SCBM302: Regenerative Neurobiology
Topic: applications of nanotechnology for regenerative medicine

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Regenerative medicine aims to develop methods to regrow, repair, or replace damaged or diseased cells, tissues, or organs based on integrated strategies.
Various nanotechnology approaches in regenerative medicine

Nanotechnology approaches

- Scaffolds
  - Retention of growth factors
  - Integration with neighboring tissues
  - Cell attachment and proliferation

- Nanomaterials
  - Conduit for growth
  - Bioactive properties
  - Preferential differentiation of cells into specific lineage

- Stem cells
  - Release of chemokines
  - Cellular support for integration of implants
  - Differentiation into specific lineages

Figure 1 Various nanotechnology approaches in regenerative medicine.
Engineered nanostructured scaffolds for human tissues

- **Organ**
  - Heart
  - Liver
  - Bone

- **Natural tissue structure**
  - Heart: Cardiomyocytes, Fibroblasts
  - Liver: Polarized cells, ECM
  - Bone: Osteon, HAP crystals, ECM

- **Engineered scaffold**
  - Grooved arrays can promote cardiomyocyte elongation and alignment.
  - Surface molecules on nanofibres can promote polarization.
  - HAP nanostructures can enhance osteogenesis.

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*Principles of Regenerative Medicine (Third Edition) 2019, Pages 485-504*
Bone regeneration using nanotechnology

Figure 2 Schematic representation of bone regeneration using nanotechnology.

Notes: Improved bone healing using (A) nanofibrous scaffold and (B) culturing MSCs on nano matrices.

Abbreviation: MSCs, mesenchymal stem cells.
Various nanotechnology-based methods for skin regeneration

Figure 3 Various nanotechnology-based methods for skin regeneration.
Dental regeneration

Figure 4 Surface modification showing improved performance and longevity of dental implants.

Abbreviation: HA, hydroxyapatite.
Applications of nanotechnology for stem cell therapy
Nanotechnology is science, engineering, and technology conducted at the nanoscale, which is about 1 to 100 nm. (National Nanotechnology Initiative (NNI))

Figure 1: Relative sizes of naturally occurring items. The nanometer length scale is thousands times smaller than bacteria and ten million times smaller than a human hair.
Does the size matter?

Quantum effect

http://en.wikipedia.org/wiki/Nanoparticle

High surface area


http://www.nano.gov
Two different approaches

Top down

Bottom up
Physicochemical properties of nanomaterials
There are very broad materials that can be used in nanomedicine. It can be biomaterials, or synthetic materials such as carbon nanostructures, quantum dots, metal particles, liposomes, and formulations based on natural and/or synthetic polymers.

The composition and architecture of these systems play an important role in their loading capacity, stability, biodegradability and overall biocompatibility, and various functional aspects.

Nanoparticles can be further functionalized with ligands for biological targeting. Such flexibility in design freedom enables researchers to tailor nanoparticle for specific intracellular applications as contrast agents, drug delivery vehicles.
Which materials should be used?

- Core: commonly use quantum dots, iron oxide, gold
  - Depending on its applications for example iron oxide nanoparticles has magnetic properties which could be used for separation for enhance contrast in Magnetic Resonance Imaging (MRI), whereas quantum dots give fluorescence signals.

- Shell: commonly use polymers, liposome
Examples of nanomaterials

Polymeric NPs

Magnetic NPs

Quantum dots

Drug delivery

Separation

Bio-imaging

Enzyme/Protein encapsulation
Quantum dots

Optical properties of nanoparticles depend greatly on its structure. Particularly, the color (wavelength) emitted by a quantum dot (a semiconductor nanoparticle) depends on its diameter.

Solutions of CdSe QD’s of different diameter

Photo credit: Nature Methods. 2008, 5(9), 763-775

Imaging of QD’s targeted on cellular structures

The quantum dots (QD) can be injected to a subject, and then be detected by exciting them to emit light

Photo credit: Nano Letters. 2008, 8, 3887-3892
Sensitivity comparison between QD and GFP, Alexa 488

QD has higher fluorescent stability comparing to GFP and organic dye such as Alexa 488.

