Engineering Nanoparticles for Biomedical Applications

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Engineering Nanoparticles for Biomedical Applications

1. Magnetic Nanoparticles
   - SPION for MRI
   - Thermally blocked NP for biodiagnostics

2. Nanoparticles for Drug Delivery
   - Multifunctional NP
   - Thermosensitive NP

3. Ferrogel for drug delivery
Definition

FDA calls it "nanotechnology" only if it involves all of the following:

1. Research and technology development, or products regulated by FDA, that are at the atomic, molecular or macromolecular levels, and where at least one dimension, that affects the functional behavior of the product, is in the length scale range of approximately 1-100 nanometers. (Man-made materials)

2. Creating and using structures, devices and systems that have novel properties and functions because of their small and/or intermediate size.

3. Ability to control or manipulate at the atomic scale.
Medical applications of nanoparticles

- Fast and more efficient biosensors
- Targeted drug delivery to specific cells
- Novel cancer therapy and hyperthermia treatments
- Magnetic resonance imaging enhancement
- Single cell study and bio-manipulation
- Novel diagnostic tools for early stage detection of diseases

Nature Nanotechnology, 2007, 2, 469-478
Nanoparticle Engineering

Nanoparticles → Assembly → Suspension → Consolidation → 2D, 3D structures
Gas Phase Synthesis

Chemical Reaction

Coagulation (coalescence)

Precursors (vapour, or fog)

Nucleation & Condensation (surface reactions)

Agglomeration & aggregation

Reactant gas molecules

Clusters

primary particles
Chemical Solution Methods for Nanoparticles synthesis

- **Precipitation**
  - Homogenous precipitation
  - Co-precipitation
  - Hydrolysis
  - Oxidative hydrolysis
  - Reductive precipitation
  - Electrochemical reduction

- **Condensation**
  - Sol-gel technique
  - Macro-molecular chemistry

- **Evaporation**
  - Spray-drying
  - Spray-pyrolysis
  - Freeze-drying
  - Aerosol technique

- **Templates**
  - Precipitation in microemulsion
  - Precipitation in presence of surfactants

- **Others**
  - Sono-chemical reactions
Design of tailored Magnetic Nanoparticles

Superparamagnetic and Thermally blocked nanoparticles with strong magnetic response

- Magnetite ($\text{Fe}_3\text{O}_4$)
- Maghemite ($\gamma\text{Fe}_2\text{O}_3$)
- Ferrites ($\text{CoFe}_2\text{O}_4, \text{ZnFe}_2\text{O}_4, \text{MnFe}_2\text{O}_4, \ldots$)
- Iron Platinum (FePt) & CoPt

Bio-compatibility and surface functionalisaiton

- Inorganic: Gold, Silica, hydroxyapatite, ...
- Organic: Dextran, PVA, PEG, mPEG, ...
TEM images (left) and the corresponding particle size histograms (right) of magnetite nanoparticles prepared by controlled coprecipitation. (A) without heat treatment and (B) after heat treatment (80°C for 1hrs)
Magnetic characterisation

VSM measurement for SiO$_2$ coated Fe$_3$O$_4$ by co-precipitation
Superparamagnetic iron oxide nanoparticles

- Average particle size = 12 nm
- XAS shows nonstochiometric phase \( \text{Fe}_3\text{O}_4-\delta \), the curve shifts to \( \text{Fe}^{2+} \).

After one year shelf storage
Surface Functionalization of Magnetic Nanoparticles

Magnetic Nanoparticles
- Oxide: magnetite, ferrite
- Metal: Fe, Co, PtFe, CoPt

Coating
- Gold
- Silica
- Hydroxyapatite
- Dextran
- Starch
- Albumin
- Sodium Oleate
- Folic acid
- L-aspartic acid
- PVA
- PEG
- mPEG
- PLLA (PDLLA)
- PCL
- PGA
Effect of surface modification
Au Coating

ESA measurement of SPION and Au@SPION prepared by μE system

DSC analysis of bare and coated nanoparticles. (a) Magnetite, and (b) Au coated SPION.
Silica Coated Magnetic Nanoparticles

Control of thickness, porosity of coating layer
Visualization

SPION: MRI Contrast Agents

Widely used current commercial T2 contrast agent (Aq. Soln. Synthesis)

Controlled Synthesis in organic liquids (at 300 C)

Qin, J. et al., Adv. Mat. 2007
Phase transfer through Surface Coating

ABA type triblock copolymer Pluronic F127

Hydrophilic poly(ethylene oxide)

