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


Document Version: Other version

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

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Biofunctional Nanomaterials Group
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

Nanotubes

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>
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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>
</tr>
<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). 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>
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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

Stem Cells/Regenerative medicine

Wound healing

Drug Delivery (Blood brain barrier, cancer, Gram-negative bacteria, HIV, in situ forming implants)
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
• \((X_1\text{-FF-}X_2)\)
• 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 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 3.1billion, 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
a) NFκB
b) IκB
IKKα/IKKβ kinases
NSAID
(PO3)−
c) IL-6
IL-8
IL-10
↓ inflammation
d) DNA
LPS
TLRs
NFκB
↓ inflammation
e) TLRs
NFκB
↓ inflammation
f) Nanofibrous scaffold supports cell growth
↑ keratinocyte migration
↑ subcutaneous fibroblast migration
g) NSAID-peptide
Selectively targets Cox-2
↓ scar-tissue formation
↑ recruitment & activation of VEGF, FGF2, HGF growth factors:
↑ angiogenesis
Multifunctional NSAID-peptide hydrogels: Design

Naproxen

Ibuprofen

Indomethacin

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, 500µM 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)=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 3rd 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): \textit{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

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