Ultrashort Peptides as Bifunctional Nanomaterials

Introduction

Biomaterials have contributed greatly to advances in modern medicine enhancing patients’ quality of life however they are regarded as foreign objects by the human body [1]. Their presence and sometimes trauma caused by insertion of a biomaterial triggers host inflammatory mediators. They also provide an ideal surface for bacterial attachment and biofilm formation. These can compromise biomaterial function and mechanical properties resulting in its failure or destruction of with associated negative effects on the patient and their outcomes [2].

Self-assembling ultrashort cationic peptides are an innovative form of antimicrobial hydrogels. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat pain and inflammation despite their systemic side effects. These can compromise biomaterial function and mechanical properties resulting in its failure or destruction of with associated negative effects on the patient and their outcomes [2].

Gelation can be tailored to occur in response to physiological infectious indicators:

- pH
- Enzymes
- Temperature

Thus facilitating targeted antimicrobial and anti-inflammatory activity at the site of infection [4]. This work focuses on the development of dual acting anti-inflammatory and antimicrobial self-assembling peptides with the incorporation of clinically used NSAIDs into our previously investigated self-assembling antimicrobial peptides with FFKK.

Methods

Diphenylalanine-diylsine peptides were synthesized on Wang resin following standard Fmoc protocols using a manual Nitrogen bubbler apparatus. The final coupling step involved addition of one of the following NSAIDs (listed below) in the same manner as to the addition of an amino acid: naproxen (Npx), ibuprofen (Ibu) and indomethacin (Ind).

Gelation was triggered by addition of sterile deionized water, raising the pH to ~pH 9 with 1M NaOH to ensure full dissolution of the peptide. 0.5M HCl titrated the pH of the peptide solution to near physiological pH (~pH 7.4). Gelation was assessed after 24 hours via the inversion method.

FTIR provided information regarding the secondary structure of the peptides. Hydrogels were prepared as detailed above but with deuterated solvents. Peaks were obtained from an average of 128 scans and wavenumbers of 4000-4000 cm⁻¹.

Hemolytic activity was determined using equine erythrocytes and cytotoxicity against (NCTC929) murine fibroblast cells. Activity against cyclooxygenase -1 and -2 (COX-1 and -2) proved anti-inflammatory activity of the peptide hydrogels.

The antimicrobial properties of each concentration of peptide hydrogel were determined by the ability to reduce viable 24 hour biofilms of both Gram-positive (Staphylococcus aureus ATCC 6538 and Staphylococcus epidermidis ATCC 35984) and Gram-negative bacteria (Escherichia coli NCTC 11303 and Pseudomonas aeruginosa PAO1).

Results and discussion

The ability to self-assemble to form hydrogels is determined by the primary peptide structure. Naproxen and indomethacin conjugated peptides formed self-supporting hydrogels. Ibuprofen conjugates due to insufficient π-π stacking, remained as viscous solutions confirmed via viscosity measurements. Rheological analysis confirmed the hydrogels as viscoelastic with G’ being one order of magnitude higher than G” for all concentrations of peptides. FTIR further confirmed assembly with shoulders in the amide I region (1570 cm⁻¹) and characteristic peaks representative of G’ being one order of magnitude higher than G” for all concentrations of peptides. FTIR further confirmed assembly with shoulders in the amide I region (1570 cm⁻¹) and characteristic peaks representative of secondary structure.

FTIR spectra for the NSAID conjugates at 2% (w/v). G’= storage modulus, G”= loss modulus

The hydrogels proved to be relatively non-hemolytic and non-toxic in vitro. Strong antibiofilm activity was demonstrated by all hydrogels. The optimal reduction in percentage viable biofilm was exhibited by IbuFFKK against Staphylococcus epidermidis. The anti-inflammatory activity of the NSAIDs was not inhibited following coupling to the peptide with hydrogels showing inhibitory activity against both COX-1 and 2 enzymes.

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Conclusion

This investigation has shown that self-assembled NSAID conjugated peptide hydrogels may have potential clinically. Dual activity means that such molecules could act as novel candidates for wound dressings and medical device coatings. Further to this, pH triggered assembly and cytotoxic investigations conducted thus far indicates the potential for targeted activity at the necessary site of action. The next step will be to characterise further the biocompatibility and enzymatic stability of these molecules.

References