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MULTIFUNCTIONAL ULTRASHORT PEPTIDE HYDROGELS FOR CHRONIC WOUND HEALING

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INTRODUCTION

Wound healing is a complex and dynamic process consisting of four main steps:

1. Coagulation & Haemostasis
2. Inflammation
3. Proliferation (granulation and contraction)
4. Remodelling

In chronic wounds the wound remains in the proliferation stage with excess inflammation occurring and fails to progress to remodeling [1].

The use of peptides represents a novel approach to enhance wound healing. Inherent antimicrobial activity, increased biocompatibility and tunable biodegradability render peptides more suitable for application to the chronic wound environment than currently administered synthetic materials [2]. Uncontrolled local inflammation and the presence of infectious pathogens result in the development of chronic non-healing wounds, leading to increased patient morbidity and the failure of standard therapies such as antibiotics [3].

The chemical versatility of the peptide motif enables three main qualities to be incorporated into a structure which is capable of self-assembly:

- Antimicrobial activity
- Pro-angiogenic properties
- Anti-inflammatory activity

Self-assembly can occur in response to a number of different infective stimuli including pH, temperature and specific enzymes to enable targeted action at the required site. The incorporation of multifunctional properties overcome limitations with existing topical therapies, which fail to address a profile of increased inflammation and microbial load, with reduced angiogenesis that combine to prevent healing.

AIM

To develop a therapeutically active hydrogel network with the potential to be employed as a dressing, where the hydrogel is applied to the wound and conforms to its shape, whilst providing a moist environment and enabling gas exchange at the wound site.

METHODS

The peptide was synthesized according to standard Fmoc-based solid phase peptide synthesis. The structure was based on a diphenylalanine-dilysine sequence (FFKK), which was previously shown to gelate and display antimicrobial properties [4]. The non-steroidal anti-inflammatory drug naproxen was conjugated to the end of the amine terminal of the peptide sequence to confer the desired anti-inflammatory properties and a heparin motif was incorporated to provide pro-angiogenic qualities.

Peptide gelation ability at various concentrations was examined via pH modulation.

Hydrogels form at concentrations above the minimum gelation concentration (MGC) (% w/v). Vial inversion assays and Scanning Electron Microscopy were employed to examine gelation.

Bacterial susceptibility assays were performed against Gram-negative Pseudomonas aeruginosa (PA01) and Gram-positive Staphylococcus aureus (NCTC 10788) using the Miles and Misra drop count method.

Haemolytic activity was measured using equine erythrocytes and (NCTC 10788) using the Miles and Misra drop count method. Vial inversion assays demonstrated the formation of peptide hydrogel at a concentration of 1.5 % w/v and above and the fibrous network was confirmed via SEM imaging with Figure 1 below showing the dense fibrous network at the highest concentration employed.

RESULTS AND DISCUSSION

The self-assembly process is dependent on the amino acids incorporated in the primary sequence of the peptide structure and the formation of intermolecular interactions including Van der Waals interactions and π – π stacking. Vial inversion assays demonstrated the formation of peptide hydrogel at a concentration of 1.5 % w/v and above and the fibrous network was confirmed via SEM imaging with Figure 1 below showing the dense fibrous network at the highest concentration employed.

Clinically significant Log10 reductions in the growth of Gram-negative P. aeruginosa and Gram-positive S. aureus was observed at higher concentrations employed, with significance denoted as at least a three Log10 reduction in viable counts since this is commonly employed to denote clinical significance [5]. P. aeruginosa and S. aureus are the most common ESKAPE pathogens isolated from chronic wounds and demonstrate increased resistance to topical antibiotics [6]. There is no necessity for a sterile environment for wound healing, however, a reduction in the bioburden can promote wound healing in the chronic case of wounds and this peptide demonstrates at least a three Log reduction against each organism at a concentration of 1.5 and 2.0 % w/v.

The MTS assay was performed to assess the viability of cells after incubation with varying concentrations of the peptide as an initial assessment of biocompatibility. The micro-molar concentrations employed had no significant effect on the cell viability whilst the higher concentrations exposed to since the peptide will slowly diffuse from the gel matrix and into the surrounding environment to exert its effect. Further studies will be necessary to assess the biocompatibility of the peptide.

The peptide was shown to be relatively non-haemolytic at the lower concentrations employed, with the haemolysis observed at higher concentrations likely to be attributed to issues with tonicity or osmolarity due to osmotic variations in the test media resulting in over-estimation of the toxicity.

CONCLUSIONS

Multifunctional ultrashort peptide hydrogels demonstrate potential as wound healing products. Peptide hydrogel dressing products may resolve issues in the case of chronic wounds which fail to heal, for example, diabetic ulcers. Future work will involve assessment of the mechanical properties of the gel structure via oscillatory rheology and wound healing properties using the in vitro wound scratch assay to assess cell migration using the human dermal endothelial cell line HMEC-1 and the human keratinocyte cell line HaCat [7]. In addition to this it will be possible to modify the peptide sequence to tailor the properties to the exact desired requirements via modification of the primary sequence and incorporation of other non-steroidal anti-inflammatory drugs.

REFERENCES