NANOTOXICOLOGY: BIOENGINEERING BASED APPROACHES TO A NEW TECHNOLOGY

Ellen Silbergeld, PhD
Johns Hopkins University
Bloomberg School of Public Health
Collegium Ramazzini 2008
Looking as we leap…or before we leap?
Topics for this presentation

- Work at Hopkins on nanobiotechnology
- State of the technology
- Signals of concern
- A nanobiological approach to assessing nanotechnology
### Why is nanotechnology relevant to biology and medicine

<table>
<thead>
<tr>
<th>Size (nm)</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 nm</td>
<td>Au atom</td>
</tr>
<tr>
<td>1 nm</td>
<td>H₂O molecule</td>
</tr>
<tr>
<td>10 nm</td>
<td>cell membrane</td>
</tr>
<tr>
<td>100 nm</td>
<td>Viruses:</td>
</tr>
<tr>
<td>1 µm</td>
<td>prokaryotic cells:</td>
</tr>
<tr>
<td>10 µm</td>
<td>animal and plant cells</td>
</tr>
</tbody>
</table>

- **Au atom**
- **H₂O molecule**
- **cell membrane**: DNA: 2.5 nm
- **Viruses:**
- **prokaryotic cells**: red blood cells:
- **animal and plant cells**
- **Quantum Dots**: multifunctional nanoparticle
- **scaffolds for tissue engineering**
- **lab-on-a-chip**
Why an Institute?

Device Fabrication
Nanoparticle Synthesis
Molecular Synthesis

Surface Engineering
Molecular Recognition/Targeting

Cell Biology

Therapeutics
Diagnostics

Animal Studies

Clinical Trials

Health/Environmental Issues

Translational Research
Graduate Training Programs
HHMI; NSF IGERT
Goal: train a new generation of multidisciplinary students

Undergraduate NanoBio Minor
(Fall 2007)

Research
>120 faculty
School of Medicine
Whiting School of Engineering
Krieger School of Arts and Sciences
Bloomberg School of Public Health

Web Site
Databases for faculty, courses, facilities, funding opportunities, NanoBio message board

Graduate Technology Commercialization Program

Graduate Training Programs
HHMI; NSF IGERT
Goal: train a new generation of multidisciplinary students

INBT Undergraduate NanoBio Summer Research Awards

Graduate VC Summer Internship

INBT Animation Studio

Undergraduate technical writing internships

Translational Research Center
Partner: Maryland DBED
Goal: new biotech businesses in MD

Industry Affiliates Program
Benefits: first look at new inventions, discount on research projects, student resume database
What is nanotechnology?

- **Engineered materials** with at least one dimension less than ≤ 100 nm
  - Nanodots, nanotubes, nanospheres
  - Approaching or at the subcellular scale

- Highly purified materials
  - Often metals

- High surface to mass ratio – high surface reactivity
  - Potential for chemical delivery/binding

- Multiple potential uses
  - Passive, active, systems
### Nano Applications

#### Current uses
- Surface treatments – “nanowhiskers”
- Water/stain repellent treatments
- Cosmetics/UV protection
- Fillers in plastics
- Semiconductors
- *Fuel additives*
- Biomedical imaging materials

#### Near term introductions
- Drug delivery systems
- Nanoenergy sources
- Environmental remediation products
- Sensors

On the drawing board: intelligent systems, complex machines
State of nanotechnology: precaution is no longer an option

The Economist 22/11/2007
Large government investments...in R&D

$44 m/yr for environment, health and safety

<2% of publications in 2006 on health and safety

Source: UNEP (2007) Geo Yearbook
Nanomaterials: Is there cause for concern?

• The existing literature on nanomaterials

• TSP → PM10 → PM5 → ultrafines

• What can happen at the nanoscale?

• The sorcerer’s apprentice…
Status of nanotoxicology

• Toxicological research

• Inferences from current knowledge

• Risk assessment?
  – Something about hazard identification (toxicity testing)
  – Very little about dose
  – Uncertain exposure assessment
Reasons for concern: Metals form the base for nanomaterials development

<table>
<thead>
<tr>
<th>Major types of nanoparticles anticipated to be commercially available in 2006-14</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Product</strong></td>
</tr>
<tr>
<td>Nickel (carbon-coated) (Ni-C) powders</td>
</tr>
<tr>
<td>Poly (L-lactic acid) (PLLA) nanofibres</td>
</tr>
<tr>
<td>Yttrium Oxide (Y₂O₃) nanopowders</td>
</tr>
<tr>
<td>Ceria (CeO₂) nanoparticles, coatings</td>
</tr>
<tr>
<td>Fullerenes</td>
</tr>
<tr>
<td>Graphite Particles</td>
</tr>
<tr>
<td>Silica (SiO₂) nanoparticles, coatings</td>
</tr>
<tr>
<td>Titanium (TiO₂) nanopowders, thin layers</td>
</tr>
<tr>
<td>Zinc Oxide (ZnO) nanopowders, thin films</td>
</tr>
<tr>
<td>Carbon black</td>
</tr>
<tr>
<td>Carbon nanotubes</td>
</tr>
</tbody>
</table>

Source: UNEP (2007) Geo Yearbook
Smaller is worse: size determines exposure: size and lung deposition

Oberdorster et al (2005) EHP
Structure determines activity and function

And toxicity?

