Ultrashort NSAID-conjugated Peptides as Bifunctional Nanomaterials


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Ultrashort NSAID-conjugated Peptides as Bifunctional Nanomaterials

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Outline

1. Ultrashort peptides
2. Self-assembling peptides
3. Ultrashort self-assembling antimicrobial peptides
4. NSAID-conjugated self-assembling peptides
What are Ultrashort Peptides?

• Ultrashort = 4-7 amino acids
• Cationic = net positive charge (+2)
• Cost effective → Upscale → Translational potential → Patient
• Numerous advantages including:
  – chemical versatility
  – immunogenicity
  – Tunable biocompatibility + biodegradability
  – Tailored self-assembly/pharmacological properties
  – Antimicrobial = innate immune response
  – Nanotechnology
Self-assembling Peptides

Peptide Amphiphiles (Stupp)

α-helices/ Coiled coils (Woolfson/ Tirrell)

β-sheets (Agelli/ Collier)

Short Aromatics (Xu/ Gazit/ Ulijn)

β-haripins (Pochan/ Schneider)
Core Technology

Self-assembled Peptides

Stimuli
- pH
- Light
- Temperature
- Ionic Strength
- Specific enzymes

Assembly

Peptide Hydrogels

Short peptide sequences

External stimuli

Hydrophobic: Hydrophilic → Hydrogel (critical gelation concentration)
Biofunctional Nanomaterials Utilising the Building Blocks of Life!

- Infection and Medical Devices
- Wound healing
- Drug Delivery
- Stem Cells/Regenerative medicine
Planktonic vs. Biofilm Bacteria

- Planktonic form: Free floating in liquid
- Biofilm form: sessile, composed of aggregated microcolonies of cells surrounded by a protective extracellular polymeric matrix
- Mature biofilms can resist 10-1000 times the concentrations of standard antibiotic regimens that are required to kill genetically equivalent planktonic forms

P. Dirckx, Centre for Biofilm Engineering, Montana State University, Bozeman

Biofilms in the Environment and Medicine

Biofilm growth on rocks in a stream (USGS) and within a kitchen pipe (MSU Center for Biofilm Engineering).

SEM Pseudomonas aeruginosa, shown here attached to an implant surface, is one of many resistant microorganisms.

University of Illinois researchers tested a prototype of a new device that can see biofilms behind the eardrum to better diagnose and treat chronic ear infections.
Antimicrobial Resistance

- Healthcare associated infections
- Medical devices: reservoir for “superbugs”
- Chronic wounds
- Persistent burden on:
  - Patient morbidity & mortality
  - Family and carers
  - Healthcare budgets
What are the solutions?

Antimicrobial Activity of Short, Synthetic Cationic Lipopeptides

Garry Laverty, Martin McLaughlin, Christopher Shaw, Sean P. Gorman and Brendan F. Gilmore

Biomaterials Research Group, School of Pharmacy, Queen’s University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, N. Ireland.

ABSTRACT: For new antimicrobial agents with activity against pathogens that are resistant to the available array of antibiotics (1,4), one class of compounds that has attracted increasing attention in the last two decades are the cationic antimicrobial peptides (CAMP) synthesized outside of the traditional antibiotic paradigm. Surfaces mediated by CAMP have shown promise as novel means of imparting resistance to bacteria. In this review, we summarise the CAMP field, discuss the advantages of CAMP, and highlight the future directions for CAMP applications.

Evolution of Antimicrobial Peptides to Self-Assembled Peptides for Biomaterial Applications

