Mechanisms of Biological Response to Engineered Nanomaterials

ICON Workshop 2: Towards Predicting Nano-Bio Interactions
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The Workshop Goal

**Identify**
- critical components of a predictive model of NanoBio Interactions
- from minimal NP data
- in a paradigm developed for chemicals, not particles, and
- the research needed to develop, refine and validate the model.
Knowledge Requirements for Predicting Nano-Bio Interactions

Structure

Descriptors of 3 dimensional structures
size, shape, surface chemistry

Function

Critical biological responses to ENM exposure
What to measure and where to measure it
Utilize cell biology and ambient particulate research
The Goal for this Discussion

Understand the characteristics of cellular and molecular endpoints that query mechanisms and pathways of homeostasis.

Good Health
Some Evidence for Engineered Nanomaterial Cellular and Molecular Toxicity

Changes in normal cell function
  cytoskeleton, calcium transport, mitochondrial function, protein denaturation, DNA damage, cell cycle regulation

Activation of protective response pathways
  oxidative stress, inflammation, immune response

Development and exacerbation of disease
  cardiac and vascular dysfunction, skewing of the adaptive immunity, asthma exacerbation, tumorigenesis
What Should be Measured?

Cellular and molecular mechanisms that
• activate in response to exposure
• neutralize and contain the exposure and
• return the system to baseline (homeostasis)

Characteristics of the Response Mechanism

The response should be
• proportional to the exposure,
• appropriate to the type of exposure,
• shut down completely when the exposure is neutralized
• not cause damage to healthy surrounding tissue
Measurement Parameters

EXPOSURE

CONTROL CENTER

SENSOR

EFFECTOR
Hierarchical Oxidative Stress Model

Response Pathway:
- Normal
- Anti-Oxidant defense
- Inflammation
- Cytotoxicity

Outcome:
- Phase II enzymes
- Cytokines
- Chemokines
- Apoptosis

Adapted from Nel, Science, 2006
Major Mechanisms and Pathways

EXPOSURE

- Oxidative Stress
- Inflammation
- Immune Response
- Apoptosis and Necrosis
- Genotoxicity and Mutagenicity
- Developmental Effects

DISEASE
Antioxidant Defense: Cellular Redox Equilibrium
Oxidative Stress: Redox Disequilibrium
Multiple Cellular Effects of Oxidative Stress

- Oxidative stress
  - Increased lipid peroxidation
  - GSH depletion
  - Cytoskeletal damage
  - Inhibition of ATP synthesis
- DNA damage
  - Direct damage to proteins
  - Rises in intracellular free Ca^{2+}
  - Membrane blebbing
- NAD(H) depletion
  - Rises in intracellular free iron
  - Membrane peroxidation and destruction
  - Increased damage to DNA, proteins, lipids
  - Metal ion release into surrounding tissues, injury to adjacent cells
Cell Death Pathways: Apoptosis and Necrosis

**APOPTOSIS**
- Caspase activation
- Inhibition of mRNA translation
- Condensation of cell and organelles
- Chromatin condensation, DNA fragmentation
- Loss of membrane asymmetry
- Membrane remains impermeable
- Cell falls apart into apoptotic bodies

**NECROSIS**
- Pro-inflammatory signaling and cytokine production
- Swelling of the cell and organelles
- Mottled chromatin condensation
- Loss of membrane asymmetry
- Rapid loss of membrane permeability
- Cell-membrane explodes
- Remains stay together
Inflammation: A Component of Multiple Response Mechanisms

Exposure

- FcεRI
- Histamine
- Leukotrienes
- Tryptase
- IL-16
- IL-5
- IL-2
- IL-4
- IL-6
- Eotaxins
- IgE
- Neutrophil
- Granules
- Eosinophil
- Major Basic Protein
- ECP
- Nitric Oxide
- Eotaxins
- Cytokines
- Bronchospasm
- Major Basic Protein
- Peroxidase
- Major Basic Protein
- Nitric Oxide
- Vasodilation
Innate Immunity: The Immediate Immune Response to Exposure
Adaptive Immunity: Antigen Specificity Takes Time

**B Cell Immunity**
- Extracellular microbes (e.g., bacteria)
- B lymphocytes
- Secreted antibody
- Neutralization
- Lysis (complement)
- Phagocytosis (PMN, macrophage)

**T Cell Immunity**
- Intracellular microbes (e.g., viruses)
- Antigen-presenting cell
- Helper T cell
- T-cell receptor
- Processed and presented antigen
- Cytokines
- Cytokine receptor
- Proliferation and activation of effector cells (cytotoxic T cells, natural killer cells, macrophages)
- Lysis of infected cell

Elsevier Press, 2002
Genotoxicity: DNA Damage and Repair

- UV
- Ionizing Radiation

![Diagram showing DNA damage and repair processes with nodes for exogenous damage, endogenous damage, nuclear DNA, mitochondrial DNA, unrepaird DNA, metabolism, damage, pathology, and disease states such as cancer, senescence, and apoptosis.]

healthy cell
rate of DNA damage = rate of repair

up to 500,000 DNA modification events per cell per day

diseased cell
rate of DNA damage > rate of repair

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Mutagenicity: Change in the DNA Sequence

**Point Mutation**

- **Normal**: T C
- **Mutation**: T C

“T” Changes to “C”

**Wild type**

- mRNA: AUGACAAGUUUGCGCUAA
- Protein: Met Lys Phe Gly Stop

**Base-pair insertion or deletion**

1. **Frameshift causing extensive missense**
   - mRNA: AUGACAAGUUUGCGCUAA...
   - Protein: Met Lys Leu Ala...
   - Missing

2. **Frameshift causing immediate nonsense**
   - mRNA: AUG AAG ACG UUG GCC CUA
   - Protein: Met Leu...
   - Stop

3. **Insertion or deletion of 3 nucleotides**: no frameshift; extra or missing amino acid
   - mRNA: AUG UUU UCG CUA
   - Protein: Met Phe Gly...
   - Missing

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Developmental Pathways and Mechanisms: Protecting Vulnerable Populations

- analogous to human pathways and mechanisms or
- sentinel for environmental change

Zebrafish Model
Kimmel et al., 203:253-310, 1995

Dioxin-induced malformation of head and heart

Images by Warren Heideman
EHP, 113(3), 2005
Summary

NANOMATERIALS

SENSOR

CONTROL CENTER

EFFECCTOR

Imbalance

Variable (in homeostasis)

Imbalance
Summary

Important Biological Mechanisms for Understanding Structure Activity Relationships

Cellular and molecular mechanisms that
• activate proportionally in response to exposure
• neutralize and contain the exposure and
• return the system to baseline (homeostasis)

Developmental pathways indicating susceptibility
Systems Biology: Does It Have a Role in Predictive Modeling?

DNA → TRANSCRIPTION → RNA → TRANSLATION → Protein

Genomics
Transcriptomics
Proteomics
Systems Biology: Functional relationships