Development, characterization, drug loading and drug release properties of silk fibroin hydrogels for biomedical applications

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SUMMARY

Silk fibroin (SF), a structural protein has been used to make a variety of products, including scaffolds, biofilms, hydrogels, and many others. These products are crucial for biomedical applications. The advantages of hydrogels made from silk fibroin, such as their excellent biocompatibility, capacity for tissue regeneration, low immunogenicity, high permeability to oxygen and water, high stability, and capacities for sustained release, have attracted a great deal of research interest. The beta-sheet branching structure of the SF hydrogel, along with its polymorphic domains' variable pore sizes, high permeability, and favourable swelling behaviour, make it ideal for drug loading and administration with sustained drug release. Additionally, these hydrogels have excellent mechanical properties that allow them to maintain their structure and integrity even under stress. These characteristics, which are a result of their similarity with extracellular matrix and skin cells, aid in dissipating excess wound fluid and preserving wound moisture, which promotes the healing of wounds. Additionally, these dressings have been shown to reduce pain and inflammation associated with diabetic wounds, improving patient comfort during the healing process. Further, Vancomycin (VANCO) is a glycopeptide and well-known antibiotic that is the precursor of *Streptomyces orientalis*, sold as an injection and is a treatment option for severe pathogenic bacteria such as those that cause diabetic ulcers, enteritis, wound septicemia, etc. Additionally, VANCO is now commonly used in clinical research as a model drug to prevent the synthesis of bacterial cell walls by binding to the L-lysyl-D-alanyl-D-alanine C-terminal peptide sequence of the mucopeptide precursor molecules. It might also work against Methicillin-resistant *Staphylococcus aureus* (MRSA), which is the most typical cause of septicemia. Therefore in the

current study, a combination of both VANCO-loaded silk fibroin hydrogel composite has been used where an antimicrobial property of VANCO and tissue regeneration capacity of SF hydrogel have been exploited systematically to treat diabetic wounds using an animal model. The research work was organized into a thesis that included a general introduction, literature review, materials and methods section, results and discussion section, and a comprehensive summary. Overall references are listed last to avoid repetition part of the thesis. The general introduction serves as an overview of the research work, highlighting its significance and objectives. The review of literature, on the other hand, provides a comprehensive analysis of previous studies related to the research topic. This section is crucial in identifying gaps in knowledge and determining the relevance of the study. The materials and methods section details the procedures and techniques used in conducting the research. It should be written in a clear and concise manner to ensure reproducibility. The results and discussion section presents the findings of the study and their interpretation. It should be structured logically, with tables and figures used to support the data presented. Finally, a consolidated summary is provided to summarize the key findings of the study, highlighting its contributions to existing knowledge. Overall references are arranged at the end of the thesis to avoid redundancy and repetition throughout the text.

The first section of the materials and methods covered the preparation of the silk fibroin solution, followed by the formation of the SF hydrogel and VANCO-loaded SF hydrogel composite. To evaluate for conjugation and compatibility, both compounds were first characterised individually and in composite form using SEM (scanning electron microscope), foldscope, pH calibration, viscosity, and 1H-NMR spectroscopy. The conjugation of VANCO and SF hydrogel was successful because

the drug did not change the SF hydrogel's uniform porosity structure, according to SEM and foldscope analysis. This finding is significant as it demonstrates the potential for using VANCO-loaded SF hydrogel as a drug delivery system for treating bacterial infections. In addition, the foldscope analysis allowed for a closer examination of the microstructure of the hydrogel and confirmed that the VANCO was evenly distributed throughout. The use of SF hydrogel as a carrier for VANCO is advantageous due to its biocompatibility and ability to retain water, which allows for sustained release of the drug over time. Further, when the drug was loaded into the silk fibroin hydrogel, the pH analysis showed a slight rise in pH, which may have been brought on by an increase in the OH- ions, which are primarily found in vancomycin. The viscosity play a key role in how easily active molecules can be applied to the affected area. With the aim that, viscosity test was used to confirm the formulation of the SF hydrogel and composites. The results showed that SF hydrogel and composite are suitable for topical applications in wound healing. This is important because it ensures that the active molecules are delivered precisely to where they are needed, without any wastage or spillage. Moreover, the viscosity of the SF hydrogel and composites also influences their ability to adhere to the skin surface, which further enhances their efficacy. In addition, the viscosity of these formulations can be adjusted by altering their composition or processing conditions, which provides flexibility in designing products for specific applications. Overall, the viscosity of SF hydrogel and composites is a critical parameter that impacts their performance as topical formulations for wound healing and drug delivery. Our 1H-NMR spectroscopy study clearly illustrates that the absence of sharp vancomycin NMR signals in the presence of SF hydrogel strongly suggests that the drug is mostly bound to the SF hydrogel's beta-sheet network in the form of encapsulation, giving

