GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Evaluation of cutaneous wound healing in mice models using non-mulberry silk fibroin derived from *Kunugia latipennis* and *Vespa affinis*

Menguzeno Nakhro [1,*], Ridashisha Rymbai [2], Dibyendu Paul [1], Shiva Shankar Chaturvedi [1] and Elareen Belljoy Donshiew [2]

[1] *Department of Environmental Studies, North-Eastern Hill University, Shillong, Meghalaya, India-793022.*
[2] *Department of Biochemistry, North-Eastern Hill University, Shillong, Meghalaya, India-793022.*

GSC Biological and Pharmaceutical Sciences, 2025, 31(03), 001–010

Publication history: Received on 06 April 2025; revised on 27 May 2025; accepted on 30 May 2025

Article DOI: https://doi.org/10.30574/gscbps.2025.31.3.0199

Abstract

Skin wounds present a persistent clinical concern, often requiring effective yet affordable treatments to ensure proper healing. Silk fibroin (SF), a natural biopolymer recognized for its excellent biocompatibility and mechanical integrity, has gained attention for wound healing applications. While *Bombyx mori* silk fibroin (BMSF) has been widely studied, there's limited research on the potential of non-mulberry silk fibroins derived from *Vespa affinis* and *Kunugia latipennis*. This study investigates the potential of *Vespa affinis* silk fibroin (VASF) and *Kunugia latipennis* silk fibroin (KLSF) films as bioactive wound dressings using a full-thickness excisional wound model in mice. The *in vivo* results showed that both silk films significantly enhanced wound healing compared to the untreated control. By day 7, early granulation tissue had formed; by day 14, re-epithelialization was initiated; and by day 21, advanced dermal remodeling was evident. Histological analysis confirmed increased vascularization and well-organized epidermal and dermal layers. VASF in particular showed superior outcomes in promoting re-epithelialization and minimizing fibrosis. Both VASF and KLSF were found to be safe, biocompatible, and effective in supporting skin regeneration. Their ability to improve healing rates, enhance epidermal differentiation—including the development of hair follicles and sebaceous glands—highlights their potential as clinical wound dressings.

Keywords: *Vespa affinis*; *Kunugia latipennis*; Silk; Fibroin; Wound healing; Biomaterial

1. Introduction

Wound healing is a complex biological process that unfolds through four sequential stages: hemostasis, inflammation, cell proliferation, and tissue remodeling. Disruptions at any stage may result in delayed recovery, chronic wounds, or fibrosis, making effective intervention crucial. The use of bioactive wound dressings is vital, as they not only enhance the healing process but also regulate extracellular matrix (ECM) deposition to minimize scarring risks. For optimal recovery and improved aesthetic outcomes, wound dressings that support all four phases are essential [1, 2]. The skin, the body's biggest organ, serves as a principal barrier against environmental hazards and detrimental substances. Any breach of this barrier may render the body susceptible to infection and other consequences. Consequently, efficient wound dressings are essential for establishing an optimal healing environment [3]. The optimal dressing must retain moisture, manage exudates, facilitate gas exchange, and safeguard against microorganisms. Conventional dressings such as lint, cotton wool, and gauze can inflict tissue damage upon removal and are frequently associated with inadequate recovery. As a result, bioactive and biodegradable dressings that enhance ECM deposition and facilitate expedited recovery are becoming increasingly favored [4].

Biomaterials such as polymers, metals, and ceramics have been employed in tissue engineering, with natural polymers offering notable advantages due to their biocompatibility and reduced immunogenicity. Among these, structural

* Corresponding author: Menguzeno Nakhro

proteins like collagen, elastin, albumin, and fibrin are widely used in scaffolds, sutures, and drug delivery systems [5, 3]. Silk fibroin (SF), a natural biopolymer derived from silkworms, is composed of fibroin (80%) and sericin (20%). SF's structural integrity, characterized by its highly oriented crystalline structure, contributes to its mechanical strength and bio-resorbable properties, making it suitable for biomedical applications [6]. Silks are naturally produced by various species, including spiders, fleas, mites, and scorpions. These silks have been adapted for medical applications due to their desirable morphological characteristics and biocompatibility [7]. SF has been approved for medical use by the U.S. Food and Drug Administration (FDA) [8]. In addition to its FDA approval, SF's remarkable mechanical strength, elasticity, and suitability for use in scaffolds, sutures, and drug delivery have been well documented [5]. Recent developments have expanded SF's application to various formats such as films, sponges, and hydrogels, which have shown promise in accelerating wound healing [9].

