Modifying short phenylalanine-phenylalanine peptide sequences to create multifunctional nanomaterials with biomaterial and drug delivery applications


Document Version:
Other version

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

Publisher rights
Copyright 2018 The Author.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.
Modifying short FF peptide sequences to create multifunctional nanomaterials with biomaterial and drug delivery applications

Dr Garry Laverty
School of Pharmacy
Biofunctional Nanomaterials Group

Our Research is Funded by
The School of Pharmacy at Queen’s has been ranked as the number 1 school of Pharmacy in the UK.
Core Technology

Self-assembled Peptides

Stimuli
- pH
- Temperature
- Ionic Strength
- Specific enzymes

Self-assembly

Peptide Hydrogels

Short peptide sequences
Non assembled
Rational Design of Antimicrobial Peptide Motif vs Self-assembly

<table>
<thead>
<tr>
<th>Antimicrobial Activity</th>
<th>Propensity to Self-assembled hydrogels</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). Binds to DNA, lipopolysaccharide to prevent pro-inflammatory response = immunomodulatory</td>
<td>Ability of peptide to form hydrogen bonds with each other and with water</td>
</tr>
</tbody>
</table>

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 (Blood brain barrier, cancer, Gram-negative bacteria, HIV, *in situ* forming implants)
- Stem Cells/Regenerative medicine
Peptide Hydrogel Nanomaterials
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
• (X₁-FF-X₂)
• More cost effective
• Hydrophobicity provided by inclusion of a naphthalene (Nap) grouping (at X₁ 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 and antibiofilm activity
- Primary amine group provides cationic charge
- Cationic amino acids vary by number of methylene units on R-group

NapFFKK: Fungal infections

Fungal viability counts (Log10 CFU/mL) of *Aspergillus niger* CABI 017454 after 24 h exposure

**Fmoc variants antibiofilm**

Percentage reduction in viability of 24 hour *E. coli* (ATCC 11303) biofilm following 24 hour exposure to Fmoc-peptides.

Percentage cell viability of NCTC clone 929 (ATCC CCL 1) cells after 24 hour exposure to varying concentrations of Fmoc-peptides.

Multifunctional NSAID-peptide hydrogels for the treatment of chronic wounds

- Chronic wounds: unable to heal fully or respond to treatment within 4 to 12 weeks. E.g. pressure wounds, diabetic ulcers, burn/surgical wounds.
- Latest UK estimates (2005-06), reported an incidence of 575,600 patients annually costing the NHS between £2.3 and 3.1 billion, 3% of yearly healthcare expenditure.
- Differ from acute wounds in that they are associated with prolonged inflammation that prevents healing fully: Non steroidal anti-inflammatory drugs (NSAIDs) showing benefit.
- Optimal multifunctional peptide: hydrogelating, biocompatible, antimicrobial, anti-inflammatory, pro-angiogenic

Prevention better than cure!!
a) NFκB

b) P. aeruginosa

c) IL-6, IL-8, IL-10 → ↓ inflammation

d) NFκB

 NSAID-peptide: ↓ inflammation, ↓ scar-tissue formation

f) Nanofibrous scaffold supports cell growth:

↑ keratinocyte migration

↑ subcutaneous fibroblast migration

DNA

LPS

tLRs

COX-2

NSAID-peptide

Selective Cox-2 inhibition:

↓ scar-tissue formation

g) Recruitment & activation of VEGF, FGF2, HGF growth factors:

up angio genesis

NFκB

IKKα/IKKβ kinases

IκB

NSAID

(PO₃)⁻
Multifunctional NSAID-peptide hydrogels: Design

Optimal multifunctional peptide:
- Hydrogelating ✓
- Biocompatible ✓
- Antimicrobial ✓
- Anti-inflammatory/immunomodulatory
  - selective COX-2 inhibition ✓
  - inhibit NFκB
  - inhibit toll-like receptors by binding to biomolecules (e.g. DNA, bacterial LPS)
- Pro-angiogenic (heparin mimetic motif)

Multifunctional NSAID-peptide hydrogels: Hydrogelating, Biocompatible

Data relating to L-isomers of 2% w/v peptide. a) Npx-FFKK-OH hydrogel. b) TEM showing Npx-FFKK-OH nanofibres. c) LIVE/DEAD assay, 500 µM Npx-FFKK-OH with NCTC929 fibroblasts.

Oscillatory frequency sweep 2% w/v NSAID-peptides. Key: black triangle: G' IbuFFKK, white triangle: G" IbuFFKK black circle: G' IndFFKK, white circle: G" IndFFKK, black square: G' NpxFFKK, white square: G" NpxFFKK.

Multifunctional NSAID-peptide hydrogels: Biocompatible & COX-2 selective (anti-inflammatory)

Cell compatibility and COX inhibition of L-isomers of X-FFKK-OH peptides. Ibuprofen (Ibu), indomethacin (Ind) and Npx conjugated at X. a) >90% cell viability, NCTC929 fibroblasts, 24 hour exposure (alamar blue assay). Key: striped:ibuFFKK, white:IndFFKK, grey:NpxFFKK, ns:no significant difference compared to negative PBS control. b) IC$_{50}$ NSAID-peptide and NSAIDs only, inhibition of COX-1 (black column) and COX-2 (white column). Selectivity (S)=$\text{COX-1:COX-2 ratio of IC}_{50}$ values. Addition of FFKK-OH to NSAIDs increases IC$_{50}$ values relative to NSAID only but significant inhibition is maintained within the µM range. NSAID-peptides possess increased COX-2 selectivity compared to NSAID only, which is promising for chronic wound therapy. COX-2 selectivity highest for NpxFFKK-OH (S=2.78) therefore it is the most promising NSAID-peptide for reducing scar tissue formation in chronic wounds.
Multifunctional NSAID-peptide hydrogels: Anti-biofilm/Antimicrobial

Bactericidal activity of NSAID-FFKK-OH. a) NpxFFKK-OH (2-0.5%w/v) shows >90% in 24 hour biofilms of *S. aureus* ATCC25923 (black column), *S. epidermidis* ATCC35984 (grey), *E. coli* ATCC11303 (striped) and *P. aeruginosa* PAO1 (white) after 24 hours (alamar blue assay). b) Log$_{10}$ reduction in *S. aureus* viable count after 24 hours, NSAID-peptides (2-0.5%w/v). Key: striped column: IbuFFKK-OH, white: IndFFKK-OH, grey: NpxFFKK-OH, dotted line: PBS control. At least a 3 log$_{10}$CFU/mL (99.9%) reduction in bacteria, employed as a threshold for efficacy was observed for all NSAID-peptides at concentrations ≥0.5% w/v compared to PBS control. A similar trend was demonstrated for NSAID-peptides against *S. epidermidis*, *E. coli* and *P. aeruginosa.*
Tuesday 3\textsuperscript{rd} September ~9.25am: Optimising phenylalanine-phenylalanine peptide nanotubes to demonstrate selective antibiofilm activity
Biofunctional Nanomaterials Group
- Dr Sreekanth Pentlavalli (Wellcome Trust Research Fellow)
- Sophie Gilmore (Dfe funded PhD student): *in situ* implants
- Rawan Huwaitat (PhD student): Selective Gram-negative antimicrobials
- Simon Porter (Dfe funded PhD student) Nanotubes
- Alyaa Albadr (PhD student) Ocular drug delivery/antimicrobial
- Marina Afami (Dfe funded PhD student) Stem cell delivery/dental

http://lavertylab.weebly.com

- The Xu Group
  Brandeis University
- The Adams Lab
  University of Glasgow