Cytotoxic effects of engineered nanoparticles at the bio-nano interface

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November 3 2011

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What happens at nanoscale?

- High specific surface area
- High reactivity
- High transportability
- More……

Quantum Mechanics (Wave Physics)

Classical Mechanics (Everyday Physics)
What could happen to NPs at biological interface?

- Adsorption
- Specific (ligand/receptor) and non-specific interactions
- Disruption (e.g., physical and chemical damage)
- Permeation/penetration

Figure 1.1. Representation of the interface between a nanoparticle and an intact lipid bilayer representative of a cell surface. Various environmental factors, particle properties, and their interrelationships are depicted. Credit: Wen Zhang. PhD thesis, Georgia Tech. 2011
My research outline

Semiconductor nanomaterials

Physical
- Interfacial forces
- Adsorption kinetics on cells
- Adhesiveness, hardness, elasticity, surface potential

Chemical
- Aggregation kinetics
- Ion release kinetics
- Reactive oxidative species

Chemical compounds:
- Fe$_2$O$_3$
- CeO$_2$
- TiO$_2$
- ZnO
- Al$_2$O$_3$
- CuO
- SiO$_2$
- QDs
- Au
- Ag
Some of the key findings on adsorption kinetics and the associated cellular impairments

Interaction force boundary layer (IFBL) model

Role of random kinetic energy (diffusivity)

Role of interfacial energy

Adsorption kinetics (i.e., rate constant)

Wen Zhang, Bruce Rittmann, and Yongsheng Chen. Size effects on adsorption kinetics of hematite NPs on E. coli cells. Environmental Science and Technology, 2011, 45 (6), 2172-2178.
Surface disruption to bacteria (E. coli) after exposure to hematite NPs

Surface disruption to human intestinal cells (Caco-2) after exposure to hematite NPs

- Microvilli disruption, membrane penetration, and adheren junction disruption.
- Possibly interpreted by depletion attraction.

Mechanisms of surface disruption from a physical perspective: Fe$_2$O$_3$ vs *E. coli*

To better understand and interpret nanotoxicity test results:

**Cellular property**

- Hardness (or softness)
- Elasticity (or rigidity)
- Adhesiveness
- Aqueous surface potential (zeta potential)
- Intrinsic surface charge

**Biomechanical**

- Observed cytotoxicity (e.g., loss of cell integrity, mortality)
Mechanisms of surface disruption from a physical perspective: Fe$_2$O$_3$ vs *E. coli*

(a) TEM image of hematite (α-Fe$_2$O$_3$) NPs with inset of SAED pattern; (b) AFM image of aggregated clusters of hematite NPs; (c) Crystal structure of hematite NPs; and (d) The size distribution

Several important features of hematite (reasons of choosing it in the test)

- Good reference nanomaterial
- Good aqueous stability
- Relatively uniform size distribution
- No toxic metal release (good chemical stability or chemically inert)
Mechanisms of surface disruption from a physical perspective: Fe$_2$O$_3$ vs *E. coli*

Several important advantages by using *E. coli* as a cell target:

- One of the basic and representative Gram-negative bacterial form that mirrors most of the bacterial properties
- Rapid toxicity screening relative to human cell lines
- A representative microorganism widely used in nanotoxicity tests
- Commonly used experimentally in molecular biology
- Comprehensive knowledge of gene library that allows us to conduct future genetic level studies
Results

1. Morphology changes of *E. coli* after exposure to hematite NPs

- **(a)**: *E. coli* cells with a few hematite NPs (white dots) attached
- **(b)**: *E. coli* cells with heavy adsorption of hematite NPs and aggregated clusters
- **(c)**: Deformed *E. coli* cells after long time exposure to hematite NPs

Wen Zhang, Joseph B Hughes, and Yongsheng Chen. Impacts of hematite nanoparticle exposure on biomechanical and surface electrical properties of *E. coli* cells. *Applied Environmental Microbiology*, Submitted.
Results

1. Morphology changes of *E. coli* after exposure to hematite NPs

Quasi-in situ imaging of *E. coli* cells in liquid by AFM.

- *E. coli* cells immobilized on a freshly cleaned silicon wafer surface treated with poly-L-lysine
- *E. coli* cells maintained a hydrated appearance
- Smooth outer surface without surface ultrastructures or flagella resolved
- *E. coli* cells and that the bacterial cells shrunk significantly.

Wen Zhang, Joseph B Hughes, and Yongsheng Chen. Impacts of hematite nanoparticle exposure on biomechanical and surface electrical properties of *E. coli* cells. *Applied Environmental Microbiology*, Submitted.
**Results**

2. Biomechanical property changes of *E. coli* cells at local scale

*Force measurement by AFM.*

- The resulting force-distance curves can be used to estimate surface hardness, elasticity, and adhesiveness.
- Hardness is indicated by the indentation of the tip engaged with the sample surface.
- Elasticity is measured by spring constant of the cell.
- Adhesiveness is directly reflected by the adhesion force between the tip and the cell surface.