Recognized for its biocompatibility, biodegradability, low immunogenicity, versatility, cost-effectiveness, ease of processing, and tensile strength, Silk fibroin (SF) extracted from the mulberry silkworm *Bombyx mori* has been extensively investigated for its role in wound healing applications and has become an established biomaterial in biomedical research. Despite this, studies exploring the potential of silk fibroin from non-mulberry silk varieties are limited [1]. To explore this potential, the present study developed non-mulberry SF films as prospective wound dressings derived from SF proteins extracted from *Kunugia latipennis* (Pine lappet moth) and *Vespa affinis* (Lesser banded hornet).

2. Material and methods

2.1. Samples and Chemicals

Cocoons of the Pine lappet moth*, Kunugia latipennis,* were collected from NEHU campus, Shillong, Meghalaya, and silk paper-nest cocoons of the Lesser banded hornet silk, *Vespa affinis*, were purchased from local markets in Nagaland. All chemicals and reagents used in this study were of analytical grade, sourced from Sigma-Aldrich Co. (St. Louis, USA), Bio-Rad Laboratories Inc. (Germany), Himedia, Thermo Scientific India Pvt. Ltd., and Sisco Research Laboratories (SRL, India).

2.2. Extraction and Purification of *K. latipennis* silk fibroin

5 gms of Silk cocoons were weighed, cut into small pieces, and treated with boiling aqueous solution of 0.02 M sodium carbonate for 60 minutes. The whole mass was repeatedly washed with distilled water to remove the glue-like sericin protein and dried in a hot air oven at 30 °C overnight. The extracted silk fibres were dissolved in 9.3 mol/L LiBr solution at 80 °C for 12 h. The dissolved solutions were dialyzed against deionized water for 3 days with a 12 kDa molecular weight cut-off dialysis tube to remove the salt. The resulting solutions were centrifuged at 5000 rpm for 10 minutes to remove aggregates and debris [10].

2.3. Extraction and Purification of *V. affinis* silk fibroin

5 gms of silk cocoons were weighed, cut into small pieces, and treated with a boiling aqueous solution of 0.02 M sodium carbonate for 15 minutes. The whole mass was repeatedly washed with distilled water to remove impurities and dried in a hot air oven at 30 °C overnight. The extracted silk fibres were dissolved in 9.3 mol/L LiBr solution at 60 °C for 6 h. The dissolved solutions were dialyzed against deionized water for 3 days with a 12 kDa molecular weight cut-off dialysis tube to remove the salt. The resulting solutions were centrifuged at 5000 rpm for 10 minutes to remove aggregates and debris [10, 11].

2.4. Preparation of Regenerated Silk Fibroin (RSF) films

The RSF solution was fabricated into films by casting 1 ml of SF solutions into petri plates to dry for 24 h in a fume hood. After drying, the SF films were immersed in 75% ethanol to induce β-sheet formation [10, 12].

2.5. Measurement of Protein Concentration

The fibroin protein concentration was measured by the Bradford protein assay [13]. The fibroin solution was added to the Bradford reagent and incubated at 30 °C for 5 minutes, and the absorbance at 595 nm was measured. The Bradford assay relies on the binding of the dye Coomassie Blue G-250 to protein. Thus, the quantity of protein can be estimated by determining the amount of dye in the blue ionic form, usually achieved by measuring the absorbance of the solution at 595 nm. Bovine serum albumin was used as a standard protein.

2.6. Scanning Electron Microscopy

The morphology of degummed silk fibres was determined by SEM at 500x magnification.

