Nanotechnology for cancer therapy: benefits, concerns and effects on the immune system

Marina A. Dobrovolskaia
Senior Principal Scientist, Immunology Section Head
Nanotechnology Characterization Lab (NCL)

November 28, 2016
marina@mail.nih.gov
Outline

Presentation outline

- Nanotechnology Definitions
- Nanoparticles in Daily Life
- Nanoparticles in Medical Applications
- Nanoparticles for Cancer Diagnosis and Therapy
  - Benefits of nanotechnology
  - Toxicity concerns
- Nanomaterials and the Immune System
What is Nano?

Nanotechnology:
“Research and technology development at the atomic, molecular or macromolecular scale leading to the controlled creation and use of structures, devices and systems with a length scale of approximately 1 – 100 nanometers (nm).” (Source: National Nanotech Initiative)

“Whether a material or end product is engineered to exhibit properties or phenomena, including physical or chemical properties or biological effects, that are attributable to its dimension(s), even if these dimensions fall outside the nanoscale range, up to one micrometer (1,000 nm)” (US FDA)
Nanoparticles

Nanoparticles in Daily Life

- **Consumer products**
  - 800+ “manufacturer identified” products from 400+ companies in 20+ countries
  - Clothing, wound dressings, washing machine liners
  - Sunglasses (lens coatings)
  - Sporting equipment

*Image: The Adidas Lone Star™ track shoe includes a lightweight spike-plate made of carbon nanotubes.*

*Image: Turtle Wax™ makes a nanotech car wax.*

*Image: Nanosilver is in supplements and used to treat clothing.*

*Image: Speedo LZR™ Racer Swimsuit is treated in a nanotech cold-plasma process that reduces water absorption.*
Nanoparticles in daily life

Nanoparticles in Daily Life

- Nearly all translucent sunscreens contain nanoscale TiO\textsubscript{2} or ZnO\textsubscript{2}

- Liposomes and emulsions are commonly used in cosmetics (L’Oreal holds more than 60 patents related to nanotechnology)

- Carbon nanotubes are used as structural materials
Nanoparticles in medical applications

Common features of Nanomedicines:
- Primary market is cancer therapy
- Intravenous administration
- <350 nm in size
- Neutral, hydrophilic surfaces
- Spherical
Nanoparticle type

Nanomedicine by Nanoparticle Type

- Dominant (> 10% of total) Investigational Nanomedicines: Liposomes > Polymeric NP > Emulsions > Solid NP
- Dominant Commercial Nanomedicines: Solid NP > Nanocomposites

Cancer Nanotechnology

- Improve solubility; act as a carrier for hydrophobic drugs.
- Multifunctional capability
- Tumor targeting (reduced toxicity)
- Robotic tasks such as sensing, computation, and actuation; triggered responses.


Benefits

Benefits: Drug Delivery and Targeting

EPR Effect = Passive Targeting
- Leaky neovasculature
- Particles cross endothelial barrier
- Particles are retained in the tumor

Active Targeting
- Surface chemistry allows functionalization w/ targeting molecules
  - Antibodies, e.g. Herceptin
  - Small molecules, e.g. folic acid
  - Cytokines, e.g. TNF-α
Reduced toxicity

Benefits: Reduced Toxicity

Traditional

Small Molecules

Nano

Therapeutic Proteins

Incorporation of conventional pharmaceuticals into nanotechnology-derived platforms helps decrease their immunotoxicity.

DIC = disseminated intravascular coagulation; PCA = procoagulant activity
**Immunotherapy**

### Benefits: Immunotherapy

**A**
- Athymic Nude
- Tumor Volume (mm³)
- Days after Cell Implantation
- Untreated
- Doxorubicin (5 mg/kg)
- Doxil (5 mg/kg)

**B**
- Balb/C
- Tumor Volume (mm³)
- Days after Cell Implantation
- Untreated
- Doxorubicin (5 mg/kg)
- Doxil (5 mg/kg)

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**The Immunotherapy Opdivo & Abraxane for Recurrent HER2-Negative Metastatic Breast Cancer**

A Phase 1, Open-Label, Multicenter, Safety Study of Nivolumab (BMS-936558) in Combination With Nab-Paclitaxel Plus or Minus Gemcitabine in Pancreatic Cancer, Nab-Paclitaxel / Carboplatin in Stage IIIB/IV Non-Small Cell Lung Cancer or Nab-Paclitaxel in Recurrent Metastatic Breast Cancer (NCT02309177)

