Nanotechnology Innovation Center of Kansas State University

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Nanomaterial Properties

- Catalytic- catalytic efficiency
- Electrical- increased electrical conductivity
- Magnetic- increased magnetic coercivity
- Mechanical- improved hardness
- Optical- increased quantum efficiency of semiconductor crystals
- Sterical- increased selectivity, hollow spheres for specific drug transportation & controlled release
- Biological- increased permeability through biological barriers (membrane, etc.), scaffolds, improved biocompatibility
Physicochemical Properties

- Size distribution
- Agglomeration state
- Shape
- Crystal structure
- Chemical composition
- Surface area
- Surface chemistry
- Surface charge
- Porosity
Physicochemical Parameters

Modify cellular uptake
Protein binding
Translocation

Interactions

Cells
Body fluids
Proteins

Ability to distribute throughout the body
NICKS - Tenure Track Faculty

Dr. Nancy A. Monteiro-Riviere
Regents Distinguished Research Scholar and University Distinguished Professor, Director, of NICKS
Biological interactions of NP/biocorona complex

Dr. Santosh Aryal
Biomedical applications and drug delivery

Dr. Seong-O Choi
Drug Delivery systems

Dr. Jeffrey Comer
Thermodynamics and kinetics of binding between NP and blood proteins

Dr. Robert DeLong
Therapeutic protein delivery of conjugates, peptides and polymers
**Inverted fluorescent microscope Olympus IX73**
Detection of fluorescently labeled NP and cell markers (455-670 emission range) by color and monochrome sensors, full live cell imaging system (controlled temperature and CO2) incorporating motorized stage and anti-vibration platform.

**CytoViva microscope**
Real time hyperspectral imaging analysis of nanomaterials in biological samples (localization of NP in cells, live cell imaging of NP uptake, toxicological assessments).

**NanoSight - Nanoparticle Tracking**
Detection and real time visualization of NP in liquids by Nanoparticle Tracking Analysis of Brownian motion (NP size, concentration, zeta potential & aggregation).

**Zetasizer Nano ZSP**
Particle and molecule size, translational diffusion, electrophoretic mobility, zeta potential of particles at high and low concentrations, viscosity and viscoelasticity of protein and polymer solutions, concentration, MW, A2, kD.

**NICKS Facility**
Skin: PORTAL of entry and TARGET for toxicity

KERATINOCYTES CAN FUNCTION AS ENVIRONMENTAL SIGNAL TRANSDUCER CONverting EXOGENOUS STIMULI INTO PRODUCTION OF PRO-INFLAMMATORY CYTOKINES

Environmental Cues
- e.g. chemical insult
- contact allergen (urushiol)
- ultraviolet light

Initiation Phase

**CYTOKINES**

IL-1
TNF-alpha

IL-6
IL-8
ICAM-1
TNF-alpha
MCAF
TGF-alpha

Recruitment of mononuclear cells & circulating leukocytes

Erythema
Edema

Blood flow
Capillary Permeability
QD-655-COOH in Dendritic Cells - 30 min


Mean HEK Viability and IL-8 Release of HEK at 24hrs MWCNT Exposure

Challenges for assessing carbon nanomaterial toxicity to the skin

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Abstract

This manuscript reviews a number of issues that must be dealt with to assure carbon nanomaterial interactions with the skin in the context of potential toxicity. The potential pathway for dermal absorption of carbon nanomaterials is discussed. The few existing studies assessing carbon nanomaterial toxicity to skin are reviewed. This paper addresses potential confounding factors in dealing with the experimental design of nanomaterial toxicity studies and their interpretation. Certain standard cytotoxicity assays are well suited to assessing the potential impact of carbon nanomaterials and should be considered in future investigations. Rationale and experimental design for a toxicological study comparing carbon effects on human keratinocyte cytotoxicity assessed by transmission electron microscopy, neutral red and MTT cell viability assays, as well as induction assessed by release of the cytokine IL-8. Such sources of carbon black particles were assessed. Conflicting results were obtained across all cytotoxicity endpoints potentially secondary to the admixing properties of carbon fibres and their direct contact with the skin. These data suggest that a single cytotoxicity assay should not be relied upon in assessing carbon nanomaterial toxicity and that carbon black may not be optimal control particles for assessing nanomaterial toxicity in epidermal cell culture systems due to the wide range of responses seen between the carbon black varieties.

