

Design, Synthesis and Biological Evaluation of Cell Adhesive Peptide for Angiogenesis in Wound Healing

Abstract of Thesis

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Abstract

The thesis entitled “**Design, Synthesis and Biological Evaluation of Cell Adhesive Peptide for Angiogenesis in Wound Healing**” consists of four chapters. The objective of the thesis have been subdivided as

- Synthesize cell adhesive peptide sequences optimized for enhanced adhesion and proliferation of skin cells.
- Investigate the biocompatibility and safety profile of synthesized peptide for topical application.
- Assess the efficacy of synthesized peptides in promoting wound healing through *in vitro* and *in vivo* study.
- Comparison of the different peptides for topical application.

Wound healing is a complex physiological process involving the coordinated interaction of various cell types, growth factors, and extracellular matrix (ECM) components. Angiogenesis, the formation of new blood vessels, plays a critical role in this process by ensuring an adequate blood supply to the wound site. Peptides derived from ECM proteins are promising agents for promoting angiogenesis and enhancing wound healing. In this thesis, cell adhesive peptides derived from ECM proteins were designed and synthesized to investigate their potential for promoting angiogenesis in wound healing. The peptides were characterized for their physicochemical properties and biological activities.

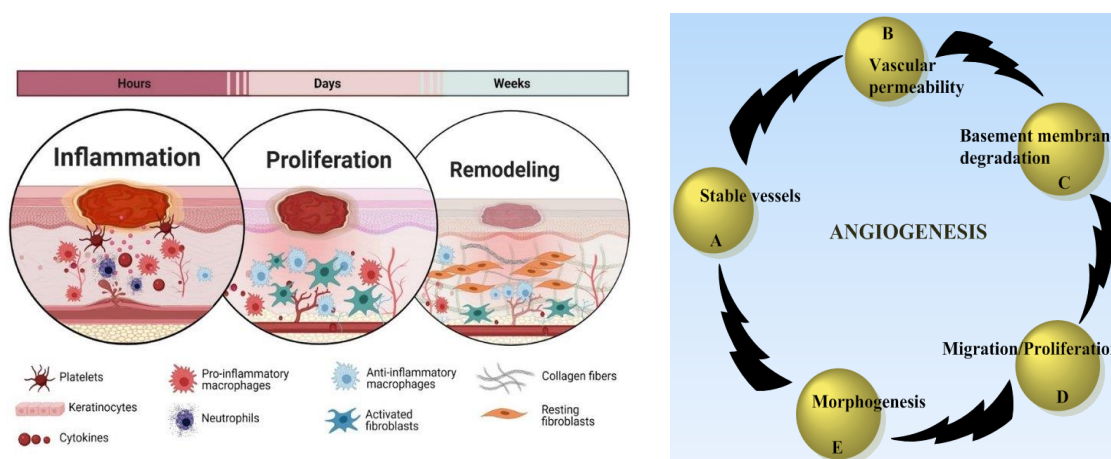


Fig. 1: Process of Wound healing

Chapter 1: A Brief Overview of Wound, Peptides, Synthesis, and their role in Wound Healing

Chapter 1 provides a comprehensive overview of wounds and their broad classification. It explores the different phases of wound healing in acute and chronic wounds, highlighting the importance of angiogenesis in the process. The chapter also delves into the structure and function of peptides, emphasizing their diverse roles as signaling molecules and wound healing agents. Furthermore, it discusses various methods of peptide synthesis, focusing on solid-phase peptide synthesis (SPPS) as a key technique.

This chapter serves as a foundation for the subsequent chapters by elucidating the fundamental importance and significance of peptides. It details the precise mechanism of solid-phase peptide synthesis, including the selection of appropriate coupling and deprotecting agents. Furthermore, the chapter elaborates on key characterization techniques essential for analyzing synthesized peptides. Lastly, explores the practical application of peptides for topical use in wound healing.

