Micro- and Nanoparticles for Drug Delivery

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IFT
To the Audience:

- General flavor of particle-based drug delivery, with examples
- Multidisciplinary research and collaboration is a *sine qua non* of this type of work
- *You* can do this
- I will be moving fast; please ask questions
- **Warning: Pictures of animal/human innards**
  - *Bon Appetit!*
Definitions

- Microparticle: $1 \, \mu m < \text{diameter} < 1 \, mm$
- Nanoparticle: $1 \, \text{nm} < \text{diameter} < 1 \, \mu m$
Differences
Surface Area/Volume Ratio

- \( V = \frac{4}{3} \pi r^3 \)
- \( SA = 4 \pi r^2 \)
- So, smaller objects have larger SA/V ratio (\( \alpha \frac{1}{r} \))
- This affects
  - Drug encapsulation
  - Release kinetics
  - Degradation time
  - Surface binding, aggregation, etc.
Fate When Injected

• Larger objects tend to stay put
• Larger objects more likely to cause embolic phenomena
• The reticuloendothelial system
  – Exactly how you formulate things has a big effect (e.g. pegylation)
Crossing Barrier

• Depends on the barrier and the particle
• Microparticles won’t cross much
• Nanoparticles can, esp. if barrier is loose or disrupted
• Modification may help
Entering cells

• Microparticles can only enter if cell is professional phagocyte
  – < 5 – 10 µm
• Nanoparticles have an easier time (pinocytosis)
• Exocytosis?
Tissue Reaction

- Depends on size
  - Phagocytosed
  - Giant foreign body cells
  - Leaving the site
Large Particles (60 µm; PLGA)

FBGCs, not phagocytosed
Small Particles
(3.6 µm ; PLGA)

Much less FBGCs
Get engulfed instead…
Nanoparticles in Mesothelial Cells
Applications
4 Basic Ways to Apply Particles

- Systemic delivery, for systemic use
  - Amphotericin B liposomes
- Systemic delivery for local use
  - Targeted entities (e.g. immunoliposomes)
- Local delivery for systemic use
  - Growth hormone, risperidone microspheres
- Local delivery for local use
  - Bupivacaine microspheres, liposomes
Local Application of Microparticles I.

Prolonged duration local anesthesia
Conventional Local Anesthetics
Tetrodotoxin (TTX)

- Site 1 sodium channel blocker
- Potent local anesthetic (blocking concentration in $10^{-8}$ to $10^{-7}$ M range)
- Duration of block is potentiated by bupivacaine
TTX + BPV

**Anesthesiology 1998; 89: 119-31**
Bupivacaine Release From PLGA MS

Anesthesiology 1996; 84: 1401-1410
PLGA (polylactic-co-glycolic acid)

- Related to what absorbable surgical sutures are made of
- Minimal inflammatory response
- PLGA breaks down to lactic and glycolic acids
- FDA approved
Single-compound Microspheres

- TTX block unreliable; bupivacaine block short
- Previously known: dexamethasone microspheres do not cause nerve block
TTX + Bupivacaine Microspheres

- Block prolongation to 1.5 days

![Graph showing duration of sensory block for TTX, Bupivacaine, and TTX + Bupivacaine]
Effect of Dexamethasone

- Effect on bupivacaine previously known (

- TTX block prolonged by dexamethasone.
Microspheres containing TTX + Bupivacaine + Dexamethasone

- Median duration of sensory block > 9 days
Nanoparticles can do this too

- A variety of liposome nanoparticles have been used
- Max expected durations will be days
- Limitation of the maximum possible duration of release
- Depending on composition of matter and site of injection, tissue reaction could be quite different.
Local Application of Microparticles II.

Focal delivery of anticonvulsants
Status Epilepticus

MRA  PET  Intra-Op
Lipid-GAG Microparticles

Brain Res 2002; 946: 206-13
Primary Cultured Cortical Neuron Death From Particles

Brain Res 2002; 946: 206-13
Lipid-GAG Particles in the Brain

Brain Res 2002; 946: 206-13
Intraventricular Lipid-GAG Particles

Brain Res 2002; 946: 206-13
Muscimol-containing Microparticles
Seizure Scores Following Treatment

Epilepsia 2002; 43: 1462-8
Cell Loss in the Hippocampus

Epilepsia 2002; 43: 1462-8
Sprouting of Mossy Fibers in Hippocampus

Epilepsia 2002; 43: 1462-8
Nanoparticles and the brain

- Can be used
- The same considerations apply
- Much enthusiasm for their use in crossing the BBB
  - A confusing story
Local? Systemic? Application of Microparticles I.