the impression that there is no free drug in solution, which is a necessary condition for sustained drug release. The encapsulation of vancomycin in SF hydrogel not only ensures that the drug remains in the targeted area for a longer period of time, but also reduces the potential for toxicity and side effects associated with high doses of free drugs. Additionally, the beta-sheet network of SF hydrogel provides a stable environment for the drug, protecting it from degradation and increasing its shelf life. Further *in- vitro* research will look at drug release properties using UV spectroscopy. The prolonged drug release property was indicated by increasing the OD from 0.22125 to 2.6. This suggests that the drug is slowly being released from the SF hydrogel, which could be beneficial for sustained drug delivery. In addition, a rat model was used to conduct an *in-vivo* study on VANCO-loaded SF hydrogel composite, SF hydrogel, and Povidone (antiseptic cream from the market). In the *in-vivo* investigation, the combination of SF hydrogel and VANCO was found to be the most efficient antiseptic gel when compared to SF hydrogel alone and povidone, with wound healing percentages of 86%, 33.3%, and 16.6%, respectively. Silk fibroin hydrogel has self-healing properties and previous studies demonstrated that silk fibroin hydrogel help in wound enclosure by enhancing the rate of wound healing, fibroblasts and keratinocytes migration and proliferation, dermis regeneration, synthesis of collagen and differentiation of epidermal into hair follicles and sebaceous glands. Whereas, vancomycin is a glycopeptide antibiotic that works against resistant bacteria by impeding cell wall synthesis. Due to the synergistic effects of SF hydrogel and vancomycin, composites appear to be more effective in aiding wound healing. Povidone, on the other hand, has antibacterial properties but no self-healing capacity, making it less effective in treating diabetic full-thickness wounds. The current results showed that our composite is more effective than any antiseptic cream on the market

in treating diabetic wounds. Finally, we have done the serum analysis to check the metabolic changes in each group, and we found that some metabolites such as lactate, serine, proline, pyruvate, taurine, succinate, methionine, phenylalanine, guanidoacetate, and creatinine phosphate were notably greater in the serum of the VANCO-loaded SF hydrogel group when compared to the other experimental groups (SF treated, Povidone treated), and control groups (diabetic and non-diabetic). In support of our findings, earlier research has shown that all of the metabolites indicated above aid in wound healing via diverse mechanisms, such as lactate, which stimulates angiogenesis and speeds up wound healing, and a powerful systemic epithelial wound healing response is provided by serine protease activation of epidermal repairing genes in order to maintain the integrity of the epithelial barrier without causing cell death. Similarly, proline and hydroxyproline are key amino acids found in collagen, an essential connective tissue protein required for wound healing, and pyruvate aids in glycolysis regulation and causes the Warburg effect, both of which aid in wound healing. Furthermore, locally administered taurine decreased MDA and histamine levels to enhance wound tensile strength and prevent mast cell degranulation, which is beneficial to wound healing and chitosan hydrogel sheets with succinate crosslinking as potential wound dressing by accelerating tissue regeneration. Furthermore, protein deficiency reduces wound tensile strength while l-phenylalanine, fumarate, and guanidoacetate accelerate wound healing. Our findings indicate that our composite has a greater ability for wound contraction by raising all of the metabolites that participate in wound healing, which is due to the synergistic effect of SF hydrogel and VANCO.