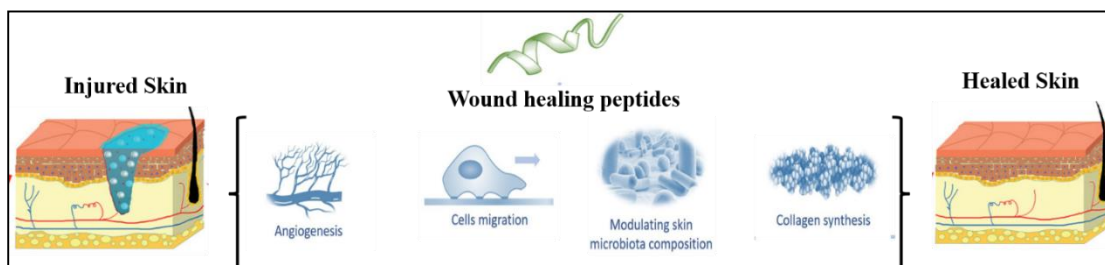


Fig. 2: Role of Peptide in wound healing

Chapter 2: Laminin-derived Peptides for Enhancing Dermal Wound Healing

This chapter of the thesis focuses about to test the efficacy of peptide loaded CMC hydrogels in improving wound healing in normal and diabetic excision wound models. The laminin α_1 and γ_1 chains derived

- (a) Native peptide IKVAV (Ile-Lys-Val-Ala-Val)
- (b) Modified angiogenic peptides [DYVRLAI (Asp-Tyr-Val-Arg-Leu-Ala-Ile) and CDYVRLAI (Cys-Asp-Tyr-Val-Arg-Leu-Ala-Ile)]
- (c) Angiogenic collagen peptides [PGPIKVAV (Pro-Gly-Pro-Ile-Lys-Val-Ala-Val) and Ac-PGPIKVAV (Ac-Pro-Gly-Pro-Ile-Lys-Val-Ala-Val)],

A total of five peptides were designed using molecular peptide-receptor docking analysis. The analysis aimed to elucidate the binding affinity, and identify amino acids involved in interactions with the respective receptor. This interaction among amino acids and receptors stabilized the conformational complex formed by the peptide and the target protein. The peptides were then synthesized, purified, characterized, and tested for *in vitro* biocompatibility studies. Moreover, peptide-loaded CMC hydrogels were successfully developed *via* cross-linking method, and their comprehensive physiochemical characterization was performed. The wound healing efficacy of the peptide loaded hydrogels was evaluated by *in vivo* studies, including wound area measurement and histopathological analysis. The peptide hydrogel showed complete epithelial regeneration with no inflammatory response, significant collagen deposition, and higher angiogenesis as compared to commercially available becaplermin (standard) gel. However, the angiogenic peptides [DYVRLAI (Asp-Tyr-Val-Arg-Leu-Ala-Ile) and CDYVRLAI (Cys-Asp-Tyr-Val-Arg-Leu-Ala-Ile)] had shown excellent angiogenesis, re-epithelization, whereas collagen peptides PGPIKVAV (Pro-Gly-Pro-Ile-Lys-Val-Ala-Val) exhibits the highest collagen deposition.

Therefore, the development of laminin peptide hydrogels emerges as a novel and potential approach for enhancing skin healing. These hydrogels efficiently promote epithelialization and collagen synthesis, providing crucial structural support to tissues. Moreover, they stimulate microvessel formation, facilitating nutrient delivery. This novel peptide hydrogel treatment holds significant promise for future clinical applications, offering enhanced wound healing effects.