2.7. Experimental Animals

The study utilized *Swiss albino* mice of the Balb/C strain, with individual weights ranging from 20 to 30 gms. These mice were maintained in a controlled environment, with the room temperature kept at 22 °C and a light/dark cycle of 12 h each. They had access to water *ad libitum* and were fed a standard mice diet, sourced from the laboratory of Amrut in Pune, India. All experimental procedures adhered to the guidelines established by the Institutional Ethics Committee (IEC) of North-Eastern Hill University, Shillong, Meghalaya (Approval date: 28.07.2021, No. IEC/MS/Misc./08)

2.8. Incision and Implantation of RSF films on animal models

The mice were distributed among three experimental groups consisting of 5 male mice at three time points: 7 days, 14 days, and 21 days. Anaesthesia was used for all the animals before and during the experimental interventions. The dorsal fur of the mice was shaved before the infliction of the experimental wounds. The surgical interventions were carried out using ketamine hydrochloride (75 mg/kg) and xylazine hydrochloride (8 mg/kg) intraperitoneally [14]. Full-thickness wounds, 1 × 1 cm in length and as deep as the panniculus carnosus layer, were excised from each side of the back of the mice. These surgical interventions were carried out under sterile conditions. The wounds covered with surgical gauze bandage were termed as the control group, while the other wound sites covered with silk biomaterial films were termed the treated group. The wound region of the treated group was compared to that of the control group. Secondary gauzes were applied to the primary dressings to protect against outer damage. The progress of wound healing was evaluated by periodic monitoring of the mice. Digital photographs were taken on days 7, 14, and 21 to evaluate wound closure rate [15, 16]. The rate of wound healing was calculated by measuring the size of wounds through analysis of the photographs using ImageJ software. The following formula was used.

$$\text{Wound closure (\%)} = A_t/A \times 100\%$$

where A_t was the area of the wound at time (t) and A was the initial wound area. Five animal models were taken for each group, and the average value was plotted.

2.9. Histopathological Examination

Tissues from the wound bed were excised on post-wounding days 7, 14, and 21 for histological examination. Briefly, tissue samples were preserved in 10% formalin, dehydrated with a series of graded ethanol, and embedded in paraffin to get sections of 5 μm thickness. Slides containing sections were then stained with haematoxylin and eosin (H & E) for general morphological observations.

2.10. Statistical Analysis.

All the *in vivo* experiments were performed for n=5 animals at each time point. Quantitative data were expressed as mean ± standard deviation as calculated by Microsoft Excel. Further data analysis was carried out using statistical software Origin 9.0 (Origin Lab Corporation, USA). The data between groups were compared at a significance level by performing one-way analysis of variance (ANOVA) followed by Tukey's test. Histological examination was performed by capturing at least 10 fields per section, and images were analyzed.

3. Results

3.1. Scanning Electron Microscopy of degummed silk fibres

Figure 1 presents SEM images of silk fibroin fibres captured at a 50 μm scale, both before and following the extraction process. In image (A), the presence of a sericin coating on the untreated silk fibre is distinctly visible. Post-extraction with 0.02 M Na_2CO_3, the sericin layer on the KL silk fibre was effectively removed, leading to the separation of individual silk filaments, as shown in image (B). Similarly, surface impurities initially observed on the VA silk fibre in image (C) were eliminated after extraction, as seen in image (D). Additionally, optimal dissolution conditions for degummed silk fibres were found to be 80 °C, with complete dissolution occurring in LiBr aqueous solution after 12 h for KLSF and 60 °C at 6 h for VASF.