**Doxil improves efficacy of cancer immunotherapeutics in CT26 mouse model of colorectal cancer**

**Abraxane is investigated in combination with a-PD-1 in clinical trials for metastatic breast cancer**
Vaccines

Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy

LETTER 16 JUNE 2016 | VOL 534 | NATURE | 397

Systemically administered lipoplex carrying tumor specific RNA:
- LPX protects RNA from degradation, enhances RNA uptake by APC in lymphatic and maturation of DC
- APC express cancer antigens and produce IFN response similar to tat during viral infection
- Induction of strong effector and memory T-cells
Lymphatic delivery

Benefits: lymphatic delivery

- i.d. injection
- Examine draining lymph nodes

Injection Site

Tail

100nm

25nm

Smaller particles travel through lymphatics. Larger particles do not.


- Particle distribution to lymph nodes after i.d. injection depends on their size
- Lymphatic delivery benefits vaccines, HIV and infectious diseases therapy
Toxicity

Concerns: Toxicity

- Both nanocarrier and API can be toxic
- Some nanocarriers contribute to toxicity of API.
Toxicity

Concerns: Toxicity

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Biodistribution</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin HCl</td>
<td>Bone Marrow</td>
<td>Myelosuppression</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>Cardiotoxicity</td>
</tr>
</tbody>
</table>

- Both nanocarrier and API can be toxic
- API toxicity can “relocate” depending on the particle biodistribution
Immune system

Nanoparticles and the immune system

- Plasma Proteins
  - Biodistribution and MPS uptake
- Effects on erythrocytes
- Blood coagulation system
  - Platelets
  - Leukocytes
  - Endothelial cells
- Allergy
  - Complement activation
  - DTH
- Cytokines
- Immunogenicity
Bidirectional communication between Nanoparticles and Proteins

- Opsonization and Uptake by MPS
- Influence on Particle Distribution
- Change in Physicochemical Characteristics
- Dissolution
- Interference with Targeting

- Interaction with Coagulation Factors
- Activation of Complement
- Formation of Amyloid Structures
- Activity Gain or Loss
- Changes to Protein Stability
- Exposure to New Epitopes

Binding of proteins to nanoparticle surface result in changes in particle properties. Properties and function of some proteins may also change after binding to the nanoparticle.
Particle size influences protein binding.

**Figure 2.** Size of proteins in the corona compared to nanoparticles of varying diameter. Nanoparticles are represented in blue and the diameter is given by the number under each particle in nm. Serum albumin is shown in red and scaled relative to the nanoparticles. High-density lipoprotein is represented by orange spheres at a size of 12.5 nm diameter.

*Cedervall Tet al, 2016. Handbook of Immunological properties of engineered nanomaterials*
Protein binding affects particle size

**BEFORE**

30 nm colloidal gold nanoparticle

**AFTER**

30 nm colloidal gold nanoparticle

“Protein Corona”

DLS

33 nm

DLS

76 nm

Incubation with human plasma increases hydrodynamic size of nanoparticles

Bidistribution

Protein Binding and biodistribution

- Particles which bind proteins are eliminated by MPS
- Particle surface protection (e.g. with PEG) reduces protein binding and MPS
- Good correlation between in vitro and in vivo
MPS uptake

- Two theories about nanoparticle distribution to the MPS
  - Capture – uptake by phagocytic cells in the tissue
  - Hijacking – uptake by circulating phagocytic cells which then take the particle to tissue

Hemolysis

Main symptoms of Acute hemolytic reaction

- Systemic: Chills, Fever
- Vascular: Hypotension, Uncontrollable bleeding
- Transfused vein: Heat sensation
- Lumbar region: Pain
- Heart: Increased heart rate
- Chest: Constricting pain
- Urinary: Hemoglobinuria, Hyperbilirubinemia

Role of Nanoparticle Size

- Anionic
- Neutral
- Cationic

Role of Nanoparticle Surface Charge
Hemolysis

Composition

Terminal groups

EDA core
PAMAM
Dendrimers
G3-G6

Succinamic Acid

\[ \text{N} \rightarrow \text{O} \]

\[ \text{O} \rightarrow \text{H} \]

Amine

Ethanolamine

Effects of Size

% Hemolysis

0 5 10 15 20 25 30

NC PC G3-NH2 G6-NH2

ND 98.9 ± 0.6%

Effects of Surface Charge

% Hemolysis

0 5 10 15 20 25 30

NC PC -NH2 -COOH OH

ND ND ND ND ND

- Cationic dendrimers are more hemolytic than their anionic and neutral counterparts of the same size
- Larger dendrimers are more hemolytic than smaller

NC = negative control; PC = positive control; EDA = ethylenediamine; PAMAM = poly(amidoamine)
Coagulation system

Nanoparticles can be engineered to avoid or specifically interact with coagulation system.