QD-tagged cancer cells (orange, upper) and GFP-labeled cells (green, lower)

Green: Alexa488 Conjugate    Red: qdot 605 Conjugate

Photo credit: Nature Biotechnology. 2004, 22, 969 - 976
The quantum dot itself (the semiconductor nanoparticle) is toxic. Therefore some typical modifications have to be made for it to become biocompatible.

1) The core consists of the semiconductor material that emits lights

2) The shell consists of an insulator material that protects the light-emitting properties of the QD in the upcoming functionalization

3) The shell is functionalized with a biocompatible material such as PEG or a lipid layer

4) Additional functionalization can be done with several purposes (e.g. embed a drug for drug delivery, or assemble an antibody to become the QD target-specific

Source: The scientist. 2005, 19, 35
Magnetic nanoparticles are normally prepare by salt co-precipitation and the common one is iron oxide nanoparticles which is very useful for MRI imaging.

Magnetic nanoparticles can be functionalized with other ligands for medical applications.

TEM images of magnetic nanoparticles

J. Mater. Chem. B. 2013, 1, 1749-1754

J. Mater. Chem. 2009, 19, 6258-6266
Polymeric nanoparticles

Polymer properties are different depending on the polymer itself, the combination of monomer in co-polymer, the formulation process of materials. It allows us to be able to tune the properties of the nanoparticles such as sustained release properties by changing the type of materials or ratio of co-polymer or mix different type of polymer.

poly(lactic-co-glycolic acid)  polyethylene glycol
polyvinyl alcohol  polycaprolactone
Polymeric nanoparticles also offer the functionality for further modify with biomolecules.

**Advantage** of polymer is easy to modify physical & mechanical properties, surface modification, and it is biodegradable.

**Disadvantage** of polymer is leachable compounds, absorb water & proteins etc., surface contamination, biodegradation and difficult to sterilize.
The use of nanotechnology to diagnose, treat, and prevent disease.

Can you think of applications in the area of healthcare that would benefit from the ability to control things at the nano level?
Nanomedicine

Nano-vehicles travel in bloodstream to the targeted tissues or organs.

Blood vessels

Gold or gold-shell nanoparticles accumulated in mice as a contrast agent or targeted-drug delivery.

Quantum dots accumulate in targeted tissue or organ, and become fluorescent after exposure to light or laser.

Nanoparticles deliver therapeutic agents to a targeted tissue.

Targeting molecule
Polyethylene glycol stalk
Receptor

Therapeutic core
Good design of nanodevice

- Good mechanical and chemical properties
- No undesirable biological effects
- Possible to process with good reproducibility
- Controllable for specific functions (programmable nanodevice)

The general design of nanoparticles should have good mechanical and chemical properties, no undesirable biological effects, possible to process, fabricate with good reproducibility, and controllable for specific functions (programmable nanodevice) to take action at the right time and the right place.

Therefore, the nanoparticles usually are constructed as a multicomponent system.
## Typical nanocarrier components

<table>
<thead>
<tr>
<th>Component</th>
<th>Functions</th>
</tr>
</thead>
</table>
| **Binder** | • Hold different components  
• Can be inert or be imaging contrasting agent |
| **Biocompatibilization** | • Make nanocarrier compatible with biological environment  
• Avoid defense mechanism |
| **Imaging contrast** | • Provide imaging properties |
| **Sensor** | • Alter the behavior of nanocarrier once it arrives at the target |
| **Targeting** | • Drive nanocarrier to desired location  
• Passive vs active targeting |
| **Therapeutics** | • Bioactive agents such as drugs |
The nano drug delivery system must contain specific molecules/biomolecules to do one or all of the following:

1. Cell targeting
2. Cell entry
3. Intracellular targeting
4. Controlled drug delivery
Construction of multi-component nanodevice in reverse order of controlling events

- Core such as QD, magnetic
- Drug or therapeutic gene
- Intracellular targeting
- Cellular targeting
Goal of nanomaterials is to protect the biopharmaceutical molecules from chemical and enzymatic degradation until it arrives at the target site.
Glucose responsive nanoparticles for insulin delivery

NPs are able to encapsulate biopharmaceutical molecules, thus providing protection against chemical and enzymatic degradation.