The illustrations are not drawn to scale.

Wen Zhang, Joseph B Hughes, and Yongsheng Chen. Impacts of hematite nanoparticle exposure on biomechanical and surface electrical properties of *E. coli* cells. *Applied Environmental Microbiology*, Submitted.
Results

2. Biomechanical property changes of *E. coli* cells at local scale

Force-distance curves as the tip approached and retracted from the contact with sample surfaces at a maximum loading force of approximately 4 nN (averaged from 6-20 replicates).

- Intact *E. coli* cells had indentation 250–450 nm, which is dependent on accurate estimation of the contact point; impaired cells only had an average indentation of 120 nm.
- No adhesion on intact untreated cells, whereas large adhesion was detected on treated cells.

Wen Zhang, Joseph B Hughes, and Yongsheng Chen. Impacts of hematite nanoparticle exposure on biomechanical and surface electrical properties of *E. coli* cells. *Applied Environmental Microbiology*, Submitted.
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2. Biomechanical property changes of *E. coli* cells at local scale

The spring constant of the cell ($k_b$) can be calculated using the equation: $k_b = k_c s / (1 - s)$

where $k_c$ is the spring constant of the cantilever and $s$ is the slope in the linear compliant region of the force-distance curve.

<table>
<thead>
<tr>
<th>Table 1 Summary of spring constants for untreated and hematite-treated <em>E. coli</em> cells.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cantilever spring constant, $k_c$, (nN/nm)</td>
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<tr>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>Untreated <em>E. coli</em> cells</td>
</tr>
<tr>
<td>Hematite-treated <em>E. coli</em> cells</td>
</tr>
</tbody>
</table>
Results

2. Biomechanical property changes of *E. coli* cells at local scale

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Results
3. Zeta potential and electrical double layer (EDL) theory

E. coli cells, when suspended in liquid, are subject to hydration and forming a EDL. The overall electrical property can be described by zeta potential.

Zeta potential ($\zeta$) is commonly in aquatic chemistry and is measured by Laser Doppler electrophoresis. The electrophoretic mobility ($\mu_E$) is directly measured and $\zeta$ potential is converted from Henry’s approximation:

$$\mu_E = \frac{2\zeta \varepsilon f(\kappa r)}{3\eta}$$

where $\varepsilon$ is the dielectric constant (or permittivity); $\eta$ is the medium’s viscosity (i.e., the viscosity of water); $\kappa r$ is the ratio of particle radius to Debye double layer thickness; and $f(\kappa r)$ refers to Henry’s function, which is 1.5 under the Smoluchowski approximation and 1 under the Hückel approximation.

3. Zeta potential and electrical double layer (EDL) theory

Mixture of hematite NPs and E. coli cells in PBS

Results

4. Surface electric potential probed by KPFM

In metals, CPD can be the energy difference between the vacuum level and the Fermi energy. In semiconductors or biomolecules, the work function may arise from the difference in energy between the vacuum level and the most loosely bound electron inside the sample.
Results

4. Surface electric potential probed by KPFM

Topographical and surface potential images of hematite NPs and intact *E. coli* cells generated by KPFM. (a) and (d) are topographical images. (b) and (e) are surface potential images. (c) and (f) are cross-sectional profiles of surface potentials taken along the dashed red lines in the images in (b) and (e). The red solid lines at the bottom right in (a) and (d) indicate scale bars of 0.5 and 1 µm, respectively.
The relation between the work function of the conductive tip, $\Phi_t$, and the sample, $\Phi_s$, is given by $\Phi_s = \Phi_t - eV_{CPD}$, where $e$ is the elementary charge and $V_{CPD}$ is the CPD or surface potential measured by KPFM. Thus, the mean work function of hematite NPs is approximately $5.71 \, \text{eV}$.

Literature reported value of the work function or Fermi level is $5.88 \, \text{eV}$ and the minor difference is probably due to the moisture on hematite surface that induced band bending.
Results
4. Surface electric potential probed by KPFM

The topographical (left column), surface potential (middle column), and phase (right column) images of *E. coli* cells exposed to hematite NPs (98 nm). Exposure times were approximately (a)-(c) 3 min; (d)-(f) 10 min; and (g)-(i) 45 min. The red scale bars at the bottom right of (a)-(c) and (d)-(i) indicate lengths of 2 µm and 0.5 µm, respectively.

- Deformation observed with hematite NPs adsorbed and the increasing adsorption time
- Surface appendage (flagella) shredded and scattered around.