Keywords: Carbon nanoparticles; Nanotoxicology; Transmission electron microscopy; Aggregation

1. Introduction

Nanomaterial toxicology remains a challenge with regard to conducting a comprehensive safety evaluation of nanomaterials. As the scale of production of nanomaterials increases, so does the threat of adverse health effects in humans and the potential for environmental damage [1-2]. An important route of exposure is by skin contact, which could lead to skin cancer, skin sensitization, skin irritation, or produce systemic effects after absorption. Little information is available on nanomaterial toxicology, and as the field grows there is an additional need to standardize and categorize the nanomaterials so that results can be compared across studies and data can be obtained for risk assessment. However, to determine and understand the toxic effects of nanomaterials, strategies and interpretation of the data must be done correctly and any assumptions taken into consideration. The purpose of this paper is to provide a review along with original research data to explain the potential problems working with carbon and carbon nanomaterials in skin.

Skin is unique because it is a potential route of both occupational and environmental exposure to nanomaterials and also provides an environment within the associated epidermis where particles could potentially lodge and not be susceptible to removal by phagocytosis. The skin or ingestion is the largest organ of the body and can serve as one of the principal portals of entry by which nanomaterials can enter the body. It also has a relatively large surface area for exposure. Skin is considered to be the barrier between the well-regulated “in vivo environment” and the outside environment. The structure of skin is heterogeneous, yet in functionally organized to yield a dynamic...
TEM of HEK Treated with 0.4 mg/ml BAA for 48 Hrs

