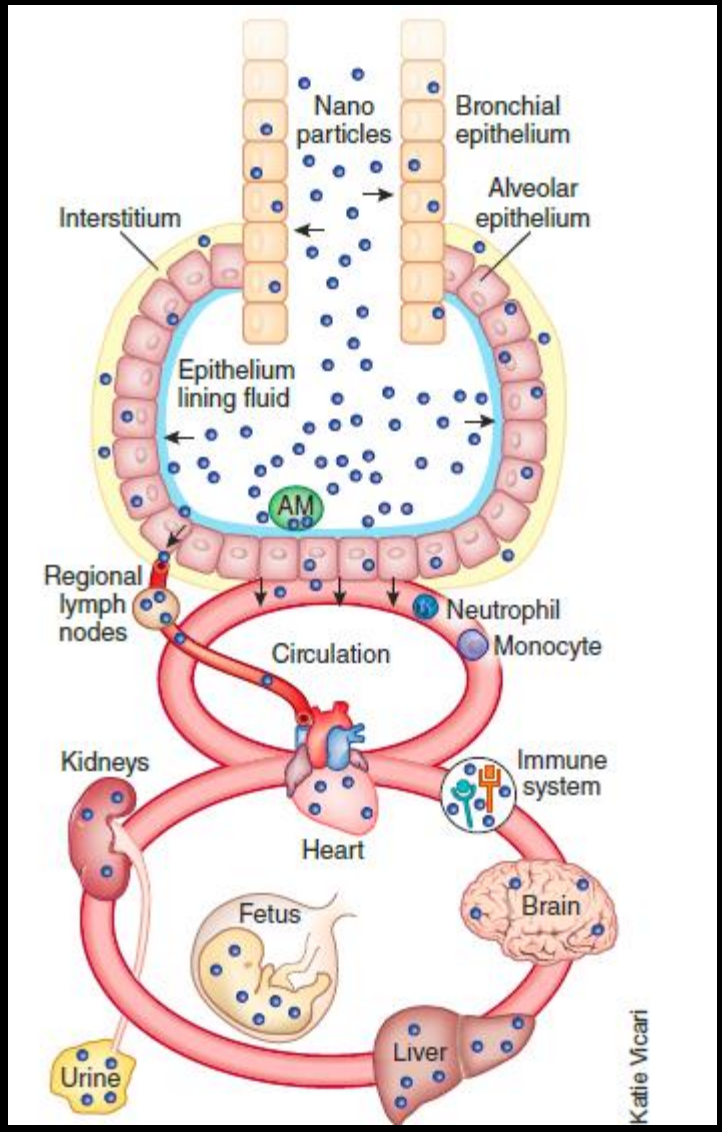
[1] Nel et al., A Multi-stakeholder Perspective on the Use of Alternative Test Strategies For Nanomaterial Safety assessment. ACS Nano 7(8), 6422-6433, 2013.

Challenges to Overcome for *In Vitro* Testing of Nanomaterials

In vitro modeling will need to address complex cellular interactions and systemic effects since some nanomaterials easily cross biological barriers and reach multiple organ systems.