Hydrophobic poly(propylene oxide)

Amphiphilic coating layer

PF127/Oleic acid (POA)
Hydrophobic-Hydrophilic Phase Transfer

Organic coating molecules

Phase transfer

Amphiphilic macromolecules with PEG section

SPION: Superparamagnetic iron oxide nanoparticles
PEG: Poly(ethylene glycol)

J. Qin et al, Adv Mat (2007)
Superparamagnetism Retained

Magnetization curve of (a) as-synthesized SPION and (b) POA@SPION
## Compare with Conventional Iron Oxide Nanoparticle Based Contrast Agents

<table>
<thead>
<tr>
<th>Particles name</th>
<th>Surface polymer</th>
<th>$r_2/r_1$ ratio (0.47 T, 310 K)</th>
<th>Mean hydrodynamic diameter (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POA@SPION</td>
<td>Pluronic F127 + Oleic acid</td>
<td>41.5</td>
<td>116</td>
</tr>
<tr>
<td>AMI-25 (Feridex; Advanced Magnetic s, Cambridge, Mass)</td>
<td>Dextran</td>
<td>4.0</td>
<td>72</td>
</tr>
<tr>
<td>AMI-227 (Combidex; Advanced Magnetics, Cambridge, Mass)</td>
<td>Dextran</td>
<td>2.2</td>
<td>19</td>
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<tr>
<td>MION-37 (R. Weissleder, Massachusetts General Hospital, Boston, Mass)</td>
<td>Dextran T10</td>
<td>2.2</td>
<td>16-28</td>
</tr>
<tr>
<td>MION-37 (R. Weissleder, Massachusetts General Hospital, Boston, Mass)</td>
<td>Dextran T10</td>
<td>2.2</td>
<td>18-24</td>
</tr>
<tr>
<td>NC100150 (Clariscan, Nycomed, Amersham, Oslo, Norway)</td>
<td>Oxidized Starch</td>
<td>1.6</td>
<td>11.9</td>
</tr>
<tr>
<td>SH U 555 A (Schering AG, Berlin, Germany)</td>
<td>Carboxydextran</td>
<td>7.1</td>
<td>65</td>
</tr>
<tr>
<td>USPIO S (Schering AG, Berlin, Germany)</td>
<td>Carboxydextran</td>
<td>2.3</td>
<td>21</td>
</tr>
</tbody>
</table>
Dose response of Fe$_3$O$_4$ nanoparticles in MRI
Thermally Blocked Nanoparticles
Magnetic Relaxation for Bio-Diagnostics

Néel relaxation

\[ \tau_N = \tau_0 e^{\frac{KV}{kT}} \]

- \( \tau_N \) = Néel rel. time
- \( \tau_0 \) = characteristic rel. time
- \( k \) = Boltzmann constant
- \( T \) = temperature
- \( K \) = magnetic anisotropy
- \( V \) = single domain volume

Brownian relaxation

\[ \tau_B = \frac{3V_H \eta}{kT} \]

- \( \tau_B \) = Brownian rel. time
- \( V_H \) = Hydrodynamic particle volume
- \( \eta \) = viscosity
Detect specific biomolecules by measuring changes in Brownian relaxation of thermally blocked magnetic nanoparticles.

shift in the maximum of the imaginary magnetic susceptibility.

Brownian relaxation process can be detected in the frequency domain

\[ M = \chi H = (\chi' - i\chi'')H \]

- \( M \) = magnetisation
- \( H \) = alternating external magnetic field
- \( \chi \) = complex magnetic susceptibility

\[ f_{\text{max}} = \frac{1}{2\pi \tau_{\text{eff}}} \]

IMEGO AB
Synthesis of Thermally blocked Magnetic nanoparticles
**Quantitative detection of PSA by Brownian relaxation frequency measurements**

- No pretreatment
- Simple mixing of fluids
- Fast
- Multiple bio molecules detection
- Practical for point of use
Detection of Brucella Antibodies in Serum

Detection limit: 0.05 µg/ml

Targeted drug delivery – Targeted drug delivery using a multicomponent nanoparticle containing therapeutic as well as biological surface modifying agents
Biocompatible Polymers

Poly lactide

Poly glycolide

Poly L-lysine

Poly(ethyl-2-cyanoacrylate)

Poly ε-caprolactone
Amphiphilic copolymer for biodegradable nanosphere fabrication

