Applications of self-assembling peptides in controlled drug delivery

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Problems associated with drug administration
The importance of Control:

• Bio-compatible
• Non-toxic, non-immunogenic
• Bio-degradable
• Easy to handle
• Protect the cargo
• Deliver small, large, hydrophilic and hydrophobic molecules

• Programmed dose and time release for as long as it is required
• Flexibility & patient compliance
• Reduced risk of side effects from overdose

Most common systems
1. Electronic devices e.g., insulin pumps
2. Particle- and matrix-based systems e.g. polymers or inorganic materials
3. Liposomes

World drug delivery market: $17 - 29 billion for 2006
$33 - 67 billion for 2009
$65 - 97 billion for 2013
The importance of Control: 1. Devices

- First controlled drug delivery systems were built in the 1960s
- Insulin reservoir (like a regular syringe)
- Controller (computer chip)
- Glucose sensor
- Infusion device (pump) to deliver insulin to the body through a needle

The importance of Control: 2. Polymer micro- & nano-particles, matrices, and hydrogels

Poly-lactic-co-glycolic acid (PLGA)
Poly-ethylene glycol (PEG)
Poly-phosphoesters
Poly-vinyl alcohol (PVA)
Poly-ethylene oxide (PEO)
....

Clinical studies show:
Immune reaction to polymer surfaces
Degradation products may be toxic
Often toxic initiators are used
Not very efficient for protein delivery

Nanoparticles – microparticles
Composite polymer materials
Micelles
Colloids
Nanogels
Emulsions
Pluronic acid micelles (PEO/PPO)
Thin films
Polymer brushes
Hydrogels
The importance of Control: 3. Liposomes

- Associated with cell toxicity effects
- Immune response in some patients
- Research started in late 1960s
- Not many unique liposome-based drugs (most contain phosphatidylcholine)
- Doxil (1993, ovarian cancer, myeloma)
- Poor industrial reproducibility

Self-assembling peptides

<table>
<thead>
<tr>
<th>Length</th>
<th>Diameter</th>
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<tbody>
<tr>
<td>5-7 nm</td>
<td>2.5 nm</td>
</tr>
<tr>
<td>12-16 aa</td>
<td>5-7 aa</td>
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5-7 nm

100-nm
Hydrogel consisting of self-assembling peptides

At physiological conditions
0.15 M NaCl & 3 < pH < 8

Liquid Gel

Hydrogel consisting of self-assembling peptides

Water solution + Drugs

Tissue specific injectable drug release system
**Release of model drugs through peptide hydrogels**

- **Phenol Red**
  - MW: 354.4

- **8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (3-PSA)**
  - MW: 524.4

- **Bromophenol blue**
  - MW: 691.5

- **Phenol 1,3,6,8-pyrenetetrasulfonic acid tetrasodium salt (4-PSA)**
  - MW: 610.4

Measure diffusion for 1 week.
Release of model drugs through peptide hydrogels

**Slow release** depending on charge and MW of the model drug

Release depending on **charge** of the model drug
Release due to specific interactions of molecules with nanofibers
**Protein drug delivery**

- FDA has approved >200 proteins for therapeutic uses
- In 2014, protein drugs returned a revenue of ca. $70 billion

Large and unstable molecules (structure held by weak forces)
Easily damaged at mild storage conditions
Rapidly eliminated by the body
Some are difficult to obtain in large quantities

**Common administration routes are not suitable for proteins**

- **Oral** Proteins/enzymes are degraded in the stomach or they are not absorbed through the intestines
- **Skin** Low permeability of large molecules
- **Inhalation** Protein delivery through the lungs is difficult (e.g., Exubera)
- **Injection, subcutaneous or intravenous** Proteins are cleared from circulation (frequent injections)
**Controlled release of proteins**

Study protein delivery through peptide hydrogels using proteins that:
- are well characterized and widely used
- have a wide range of MW and pI
- have therapeutic interest

