Nanoparticles for Drug Delivery

- Introduction – Systemic Drug Delivery
- Nanoparticles
  - Challenge 1: Stabilization
  - Challenge 2: Extended Circulation
  - Challenge 3: Targeting
- Examples:
  - Liposomes for chemotherapeutic delivery
  - Cyclodextrin particles for gene delivery

Methods of Drug Delivery

- Oral Delivery
- Inhalation
- Transdermal
- Implantation
- Injection

Advantages of Nanoparticles for Drug Delivery

- Oral Delivery
- Inhalation
- Transdermal
- Implantation
- Injection

Examples of Nanoparticles for Drug Delivery

Liposomal Amphotericin, sold by Gilead (Ambisome) and Enzon (Abelcet)

- Amphotericin treats fungal and parasitic infection
- Most commonly used in patients with depressed white blood cell count (cancer and chemotherapy patients, HIV-infected patients, elderly patients).
- Liposomal formulation is preferred because of decreased side effects and prolonged drug exposure (due to slow release).

Applications: Non-resistant cancers

- In hepatic metastases model, the reduction in number of metastases was greater with Dox-loaded nanospheres than free dox. (Why hepatic model?)
- No special affinity for tumor tissue detected. Most nanospheres located within Kupffer cells. See proposed mechanism of action
- Note that this approach reduces side effects and toxicity! Small molecules are distributed throughout the body.

Examples of Nanoparticles for Drug Delivery

Applications: Intracellular Infections

Antibiotic-loaded nanoparticles

Resistance of many microorganisms to antibiotics is often related to low uptake of antibiotics or reduced activity in acidic pH of lysosomes.

1. Ampicillin-loaded nanoparticles for Listeria treatment. Dramatic improvement over free drug; bacterial counts in liver reduced at least 20-fold.
2. Ampicillin-loaded nanoparticles for Salmonella treatment. Drug alone – required 32 mg per mouse; with nanoparticle, only 0.8 mg ensured survival.


Drug Carrier Systems

<table>
<thead>
<tr>
<th>Generation</th>
<th>Size (µm)</th>
<th>Definition</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>&gt; 1</td>
<td>Able to release a drug at the target site but needing a particular type of administration</td>
<td>Microspheres and microcapsules for chemosensitization</td>
</tr>
<tr>
<td>Second</td>
<td>&lt; 1</td>
<td>Carriers that can be given by a general route able to transport a drug to the target site</td>
<td>Liposomes, nanoparticles, polymer drug carriers</td>
</tr>
<tr>
<td>Third</td>
<td>&lt; 1</td>
<td>Carriers able to recognize a specific target</td>
<td>Monoclonal antibodies; second-generation carriers with targeted antibodies or other ligands</td>
</tr>
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Metal Nanoparticles


Biological nanoparticles

Polymer-based nanoparticles

Metal Nanoparticles for Drug Delivery

Metal Nanoparticles

Lipid-based nanoparticles for Drug Delivery

Deliverables:
- Small molecules (Amphotericin B, Daunorubicin, Doxorubicin – all approved and marketed drug formulations) Gilead, Alza
- Viruses and bacteria (as vaccines) – in development
- Nucleic acids – in development

Many formulation methods --
1. Mixing lipids together in organic solution.
2. Remove solvent by evaporation
3. Hydration with aqueous solvent containing drug to form multilamellar vesicles
4. Sonication or extrusion are common methods to reduce the size of the liposomes

Lipid-based nanoparticles for Drug Delivery

Drug properties and Liposome association

- **Hydrophilic** Retained in aqueous interior ~may be difficult to get high loading Slowly released over several hours-several days
- **Hydrophobic** Inserted into hydrophobic interior of the liposome bilayer ~can disrupt liposome at high concentrations Excellent retention
- **Intermediate** Rapidly partition between lipid bilayer and aqueous phase Rapid release from liposomes but pH manipulation or formation of molecular complexes can result in good retention

Polymer-based nanoparticles for Drug Delivery

- Poly(alkylcyanoacrylates) used extensively for tissue adhesives for skin wounds and surgical glue (late 1960’s)
- Application as drug nanoparticulate carriers (1980s)
- For detailed review, see:

DEGRADATION OF NANOPARTICLES

Hydrolysis of ester bond; degradation products (alkylolcohol and poly(cyanacrylic acid)) are eliminated by kidney filtration.

Stratagene, Inc.
1. Attractive van der Waals forces and random Brownian motion cause particle flocculation.

2. Stabilization of colloids by electrostatic stabilization (DLVO theory):
   - Charged particles have a counter-ion layer. The charged surface and counter-ion layer is called the "electrostatic double layer". For homogenous colloid suspensions, this electrostatic double layer acts as a repulsive force between particles.
   - The sum of the van der Waals force and the double layer repulsion force gives the DLVO interaction potential:
     - higher ionic strengths collapse this boundary layer
     - at high salt concentration, no stable region
     - physiologic salt concentration ~150 mM

3. Stabilization of colloids by steric stabilization:
   - Add polymers to the surface of particles to prevent the particles from coming in close proximity to each other. At these distances, there is not enough attractive force for flocculation to occur.
   - Note that this phenomena is solvent dependent (affects the structure and interaction of the surface polymers)

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Steric Stabilization
Steric Stabilization

Chemical stability
- Reducing oxidation – addition of antioxidants, storage at low temperatures and pH 6.5
- Removal of water – spray drying or lyophilization (but both have to occur under controlled and optimized conditions)

Physical stability
- Electrostatic stabilization
- Steric stabilization
- Cream or hydrogel incorporation

Liposome Stability Optimization

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Mechanisms of Removal from Circulation

- Fast removal from circulation
  - Binding to cells, membranes, or plasma proteins
  - Uptake by phagocytes (macrophages)
  - Trapping in capillary bed things
- Renal clearance
  - Size restriction for kidney glomerulus is ~30-35 kDa for polymers (~20-30 nm)
- Extravasation
  - Depends on the permeability of blood vessels
  - Capillaries are thought to be more permissive to extravasation
  - Note: for cancer applications this works to our advantage: EPR!
Drug Carrier Systems: Influence of Physicochemical Properties

- **Molecular weight**
  - Macromolecules smaller than renal threshold are rapidly eliminated.
  - For larger, non-degradable molecules, excretion is useful to decrease toxicity.