Lactide (3,6-dimethyl-1,4-dioxane-2,5-dione)
- Biocompatible
- Degradable under physiological condition
- Applicable as a hydrophobic segment in amphiphilic copolymer

Poly ethylene glycol (PEG)
- Biocompatible
- Applicable as a hydrophilic segment in amphiphilic copolymer

copolymerization
Strategies for Drug Delivery Systems

- **Hydrophobic drug** i.e. steroids
- **Emulsion/evaporation** (o/w)
- **Hydrophilic drug** i.e. proteins
- **Modified-double-emulsion** (w/o/w)

**Surface modification**
and activation
Procedures for preparation of drug-loaded Nanospheres

Drugs, Fe₃O₄, & Quantum dots loaded in the cavity
Compositions of PLA-mPEG diblock copolymers

<table>
<thead>
<tr>
<th>Code</th>
<th>PLA Enantiomers</th>
<th>Mw [kDa]</th>
<th>mPEG Mw [kDa]</th>
</tr>
</thead>
<tbody>
<tr>
<td>M90</td>
<td>PDLLA(P-meso-LA)</td>
<td>90</td>
<td>5</td>
</tr>
<tr>
<td>M45</td>
<td>PDLLA(P-meso-LA)</td>
<td>45</td>
<td>5</td>
</tr>
<tr>
<td>M25</td>
<td>PDLLA(P-meso-LA)</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>M5</td>
<td>PDLLA(P-meso-LA)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>L45</td>
<td>PLLA</td>
<td>45</td>
<td>5</td>
</tr>
<tr>
<td>D45</td>
<td>PDLA</td>
<td>45</td>
<td>5</td>
</tr>
<tr>
<td>R45</td>
<td>PLLA+PDLA(P-rac-LA)</td>
<td>45</td>
<td>5</td>
</tr>
</tbody>
</table>

![Graph showing relative integration vs mean diameter]
TEM images of BSA-loaded nanospheres

BSA-loaded PLA-mPEG nanospheres

BSA and Fe₃O₄-loaded PLA-mPEG nanospheres
Thermo-sensitive Drug delivery system

- Stable suspension at temperatures **below** body temperature
- Unstable at temperatures **above** body temp.
  - Use body increase of temp to trigger release
  - Use external heating source - hyperthermia
‘Smart’ polymeric Nanoparticles Systems

Poly(N-isopropylacrylamide)

Drug

T<LCST

LCST : Lower critical solution temperature

T>LCST

Under the condition that temperature exceeds the LCST, amphiphilic micelles are collapsed so as to start to release the entrapped drug.


Qin et al (2006)
Schematic View of Modified-Double-Emulsion-Method (MDEM)

Aqueous solution (containing BSA, SPION suspension, etc.)

Emulsify

PNIPAAm-PDLA chloroform solution

BSA loaded PNIPAAm-PDLA single-shell spheres

Emulsify

PLL-PEG@PNIPAAm-PDLA dual-shell spheres in PVA aqueous solution

BSA: Bovine serum albumin
PEG: Poly(ethylene glycol)
PDLA: Poly(d, d-lactide)
PLLA: Poly(L, L-lactide)
PNIPAAm: Poly(N-isopropylacrylamide)
PVA: Poly (vinyl alcohol)
SPION: Superparamagnetic iron oxide nanoparticles
(a) transparency change of the PNIPAAm-PDLA solution
(b) differentiated curve of (a)
(c) w/o/w’ emulsion
Thermogram of Polymeric Structures

Tg of PLLA segment

Tg of PEG segment

LCST: 36.4 °C
Schematic View of the “Shell-in-Shell” Structure

Below LCST

Aqueous solution (containing BSA, SPION suspension, etc.)

PNIPAAm: hydrophilic, stable

Above LCST

Aqueous solution (containing BSA, SPION suspension, etc.)

PNIPAAm: hydrophobic, unstable  
Drug release

PNIPAAm-co-PDLA  PLLA-co-PEG

LCST: Lower critical solubility temperature
Temperature-dependent Bovine Serum Albumin (BSA) Release

Conditions: PBS
(a) 37 °C
(b) 22 °C
Membrane Mw cut-off: 100 kDa

Toxicity Assay
Au@PLLA-PEG@PNIPAAm-PDLA
and PLLA-PEG@PNIPAAm-PDLA

MTS: 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
TCCV: Two-color cell fluorescence viability

Green spots = living cells
Red spots = dead cells
Mathematical modeling of controlled release rate

\[ \frac{\partial c}{\partial t} = D \left( \frac{\partial^2 c}{\partial r^2} + \frac{2}{r} \frac{\partial c}{\partial r} \right) \]