Alice P. McCloskey, Brendan F. Gilmore and Garry Laverty

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ABSTRACT: Self-assembling dipeptides conjugated to naphthalene show considerable promise as nanomaterial structures, biomaterials, and drug delivery devices. Biomedical infections are responsible for high rates of patient mortality and morbidity. The presence of biofilm bacteria, which thrive on implant surfaces, is a huge burden on healthcare budgets, as they are highly resistant to current therapeutic strategies. Ultrashort cationic self-assembled peptides represent a highly innovative and cost-effective strategy to form antibacterial nanomaterials. Lysine conjugated variants display the greatest potency with 2% w/v NapFPK5 hydrogels significantly reducing the viable Staphylococcus aureus biofilm by 94%. Reducing the size of the R-group methylene chain on cationic moieties resulted in reduction of antibacterial activity. The primary amines of the promoting R-group tail may not be as readily available to interact with negatively charged bacterial membranes. Cys-PEM, FTR, QD spectroscopy, and oscillatory rheology provided evidence of supramolecular hydrogel formation at physiological pH (pH 7.4). Cytotoxicity assays against murine fibroblast (NCTC 929) cell lines confirmed the gels possess reduced cytotoxicity relative to bacterial cells, with limited hemolysis upon exposure to equine erythrocytes. The results presented in this paper highlight the significant potential of ultrashort cationic naphthalene peptides as future biomaterials.

SOJ Microbiology & Infectious Diseases

Cationic Antimicrobial Peptide Cytotoxicity

Garry Laverty* and Brendan Gilmore

Anti-biofilm activity of ultrashort cinnamic acid peptide derivatives against medical device-related pathogens

Garry Laverty, *Alice P. McCloskey, Sean P. Gorman and Brendan F. Gilmore
## Rational Design of Antimicrobial Peptide Motif vs Self-assembly

<table>
<thead>
<tr>
<th>Antimicrobial Activity</th>
<th>Propensity to Self-assemble</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrophobic/Hydrophilic (Charge) ratio (more important with regard to antimicrobial activity than size)</td>
<td>Hydrophobic/Hydrophilic balance</td>
</tr>
<tr>
<td>Interactions with microbial extracellular membranes</td>
<td>Non Covalent intermolecular interactions (e.g. Van der Waal’s, π-π stacking)</td>
</tr>
<tr>
<td>Interaction with intracellular targets/processes (DNA, RNA, enzymes, protein synthesis)</td>
<td>Ability of peptide to form hydrogen bonds with each other and with water</td>
</tr>
</tbody>
</table>

Self-assembled Ultrashort Peptide Gels

• Successful library of ultrashort peptides: self-assembled at physiological pH
• \((X_1\text{-FF-X}_2)\)
• Hydrophobicity: naphthalene (Nap) grouping (at \(X_1\)) and varying quantity of phenylalanine (F) in primary structure
• Minimum of 2 charged units required for antimicrobial activity
• Primary amine group provides cationic charge
• Cationic amino acids vary by number of methylene units on R-group

Dual Antimicrobial Anti-inflammatory Nanomaterials

- Hydrophobicity provided by NSAID structure
- High in aromaticity
- Display self-assembly and gelation characteristics
- Potential applications in chronic infected wounds

Self-assemble to Hydrogel Networks

NpxFFKK 0.5 %

IbuFFKK 2 %

IndFFKK 1 %

NpxFFKK 2% (w/v)

IndFFKK 2% (w/v)
Confirmation of β-sheet Hydrogel Networks

Oscillatory rheology

FTIR

- NpxFFKK 2% G''
- NpxFFKK 2% G'
- IbuFFKK 2% G''
- IbuFFKK 2% G'
- IndFFKK 2% G''
- IndFFKK 2% G'

Transmittance (%)
Percentage reduction of mature 24h biofilm treated with 2% w/v NSAID-conjugated hydrogels utilizing an alamarBlue assay.

Percent inhibition of COX 1 and 2 enzyme by NSAID self-assembled hydrogels and by the model COX inhibitor DuP-697 using a COX Fluorescent Inhibitor Screening Assay Kit.
Conclusion

• Developed a library of ultrashort self-assembling bifunctional peptides
• Vast potential for use against Biomaterial/Medical Device/Implant Infections
• Wound healing/surgical gel: Increased healing as mimics natural tissues
• Platforms/vehicles to deliver existing antimicrobials, extend spectrum of activity to Gram-negatives
• Translatable and economically friendly form of nanotechnology for patient benefit
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