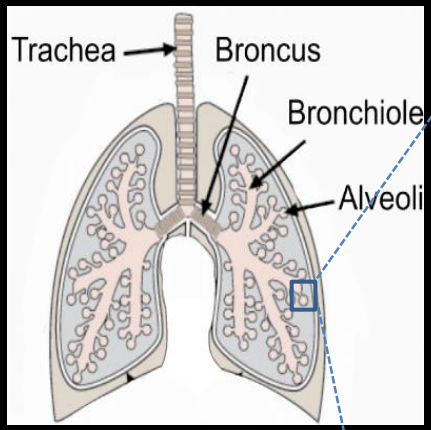


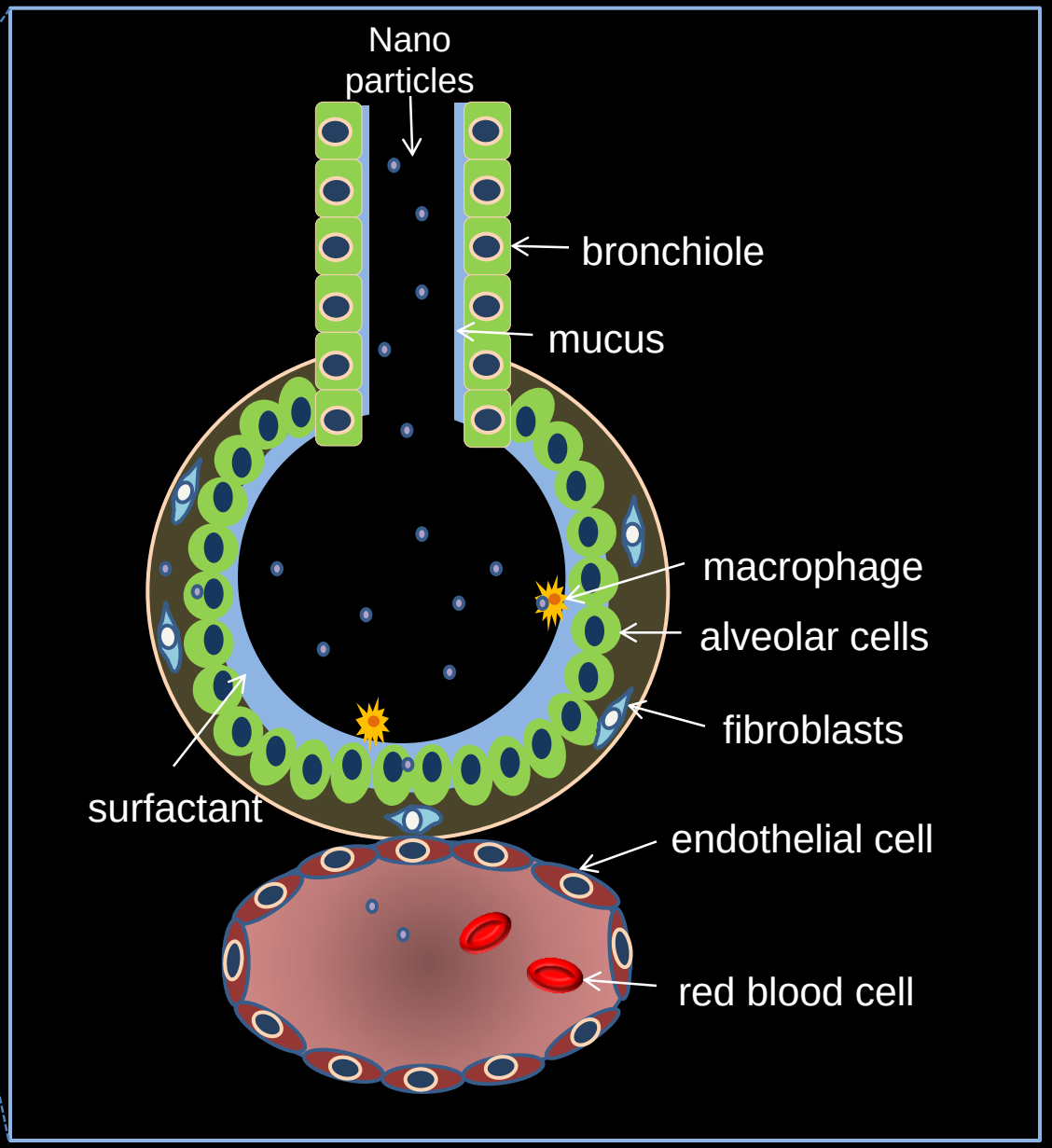
Nel et al., ACS Nano, 2013 7(8): 6422-6433.



*Kreyling et al. (2010). Nat Biotechnol **28**(12): 1275-6.*

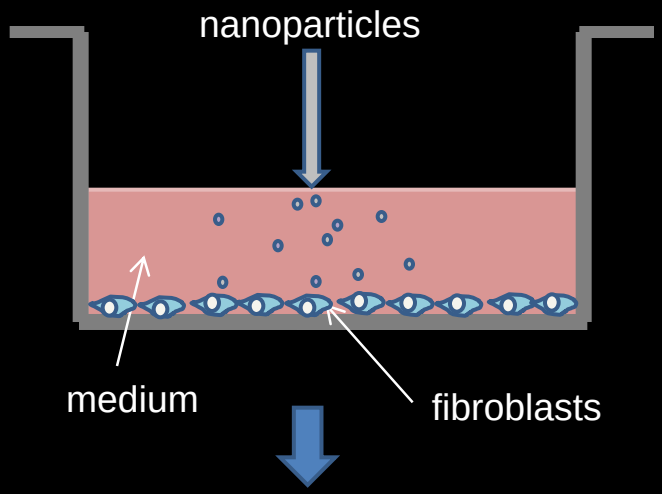
Modeling the Alveolar Region of the Lung





