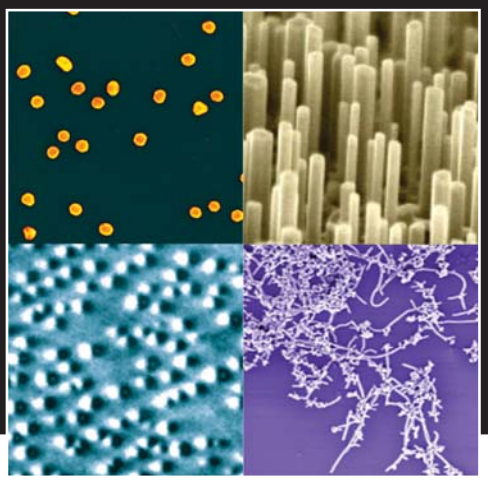
Cell Platforms for Quantifying Nano/Bio Interactions

BIOMATERIALS

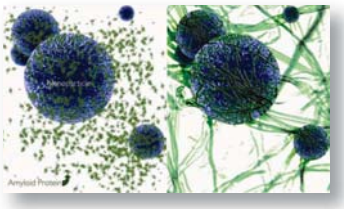
Objective

We are developing *in vitro* tools to characterize the interaction of nanoparticles with cells and the reliability of nanoparticles in biological environments. We focus on measures of nanoparticle uptake (bioavailability), the response of model neural cells and neural stem cells grown within tissue-mimicking hydrogel scaffolds, and the characterization of nanoparticle surfaces post-exposure.



Impact and Customers

- Over 300 US companies are involved in nanoparticle-based product development. Nanotechnology innovations are expected to be a major driver of the world economy in the next decade. Market size estimates range from conservative ($5B) to astronomical ($3T).
- Products utilizing nanomaterials are already on the market despite very limited environmental health and safety data. Perceptions (real or imagined) of health and safety risks can derail further product development. Media coverage of a 2008 Nature Nanotechnology research article included headlines such as "Are nanotubes the next asbestos?" and "Cancer risk seen in nanotechnology."

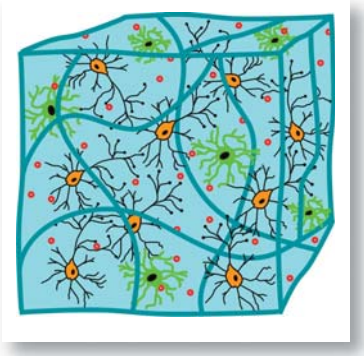


- Toxicologists need standardized, high-throughput measurement tools to ensure worker health, certify the safety of consumer products, assess environmental impact, and aid scientists in developing benign alternatives. Such tools do not exist: cell culture assays do not mimic the essential components of a biological system; whole animal studies are expensive, slow, and increasingly unpopular.

Approach

Two-dimensional cell cultures grown on the surface of a quartz crystal microbalance (QCM) offer the possibility of rapidly assessing nanoparticle uptake by monitoring the shift in resonant frequency. This approach will be validated by quantitative measurements of fluorescent nanoparticles and ultimately applied to nanoparticles that cannot be optically probed.

Three-dimensional cell cultures, consisting of cell-seeded polymer scaffolds, offer a unique medium for toxicology studies because they can be engineered to mimic specific biological systems. They enable long-term studies in which nanoparticles are encapsulated with a population of cells for real-time and end-point analyses of cell health and differentiation. This analytical strategy targets subtle developmental effects rather than acute cytotoxicity.



Nanoparticle surface chemistry is critical to cellular interactions, influencing protein adsorption, particle uptake, sub-cellular targeting, and dissolution or agglomeration. By quantifying properties such as ligand density and stability and zeta potential after exposing nanoparticles to biological systems with increasing complexity, we will identify material properties that impact cell response.

Accomplishments

Encapsulation of Neural Cells

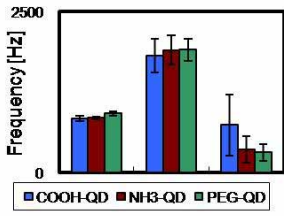
Animal studies suggest that nanoparticles interact with numerous organ systems including the lung (inhalation), liver and kidney (ingestion), and brain (translocation). Our focus is the developing neurological environment. Both model neural cells (PC12s) and neural stem cells (NSCs) isolated from the embryonic rat cortex are under investigation.