Procoagulant
Hemostasis
Anticoagulant

Blood Flow
Coagulation Factors
Endothelial Cells, Platelets

GOOD
BAD
BAD
Undesirable effects
Platelet aggregation was induced by cationic PAMAM dendrimers, and increased with increasing particle size and greater number of surface amines. Anionic and neutral dendrimers, irrespective of size and number of surface groups, did not induce platelet aggregation in vitro.
Zeta potential

Platelets: role of zeta potential

Zeta Potential is important. Less surface amines = less platelet aggregation.
Platelets: effect of composition

Triazine dendrimers are less potent in inducing platelet aggregation than their PAMAM counterparts.

Triazine dendrimers were kindly provided by Dr. Eric Simanek, Texas Christian University.
Leukocyte Procoagulant Activity

Key events (occur through multiple mechanisms)
1. Exposure of phosphatidylserine (PS) on cell surface
2. Expression and/or de-encryption of tissue factor (TF)

Activation of extrinsic plasma coagulation cascade

- Cationic, but not anionic or neutral PAMAM dendrimers induced PCA in vitro
- Large particles are more reactive
Endothelial cells

Effects on endothelial cells

Nanomaterial

Direct toxicity

Inflammatory response

EC

Type I activation

Type II activation

Injury

EC dysfunction

EC death

Simak J. Handbook of Immunological properties of Engineered Nanomaterials (2016), Vol 2
Allergenicity

Table 2. Main characteristics of the different types of hypersensitivity reactions.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Type I (anaphylactic or immediate hypersensitivity)</th>
<th>Pseudoallergy</th>
<th>Type II (cytotoxic hypersensitivity)</th>
<th>Type III (immune complex mediated hypersensitivity)</th>
<th>Type IV (delayed hypersensitivity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main mediators</td>
<td>IgE, mast cells</td>
<td>Complement</td>
<td>Mainly IgG, natural killer cells, complement</td>
<td>IgG, IgM, complement</td>
<td>T helper cells and macrophages</td>
</tr>
<tr>
<td>Antigen</td>
<td>Exogenous</td>
<td>Exogenous</td>
<td>Cell surfaces minutes–hours</td>
<td>Soluble antigens</td>
<td>Bacteria, tissues</td>
</tr>
<tr>
<td>Time</td>
<td>15–30 min</td>
<td>Skin prick negative</td>
<td></td>
<td>3–8 h</td>
<td>48–72 h</td>
</tr>
<tr>
<td>Skin reaction</td>
<td>Skin prick positive wheal and flare</td>
<td>Urticaria, angioedema</td>
<td></td>
<td>Intradermal injection (swelling and redness)</td>
<td>Mantoux-positive (erythema and induration)</td>
</tr>
<tr>
<td>Clinical</td>
<td>Allergic asthma, hay fever, anaphylactic shock</td>
<td></td>
<td>Pemphigus, nephritis, autoimmune haemolytic anaemia, Goodpasture's syndrome</td>
<td>Serum sickness, fever, glomerulitis, vasculitis</td>
<td>Tuberculin test, poison ivy, contact dermatitis, maculopapular rashes, granuloma</td>
</tr>
<tr>
<td>manifestations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


- Nanoparticles can be engineered to inhibit allergy (tolerogenic and drug-carrying nanoparticles)
- Some nanoparticle can exaggerate allergy to traditional allergens
- Pseudoallergy is the most common and best studied reaction to nanomaterials
- Rare example of cell-mediated allergy to dendrimers
Allerginicity

Allerginicity: CARPA to PEG-Liposomes

Complement activation is dose limiting toxicity of PEGylated liposomes

Complement activation

**Complement activation: take home message**

**Complement**
- Enhances B-cell responses to antigen
- Promotes T-cell activation
- Promotes DC activation

Nonspecific clearance of pathogens
- Hypersensitivity reactions
- Anaphylaxis

Humoral immune response
- Cellular immune response

Nanotechnology-based pharmaceuticals

If particles are intended for systemic administration, complement activation should be avoided.

If particles are intended for s.c. & i.d. routes, complement activation may benefit vaccine efficacy.