In Vitro Cell Systems for Testing Nanoparticle Toxicity

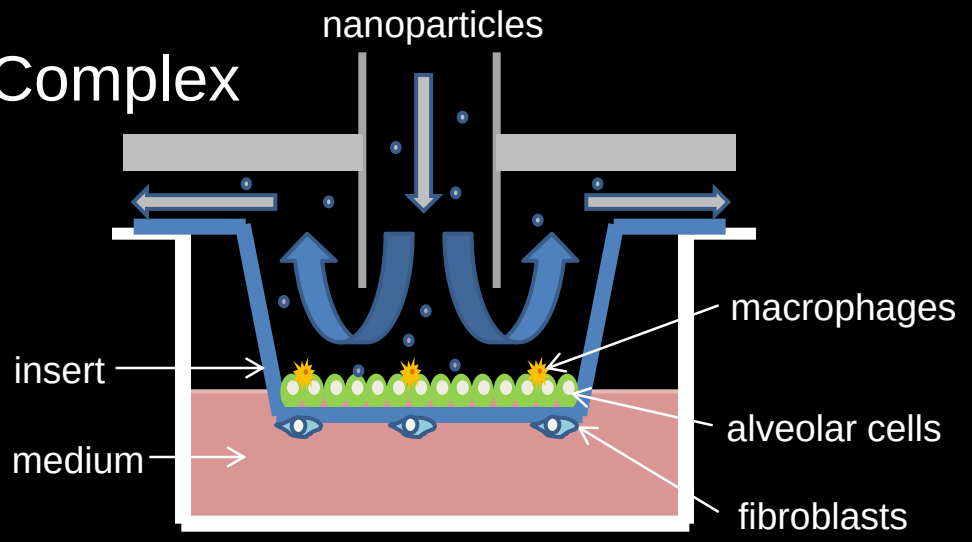
Simple

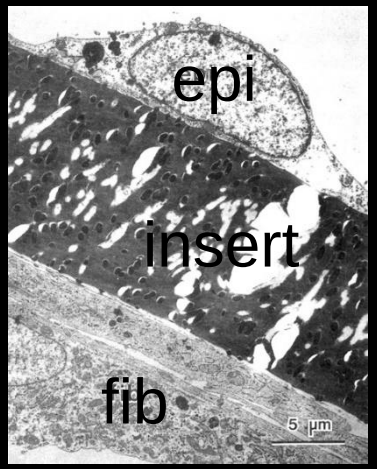


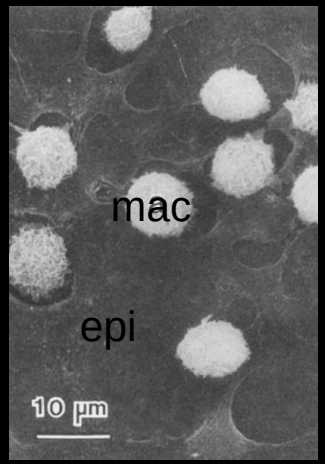
Collect Cells & Media

Measure:
Cytotoxicity, Proliferation,
Collagen, Cytokines,
Lipid Mediators, ROS, etc.

More Complex







Co-culture of primary rat alveolar type I cells, rat lung fibroblasts, and rat alveolar macrophages. (Mangum, Everitt, Bonner et al., *In Vitro Cell Dev. Biol*. 26:1135-1143, 1990).

Practical Considerations

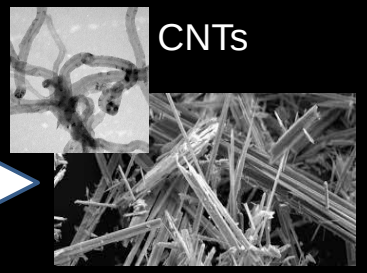
1) Structure-Activity Relationships (SARs) are useful. "*If it looks like a duck, acts like a duck, and swims like a duck…*"

2) Probability of Human Exposure: Which ENMs will represent the highest consumer exposures? (Ag in clothing, ZnO in suncreens, CNTs as flame-retardants). (Powers et al., Tox. Sci. 141:6-17, 2014).

