Ultrashort NSAID-conjugated Peptides as Bifunctional Nanomaterials

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)
- β-haripins (Pochan/Schneider)

Short Aromatics (Xu/Gazit/Ulijn)
Core Technology

Self-assembled Peptides

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

Assembly

Peptide Hydrogels

Short peptide sequences

External stimuli

Hydrophobic: Hydrophilic \( \rightarrow \) 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).

Biofilm formation on a voice prosthesis implant.

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*

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Pathogens 2014, 3, 791-821; doi:10.3390/pathogens3040781

Review

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

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Research Article

SOJ Microbiology & Infectious Diseases

Cationic Antimicrobial Peptide Cytotoxicity

Garry Laverty* and Brendan Gilmore

Ultrashort Cationic Naphthalene Derived Self-Assembled Peptides As Antimicrobial Nanomaterials

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Supporting Information

ABSTRACT: Self-assembling dipeptides conjugated to naphthalene show considerable promise as nanomaterial structures, biomaterials, and drug delivery devices. Biomaterial infections are responsible for high rates of patient mortality and morbidity. The presence of biofilm bacteria, which thrive on implant surfaces, are 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. Linear conjugated variants display the greatest potency with 2% w/v NapFPKK hydrogels significantly reducing the viable Staphylococcus epidermidis biofilm by 94%. Reducing the size of the R-group methylene chain on cationic moieties resulted in reduction of antibiotic activity. The primary amine of the promoting R-group tail may not be as readily available to interact with negatively charged bacterial membranes. Cryo-SEM, FTR, CD spectroscopy, and oscillatory rheology provided evidence of supramolecular hydrogen formation at physiological pH (pH 7.4). Cytotoxicity assays against murine fibroblast (NCTC 929) cell lines confirmed the gels possessed 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.
### Rational Design of Antimicrobial Peptide Motif vs Self-assembly

<table>
<thead>
<tr>
<th>Antimicrobial Activity</th>
<th>Propensity to Self-assemble</th>
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</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 %

 NxFFKK 2% (w/v)

Queen's University
Belfast
Confirmation of β-sheet Hydrogel Networks

Oscillatory rheology

FTIR
Dual action

Antimicrobial

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

Anti-inflammatory

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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The Adams Lab, Department of Chemistry, University of Liverpool

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