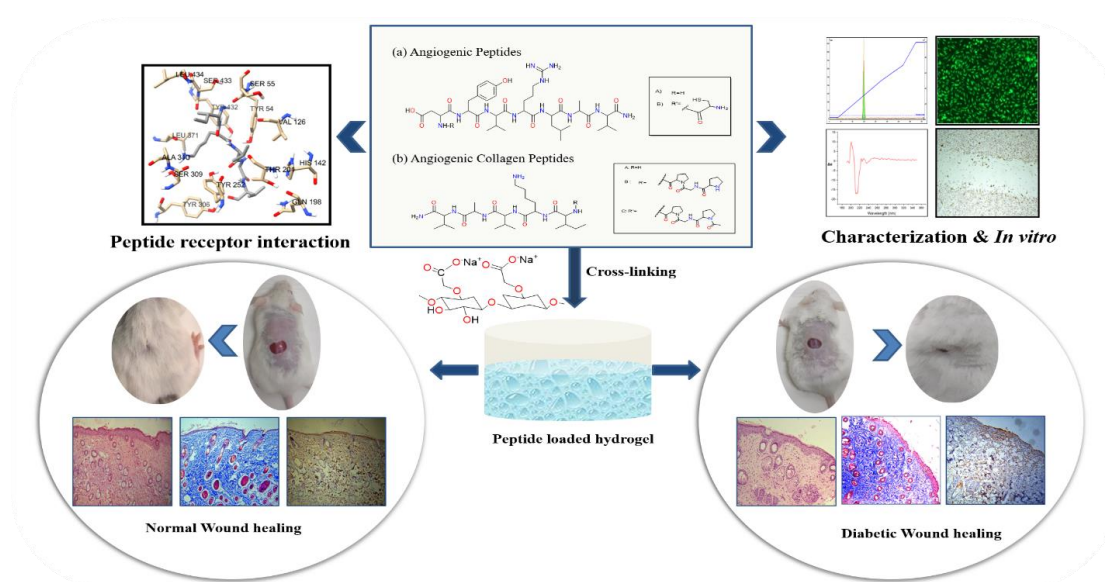


Fig. 3: Outline of work

Chapter 3: Peptide hydrogels (DO3A-PGPIKVAV, N₂S₂-PGPIKVAV) in non-invasive imaging of full-thickness excision wound

This chapter of the thesis focuses on the importance of hydrogel adherence in promoting wound healing. Hydrogels need to maintain crucial properties at the wound site, such as moisture retention, infection prevention, exudate absorption, and delivery of bioactive components. Non-invasive imaging techniques, particularly SPECT imaging, was used to assess the retention of hydrogels at the wound site over time. The study aimed to develop a SPECT imaging probe using a modified angiogenic collagen peptide [PGPIKVAV (Pro-Gly-Pro-Ile-Lys-Val-Ala-Val)] conjugated with acyclic (N₂S₂) and cyclic (DO3A) chelators for radiolabeling with ^{99m}Tc. The peptide conjugate showed radiolabelling efficiency >95%. SPECT imaging and biodistribution analysis confirmed significant retention of the peptide hydrogel at the wound site for up to 24 hours, indicating its potential for sustained peptide release and improved wound healing outcomes.

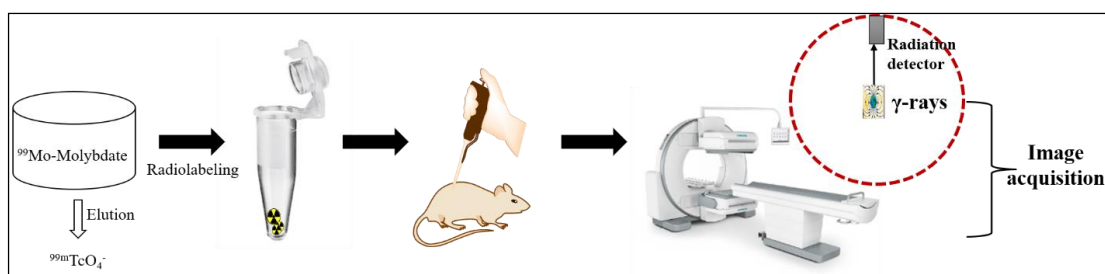


Fig. 4: SPECT imaging

Chapter 4: Design, and development of a biocompatible self-assembling peptide and its silver and copper nanocomposite for antibacterial activity in wound healing

This chapter of the thesis focus on to develop a self-assembling peptide nanocomposite that can accelerate healing with the help of bioactive peptides while protecting against bacterial infections, ultimately improving the wound-healing process. A diphenylalanine (FF) modified, self-assembling peptide [(NapFFRGDC) (Nap-Phe-Phe-Arg-Gly-Asp-Cys)] was designed based on diphenylalanin's inherent ability to self-assemble into nanostructure. The sequence of self-assembling peptides (Nap-FFRGDC) exhibited two distinct moieties: one hydrophobic and the other hydrophilic. The hydrophobic moiety [NAP (naphthalene)] facilitate the self-assembly process that leads to the formation of nanofibers. The hydrophilic moiety displays many carboxylic acid and thiol groups, which can serve as metal ion binding sites for the nanocomposites