Quantum dots (QDs) with a CdSe core, ZnS shell and three different surface chemistries - amine (NH_3-QD), carboxyl (COOH-QD) or poly(ethylene glycol) (PEG-QD) - have been the focus of recent investigations. TEM verifies spherical particles of approximately 4.7 nm diameter that can be tracked by their fluorescence at 565 nm.

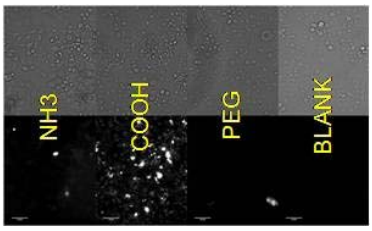
Nanoparticle Uptake

COOH-QDs are incorporated by PC12s to a greater extent than NH_3-QDs or PEG-QDs. Uptake also depends on the exposure conditions (concentration, presence of sugars) and exposure time. Viability staining of individual cells reveals that dead cells exhibit similar levels of fluorescence to live cells.

QCM frequency shifts are largely attributable to mass gain or loss. Upon the addition of media or cells to the crystal, frequency shifts are quite consistent over multiple samples. Upon exposure to QDs, the frequency shifts suggest greater incorporation of COOH-QDs compared to NH_3-QDs or PEG-QDs.



QCM frequency shifts for media (left), cells (center), and QDs (right)

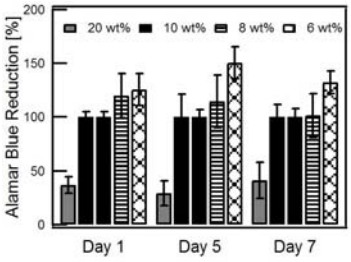


PC12s exposed to 48 nM QDs

Live cells rapidly lose their QDs under ordinary culture conditions (within 48 hr). Therefore polymer scaffolds might serve two purposes: creating a tissue-like environment for normal cell development and entrapping a population of nanoparticles. Since particles are expected to change over time in biological systems, cell response may change over time (e.g., if the particles degrade).

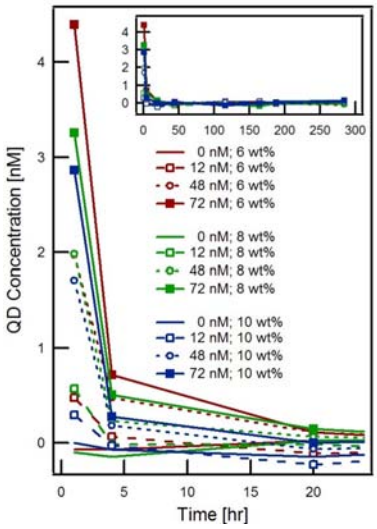
Cell and Nanoparticle Encapsulation

Normal brain tissue is compliant compared to other tissues. We quantified PC12 metabolism in a series of PEG hydrogels with increasing compliance, formed by decreasing PEG weight fractions.



PC12 metabolic activity in PEG hydrogels

Compressive modulus (at 10% strain) decreases from 5 kPa for 10 wt% hydrogels to 0.6 kPa for 6 wt% hydrogels. Since modulus is linked to mesh size (lower modulus equals larger mesh size), nanoparticle leaching may be a concern.

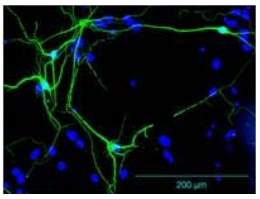


COOH-QD leaching from PEG hydrogels

COOH-QDs incorporated into 6 - 10 wt% hydrogels do leach. However leaching falls below the detection limit after 24 hr of culture; these measurements verify that most QDs remain entrapped in the hydrogels.

Neural Cell Differentiation

NSCs can be induced to differentiate into mature neurons or astrocytes by manipulation of their culture environment.



PC12 metabolic activity in PEG hydrogels

Preliminary evaluation of βIII-tubulin (a neuronal marker) indicates that QD exposure may inhibit normal differentiation.

Learn More

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Publications

Oreskovic TL, Goldstein NS, Jeerage KM, *Neural Stem Cell Differentiation after Exposure to Quantum Dots*, Society of Toxicology, Salt Lake City (2010)

Mansfield E, Oreskovic TL, Jeerage KM, *Two and Three-Dimensional Measurement Platforms for Nanoparticle Screening*, Materials Research Society, Boston (2009)

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