Nature Reviews. 2015, 4, 45-57
Two action modes for therapeutical nanoparticles

Passive Targeting

Based on retention effect of particle of certain hydrodynamic size in cancerous tissues

Active Targeting

Based on nanoparticle functionalization for specific targeting of cancerous cells
A. Tumorous tissues suffer of Enhanced Permeability and Retention effect

B. Nanoparticles injected in the bloodstream do not permeate through healthy tissues

C. Blood vessels in the surrounding of tumorous tissues are defective and porous

D. Nanoparticles injected in the bloodstream permeate through blood vessels toward tumorous tissues, wherein they accumulate

Active targeting of nanoparticles

A. A dual Nanoparticle, the targeting ligand allow it to diagnose if a cell is healthy or sick, and bind specifically to the tumorous cell.

B. Once inside the cell, the polymeric nanoparticle degrades and the anticancer agent is set free.

C. An imaging agent can be added as well.

Active Targeting mechanism

Because of their small sizes, nanoparticles are taken by cells where large particles would be excluded or cleared from the body.

1) nanoparticle carries the pharmaceutical agent inside its core, while its shell is functionalized with a ‘binding’ agent

2) Through the ‘binding’ agent, the ‘targeted’ nanoparticle recognizes the target cell. The functionalized nanoparticle shell interacts with the cell membrane.

3) The nanoparticle is ingested inside the cell, and interacts with the biomolecules inside the cell.

4) The nanoparticle particles breaks, and the pharmaceutical agent is released.

Source: https://cancer.osu.edu/
Nanoparticles-based drugs

<table>
<thead>
<tr>
<th>Commercial name</th>
<th>Platform</th>
<th>Targeting ligand</th>
<th>Active pharmaceutical ingredient</th>
<th>Indication</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>DaunoXome</td>
<td>liposomes</td>
<td></td>
<td>daunorubicin</td>
<td>Kaposi’s sarcoma</td>
<td>approved</td>
</tr>
<tr>
<td>Myocet</td>
<td>liposomes</td>
<td></td>
<td>doxorubicin</td>
<td>combinational therapy of recurrent breast, ovarian cancer refractory</td>
<td>approved</td>
</tr>
<tr>
<td>Doxil/Caelyx</td>
<td>PEG-liposomes</td>
<td></td>
<td>doxorubicin</td>
<td>Kaposi’s sarcoma, recurrent breast, ovarian cancer relapsed</td>
<td>approved</td>
</tr>
<tr>
<td>Onco TCS</td>
<td>liposomes</td>
<td></td>
<td>vincristine</td>
<td>aggressive non-Hodgkin’s lymphoma metastatic breast cancer</td>
<td>approved</td>
</tr>
<tr>
<td>Abraxane</td>
<td>albumin-bound paclitaxel nanoparticles</td>
<td>paclitaxel</td>
<td></td>
<td></td>
<td>approved</td>
</tr>
<tr>
<td>Genexol-PM*</td>
<td>PLA-PEG micelle</td>
<td></td>
<td>paclitaxel</td>
<td>metastatic breast cancer</td>
<td>approved</td>
</tr>
</tbody>
</table>

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<th>Status</th>
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</thead>
<tbody>
<tr>
<td>CALAA-01</td>
<td>cyclodextrin-containing polymeric nanoparticle liposome</td>
<td>transferrin</td>
<td>siRNA</td>
<td>solid tumor</td>
<td>Phase I</td>
</tr>
<tr>
<td>MBP-426</td>
<td></td>
<td>transferrin</td>
<td>oxaliplatin</td>
<td>gastric, esophageal, gastricesophgeal adenocarcinoma</td>
<td>Phase Ib/II</td>
</tr>
<tr>
<td>MCC-465</td>
<td>liposome</td>
<td>F((ab)')(_2) fragment of human Ab GAH</td>
<td>doxorubicin</td>
<td>metastatic stomach cancer</td>
<td>Phase I (not continued)</td>
</tr>
<tr>
<td>BIND-014</td>
<td>PLGA-PEG nanoparticle</td>
<td>PSMA-specific peptide</td>
<td>doxetaxel</td>
<td>solid tumor</td>
<td>Phase I</td>
</tr>
<tr>
<td>SGT53-01</td>
<td>liposome</td>
<td>transferrin receptor-specific-scAb</td>
<td>p53 gene</td>
<td>solid tumor</td>
<td>Phase I</td>
</tr>
</tbody>
</table>

