Antimicrobial Activity of Naphthalene Lysine Conjugated Peptide Hydrogels
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BACKGROUND
An important consideration in dental tissue engineering is the suitability of biomaterials to allow regeneration of new tissues. Current interventions for dental pathologies, such as root canal treatments, are based on the use of filling materials which replace the living dental pulp tissue after its removal rather than promote its regeneration. The use of hydrogel scaffolds may provide a more promising approach in regenerative endodontics to allow repair and regeneration which will improve the outcome for the tooth.

One of the issues to be resolved is that the dental pulp tissue is inevitably infected prior to the need for therapeutic intervention. Therefore the use of standard hydrogels for stem cell growth does not treat the underlying infection in the tissue. Ultrashort peptide hydrogels are becoming an interesting class of biomaterials because of their ease of synthesis and their ability to be customised to tailor antimicrobial activity. An ultra-short naphthalene lysine conjugated peptide, NapFFK’, containing naphthalene (Nap) as a molecule of high aromaticity for gel strength, as well as phenylalanine (F) and epsilon variant lysine (K’) has previously been shown by us to self-assemble to form hydrogels that have inherent antimicrobial properties against a limited number of pathogens tested [1].

AIMS & OBJECTIVES
The aim of this work was to extend the antimicrobial activity studies on NapFFK’ to include pathogenic bacteria associated with dental infections; Gram positive aerobic bacteria Staphylococcus aureus, Enterococcus faecalis and gram negative anaerobic bacterium Fusobacterium nucleatum .

METHODS
➢ Fmoc – Solid Phase Peptide Synthesis of naphthalene lysine conjugated peptide variants
NapFFK’, was synthesised from its C- to its N-terminus following standard Fmoc solid phase protocols on Wang resin, as a solid support, using a manual nitrogen bubbler apparatus. Peptide purity was analysed by mass spectrometry prior to hydrogel formulation.

➢ Hydrogel Formulation
NapFFK’ hydrogels were prepared at peptide concentrations of 1%, 1.5% and 2% w/v by a process of pH-triggered induction. Peptide was initially suspended in aliquots of sterile deionized water. Titration with 1M NaOH increased the pH of the solution to pH 9 and resulted in deprotonation of the terminal carboxylic acid moiety and enabled dissolution of the peptide. Aliquots of deionised water were added again and subsequent titration with 0.5M HCl lowered the pH to pH ~7 resulting in protonation of the terminal carboxylic acid and formation of a homogeneous gel. Hydrogels were allowed to develop for 24 hr at 4°C.

➢ Bacterial Susceptibility Assay
The ability of NapFFK’ hydrogels to reduce bacterial viability was tested using a colony counting method. E. faecalis (NCTC 12697) and S. aureus (ATCC 25923), and F. nucleatum (NCTC 10562) were subcultured and left to grow, under appropriate conditions, until their mid-log phase. Aerobic bacterial inoculums were adjusted to an optical density (OD) of 0.3 in PBS at 600 nm which were further diluted to 2x10⁶ CFU/ml in aerobic broth. Anaerobic inoculum was adjusted to an OD of 0.3 at 590 nm and further diluted 1:50, both steps in anaerobic broth. 100 μl of the diluted inoculums were plated on top of 100 μl of prepared hydrogels and incubated for 24 hours at 37°C under appropriate conditions. Supernatants were then serially diluted in PBS and transferred onto Columbia blood agar or anaerobic plates for colony counting using the Miles and Misra method.

RESULTS
➢ Chemical structure of the synthesised NapFFK’ peptide
Increased aromaticity due to Nap molecule confers improved structural integrity and superior gel strength via π-π interactions. Intermolecular interactions provided by the peptide backbone ensure integrity of the formulated hydrogel.

➢ Bacterial Susceptibility Assay
Antimicrobial activity was determined by the number of colonies formed after 24 hour incubation. The hydrogel with 1% NapFFK’ concentration was most effective against both S. aureus and E. faecalis whereas the hydrogels with 2% NapFFK’ concentration was most effective against F. nucleatum.

CONCLUSIONS
➢ Given the efficacy of the self-assembling NapFFK’ peptide hydrogels against oral pathogens, they may have potential use in tissue engineering approaches for regenerative endodontic treatments.

Reference