Wen Zhang, Joseph B Hughes, and Yongsheng Chen. Impacts of hematite nanoparticle exposure on biomechanical and surface electrical properties of *E. coli* cells. *Applied Environmental Microbiology*, Submitted.
Surface became more negatively charged as more hematite attached.

Wen Zhang, Joseph B Hughes, and Yongsheng Chen. Impacts of hematite nanoparticle exposure on biomechanical and surface electrical properties of E. coli cells. Applied Environmental Microbiology, Submitted.
Results

4. Surface electric potential probed by KPFM

My previous work on basis of KPFM focused on differentiation of interacting entities (DNA and QDs) at nanoscale.

Results

4. Surface electric potential probed by KPFM

The topography and surface potential differences enable us to better differentiate and quantify QDs bound with DNA.

Concept of NanoID

Brief conclusion

• The *E. coli* cell surface became coarser, stiffer, and more adhesive with hematite NPs attached.

• Surface potential shifted to more negatively charged with the attachment of hematite NPs, observed by both zeta potential and KPFM.
Mechanisms of surface disruption from a chemical perspective

- High surface area of NPs provides more reactive sites for ROS production
- ROS formed in NP suspension usually consist of superoxide radical (O$_2$•$^-$), hydroxyl radicals (•OH), and singlet oxygen (¹O$_2$)
- Representative reaction stochiometry (TiO$_2$ as an example):

  TiO$_2$ →$^{hv}$ TiO$_2$(h$_{vb}^+$ + e$_{cb}^-$)
  O$_2$ + e$_{cb}^-$ → O$_2$•$^-$
  O$_2$•$^-$ + e$_{cb}^-$ + 2 H$^+$ → H$_2$O$_2$
  O$_2$•$^-$ + H$_2$O$_2$ → •OH + OH$^-$ + O$_2$
  e$_{cb}^-$ + H$_2$O$_2$ → •OH + OH$^-$
  h$_{vb}^+$ + OH$^-$ → •OH
  h$_{vb}^+$ + H$_2$O → H$^+$ •OH
  2•OH → H$_2$O$_2$

Implications:
- Dissolution of metal ions (potentially hazardous to cells);
- Oxidant injury of cells, lipid peroxidation, enzyme or protein oxidation, membrane pitting, changes in membrane permeability, etc.
Almost all types of engineered NPs were reported to produce ROS!

<table>
<thead>
<tr>
<th>NPs</th>
<th>DLS size in diameter (nm)</th>
<th>TEM Size in diameter (nm)</th>
<th>Results</th>
<th>NPs</th>
<th>DLS size in diameter (nm)</th>
<th>TEM Size in diameter (nm)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiO₂</td>
<td>364 175 826-2368 800-1900 79 79</td>
<td>612 15-45 20±3 2610 6, 12, and 1000 14</td>
<td>Y N Y Y</td>
<td>SiO₂- NH₄OH/NaCl l/Na₃AlO₃ 590±10</td>
<td>13.3, 15.3</td>
<td>15±5</td>
<td>Y</td>
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<td></td>
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<td>Si- NH₂    1.6±0.2 (only core)</td>
<td>1.6±0.2 (only core)</td>
<td>Y</td>
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<td></td>
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<td>Si- N₃     1.6±0.2 (only core)</td>
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<td>N</td>
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<td>Si- COOH   15, 100</td>
<td>5-10</td>
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<td>Ag         15</td>
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<td>Ag- PVA    15</td>
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<td>γ-Al₂O₃     15</td>
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<td>α-Al₂O₃     15</td>
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<td>AuNPs- TPPMS 1.4</td>
<td>22.1±1.9</td>
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<tr>
<td>ZnO</td>
<td>50-300 413 800 93</td>
<td>20</td>
<td>Y</td>
<td>AuNPs      11.4 (by SSA)</td>
<td>22.1±1.9</td>
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<td>Co₃O₄      11.4 (by SSA)</td>
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<td>ZnO/PEG</td>
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<td>Fe₂O₃      11.4 (by SSA)</td>
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<tr>
<td>Fe₂O₃</td>
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<th>TEM Size in diameter (nm)</th>
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<tbody>
<tr>
<td>Mn$_3$O$_4$</td>
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<td>CdTe QDs</td>
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<td>InP/ZnS QDs</td>
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<tr>
<td>CdS QDs-mercaptoacetic acid (MPA)</td>
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<td>CdSe QDs-MPA</td>
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<tr>
<td>CdSe/ZnS QDs-mercaptoacetic acid (MPA)</td>
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<tr>
<td>CdTe QDs-biotin</td>
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<tr>
<td>CdTe QDs-MPA</td>
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<td>Polystyrene-NH$_2$</td>
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<tr>
<td>Polystyrene-NH$_2$</td>
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</tbody>
</table>

Results:
- •OH
- O$_2^-$
- ROS
Example 1: Photoleaching or photo-oxidation of QDs, a chemical reaction driven by ROS production

- Environmental effects (irradiation intensity, temperature, dissolved oxygen, and dissolved organic matter)
- Coating effect

Photochemical Experiments

UV (254 nm)

QDs Solution

Membrane with nominal pore size 1-2 nm

Centrifugal Separation

• ions • particles

Ion Concentration Measurement with ICP-MS
Characterization of QDs

(a)-(c) HR-TEM images of Polydiallyldimethylampphounium chloride (PDDA)-, PEG-carboxylic acid-, and PEG-amine-coated QDs, (d) FTIR spectra, (e) Intensity-averaged PSD diagrams, and (f) zeta-potentials as a function of pH for the three types of QDs.