A) isolation of target cells out of the heterogeneous cell populations
B) \textit{in vitro} instruction of therapeutic cells with nanomaterials as delivery vehicles, functional modules and artificial ECMs with nanotopological cues
C) delivering nanoengineered cells to target tissues for regenerative therapy.
D) In addition to therapeutic applications, nanoengineered cells can also be used to build \textit{in vitro} disease models for understanding disease pathology and drug screening.
E) \textit{In situ} cell engineering, an emerging strategy to manipulate cell functions using nanomaterials directly within native tissue \textit{in vivo}.
Methods for evaluation of cell

Invasive technique: tissue biopsy

Noninvasive technique: magnetic resonance imaging (MRI), positron emission tomography (PET), fluorescence spectroscopy

Rely on contrast agent, lack of specificity

Nanoparticles are developed such as quantum dots, magnetic nanoparticles, gold nanoparticles
Fixed breast cancer SK-BR-3 cells were incubated with (A) monoclonal anti-Her2 antibody and goat anti-mouse IgG conjugated to QDs (QD 535–IgG) (B) When cells were incubated with normal mouse IgG and QD-IgG.

(A) Microtubules were labeled with monoclonal anti-α-tubulin antibody, biotinylated anti-mouse IgG and QD 630–streptavidin (red). (B) Control for (A) without primary antibody.

The nucleus of a 3T3 cell was stained with ANA, anti-human IgG–biotin, and QD 630–streptavidin (red). The microtubules were labeled with mouse anti-α-tubulin antibody, anti-mouse IgG–biotin, and QD 535–streptavidin (green).

It is possible to overlap X-ray images with infrared images to localize a tumor. The X-ray images give the images an anatomical context, while the infrared images detect the QD’s emission, which correlates to the tumor location.
Carbon nanotube for imaging

Intrinsic properties of carbon nanotube for imaging are infrared radiation and Raman scattering.

Fluorescence image of one macrophage-like cell incubated with SWNTs, showing emission detected from 1125 to 1600 nm with excitation at 660 nm.

SWNT emission spectra in an aqueous Pluronic F108 suspension (blue trace) and in macrophage cells incubated in SWNT suspension and then washed (red trace). Samples were excited at 660 nm.

Fluorescence image of one macrophage-like cell incubated with SWNTs, showing emission detected from 1125 to 1600 nm with excitation at 660 nm.

Magnetic Resonance Imaging (MRI) is one of the most commonly used tests in neurology and neurosurgery. MRI has an advantage over CT in being able to detect flowing blood and cryptic vascular malformations. Tissue can be characterized by two different relaxation times – T1 and T2.

- T1 (longitudinal relaxation time) is the time constant which determines the rate at which excited protons return to equilibrium. It is a measure of the time taken for spinning protons to realign with the external magnetic field.

- T2 (transverse relaxation time) is the time constant which determines the rate at which excited protons reach equilibrium or go out of phase with each other. It is a measure of the time taken for spinning protons to lose phase coherence among the nuclei spinning perpendicular to the main field.
Magnetic Resonance Imaging (MRI)

In vivo MR imaging showed T1-positive contrast effects 1 hour after injection at the tumor sites. Cy5.5-chitosan nanoparticle-Gd(III) nanoparticles were injected into the SCC7-bearing mice, and the mice were visualized by using MR imaging.

International journal of nanomedicine. 2014:9(1), 711—726

Photocredit: David C Preston, MD.
http://casemed.case.edu/clerkships/neurology/Web%20Neurorad/MRI%20Basics.htm
Mesenchymal stem cells (MSC) show efficient uptake of iron oxide particles (b) in comparison to nonlabeled cells (a): Magnification 400×, Prussian blue and eosin staining.