<table>
<thead>
<tr>
<th>Protein</th>
<th>MW</th>
<th>pI</th>
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<tbody>
<tr>
<td>Lysozyme</td>
<td>14.7 kDa</td>
<td>11.0</td>
</tr>
<tr>
<td>Trypsin inhibitor</td>
<td>20.1 kDa</td>
<td>4.6</td>
</tr>
<tr>
<td>BSA</td>
<td>66.0 kDa</td>
<td>5.3</td>
</tr>
<tr>
<td>Human IgG</td>
<td>145.0 kDa</td>
<td>7.2</td>
</tr>
</tbody>
</table>

**Fluorescence Correlation Spectroscopy (FCS)**

- High sensitivity and resolution (time/spatial)
- Detect molecules inside the hydrogel & in the supernatant
- Measure diffusion coefficients and concentration inside and outside the gel

Volume of detection is 1.5 fL
Protein release experiment

Water solution + Protein = Gel + Protein

Protein release experiment

PBS

Protein release through (RADA)$_4$ hydrogel

慢速释放，主要取决于蛋白质的分子量(MW)
Protein release through (RADA)$_4$ hydrogel

Protein release through peptide hydrogels

(RADA)$_4$ 1.0 %
(KLDL)$_3$ 0.6 %
(RADA)$_4$ 1.0 % (core) + (KLDL)$_3$ 0.6 % (shell)
Protein release through peptide hydrogels

**Question:**
What is the state of the proteins after being released from the hydrogels?

**Study the released proteins:**
- Spectroscopy (CD, Fluorescence)
- Activity tests, bioassays

Protein release through peptide hydrogels: CD spectroscopy
Protein release through peptide hydrogels: Fluorescence

Protein release through peptide hydrogels: Biological activity

Measure the hydrolysis of the cell membrane of *M. lysodeiktkus*

Measure the suppression of trypsin activity
Protein release through peptide hydrogels: Biological activity

Measure binding efficiency of monoclonal IgG against antigen

Protein release through peptide hydrogels: Biological activity

Native or released antibodies

Immobilized antigen
Smart hydrogel material for controlled release

Lipid-like self assembling peptides

ac-A₆K-NH₂ (acetyl-AAAAAK-CONH₂)

2.6 nm

KA₆-NH₂ (KA AAAA-CONH₂)

2.4 nm

ac-A₆D-OH (acetyl-AAAAAAD-COOH)

2.6 nm

DA₆-COOH (DAAAAA-COOH)

2.4 nm
Lipid-like self assembling peptide nanovesicles: AFM

![AFM images of nanovesicles](image1.png)

![AFM images of nanovesicles](image2.png)
Lipid-like self assembling peptide nanovesicles: Carboxyfluorescein encapsulation and release

![Graph showing the cumulative CF release over time.]

Lipid-like self assembling peptide nanovesicles: Nile Red integration in the peptide bilayer

![Graph showing the cumulative Nile Red release over time.]

![Fluorescence intensity vs. wavelength graph.]
Lipid-like self assembling peptides: Epithelial cell proliferation

![Graph showing cell proliferation over time with different concentrations of peptides.]

Lipid-like self assembling peptides: Drug absorption

Caco-2 cell monolayer model and rat intestine (everted sacs) are used to predict drug absorption (oral delivery).

![Graph showing FITC-dextran transport over time and permeability to FD-4.]

50% increase
**Conclusions**

**Injectable peptide hydrogel**
- Release small molecules & proteins of broad MW (e.g., Lsz, IgG)
- Hydrogel contains up to 99.5% water, active compound loading depends on the solubility of the drug
- Release kinetics depend on hydrogel density
- Functionality assays show that released proteins are active

**Lipid-like peptide nanovesicles**
- Release of hydrophilic and hydrophobic drugs
- Release kinetics depend on amino acid sequence and drug properties
- Peptides are not cytotoxic
- Increase transport through the epithelial layer

Self-assembling peptides are biocompatible, biodegradable, non-toxic, non-immunogenic, transparent which are ideal for biomedical applications