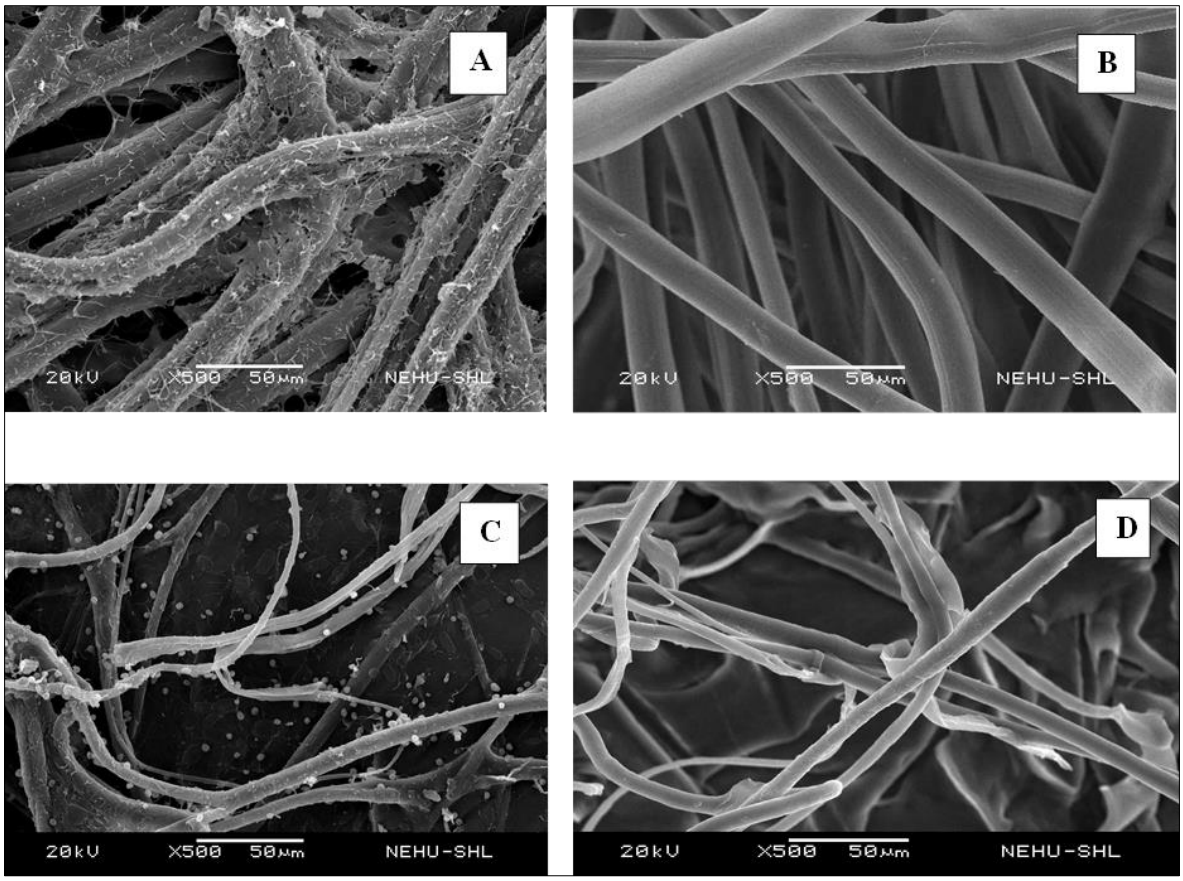

Figure 1 Scanning electron micrographs of KL and VA silk fibres at 500x. (A) Before the extraction of KL silk fibre. (B) After the extraction of KL silk fibre. (C) Before extraction of VA silk fibre and (D) After extraction of VA silk fibre

3.2. Protein Estimation

The protein concentration in KLSF and VASF was calculated using a standard curve of BSA with equation of y = 0.0353x + 0.1995. A direct correlation between the concentration of silk fibroin and protein content, with protein levels rising proportionally as the silk fibroin concentration increases, is demonstrated in Figures 2A and 2B.