- **Diffusion model**

\[ \frac{\partial c}{\partial t} = D \left( \frac{\partial^2 c}{\partial r^2} + \frac{2}{r} \frac{\partial c}{\partial r} \right) \]

\[ \frac{c_1}{c_{1\infty}} = 1 - \sum_{n=1}^{\infty} \frac{6(\alpha+1)\alpha}{(9+3\alpha+q_n^2\alpha^2)} e^{-\frac{q_n^2 R^2}{D_t}} \]

- **Dissolution model**

\[ r_d = -\frac{dc}{dt} = k(c - K_p c_1) \]

\[ c_1 = \frac{c_0}{K_p(\alpha+1)} \left[ 1 - \exp\left( -\frac{\alpha+1}{\alpha} kt \right) \right] \]

Self-assembly of gold nanoparticles on the surface of PLA-mPEG nanospheres

Silanization

Gold nanoparticle self-assembly

TEM images of ‘shell-in-shell’ structure nanoparticles

(a) PLLA-PEG micelle
(b) ‘shell-in-shell’ structures covered with Au nanoparticles in part
(c) - d) ‘shell-in-shell’ structures fully covered with Au nanoparticles

Drug Release Profile

Dissolution regime

![Graph showing drug release profile with time (hrs) on the x-axis and c1∞Vr/c0 on the y-axis. The graph includes data points and lines representing diffusion model, dissolution model, and DL1.](image-url)
Drug Release Profile
Dissolution- Diffusion Regimes

![Graph showing drug release profile with time in hours on the x-axis and c1∞V_r/c_0 on the y-axis. The graph includes data points and curves for diffusion and dissolution models, with an indication of LL2.]
in vivo test: estrogen release by PLA-PEO DDS

Group D
PLA-PEO DDS (loading 17\(\beta\)-estradiol) injected
Dosage;
(39.6 \(\mu\)g / 39.6 \(\mu\)g / 550 \(\mu\)L = 17\(\beta\)-estradiol / PLA-PEO DDS / PBS buffer solution)

Group N
550 \(\mu\)L of PBS buffer solution injected

Group P
P-I; 550 \(\mu\)L of positive control solution (17\(\beta\)-estradiol dissolved in olive oil) injected
P-II; 17\(\beta\)-estradiol tablet implanted

Androgen precursor \Star

Aromatase P450

Estrogens

Aromatase P450 (P450arom) Knocked-Out (ArKO) mice used in this work
Injectable Drug Delivery Systems

Hydrogels - Ferrogels
Injectable Drug Delivery Systems

- Biocompatible
- Liquid at Room temperature
- Solid at Body temperature
- Can be loaded with drugs
- Controlled drug release

Pluronic F127

Pluronic F127 copolymer undergoes reversible sol-gel transition at different $T$ as a function of concentration
Effect of Concentration on Gelation Temperature

- (a) 35%
- (b) 34%
- (c) 33%
- (d) 8.75%
- (e) 17.5%

![Graphs showing the relationship between gelation temperature and concentration](image-url)
Injectable Drug Delivery Systems
Magnetic-Sensitive Ferrogel

Pluronic F127

![Diagram]

- Working Range
- Transition point

**Concentration (%)**

**Temperature (deg. celcius)**

**Temperature (°C)**

**G', G'' (Pa)**
Reversible sol-gel transition

Magnetic-Sensitive Ferrogel

(a) SPION in organic solvent and
(b) embedded in the ferrogel
Sensitive to the External Magnetic Field

![Graph showing magnetization vs. applied field]
Magnetic Sensitive Release rate

![Graph showing magnetic sensitive release rate over time, with total released (%) on the y-axis and time (h) on the x-axis. The graph includes markers indicating 'off' and 'on' states at various time points.](image-url)
Magnetic Enhanced Drug delivery

Drug release profiles

Summary

- Nanomaterials and Multifunctional Nanoparticles have tremendous potentials for developing smart and novel medical treatments.

- MFNP

  - can carry different Payloads: pharma, genes, DNA, etc.
  - can be designed to be environmentally responsive:
    - Magnetic,
    - Temperature,
    - Concentration of biomolecules (sugar, pH,..)
  - Targeting devices can be attached for delivery at specific sites.

- Injectable hydrogels offers new ways for introducing DDS without surgical operation.
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Royal Institute of Technology (KTH)
Kista campus - Stockholm

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