TEM – Engulfing 120nm Silica Coated Ag NP

<table>
<thead>
<tr>
<th>Name</th>
<th>Function</th>
<th>Final conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nocodazole</td>
<td>disrupts microtubule (cytoskeleton)</td>
<td>10.0 µg/ml</td>
</tr>
<tr>
<td>CytD</td>
<td>inhibit F-actin polymerization (cytoskeleton)</td>
<td>10.0 µg/ml</td>
</tr>
<tr>
<td>MβCD</td>
<td>cholesterol-depletion reagent (caveolae/lipid rafts)</td>
<td>5.0 mg/ml</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>cholesterol depletion (caveolae/lipid rafts)</td>
<td>10.0 µg/ml</td>
</tr>
<tr>
<td>Genistein</td>
<td>inhibit F-actin recruitment to clathrin pits ^a (clathrin)</td>
<td>10.0 µg/ml</td>
</tr>
<tr>
<td>CPM</td>
<td>inhibitor of Rho GTPase (clathrin)</td>
<td>10.0 µg/ml</td>
</tr>
<tr>
<td>Y-27632</td>
<td>related to cytoskeleton and melanosome transfer ^b</td>
<td>10.0 µg/ml</td>
</tr>
<tr>
<td>NaN3</td>
<td>ATP inhibitor (ATP)</td>
<td>3.0 mg/ml</td>
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<tr>
<td>DMA</td>
<td>Na^+/H^+ exchanger inhibitor (macropinocytosis)</td>
<td>10.0 µg/ml</td>
</tr>
<tr>
<td>WMN</td>
<td>PI3K inhibitor (macropinocytosis)</td>
<td>100.0 ng/ml</td>
</tr>
<tr>
<td>LY</td>
<td>PI3K inhibitor (macropinocytosis)</td>
<td>10.0 µg/ml</td>
</tr>
<tr>
<td>PTX</td>
<td>inhibitor of G_i α subunit (GPCR)</td>
<td>100.0 ng/ml</td>
</tr>
<tr>
<td>CTX</td>
<td>activator of G_iα subunit (GPCR)</td>
<td>1.0 µg/ml</td>
</tr>
<tr>
<td>U-73122</td>
<td>PLC inhibitor (down stream of GPCR)</td>
<td>4.0 µg/ml</td>
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<tr>
<td>SRP</td>
<td>PKC inhibitor (down stream of GPCR)</td>
<td>1.0 µg/ml</td>
</tr>
<tr>
<td>TrpI</td>
<td>inhibitor of PAR-2 pathway (melanosome)</td>
<td>1.0 mg/ml</td>
</tr>
<tr>
<td>NCM</td>
<td>melanosome inhibition (melanosome)</td>
<td>122.1 µg/ml</td>
</tr>
<tr>
<td>BMA1</td>
<td>inhibits endosome acidification (endosome)</td>
<td>100.0 ng/ml</td>
</tr>
<tr>
<td>CRQ</td>
<td>inhibits endosome acidification (endosome)</td>
<td>125.0 µg/ml</td>
</tr>
<tr>
<td>BFA</td>
<td>interferes with Golgi, endosome and lysosome</td>
<td>10.0 µg/ml</td>
</tr>
<tr>
<td>PolyI</td>
<td>scavenger receptor inhibitor</td>
<td>10.0 µg/ml</td>
</tr>
<tr>
<td>FCD</td>
<td>scavenger receptor inhibitor</td>
<td>10.0 µg/ml</td>
</tr>
<tr>
<td>LDL</td>
<td>ligands for LDL receptor</td>
<td>10.0 µg/ml</td>
</tr>
<tr>
<td>AcLDL</td>
<td>ligands for scavenger receptor</td>
<td>10.0 µg/ml</td>
</tr>
</tbody>
</table>

**Table 1. Inhibitors and their function and concentration.**

^a Genistein inhibits tyrosine kinase activities that can phosphorylate actin and recruit the cytoskeleton proteins to clathrin-coated pits and enhance the internalization of clathrin mediated endocytosis.

^b Y-27632 is the inhibitor of Rho-associated kinase (ROCK) and abolishes actin filaments. It is also used to inhibit melanosome transfer to keratinocytes via protease-activated receptor 2 (PAR-2) pathway.
Role of inhibitors in the cell endocytic pathways

G-protein coupled receptor related pathway

The BioCorona Paradigm

The nanoparticle–protein complex as a biological entity; a complex fluids and surface science challenge for the 21st century

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# Biophysical Chemistry, Lund University, 221 00 Lund, Sweden

Fig. 2. Protein–nanoparticle complex. Examples of the range of proteins contained in human plasma or serum. Depending on the affinity of the proteins for the nanoparticles, and the lifetimes of the associations, the nature of the protein–particle complex can be considered as a dynamic state which changes over time and as a function of the nanoparticle’s location.
Protein Corona Formation: A Dynamic Process


Proteins Associated with > 10% of Adsorbome of a Biocorona

- Antithrombin-III
- Apoliprotein B-100
- Apoliprotein E
- Fibrinogen
- Histidine-rich glycoprotein
- Albumin
- Transferrin
- Apoliprotein A-I
- Apoliprotein C-II
- Clusterin
- Haptoglobin
- Paraoxonase-1
- Inter-α-trypsin inhibitor H1
- Apoliprotein A-IV
- Apoliprotein C-III
- Complement C3
- Hemoglobin
- IgG
- Mannose-binding protein C
Biocoronas modulate cellular uptake of 20 and 100 nm AgNP with two coatings pre-incubated with three different proteins.

TEM Silica-Coated AgNP

Albumin

Transferrin

IgG

Control
Citrate and Lipoic Acid Au NP- HSA Protein Complexes

Absorption

• Relates to the amount of chemical that penetrates the skin and then absorbed into the bloodstream to have a systemic effect.
• Detected by flux into the perfusate or blood.