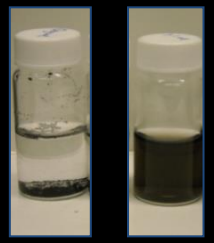
3) Dosimetry: Different sedimentation rates for ENMs or different functionalizations of the same ENM in aqueous media in cell culture systems (DeLoid et al., Nat. Commun. 5:3514, 2014).

4) Inter-Laboratory Reproducibilty: Harmonized protocols for *in vitro* assays is key for reliably predicting *in vivo* outcomes (Xia et al. EHP, 121:683-690, 2013).

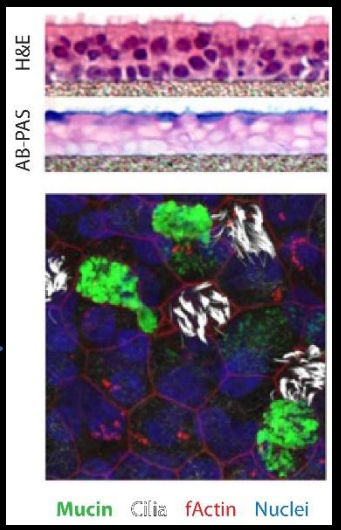
5) Cell Types: Immortalized cell lines are readily available, but often do not behave like cells *in vivo*. Primary cells are superior, but not always available. (Fulcher & Randell, Methods Mol. Biol. 945:109-121, 2013).