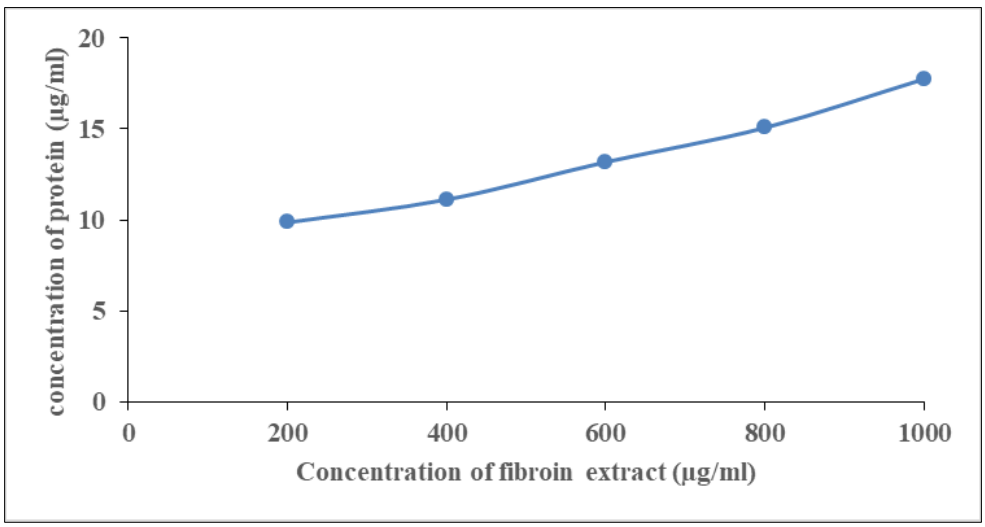

Figure 2A Protein estimation of *K. latipennis* silk fibroin

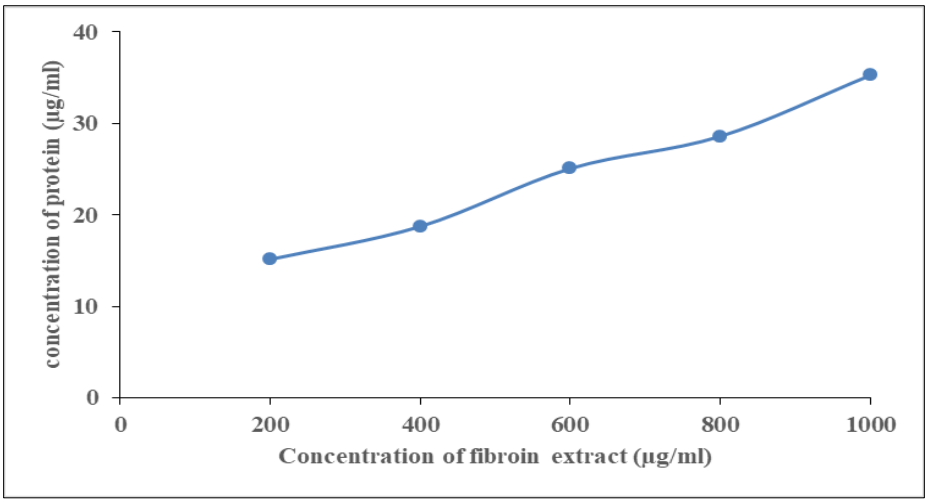

Figure 2B Protein estimation of *V. affinis* silk fibroin

3.3. Assessment of Wound Contraction

The healing progression of full-thickness dorsal skin wounds treated with different dressings was evaluated by analyzing gross wound images. KLSF and VASF films were applied as primary dressings over the wounds. As depicted in Figure 3A, the wounds exhibited varied healing responses depending on the treatment type. Notably, wounds treated with VASF films displayed a markedly accelerated healing rate compared to both KLSF-treated wounds and the untreated control group. Quantitative analysis of wound area further supported these findings, with significant differences observed in wound size between the silk fibroin-treated groups and the untreated control group (Figure 3B). By day 21, wound area measurements revealed distinct outcomes: KLSF-treated wounds and the untreated control group showed 9.87 ± 1.3 % and 31.9 ± 1.84% remaining wound area, respectively. In contrast, VASF-treated wounds exhibited a higher reduction, with only 0.7 ± 0.34% of the wound area remaining, outperforming the other treatment group. Both silk-treated groups appeared to initiate wound contraction from Day 7, potentially due to the intrinsic wound-healing properties of silk biomaterials, which may contribute to early contraction. These findings collectively highlight the positive role of silk fibroin films in enhancing the wound healing process.