Vaccine adjuvant
Uptake of FITC-albumin

A

FITC Positive (%)

0 20 40 60 80 100

0 0 10 20

B

Mean Fluorescence Intensity

0 50 100 150 200 250

0 10 15 20

FITC-alb concentration (ng/mL)
Antigen Presentation
Nanoparticles and vaccination

- It’s been done with a variety of approaches
- It works
- Some concerns, given the fact that nanoparticles can be taken up by *any* cell
  - Use of microspheres = passive targeting
Local? Systemic? Application of Microparticles II.

Peritoneal delivery
Adhesions
Adhesions: the problem

- Approx. 80% of intra-abdominal surgeries result in adhesion formation
- Abdominal and pelvic pain, infertility and bowel obstruction which could lead to intra-abdominal catastrophes
- Cost of treatment: $1.33 billion
- With related costs: $3.22 billion/year for medicare alone
Drugs

- Glucocorticoids
- NSAID
- Antihistamines
- PDE inhibitors
- Fibrinolytics
- Anticoagulants
- Many others
Underlying hypothesis

• The reason drugs don’t work well is that they are rapidly cleared
• The reason barriers don’t work well is that they don’t address the basic biological problems
• Controlled drug release could do both
Key Points for Eventual Use of PLGA in the Peritoneum

• Great for drug delivery
• Widely accepted for drug delivery
  – (The default vehicle)
• Inflammation and residue eventually disappear completely
• So, we were optimistic
Parameters

- Particle size
- PLGA molecular weight
- Mass injected
- Sterilization method
- All injected IP

Results
Residue

Not necessarily associated with injection site
Dependent area (important)
Adhesions

Not necessarily associated with injection site
Dependent area (important)
Table 2. Dissection results from PLGA (90 kDa) microparticles.

<table>
<thead>
<tr>
<th>Sterilization Method</th>
<th>Particle diameter (μm)</th>
<th>Particle mass (mg)</th>
<th>Days to dissection</th>
<th>n</th>
<th>Residue</th>
<th>Adhesions</th>
<th>Residue %</th>
<th>Adhesions %</th>
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</table>
Histology
Nanoparticles

Dissection results from PLGA (90 kDa) 265 nm nanoparticles, sterilized by UV irradiation. n = 4 in all cases.

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<th>25</th>
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<tbody>
<tr>
<td>Particle mass (mg):</td>
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<tr>
<td>Days to dissection:</td>
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<td>14</td>
<td>2</td>
<td>14</td>
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<td>Adhesions:</td>
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<td>0</td>
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<td>Splenomegaly:</td>
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<td>3</td>
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<tr>
<td>Adhesions (%):</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Splenomegaly (%):</td>
<td>0</td>
<td>100</td>
<td>50</td>
<td>75</td>
</tr>
</tbody>
</table>
Nanoparticles

Spleen 2 days

Spleen 14 days

Liver 2 days

Liver 14 days
Lower Mw polymers

Dissection results from 50 mg of 5 μm PLGA (lower Mw) microparticles, 14 days after injection.
Particles sterilized by low-energy gamma irradiation. N = 4 in all cases.

<table>
<thead>
<tr>
<th>Mw (KDa)</th>
<th>Residue</th>
<th>Adhesion</th>
<th>Residue %</th>
<th>Adhesion %</th>
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<tbody>
<tr>
<td>57</td>
<td>3</td>
<td>1</td>
<td>75</td>
<td>25</td>
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<tr>
<td>54</td>
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<td>10</td>
<td>2</td>
<td>1</td>
<td>50</td>
<td>25</td>
</tr>
</tbody>
</table>
Conclusions re PLGA in the peritoneum:

- Any formulation *can* cause adhesions
  - Accumulation in dependent areas?
- Smaller microparticles may be worse
- Lower MW polymers may be better
- Nanoparticles don’t cause adhesions…
  …but that is because they leave
Particle-hydrogel hybrids

Peritoneal delivery
Crosslinkable Hyaluronic Acid Gel (HAX gel)

- Hydrazide
- Aldehyde

• Hydrazide bond
Rabbit Sidewall Defect
+ Cecum Abrasion Model

3×4 cm defect comprising parietal peritoneum and a layer of muscle (~1 mm thick)

abrasion on seven haustra of cecum (from 6th distal to the ileocecal junction to the 12th)
HAX Gels Score 3 Adhesions

- Control (no treatment): 10/12 (83.3 %)
- HAX: 2/8 (25%)
Hybrid system

- Hydrogels: poor drug delivery, good biocompatibility
- Polymeric microspheres: the opposite
- Hybrid: best of both worlds – polymeric particles within a hydrogel
Normalized cell viability over time:

- **Medium only**
- **Medium with hyaluronidase**

<table>
<thead>
<tr>
<th>Day</th>
<th>Medium only</th>
<th>Medium with hyaluronidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>110</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>120</td>
</tr>
</tbody>
</table>
HAX-NP (“Hybrid”)

- Still there two weeks after injection
- Note normal spleens
- Some adhesion (see over)
Rabbit Sidewall Defect + Cecum Abrasion Model

3×4 cm defect comprising parietal peritoneum and a layer of muscle (~1 mm thick)

abrasion on seven haustra of cecum (from 6th distal to the ileocecal junctiion to the 12th)
Rabbit sidewall defect-cecum abrasion model scoring system

- **Score 0**: No adhesion
- **Score 1**: Tissues separate with gravity
- **Score 2**: Tissue adherence separable by gentle blunt dissection
- **Score 3**: Adhesion requiring sharp dissection
### In vivo barrier efficacy of HAX and hybrid gel
rabbit sidewall defect-cecum abrasion model

<table>
<thead>
<tr>
<th></th>
<th>no treatment</th>
<th>HAX (20mg/ml)</th>
<th>Hybrid (20mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>% weight change</td>
<td>-5.7 ± 4.6</td>
<td>-9.0 ± 7.4</td>
<td>-5.5 ± 3.6</td>
</tr>
<tr>
<td>score 3</td>
<td>10</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>score 2</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>score 1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>score 0</td>
<td>2</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>% score 3 adhesion</td>
<td>83.3</td>
<td>25.0</td>
<td>0</td>
</tr>
</tbody>
</table>

**score 3**: adhesion requiring sharp dissection  
**score 2**: tissue sticks but separable by gentle blunt dissection  
**score 1**: tissues separate with gravity  
**score 0**: no adhesion
In vivo barrier efficacy of HAX and hybrid gel rabbit sidewall defect-cecum abrasion model

<table>
<thead>
<tr>
<th>no treatment</th>
<th>HAX</th>
<th>Hybrid</th>
</tr>
</thead>
</table>

**surgery**

**1 week later**

AW=abdominal wall; C=cecum
Hybrid System

- Good biocompatibility
  - HA mitigates access of tissue to PLGA and vice versa
  - Nature of PLGA is such that even if contacts tissue, will not cause adhesions (cleared).
- Potential for good drug delivery if needed
- Therapeutic effect vs. adhesion even in the absence of drug release
t-PA
Dramatis Personae

• Dept. of Chemical Engineering, MIT
  – Robert Langer, Sarah Smith, Peter P. Ghoroghchian, Ying Chau, Dan Anderson, Steve Little, Julie Tse, Yoon Yeo
• Depts of Anesthesia, CHMC & BWH
  – Charles B. Berde, Nu T. Lu, Gary Strichartz
• Stroke and Neurovascular Regulation Lab, MGH
  – Michael Moskowitz, Nick Plesnila, Dean Le, Ellen Grant
• Depts of Pathology, CHMC, MGH, BWH
  – David Louis, Robert Padera
• Depts of Neurology and Biostatistics, CHMC
  – Gregory Holmes, Byung Ho Cha, David Zurakowski
• Dana Farber Cancer Institute
  – W. N. Haining, L. Nadler