Asbestos

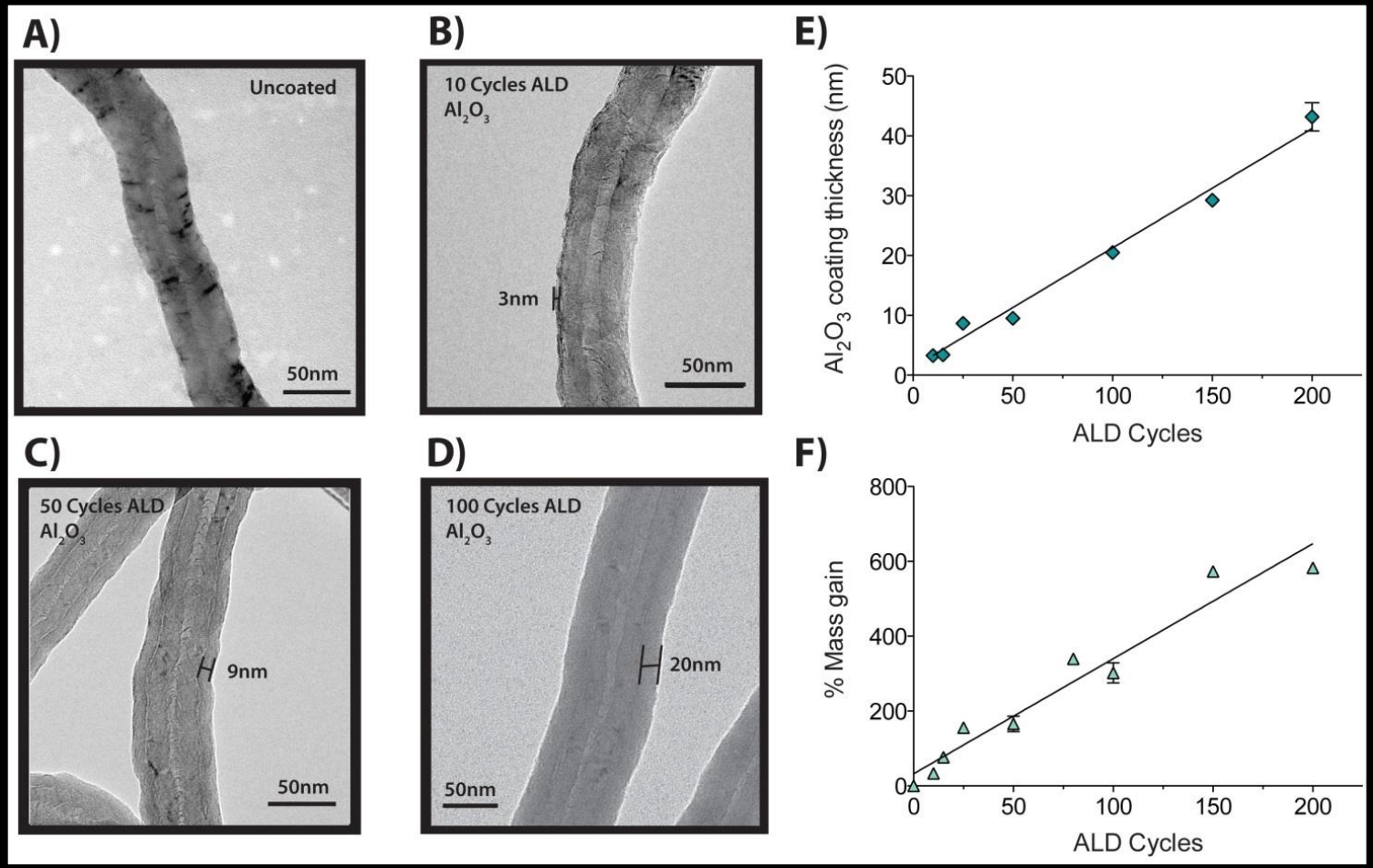


CNT *f*-CNT



Human bronchial epithelial cells in air-liquid interface

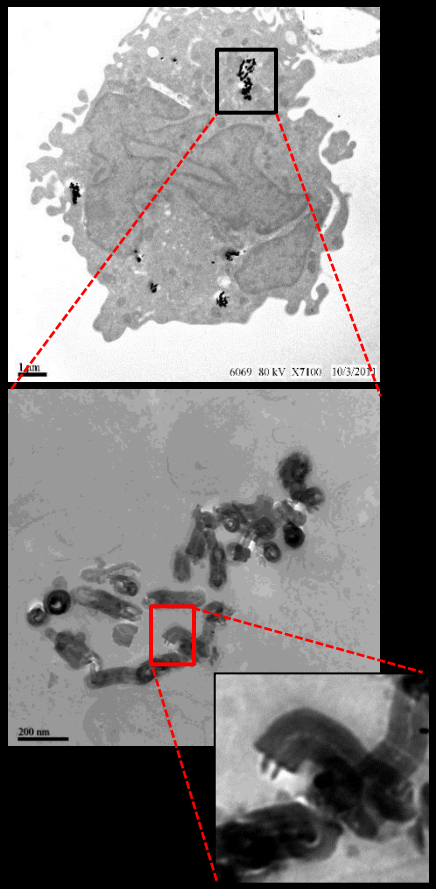
Example: Predicting how atomic layer deposition (ALD) coating of CNTs will influence severity of lung fibrosis using in vitro cell culture models

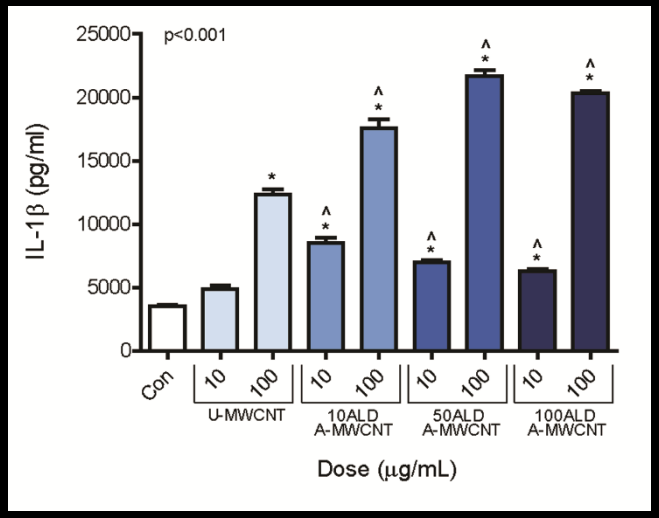


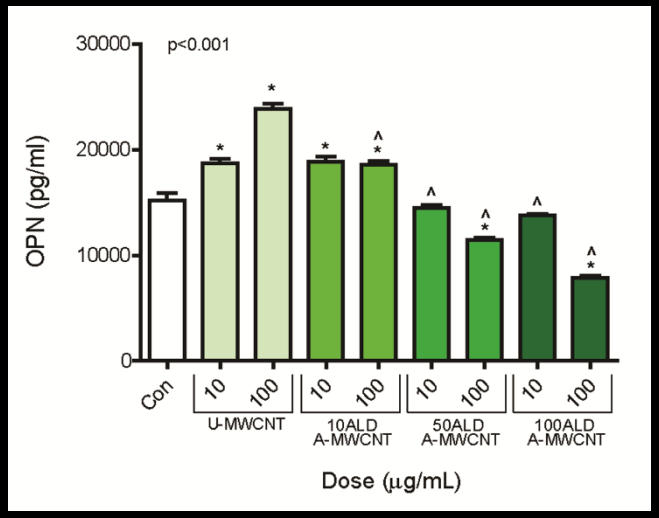
Al_2O_3 coating on multi-walled carbon nanotubes

