Engineering Biomimetic Peptides for Targeted Drug Delivery

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Medical advancements have been limited by serious adverse effects associated with many of these medicines.

The effectiveness of many drugs (genes, peptides, proteins) could be greatly enhanced or even enabled if two conditions are met:
- the drugs are selectively targeted to the diseased cells
- the drugs are delivered inside the cells to the site of their pharmacological activity.

**Targeted Stealth Liposomes**
“inert” & pH-sensitive

**Targeted Polymersomes**
“inert” & biodegradable
Targeted Delivery of Nanoparticles

-Video-
The TAT peptide (GRKKRRQRRRPQ) is derived from the Trans-Activator of Transcription (TAT) protein of human immunodeficiency virus (HIV-1) and is a cell penetrating peptide.

One of the major obstacles in using the TAT peptide is its lack of selectivity (it will penetrate any cell).

Solution: Multifunctional “smart” liposomes with temporarily “hidden” function, for example TAT, and “shielding” polymeric coat with or without targeting antibody attached to it.

Collagen-mimetic peptides have been developed to target the CD44 receptor that is over-expressed in many tumor cells.

CD44 receptors bind to a specific amino acid sequence from type IV collagen α1(IV)_{1263-1277} (GVKGDKNPGWPGAP), called IV-H1, and more importantly, binding is highly dependant on the triple helical structure of the sequence (Lauer-Fields et al., J. Biol. Chem., 2003).

IV-H1 peptide-amphiphiles were incorporated into stealth liposomes, targeted to M14#5 metastatic melanoma cells, and promoted specific ligand/receptor interactions where as non-targeted liposomes showed no binding (Rezler et al., J. Am. Chem. Soc., 2007).
Mimicking Multidomain Binding: Fibronectin-Mimetic Peptides

α5β1 integrin has impact on processes such as:
- accelerating wound healing
- promoting angiogenesis
- mediating adenovirus infection
- protection mechanism against Alzheimer’s disease
- promising target for breast, colon, rectal & prostate cancer

Adapted from Tirrell et al., Surf. Sci., 2002
Fibronectin-Mimetic Peptides

Spacer designs by others focused on the distance between PHSRN and RGD:

- $G_3$, $G_6$, $G_9$, $G_{12}$ – Kim et al., *Biotech. Let.*, 2002
- $G_{13}$ – Benoit & Anseth, *Biomaterials*, 2005
**Fibronectin – Mimetic Peptides**

Kim *et al.*, *Biotech. Let.*, 2002

**1hr incubation**

![Graph showing turbidity at 570 nm against substrate concentration (µM)](image)

- **Designs compared to FN showed smaller adhesion**
- **Surfaces used in other applications (e.g., tissue engineering) should be optimized to promote cell adhesion and ECM production**

**Benoit & Anseth, Biomaterials, 2005**

![Graph comparing cell density on different surfaces](image)
Cell Adhesion and Function:
Peptides versus Proteins

For a surface saturated with a peptide versus a surface saturated with the protein:

- More active binding sites on the peptide interface
- Easily control peptide orientation
- Prevent peptide denaturation
Importance of Hydrophobic/Hydrophilic Interactions


**Our hypothesis:** Length & hydrophobicity/hydrophilicity of linker can affect integrin affinity for the biomimetic peptide (Mardilovich & Kokkoli, *Biomacromolecules*, 2004).
Endothelial Cell Adhesion

- Serum-free environment
- GRGDSP and 50%PHSRN-50%GRGDSP fail after 24 hr
- The PR_b peptide-amphiphile surfaces outperform all other peptide surfaces and compared to FN surfaces give higher adhesion for 1-24 hr and similar adhesion for 48-72 hr

Mardilovich et al., Langmuir, 2006
Endothelial Cell Cytoskeletal Organization

- Red – actin
- Green – vinculin
- Blue – nucleus

GRGDSP and GRGESP collapse after 24 hr

Highly-developed cytoskeletal structure on PR_b: elongated actin stress fibers & sharp spikes of vinculin at termination points and across actin

Mardilovich et al., Langmuir, 2006
α5β1-Targeted Delivery with PR_b Functionalized Liposomes

PR_b peptide “bullet” that binds to the α5β1 integrin
Gene Therapy for Metastatic Colorectal Cancer (Stage 4)

Unpublished Data
Conclusions

Summary

- Targeted drug delivery offers many advantages, such as, specific delivery to the tumors, less side effects, and use of less drugs.

- Biomimetic peptides are promising “bullets”.
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