Ultrashort Self-assembled Peptides for Biomaterial Applications

No. 1 Again!!
The School of Pharmacy is ranked as the No.1 Pharmacy School in the UK by the 2016 Times and Sunday Times Good University Guide (for the second successive year)
Current Peptide Interests

Antimicrobial Activity of Short, Synthetic Cationic Lipopeptides

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Antimicrobial peptide incorporated poly(2-hydroxyethyl methacrylate) hydrogels for the prevention of Staphylococcus epidermidis-associated biomaterial infections

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Biofilm Eradication Kinetics of the Ultrashort Lipopeptide C12-OOWW-NH2 Utilizing a Modified MBEC Assay

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Cationic antimicrobial peptides exist throughout the nature as defense mechanisms in both prokaryotic and eukaryotic organisms. Antimicrobial peptides have evolved over millennia to become inherent antimicrobial molecules and effective mediators of the innate and adaptive immune responses (1). They have been proven to be effective at neutralizing Gram-negative bacterial lipopolysaccharide endotoxin and aid the process of wound healing (2,3). Variations in the length of their primary sequence frequently

Review

The Potential of Antimicrobial Peptides as Biocides

Garry Laverty 1,*, Sean P. Gorman 2 and Brendan F. Gilmore 3

Cationic Antimicrobial Peptide Cytotoxicity

Garry Laverty* and Brendan Gilmore
### Rational Design of Antimicrobial Peptide Motif vs Self-assembly

<table>
<thead>
<tr>
<th>Antimicrobial Activity</th>
<th>Propensity to Self-assembled hydrogels</th>
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<tr>
<td>Hydrophobic/Hydrophilic (Charge) ratio (more important with regard to antimicrobial activity than size)</td>
<td>Hydrophobic/Hydrophilic balance</td>
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<tr>
<td>Interactions with microbial extracellular membranes</td>
<td>Non Covalent intermolecular interactions (e.g. Van der Waal’s, π-π stacking)</td>
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<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>
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Core Technology

Self-assembled Peptides

Stimuli
- pH
- Temperature
- Ionic Strength
- Specific enzymes

Non assembled

Short peptide sequences

Self-assembly

Peptide Hydrogels

Peptide Nanotubes
Advantages of Ultrashort Peptides

- Successful in producing a series peptide sequences of that self-assemble to form hydrogels or nanotubes in response to physiological stimuli
- Ultrashort peptides (\(< 7\) amino acids) → More cost effective → Upscale by Pharmaceutical Industry → Increased translational potential → Patient benefit
- Numerous advantages over current synthetic materials including:
  - Increased chemical versatility
  - Minimal immunogenicity and enhanced biocompatibility
  - Tunable biodegradability
  - Tailored self-assembly/pharmacological properties (e.g. antimicrobial) in response to stimuli
Biofunctional Nanomaterials Utilising the Building Blocks of Life!

- Infection and Medical Devices
- Wound healing
- Drug Delivery
- Stem Cells/Regenerative medicine
Antimicrobial Resistance

• Medical device related infections
• Increased reservoir of “superbugs”
• Persistent burden on:
  • Patient morbidity & mortality
  • Family and carers
  • Healthcare budgets
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

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.
Self-assembled Ultrashort Peptide Gels

• 2013 Research Placement Prof. Bing Xu Lab, School of Chemistry, Brandeis, Waltham, Boston
• Successful in producing a series of ultrashort peptides (< 7 amino acids) that self-assembled at physiological pH
• \((X_1\text{-FF-}X_2)\)
• More cost effective
• Hydrophobicity provided by inclusion of a naphthalene (Nap) grouping (at \(X_1\) position) and varying quantity of phenylalanine in primary structure
Ultrashort Cationic Variants: Primary Structures

- Charge: Inclusion of cationic amino acids
  - 1) Lysine
  - 2) Ornithine
  - 3) epsilon (ε) Lysine
- 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

Ultrashort Cationic Variants: Self-assembly

- Form Self supporting hydrogels at pH 7.4: pH triggering method

Optical images of gel (A) NapFFOO, (B) NapFFKK, (C) NapFFεKεK, at a concentration of 1% w/v and pH of 7.4 in water

Transmission electron microscopy (TEM) images of (A) NapFFOO, (B) NapFFKK, (C) NapFFεKεK, at a concentration of 1% w/v and pH of 7.4 in water

Anti-Biofilm Activity Gram-positive Bacteria

Percentage reduction of mature 24 hour *Staphylococcus aureus* (ATCC 29213) biofilm after 24 hour incubation with naphthalene derived self-assembled hydrogels utilizing an alamarBlue assay. Results are displayed as a mean of 8 replicates.

Anti-Biofilm Activity Gram-negative Bacteria


Percentage reduction of mature 24 hour *Escherichia coli* (NCTC 11303) biofilm after 24 hour incubation with naphthalene derived self-assembled hydrogels utilizing an alamarBlue assay. Results are displayed as a mean of 8 replicates.
Toxicity: Tissue Culture & Haemolysis

Percentage viability of CCL 1 [NCTC clone 929]-murine fibroblasts subcutaneous connective tissue monolayer cells after 24 hour exposure to naphthalene derived self-assembled hydrogels utilizing an alamarBlue assay. Results are displayed as a mean of 8 replicates.

Percentage hemolysis of the naphthalene peptides against equine erythrocytes. Each value is expressed as the mean of six replicates, incubated at 37 °C for 1 hour.

Data demonstrates biocompatibility (NapFFKK) and reduction in bacterial load with *S. aureus* (ATCC 29213) *S. epidermidis* (ATCC 35984), *E. coli* (NCTC 11303) and *Pseudomonas aeruginosa* (PAO1).
Bacterial counts (Log CFU/mL) from haemolymph extracted 72 hour following inoculation with 20μL of $1 \times 10^5$ bacteria and treatment 2 hours later with 20μL NapFFKK.
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

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.
Dual Antimicrobial Anti-inflammatory Nanomaterials

Oscillatory rheology

TEM-Naproxen-FFKK-OH
Potential Applications: Antimicrobial Platforms

- Prevention of Biomaterial/Medical Device/Implant Infections:
  - The medical device industry has an estimated worth of US$75 billion worldwide
  - Urinary catheters
  - Intravascular catheters
  - Hip replacements
  - Cardiac devices
  Implant surface provides perfect environment for growth of resistant pathogens!

- Wound healing/surgical gel: Increased healing as mimics natural tissues

- Platforms/vehicles to deliver existing antimicrobials, extend spectrum of activity to Gram-negatives
- Translation for patient benefit

Urinary catheter encrustation
Thank You!

- Alice McCloskey (DEL funded PhD student): Biomaterials
- Rawan Huwaitat (PhD student): Gram-negative nanotubes
- Simon Porter (DEL funded PhD student): Nanotubes cancer
- Alyaa Albadr (PhD student): Ocular nano
- Giovanni Cristini (Erasmus student)
- Dr Hema Nagaraj (Visiting Research Fellow)
- Lucia Murias (Visiting Research Assistant)
- Sophie Gilmore (Sfram: Students into Work)
- Merissa Lee (Sfram: Students into Work)

http://lavertylab.weebly.com

- The Xu Group, School of Chemistry, Brandeis.
- The Adams Lab, Department of Chemistry, University of Liverpool