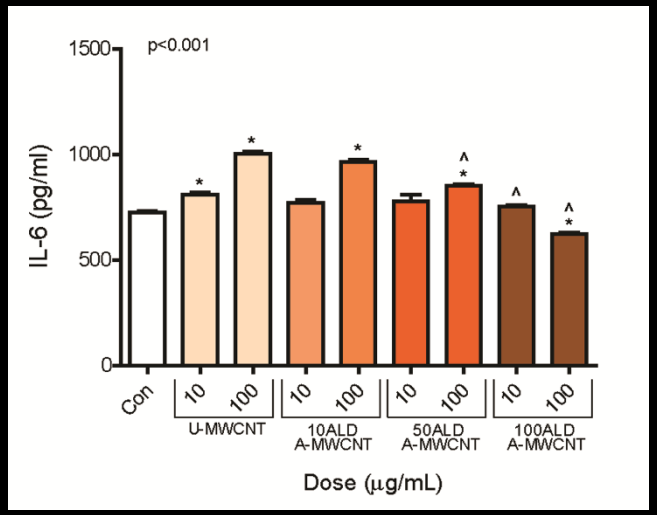
Taylor et al., PLoS One. 2014 Sep 12;9(9):e106870.

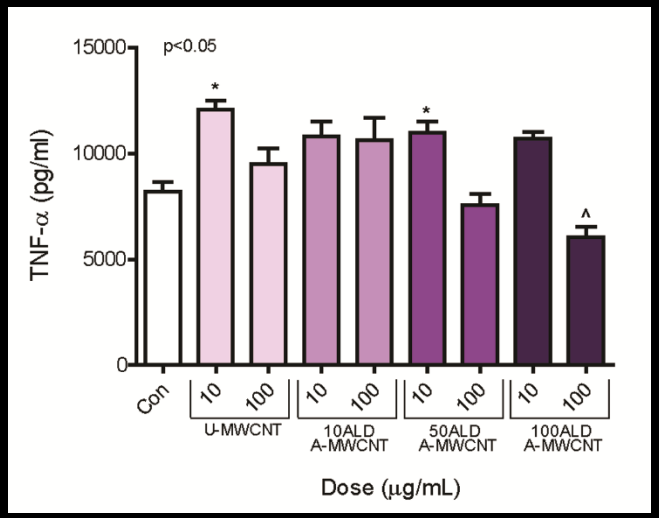
Cytokine Production by THP-1 Monocytic Cells Exposed to Al_2O_3-coated Carbon Nanotubes