Nel et al. Science 2006 (Feb 3)

Table 1. Particle number and particle surface area for 10 µg/m³ airborne particles (5).

<table>
<thead>
<tr>
<th>Particle diameter (µm)</th>
<th>Particles/ml of air</th>
<th>Particle surface area (µm²/ml of air)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>0.5</td>
<td>153</td>
<td>120</td>
</tr>
<tr>
<td>0.02</td>
<td>2,390,000</td>
<td>3000</td>
</tr>
</tbody>
</table>
Hazard identification The dose makes the poison

Paracelsus, Father of Toxicology
How to prudently evaluate the safety/risks of nanomaterials … and can we do it in time?

• Conventional toxicology
• Exposure assessment
• Fate and transport
• Predictive models
• *Let biology guide*
• *Alternatives: Nanoscale toxicology and modeling*
Nanostructure and function of human artificial chromosomes

Toxicity at the nanoscale: nanospheres and DNA

Zhao et al (2005) Biophys J
Immunotoxicity at the nanoscale: uncovering cryptic epitopes

Nanoparticles (large spheres) cause proteins to change shape, revealing regions that trigger antibody-mediated immune response.

Nanowire delivery systems in nanomedicine
Start with what we know about biology at the nanoscale: cellular and subcellular structures

Nanoscale events in intercellular communication

Nanotubes connecting macrophages, NK cells, B cells

Onfelt B et al (2005)
STKE Science
Nanostuctural events during early development

Role of tubular structures throughout early development in *Drosophila*

Development
Nanoscale regulation of gene expression through chromatin binding molecules

<table>
<thead>
<tr>
<th>Property</th>
<th>Histone H1</th>
<th>MeCP2</th>
<th>MENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcriptional repression</td>
<td>Rarely <em>in vivo</em></td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Preferred binding site</td>
<td>Near linker entry–exit</td>
<td>Methylated DNA</td>
<td>Linker DNA</td>
</tr>
<tr>
<td>Chromatin compaction</td>
<td>Yes, zigzag conformation</td>
<td>Yes, high compaction</td>
<td>Yes</td>
</tr>
<tr>
<td>Interarray interactions</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Think about unintended consequences of engineered functions of nanomaterials

- Nanomaterials as drug delivery systems...

“The Sorceror’s Apprentice” – Walt Disney
Developing nanomaterials as drug delivery nanosystems

What if nanomaterials pick up environmental contaminants?

Try to think like a nanobiologist and a nanotechnologist

• Use biological methods to visualize nanoparticles
  – In vitro assessment of cellular interactions with flow cytometry
  – NPs tagged with embedded dyes

• Study cellular reactions to nanoparticles in real time
  – FACS real time assessment of population responses
  – Biological markers of cellular response
Toxicology at the nanoscale: A collaboration between materials science and toxicology

- Splenic lymphocytes isolated from BALB/c mice
- Cell cultures \((10^6)\) established
- NPs tagged by embedded dansylamide dye
- FACS staining for activation markers for lymphocyte subsets and key events: iNOS, TLR4, CTLA-4, Tim-3; apoptosis (annexin V and 7-AAD)
How FACS works

[Diagram showing the process of FACS (Flow Cytometry).]

1. A mixture of cells in liquid is exposed to a laser.
2. The cells are then directed into new drops of liquid.
3. Cells with positive charges are selected and can be detected by a photomultiplier tube.
4. The selected cells are then analyzed by a computer.

[Graph showing frequency of intensity.

- Red intensity plot shows distribution of red cell intensity.
- Green intensity plot shows distribution of green cell intensity.
]
Synthesis of Dansylamide-containing Silica Nanoparticles

TEOS = tetraethoxysilane
Poly(dimethylsiloxane) and Dow 190 surfactant added
Properties of Dansylamide-embedded Nanoparticles

Dansylamide nanoparticles (particle size: 150 ~ 200nm)

Fluorescence of Dansyl Dye & Nanoparticles

- **Dye (500nm)**
- **NP (455-80nm)**

Wavelength (nm)

Intensity

Intensity range: 0.E+00 to 9.E+05
These NPs are pure
Spectral overlap of common FACS dyes
NPs enter B cells, T cells, and macrophages
Activation of cell subsets by nanoparticles: quantifiable results from real time FACS analyses
Biological effects of NPs in vitro

• NPs can readily enter cells

• No increase in apoptosis or cell loss

• NPs affect immune function at much lower levels than immunoactive chemicals in solution (LPS; lead; mercury)

• NPs increase expression of CTLA-4, marker of T regulatory cell activation

• NPs increased expression of CD86, marker of B cell activation

• NPs increase CD86 and TLR-4, markers of macrophage activation
Current studies

• Time course
• Effects of size and functionalization
• Other markers and signals of cell response
  – Sort cells to examine specific population response
  – Cytokine/chemokine production
  – Gene expression
Some reflections on experience in nanotoxicology

- Of course these are new materials – that’s why they are being made!
- “Old toxicology” will not meet the challenge and is probably often irrelevant to the mechanisms of toxicity
- Close collaborations between nanotechnologists and nanotoxicologists are essential
- Think about the intended activity of a nanomaterial and then consider how this might be a hazard
Looking as we leap...or before we leap?