- **Charge**
  - Positively charged macromolecules will interact with cells and membranes (remember the negatively charged proteoglycans?).
  - Negatively charged macromolecules are picked up by macrophages such as Kupffer cells, that contain polyanion scavenger receptors on their surface.

- **PEGylation**
  - May increase circulation by reducing non-specific protein binding.

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Biodistribution of Lipid-based Nanoparticles

**PEGylation**

- Non-PEGylated liposomes
- Why is there a dose-dependency?

- PEGylated liposomes

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Non-specific targeting: EPR Effect

- Tumors generally can’t grow beyond 2 mm in size without becoming angiogenic (attracting new capillaries) because difficulty in obtaining oxygen and nutrients.
- Tumors produce angiogenic factors to form new capillary structures.
- Tumors also need to recruit macromolecules from the bloodstream to form a new extracellular matrix.
- Permeability-enhancing factors such as VEGF (vascular endothelial growth factor) are secreted to increase the permeability of the tumor blood vessels.
- This effect is called the “enhanced permeability and retention effect” (EPR).

Targeting Ligands

- Small Molecules
  - Galactose/Glucose/Mannose
  - Folate
- Peptides
  - RGD
- Proteins
  - Transferrin
  - Antibodies
  - LDLs

Targeting Nanoparticles for Drug Delivery

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Examples of Nanoparticles for Drug Delivery

DOXIL

Proposed Mechanism of Action

Formulation
1. Doxorubicin-containing core
2. Lipid Bilayer membrane
3. PEG-coated surface
4. <100 nm

Examples of Nanoparticles for Drug Delivery

DOXIL Pharmacokinetics

Rats

Dogs


DOXIL Toxicity

- Mild white blood cell depression
- Skin toxicity (unique to Doxil) with full recovery. (Long distribution time?)
- Cardiac toxicity – insignificant up to 1500 mg/m²; Much lower toxicity than doxorubicin (lower peak plasma level; decrease availability to cardiac muscle)
- Hair loss – rare; only seen in ~6% of patients
- Mucositis – ulceration of oral mucosa. Dose-limiting toxicity

Examples of Nanoparticles for Drug Delivery

DOXIL Efficacy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Doxorubicin (1 mg)</th>
<th>Doxil (5 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to progression</td>
<td>18.4 wks</td>
<td>18.3 wks</td>
</tr>
<tr>
<td>CR</td>
<td>4*</td>
<td>5*</td>
</tr>
<tr>
<td>PR</td>
<td>16*</td>
<td>12*</td>
</tr>
<tr>
<td>TTP Plat Res</td>
<td>12.3 wks</td>
<td>6.5 wks</td>
</tr>
<tr>
<td>TTP Plat Sens</td>
<td>28.4 wks</td>
<td>28.8 wks</td>
</tr>
<tr>
<td>OS Plat Res</td>
<td>33.4 wks</td>
<td>37.3 wks</td>
</tr>
<tr>
<td>OS Plat Sens</td>
<td>86.1 wks</td>
<td>63.3 wks</td>
</tr>
</tbody>
</table>

Expressed as percentage.

**p = .01.

TTP Plat Res = time-to-progression platinum resistant;
TTP Plat Sens = time-to-progression platinum sensitive; OS = overall survival.

Examples of Nanoparticles for Drug Delivery

DOXIL Future Improvements?
**IDEAL SYSTEMIC DELIVERY VECTOR**

- Non-toxic vehicle
- Non-immunogenic
- Condensation of DNA
- Intracellular delivery
- Targeting ligand
- Stabilization of particles

**CYCLODEXTRIN POLYMER**

- Ave. MW 5.8 kDa, polydispersity index Mw/Mn = 1.12
- Degree of polymerization ~ 5 (10 charges/chain)
  verified by end group analysis and light scattering

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**PARTICLE ASSEMBLY**

(a) 

(b) 

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**TRANSFECTION COMPARISON**

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**TOXICITY COMPARISON TO BHK-21 CELLS**

Polymer PEI
IC$_{50}$=23 μM

Lipid
Lipofectamine
IC$_{50}$=6.4 μM

Polyethyleneglycol
Superfect
IC$_{50}$=11 μM

Cyclodextrin
polymer
IC$_{50}$=120 μM

$IC_{50}$ values reported at charge concentrations in the presence of DNA

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**INCLUSION COMPLEX FORMATION**

Association Constant is $10^5$–$10^6$
NEW METHOD OF SURFACE MODIFICATION

TEM IMAGES OF PARTICLES

Targeted, Stabilized Particle

Pre-formed Particle

Targeted Particles

Nucleic acid delivery

Nanoparticle delivery

Tumor uptake

A. Polyplexes in water
B. PEGylated polyplexes in water
C. Polyplexes in 50 mM NaCl
D. PEGylated polyplexes in 50 mM NaCl

Space bar is 100 nm except in C where it is 1000 nm