T₁-weighted MR images of a mouse injected with SPIONs. Heart (red arrow), vena cava (green arrow), and bladder (yellow arrow)
Using nanomaterials to deliver cells or biomolecules to the targeted sites to enhance the regenerative response of tissues *in vivo*

A) Development of bioactive peptide amphiphiles for therapeutic cell delivery. Left) bioactive peptide amphiphile formed beta-sheets and self-assembled into nanofibers. Right) quantification from *in vivo* bioluminescent imaging of transplanted luciferase-expressing BMNCs injected subcutaneously, the viability of cells significantly increased in the binary RGDS system (left mouse) comparison to the diluent peptide amphiphiles (middle mouse) and the saline control (right mouse)

B) Self-assembled collagen-human mesenchymal stem cell microspheres for regenerative medicine. Left) SEM image showing the internal and external structure of the microspheres. Middle) Fate of collagen-hMSC microspheres upon subcutaneous implantation in NOD/SCID mice, dark brown color indicated immunopositive staining of the human antigen; slender arrows showed the periphery of a microsphere; block arrows showed individual hMSCs with immunopositive staining. Right) 2 days after implantation, dark brown color indicated immunopositive staining of the human antigen; block arrows showed hMSCs with immunopositive staining (20 ×)
Nanotechnology for biomaterial control

- Biocompatible scaffolds

  Provide temporary structural support: cell growth, transport nutrients, form functional tissues and organ

  Biomedical implants or wound healing response
Implants to repair bone defects

After inflammation a hematoma is generated.

In the first stage of the reparative phase, the initial fibrin is gradually replaced by cartilaginous tissue and woven bone starts to form.

In a later stage of the reparative phase, the cartilaginous tissue mineralizes, more bone is formed and volume of granulation tissue substantially decreases.

Eventually, once the bone is bridged, remodeling restores the original cortex.
Healing factors

The optimal mechanical and biological stimuli would result in fast and uncomplicated healing; an inappropriate stimulus leads to impaired/delayed healing.

Biotechnology Advances. 2013. 31(5), 638-653
A segmental bone defect occurs.

A collagen scaffold is implanted into the defect site.

Endogenous MSCs invade the implanted scaffold.

A BMP gene is injected into the MSC-populated scaffold.

An ultrasound pulse is applied to the defect site, leading to DNA uptake by MSCs. BMP secretion leads to fracture repair.

https://transbiotex.wordpress.com/2013/11/14/a-new-take-on-efficient-delivery-in-regenerative-medicine/
Nanomaterials for better bone fracture repair

- Nanomaterials may precisely mimic the hierarchical and nanoscale features of bones.
- Magnetic nanoparticles may provide mechanical stimuli as needed or provide unique ‘smart’ functions.
<table>
<thead>
<tr>
<th>Requirements of nanomaterials for bone fracture repair</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biocompatibility</td>
</tr>
<tr>
<td>• Should not suppress activity of normal cells nor</td>
</tr>
<tr>
<td>toxicity during and after implantation</td>
</tr>
<tr>
<td>• promote adhesion and proliferation of osteoblast</td>
</tr>
<tr>
<td>or MSCs to form ECM</td>
</tr>
<tr>
<td>Mechanical property</td>
</tr>
<tr>
<td>• Provide mechanical strength and transfer properties.</td>
</tr>
<tr>
<td>Vesicular structure</td>
</tr>
<tr>
<td>• Porous diameter in at least 100 μm, to ensure the</td>
</tr>
<tr>
<td>transportation of nutrients and oxygen.</td>
</tr>
<tr>
<td>Bioabsorbability</td>
</tr>
<tr>
<td>• Should be degraded in vivo at a certain time, with</td>
</tr>
<tr>
<td>a controllable absorption rate that provide a space</td>
</tr>
<tr>
<td>for new bone generation.</td>
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<tr>
<td>Angiogenesis</td>
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<tr>
<td>• Promote the angiogenesis due to higher blood</td>
</tr>
<tr>
<td>demands in the bone tissues.</td>
</tr>
</tbody>
</table>
Scaffold

Tri-modal macro/micro/nano-porous scaffold loaded with rhBMP-2 for accelerated bone regeneration.

Bone Research (2016) 4, 16050
SEM images of (a) alginate, (b) alginate-chitosan, (c) chitosan, (d) chitosan-collagen, mesenchymal stem cells cultured on (e) poly(lactic-co-glycolic acid) scaffold (f) porous hydroxyapatite (HA) scaffold.

Scale bars (a–d, f) 100 μm, (e) 500 μm.
Biomimetic spiral-cylindrical scaffold based on hybrid chitosan/cellulose/nano-hydroxyapatite membrane.
• The most significant impact nanotechnology will have on regenerative medicine is that it will help in providing a detailed understanding and control of biology. This field has demonstrated significant advances over traditional imaging, sensing, and structural technologies.
Recommended Reading