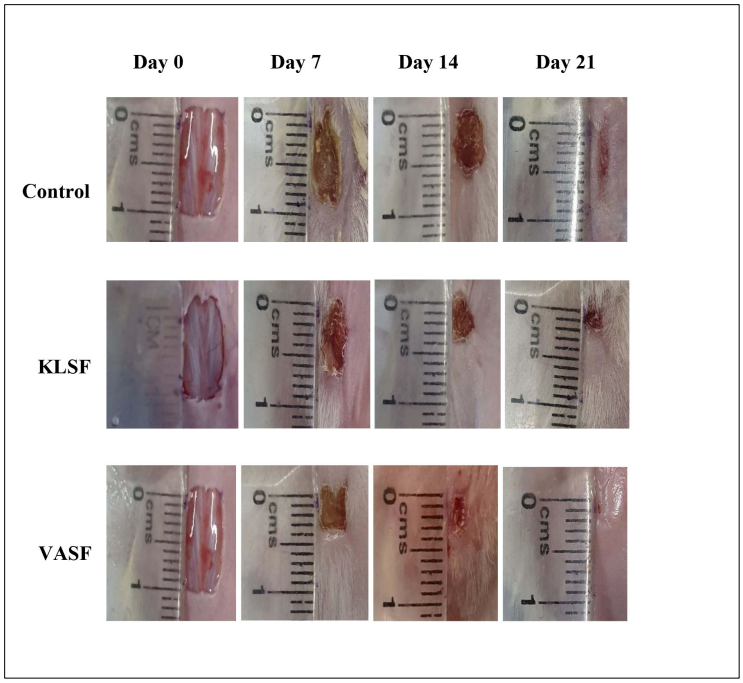

Figure 3A Gross images of wounds on mice showing extent of wound healing on day 7, 14 and 21

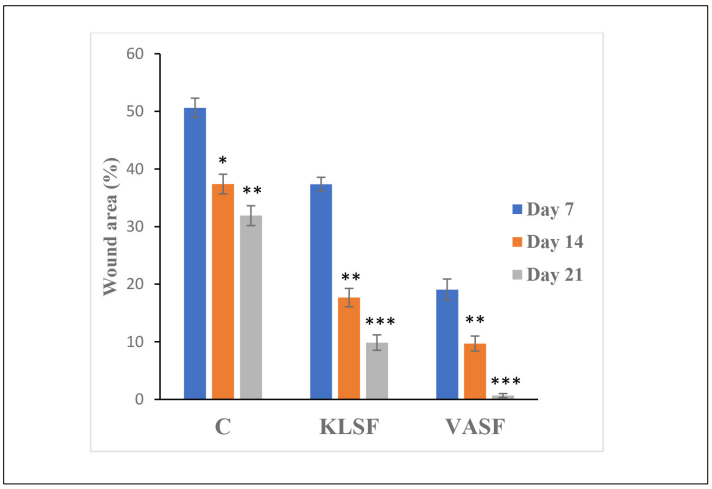

Figure 3B Changes in wound size with KLSF and VASF treatment compared to the control group over a period of 21 days. Wound area percentage determined by calculating the area of wounds at particular time point using ImageJ software. *,** and *** indicates the significance level at $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ respectively against untreated control