formation. The RGD sequence was incorporated to facilitate cell adhesion and migration, thereby promoting angiogenesis. The enhanced angiogenesis led to improved blood flow to the wound site, providing essential nutrients and growth factors for tissue regeneration. A cysteine residue was added to the N-terminus of the peptide sequence to enable the attachment of silver (Ag) and copper (Cu) nanoparticles, which are known for their antimicrobial properties and reduce the risk of infection at the wound site. The resultant Ag-NapFFRGDC and Cu-NapFFRGDC nanocomposites, of the NapFFRGDC peptide, were developed, and tested as potential antibacterial agents in wound healing applications. The peptide and its nanocomposites showed significant swelling capacity, sustained release behavior, low cytotoxicity, and biocompatibility. The NapFFRGDC peptide and its nanocomposites, Ag-NapFFRGDC and Cu-NapFFRGDC, demonstrated superior healing properties compared to standard betadine in a full-thickness excision wound model. Histological analyses showed increased epithelialization, presence of hair follicles and sebaceous glands, and better collagen deposition, indicating improved tissue regeneration. Additionally, the peptide and its nanocomposites enhanced angiogenesis by promoting microvessel formation. These results underscore the potential of the NapFFRGDC peptide and its nanocomposites as promising agents for wound healing, offering a multifaceted approach in restoring skin integrity, minimize infection and promoting tissue repair.

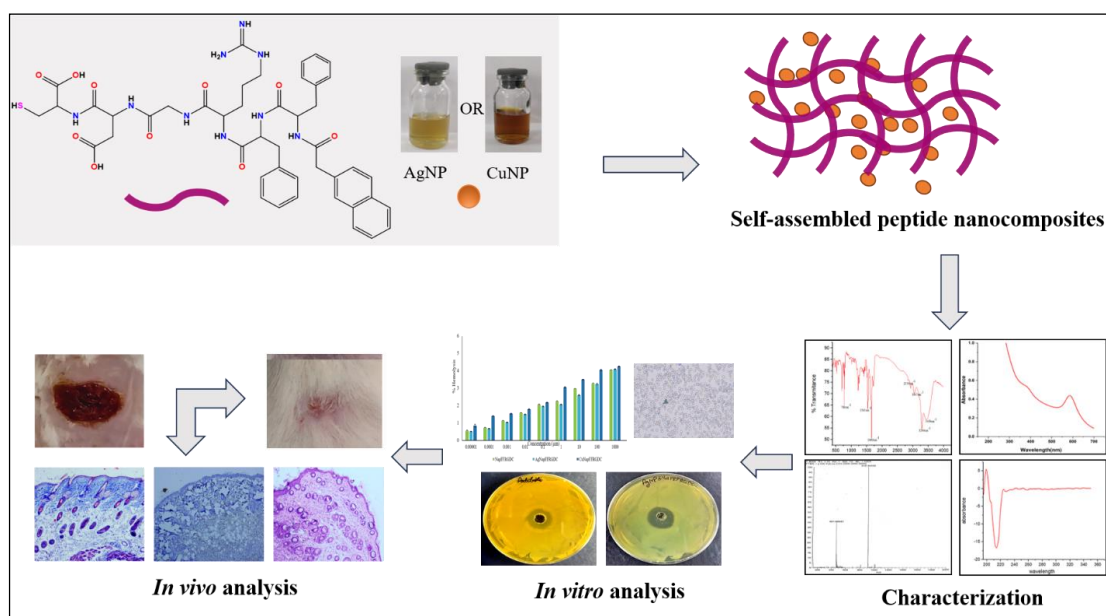


Fig. 5: Outline of work

Conclusion:

A total of eight extracellular matrix (ECM) derived peptides were designed and evaluated for their potential application in wound healing. These peptides were synthesized using solid-phase peptide synthesis (SPPS) *via* the Fmoc strategy, with HOBt and DIC as coupling agents. The peptides demonstrated good biocompatibility in *in vitro* studies and showed remarkable results in *in vivo* studies using full-thickness excision wounds. The peptides exhibited significant effects on angiogenesis, collagen synthesis, and providing structural support to the tissue. Further research into their mechanisms of action, and clinical trials could make provision for their translation into effective treatments, offering new avenues for enhanced wound care.