DC = dendritic cell; s.c. = subcutaneous; i.d. = intradermal
Allergenicity

Allergenicity: DTH to dendrimers

A case of toxic epidermal necrolysis-like dermatitis evolving from contact dermatitis of the hands associated with exposure to dendrimers

Contact Dermatitis 2008: 59: 122–123
Department of Dermatology, Course of Integrated Medicine, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

- Only one case of necrotizing dermatitis (type IV reaction) in response to dendrimers is reported in the literature: fever, chills, exudative erythema and fused bullae (Nikolsky’s reaction)
- The mechanism is unknown
Cytokine storm:
Lessons from biotechnology products:

Preclinical studies in NHP and rodents did not reveal cytokine storm

Phase I clinical trial: 6 of 6 volunteers experienced cytokine storm which lead to multiple organ failure

In vitro experiments using human PBMC showed high TNF levels in response to TGN1412

TGN1412 = CD28 Super-MAB
Cytokine storm

Cytokine Storm to nanomaterials can be predicted in vitro

In vivo

Control: Normal spleen
NP2: congestion in spleen

In vitro (human PBMC)

- Endotoxin is undetectable in NP1 and NP2 by gel-clot LAL
- NP2 but not NP1 is toxic
- Necropsy reveals congestion and multiple organ damage similar to that seen in septic shock
- Analysis of plasma samples reveals elevated cytokines

Results are reproduced in vitro using human PBMC

NP = nanoparticle; PBMC = peripheral blood mononuclear cells; IL- interleukin; TNF = tumor necrosis factor; LAL = limulus amebocyte lysate
Lipid based particles induce IL-8

- Induction of IL-8 by liposomes follows induction of oxidative stress and can be prevented by antioxidant N-acetyl cysteine
Oxidative stress

Mechanism of IL-8 induction: oxidative stress

- Lipid carrier
- Oxidative stress
- ROS
- MAPK p38
- Cremophor-EL
- Cremophor-EL-NAC
- NAC
- Cre
- Cre + p38 inhibitor
- mRNA stabilization
- IL-8 protein
- IL-8 mRNA

- dotted – isotype filled – NC solid line -treated
Fibrous and Cationic Nanoparticles induce IL-1

Particle and Fibre Toxicology

Research
Particle length-dependent titanium dioxide nanomaterials toxicity and bioactivity
Raymond F Hamilton Jr1, Nianqiang Wu2, Dale Porter3, Mary Buford1, Michael Wolfarth3 and Andrij Holiak*1

- Long fibrous TiO₂ nanoparticles enhanced endotoxin-mediated IL-1
- Cationic dendrimers have similar property
- Enhancement of endotoxin-mediated inflammation is a serious safety concern due to common contamination of nanomaterials with bacterial LPS
IL-1

Mechanism of IL-1 induction: Proton sponge effect

Cationic particles induce IL-1β through activation of NLRP3 inflammasome triggered by a proton-sponge mechanism.
Immunogenicity

Not immunogenic even in the presence of strong adjuvant

- Other C60-derivatives
- Gold colloids
- Other PAMAM dendrimers

Immunogenic after conjugation to a protein carrier

BSA = bovine serum albumin; RSA = rabbit serum albumin; TG = thyroglobulin
Immunogenicity

- Nanoparticles Can Be Engineered To:
  - Be (non)immunogenic
  - Reduce immunogenicity of therapeutic proteins
  - Enhance immunogenicity of proteins/peptides
- Accidental Nanoparticles ≠ Nanomedicines

Accidental particles contributing to antigenicity of ThPr
- Glass fibers
- Cellulose fibers
- Tungsten
- Silicon Oil
- Rubber
- Stainless steel
- Fluoropolymers

ENM approved for clinical use which resulted in antigenic response
- None

ENM carrying ThPr and resulting anti-ENP response
- None

ENM carrying ThPr and resulting in anti-ThPr response
- None

- Antibodies were generated ONLY after conjugation to protein carrier and injection in the presence of strong adjuvants
- ENM = engineered nanomaterials; ThPr = therapeutic protein; SWCNT = single wall carbon nanotubes; PAMAM = polyamidoamine; TNF = tumor necrosis factor

Immune system
Take home message

Take home messages

- Nanotechnology can benefit cancer therapy by improving formulation of traditional drugs (SM, biotechnology products and immunotherapeutics)
- Nanoparticles physicochemical properties determine particle toxicity
- Nanoparticles can be engineered to either specifically interact with or avoid the immune system
- Nanoparticle interaction with the components of the immune system can be desirable or undesirable
- Desirable interactions can benefit therapies of many disorders including cancer