3.4. Histological Examination of Wounds

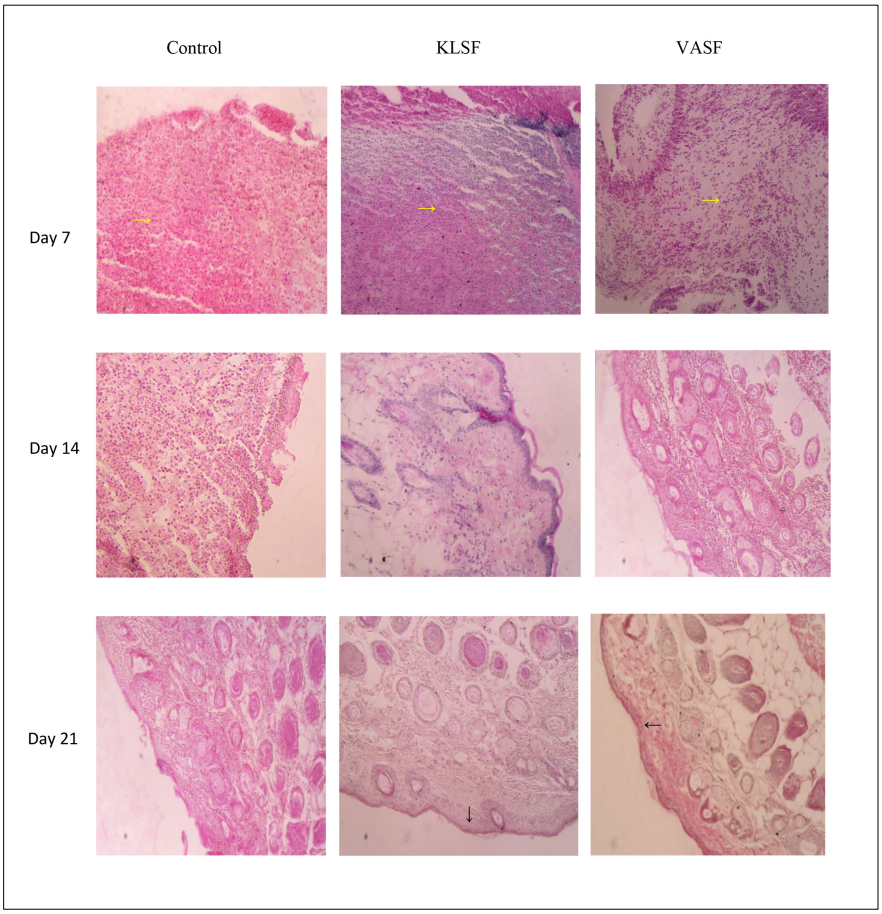

Figure 4 Histological analysis of H&E-stained sections of wounded tissue, depicting the extent of wound healing by KLSF and VASF treatment. Day 7 showed granulation tissue development in the treated wounds. Day 14 showed commencement of re-epithelialization in the wounds, and Day 21 showed regeneration of mature dermal and epidermal layers in the treated wounds as compared to the untreated control. All images are viewed at 20x magnification. The yellow arrow represents inflammatory cells, and the black arrow represents the newly formed epidermis

To further assess the wound healing process, histological evaluations were carried out on tissue samples collected at different intervals—days 7, 14, and 21. Morphological changes in the wound tissue were examined using hematoxylin and eosin (HE) staining across the various treatment groups (Figure 4). By day 7, wounds treated with silk dressings exhibited active granulation tissue formation within the wound cavity, unlike the control group, where the wounds remained covered with dried scab tissue. In untreated wounds, healing was significantly delayed, with granulation tissue absent and necrotic scab still present. Inflammatory cell infiltration was observed in all groups at this stage. On day 14, residual inflammatory cells were still present in the treated wounds; however, fibroblast infiltration and the presence of sebaceous glands were evident in both KLSF and VASF treated groups, indicating a superior healing response compared to the untreated control. Reformation of both the epidermal and dermal layers had commenced in the silk-treated groups. Wounds covered with silk film dressings showed moderate to near-complete epidermal regeneration, whereas the control group continued to lack proper epidermal covering, suggesting delayed tissue repair. By day 21, VASF-treated wounds displayed a fully regenerated and thickened epidermis. KLSF-treated wounds also showed a well-developed epidermis, albeit to a lesser extent than the VASF-treated group. In contrast, the untreated wounds remained partially covered with a thin, incomplete epidermis. These histological findings highlight the regenerative benefits of silk films, particularly their role in promoting early neo-vascularisation and enhancing the recruitment of endothelial cells during the initial stages of wound repair.

4. Discussion

Effective wound healing requires a well-regulated environment that supports cell migration, ECM deposition, and infection control. As such, selecting appropriate wound dressing materials is crucial. Successful biomaterial dressings must effectively combat infection and inflammation while guiding cellular activity to enhance healing and minimize scar formation. Additionally, these materials should promote the repair of the wound microenvironment and ensure stable ECM deposition for structural and functional skin regeneration [17]. Wound dressings serve as temporary coverings that protect the wound cavity and facilitate healing [18].