1. CdSe/ZnS as the core
2. 3-5 nm in diameter (TEM)
3. Different surface coating and surface charge

Example 1: ROS measurement, an indirect indication using scavengers

Scavenging experiments:
- t-BuOH (30 mM), l-histidine (80 mM) and superoxide dismutase (SOD) (2000 unit/L) from *Escherichia coli* were used to scavenge •OH, \(^1\)O\(_2\), and O\(_2\)\(^{•−}\), respectively.
- Monitoring the release rates of Cd and Se to indicate the presence of ROS (O\(_2\)\(^{•−}\)).

![Graphs showing ROS measurement](image)

(a) Dissolution kinetics of PDDA-coated QDs in the presence and absence of scavengers (initial concentration of QDs 140±3 µg-Cd/L, t-BuOH 30 mM, l-histidine 80 mM, and SOD 2,000 unit/L), (b) UV-Vis absorption spectra of PDDA-coated QDs in the presence of XTT as a function of irradiation time (initial concentration of QDs 1.4±0.03 mg -Cd/L and XTT 0.15 mM)

**Example 2: Another indirect ROS measurement method using indicators**

<table>
<thead>
<tr>
<th>Methods of probing ROS generation from different types of NPs</th>
<th>( \cdot \text{OH} )</th>
<th>( ^1\text{O}_2 )</th>
<th>( \text{O}_2^- )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>HPLC</td>
<td>HPLC</td>
<td>UV-Vis (430 nm)</td>
</tr>
<tr>
<td>Indicator</td>
<td>( p \text{CBA} )</td>
<td>FFA</td>
<td>XTT</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Preliminary results</th>
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<tbody>
<tr>
<td>NPs</td>
</tr>
<tr>
<td>( \text{TiO}_2 )</td>
</tr>
<tr>
<td>( \text{CeO}_2 )</td>
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<tr>
<td>( \text{SiO}_2 )</td>
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<tr>
<td>( \text{ZnO} )</td>
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<td>( \text{CuO} )</td>
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<tr>
<td>AuNPs</td>
</tr>
</tbody>
</table>
ROS generation on different NPs (TiO$_2$, CeO$_2$, and ZnO as examples)

- TiO$_2$ NPs produces three types of ROS under UV.
- Under room light or dark environment, no significant ROS were detected.

ROS generation on different NPs (TiO$_2$, CeO$_2$, and ZnO as examples)

- CeO$_2$ NPs were found to produce O$_2$$^•$ only.
- ZnO NPs produced •OH and O$_2$$^•$ only.

Quantitative relationship between ROS production and toxic potential (ongoing research)

Now, I propose to assign the weight factors and calculate the “ROS index” for each type of NPs that is further used to draw a relationship with mortality rates (as an indicator of toxic potential).

<table>
<thead>
<tr>
<th>Different ROS/NPs</th>
<th>•OH</th>
<th>(^1)O(_2)</th>
<th>O(_2)^{−}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>A1</td>
<td>A2</td>
<td>A3</td>
</tr>
<tr>
<td>ROS production rate constant (s(^{-1}))</td>
<td>k1</td>
<td>k2</td>
<td>k3</td>
</tr>
<tr>
<td>ROS index=</td>
<td>A1·k1+A2·k2+A3·k3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Yang Li, Wen Zhang, Junfeng Niu, and Yongsheng Chen. Reactive oxidative species generation from engineered nanoparticles: kinetics and toxicity implications. *In preparation.*
Take-home messages

- Physical and chemical insight into nanotoxicity.

- Changes in biomechanical and electrical properties may interpret the surface disruption.

- ROS leads to the chemical irritation for cells and is widely detected in many types of engineered NPs.
Acknowledgements

Advisor: Dr. Yongsheng Chen
PhD student:
Kungang Li, Jia Yang, Wei Zhang,
Jin Gi Hong
Research scientist:
Wen Zhang
Exchange student:
Yang Li
Visiting scholar:
Ying Chen

Funding agency:
➢ U.S. Environmental Protection Agency Science to Achieve Results Program Grant RD-83385601;
➢ Semiconductor Research Corporation (SRC)/ESH grant (425.025).