Protein Nanocapsules for Therapeutic Applications

Yi Tang

Department of Chemical and Biomolecular Engineering
Department of Chemistry and Biochemistry
University of California, Los Angeles
Delivery of Therapeutic Molecules to Biological Systems

Requirements

Effective
Efficient
Safe
Controlled Release
Gene therapy to treat congenital eye diseases

University of Pennsylvania Medical School successfully developed gene delivery methods to treat Leber's congenital amaurosis (LCA), which is a rare disease that leads to severe vision loss or no vision (Lancet, 2009, pg 1597).

One form of the disease LCA2 is caused by mutation in the gene RPE65, causing loss of 11-cis retinal and damage to light receptors required for vision. No treatment for LCA is available. Poor vision at birth becomes complete loss of eye sight in adulthood.

Use an adenoviral vector, the Penn team successfully delivered a normal copy of the RPE65 into the eyes of children with LCA2 in a phase I clinical trial. The introduced normal copy produced 11-cis retinal and restored vision of all 12 patients. Half of them were no longer classified as legally blind.

Credit: Moorfields Eye Hospital & University College London

NEJM, 2008, p2240
Drug delivery using Albumin Nanoparticles

Paclitaxel is a small molecule mitotic inhibitor that is used for treating different cancers. Its poor water solubility and toxicity to normal tissues however, had resulted in poor bioavailability and major side effects.

By conjugating paclitaxel containing nanoparticles to human serum albumin. It is more water soluble and more efficiently transported to tumor cells through albumin-mediated transcytosis. The resulting formulation (Abraxane®) is significantly more effective compared to paclitaxel itself.

*Journal of Controlled Release, 2012*
Protein-based Therapeutics

• **Advantages**
  - Specificity
  - Biocompatibility
  - High potency
  - Unique in form and function

• **Approved Protein Therapeutics**
  - Diabetes (Insulin: Humulin, Novolin, Symlin…)
  - Cancer (Herceptin, ELSPAR, Avastin, Vectibix…)
  - Cardiovascular (Natrecor, Angiomax, Retavase…)
  - Immunoregulation (Adagen, Infergen, Intron A…)
  - Growth regulation (Sandostatin, Kepivance…)
  ...~$77 billion in 2010

Nearly all current protein therapeutics act on extracellular targets

**Intracellular Protein Delivery Opportunities**

• **Potential Applications**
  – Catalyze intracellular reactions
  – Restore loss-of-function genetic conditions
  – Maintain normal cellular life cycles
  – Artificial control of gene expression levels
  – Imaging
  – Vaccination

Protein delivery adds functions to cells without modifications to the host genome

Extracellular Challenges

- Intrinsically unstable (aggregation/denaturation)
- Prone to proteolysis
- Rapid clearance of small proteins (< 30kDa)
- Elimination by immune system
- Targeting specific cells

Entry Challenges

- Surface charge (negative)

Intracellular Challenges

- Escape from delivery vehicles
- Release into cytosol
- Maintain structure and function

Nanocarriers

- Increase protein stability
- Shield protein from protease
- Increase circulation time
- Concealing immunogenic epitopes
- Allow surface modification
- Tailor carrier surface charge

Many of the delivery challenges can be addressed using nanocarriers

Choice and design of vehicle is crucial
Nanocarriers for Intracellular Delivery

Engineering Protein Nanocapsule

Outline of the Different Generations of Nanocapsules Designed in Our Lab

Surface modified NCs

Self-degradable NCs

Photo-caged self-degrading NCs

- covalent modification
- non-covalent modification

Endoprotease-degradable NCs

Redox-responsive NCs

Degradable

Controllable

Generalizable
Single-Protein Based Nanocapsules


Uniform size
Increased stability
Efficient cell entry

monomer
positive monomer
crosslinker

50°C

Fluorescence intensity (%)

Time (hrs)
Degradable Nanocapsules

Shown here is the delivery of a protein that can cause apoptosis in cells.
**Endoprotease-degradable Nanocapsules**

For cytosolic delivery, the actions of *intracellular* proteases can be explored

**Furin** – Proprotein convertase

- Required for the maturation of various cellular proteins.
- Found in various intracellular locations, including cell membrane, endosomal compartments, and trans-Golgi network.
- Increased furin expression in breast, ovarian, brain, lung and neck cancers.

Furin-mediated cleavage of papillomaviruses is necessary for dissociation of capsid, release of viral DNA and infection. Natural role of furin facilitated release of foreign cargo.

Can we mimic some of these natural infection processes in our carrier design?

Biswa *et al*, ACS Nano. 2011

Richards *et al*, PNAS. 2002
Furin-Mediated Release of Protein Cargo


Before adding furin

10 hr after adding furin
Strategy for Chemo-Degradation

- The difference in the redox environment between extracellular (oxidizing) and intracellular (reducing) can also be explored for degradation of the polymeric shell.

- The high intracellular concentrations of glutathione (GSH) can rapidly trigger reduction of disulfide (S-S) crosslinked matrices.

Redox-Responsive Nanocapsules

Before GSH Treatment

After GSH Treatment

Zhao, M. et al, Biomaterials, 2011

Gene delivery from redox degradable polyplexes

Engbersen and coworkers, Bioconjug. Chem. 2007
Redox-Responsive Nanocapsules

Time course of GSH-mediated degradation

S-S eGFP NC Trafficking in HeLa

Zhao, M. et al, Biomaterials, 2011
Application: Apoptin

Apoptin induces apoptosis selectively in tumor cells, but not in normal cells.

Potential antitumor therapeutic if the correct delivery vehicle can be designed.

Delivery of Apoptin to Selectively Kill Tumor Cells

Nanocapsule delivered-apoptin localization

Nanocapsule can slow down tumor growth in mouse MCF-7 breast cancer xenografts.

Normal Cell (HFF)  Cancer Cell (MCF-7)

No Treatment  IV PBS  IT S-S APO NC
Application: Transcription Factors

- Regenerative medicine aims to help repair diseased or damaged tissues by replacing affected cells with healthy functional cells.
- Transcription factors are driving forces for directing cellular fate.
- Effective TF delivery requires efficient nuclear delivery of proteins in active form.

Delivery of MyoD

MyoD is one of myogenic regulatory factors which act sequentially in myogenic differentiation.

Key role of MyoD is to commit mesoderm cells to a skeletal lineage, and then to regulate that process.

MyoD nanocapsule drives myotube formation

MyoD nanocapsule is resistant to protease

![Green: MyHC Ab; Blue: DAPI-stained nuclei](chart.png)

redrawn by S. Pearson-White from Olson, Konieczny reviews
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