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 infective 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.

**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 hydrogel formation. The optimal reduction in percentage viable biofilm was exhibited by IndFFKK against Staphylococcus epidermidis.

The antimicrobial properties of each concentration of peptide hydrogel were determined by the ability to reduce viable 24 h 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 PA01).

**Methods**

Diphenylalnine-dilysine peptides were synthesized on Wang resin following standard Fmoc protocols using a manual Nitrogen bubble 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)
- 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.

The viscoelastic nature of the peptides was characterized by oscillatory rheology. Hydrogels with a storage modulus (G′) at least one order of magnitude greater than the loss modulus (G′′) were classified as being viscoelastic.

Cryo-scanning electron microscopy (cryo-SEM) and transmission electron microscopy (TEM) confirmed the presence of nanofibrous networks. This was further confirmed with evidence of beta stacking in anti-parallel arrangement determined by FTIR.

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 characterize further the biocompatibility and enzymatic stability of these molecules.

**References**