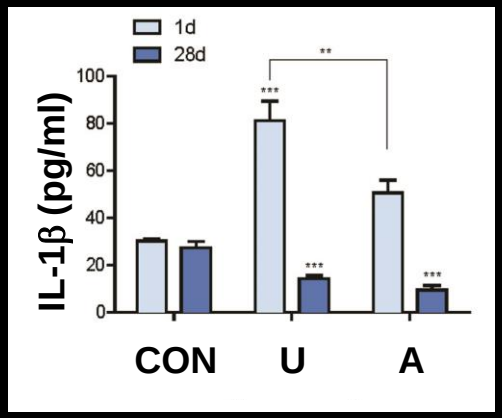


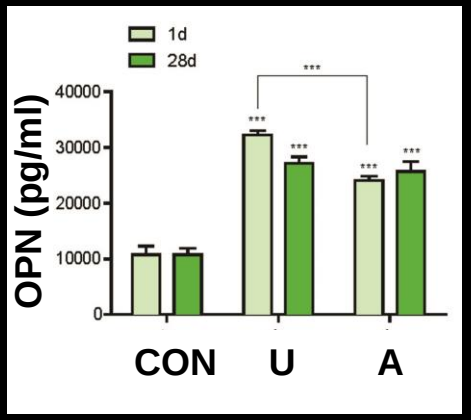


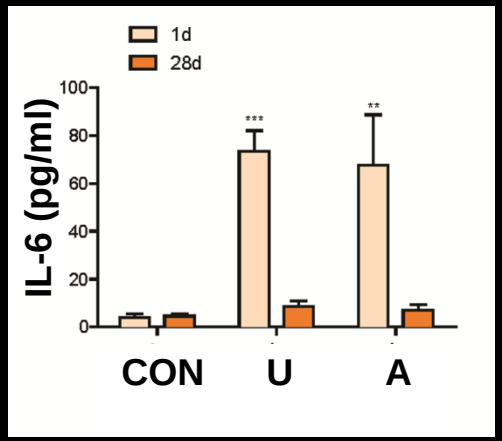
Taylor et al., PLoS One. 2014 Sep 12;9(9):e106870.

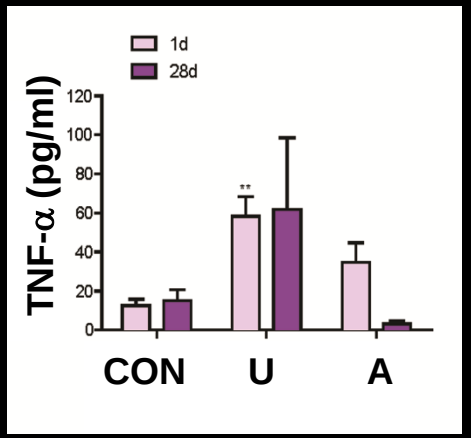
Atomic Layer Deposition Coating of Carbon Nanotubes Alters the Pro-Fibrogenic Response in Mice