Penetration

• Relates to the amount of chemical that gets to targets within the skin and could be available for local cutaneous activity.
• Detected by confocal microscopy, transmission electron microscopy, special stains, biopsies and tape stripping.
Topical occupational exposure with very fine carbon nanotube dust or consumer products such as cosmetics or sunscreen

Is there a safety issue?
TEM – In Vivo Porcine Skin-TiO$_2$ CM 630

a: No UVB- treated with CM 630 TiO$_2$ - on surface 48hrs.
b: UVB-exposed CM 630 TiO$_2$. Ti was present up to 6-8 cell layers deep - but most showed 7-11 cell layers 48hrs.

Time of Flight SIMS
TOF-SIMS - In Vivo Skin - TiO$_2$ CM 634 - 48hrs

(a) normal skin (b) UVB skin (c) CM 634; (d) UVB-CM 634 (e) intensity bar. Bar = 100μm
Human Skin - QD565-PEG - 8 hr

Panels D-E-F - Tape stripped 30X
QD565-COOH
Abraded-Rat

Bar = 100 µm
Flexing of Baa (FITC)-NLS over 8 hr

Control  
60 min Flex  
90 min Flex

Scale bars = 50 µm
Biodistribution

- What happens to any nanoparticles that enter the vasculature?
- We studied skin distribution of QD after arterial infusion into skin.
- **NOT** studying dermal absorption
IPPSF Surgery and Perfusion Systems
SF 3181 – QD-COOH 3.33 nM
QD-COOH-621 IPPSF at 6.67nM
TEM and SEM of AgNP PLCL Scaffolds

Microneedles for Drug Delivery
Micro/NanoScale Biomedical Devices

Micro/nano-scale biomedical devices

- **Drug delivery systems**
  - Microneedle-based transdermal/intracellular delivery eg., vaccinations
  - Nanoneedle array for high throughput intracellular delivery
  - Micro-implant for controlled drug delivery

- **Functional 3-D micro/nano-structures**
  - 3-D micro/nanoelectrode arrays for bio and chemical sensing
  - 3-D re-entrant structures for superomniphobic surfaces

Capabilities

- **Fabrication technology**
  - Unconventional lithography
  - Micromolding
  - Nanoimprint lithography
  - Electroplating

- **In vitro/In vivo test**
  - Histology
  - Pharmacokinetics/pharmacodynamics in animal models
  - Cell analysis (flow cytometry, confocal microscopy, etc.)
  - Quantitative assays (ELISA, BCA, etc.)
An Atomic-Scale Look at Nanomaterial Surface Interactions

Molecular simulation can predict adsorption affinities of small molecules on nanomaterials.

Structure and dynamics of the nanoparticle corona

Passive transport of nanoparticles through biological membranes

Hydroxylated CNT

Adsorption constants for small organics on hydroxylated CNT: Calculation vs experiment

Dr. Comer
Bionanomaterials

Chemistry

- Multifunctional Polymer
- Paclitaxel-PLGA conjugate
- Pt (IV) Prodrug
- Drug Conjugates

Materials Chemistry

Engineering

- Multifunctional Nanoparticle
- Cells
- RBC

Engineering Cellular Vehicles

Biomedical Applications

- Drug Delivery System
- MR
- PET-CT
- Multimodal Imaging

Multifunctional Fibrous Matrix/Scaffolds

Blood Vessels

Tissue

Materials

Dr. Aryal
Effect of Bioelement Nanomaterials on RNA, Protein Structure, Function and Delivery

Anti-cancer nanoparticles

Biomolecular nanomaterial interactions

Fluorescence and photoluminescence characterization

ZnO

ZnO +CPP

Dr. DeLong
Still Sitting Down on the Job!

Thank You