BALF Cytokines



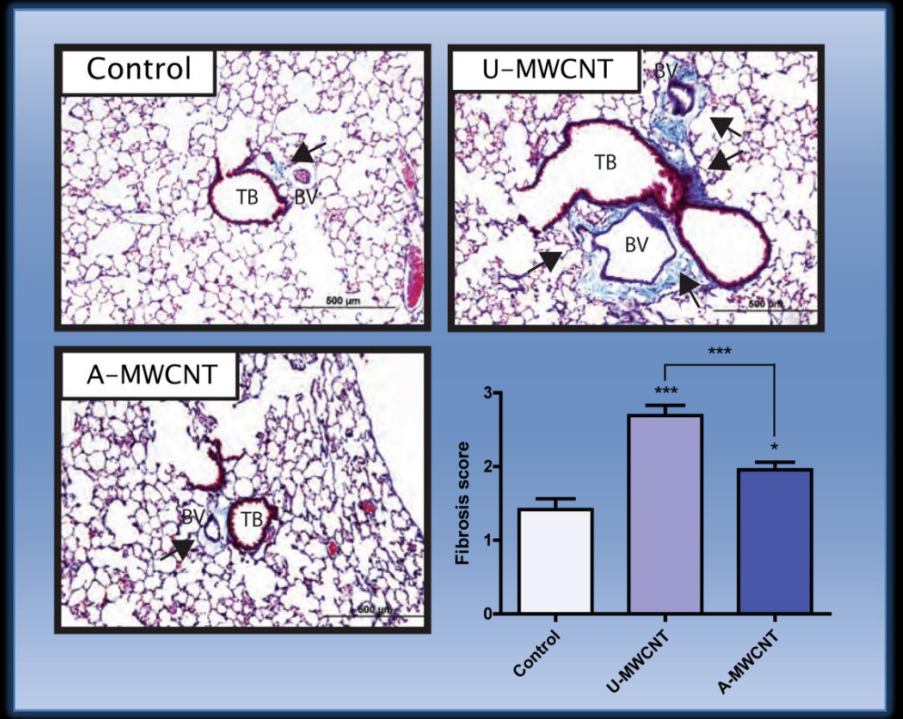






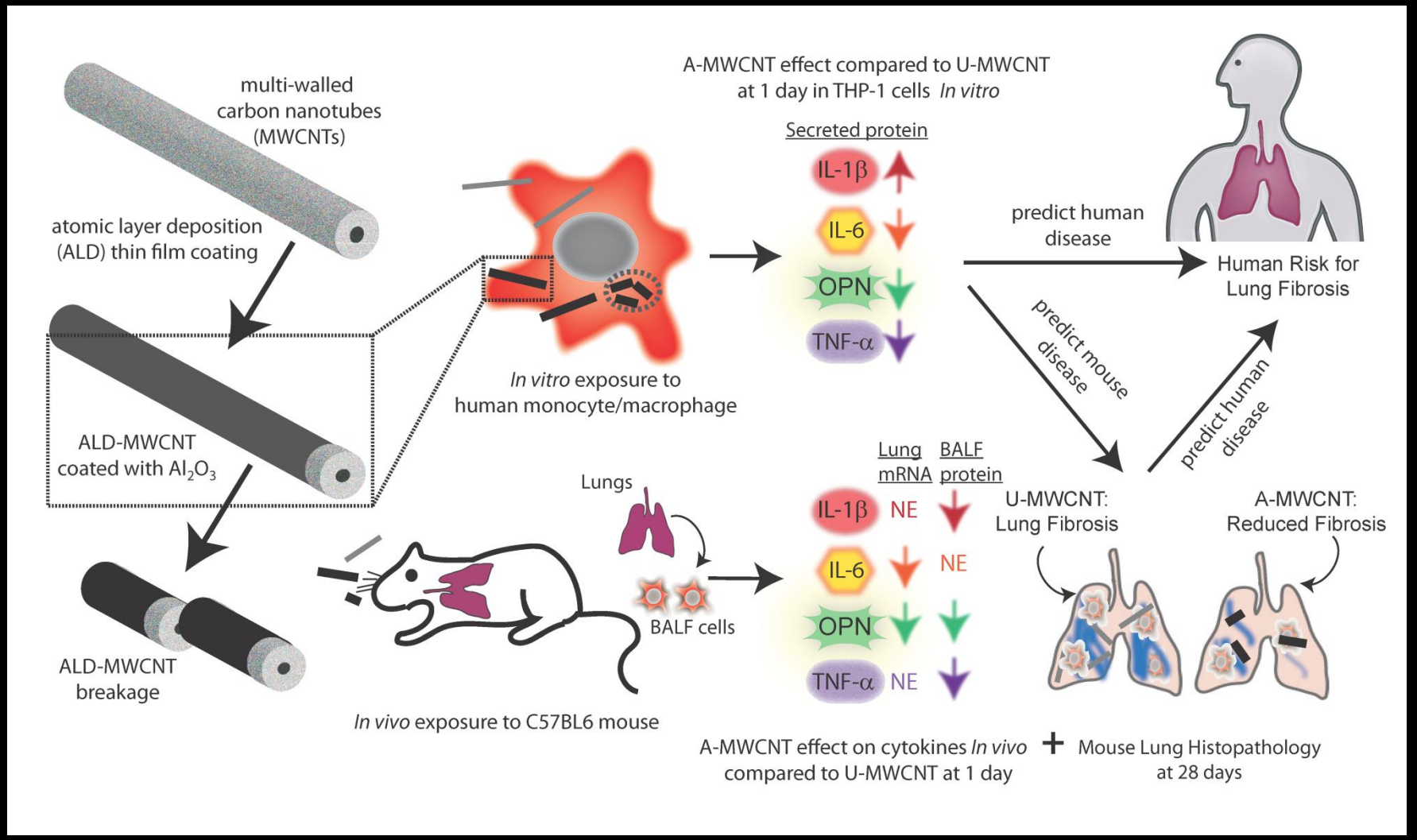
(U = uncoated, A = ALD-coated)

Pathology Scoring



Taylor et al., PLoS One. 2014 Sep 12;9(9):e106870.

Predicting Human Health Impact of Carbon Nanotubes Functionalized by Atomic Layer Deposition



Taylor et al., PLoS One. 2014 Sep 12;9(9):e106870.

Predicting Relative Risk and Assumptions

Reality

Assumed

Reality

Questions for Discussion

1) How do we begin to address the complexity of a chronic human disease (e.g., pulmonary fibrosis) caused by nanomaterial exposure using in vitro cell culture models?

2) What in vitro cell systems are available for predicting nanomaterial toxicity and are these good enough to predict disease in mice and men?

3) What gaps in knowledge will we need to overcome with in vitro testing to continue to reduce, refine, and replace (3 R's) the use of animals for nanomaterial testing?