The application of silk in healing cutaneous wounds began decades ago with the use of silk sutures. Historically, silk fibres isolated directly from cocoons were employed as sutures. However, these early silk threads, composed of both fibroin and sericin proteins, triggered immune responses in some patients. Virgin silk has been associated with type I allergic reactions, asthma, and elevated IgE levels, likely due to sericin's immunogenic properties. These sutures, known as virgin silk sutures, were later modified to remove sericin content to reduce immunogenicity. Consequently, both silk sericin and silk fibroin are now separated from silk fibres and processed individually to develop distinct matrices. While both materials are considered suitable for wound healing, silk fibroin's superior physical properties offer additional advantages in matrix development [18]. The effectiveness of the degumming process, which involved removing sericin from the silk fibroin of KLSF and impurities from VASF, has been confirmed using SEM imaging (Figure 1). This process significantly reduces immunological responses when silk fibroin is used *in vivo* [19]. The superior properties of silk fibroin films as primary wound dressings, coupled with their excellent biocompatibility, motivated us to evaluate their efficacy in treating skin defects *in vivo*. This study aimed to develop regenerated silk fibroin films and assess their safety and effectiveness in skin repair. Using an *in vivo* cutaneous excisional mouse wound model, regenerated silk films were shown to improve wound healing rates, enhance re-epithelialisation, promote dermis proliferation, and support epidermal differentiation into hair follicles and sebaceous glands, while also reducing scar formation when compared with untreated wounds and controlled treatments.

Histological analysis of the wounded tissues further validated these outcomes. Silk films aided the early development of granulation tissue by day 7, initiated re-epithelialization by day 14, and by day 21, distinct differences in regenerated skin tissue could be observed between various treatments based on re-epithelialization and dermal organisation (Figure 4). Both VASF and KLSF, as novel silk fibroin-based materials, demonstrated effective wound healing outcomes, with VASF exhibiting notably improved performance in promoting re-epithelialization and reducing scarring. However, both silk films contributed positively to accelerating healing. Importantly, several previous studies provide insights that align with our observations and further clarify silk fibroin's involvement in multiple stages of the wound healing process. For instance, Park *et al*. [20] demonstrated that BMSF promotes wound healing through NF- κB signalling, as confirmed by microarray analysis and scratch assays, highlighting silk's ability to stimulate cell migration and recruit cells toward the wound site. This property was linked to silk's regulation of vimentin, cyclin D1, VEGF, and fibronectin, key markers of cell proliferation. Silk fibroin's haemostatic potential has also been demonstrated in previous research, where regenerated silk films showed fibrinogen-binding capabilities, confirming SF's ability to contribute to blood clotting [21]. Additionally, this natural fibrinogen-silk interaction was utilised in a study where fibrinogen and thrombin were combined with silk solution and cast into porous sponges, forming a haemostatic matrix that delivered these coagulation agents gradually, aiding clotting [22]. Moreover, extensive *in vivo* and clinical studies have demonstrated that silk is minimally immunogenic. While silk-based matrices elicit an initial immune response, this reaction subsides

during the later phases, indicating signs of graft acceptance. This controlled immune response, characterised by regulated macrophage activity and fibroblast recruitment, underscores silk fibroin's ability to facilitate a balanced inflammatory response conducive to optimal healing [23, 24]. Silk has also been shown to support cell migration and growth, allowing it to integrate effectively with the wound bed during the initial healing stages. As the underlying tissue regenerates over time, the silk matrix naturally dries up and detaches, ensuring automatic removal without the need for additional intervention [24, 25]. Re-epithelialization, a major step in wound healing that seals the wound cavity, is also improved by silk fibroin. Studies have linked this enhancement to silk's cell migratory effects mediated by NF- κB signalling, which accelerates keratinocyte migration and re-epithelialization [20]. Additionally, wounds treated with silk fibroin films have demonstrated improved collagen regeneration, reduced inflammation, and less lymphocyte infiltration, further confirming SF's role in promoting effective wound healing. Silk protein film has also been reported to be a safe biomaterial with no observed skin-sensitising effects or irritation and no impact on serum biochemical profiles following dermal application [26]. Notably, the main component of silk, silk fibroin (SF), has been widely studied not only for its ability to promote adhesion, proliferation, and differentiation of stem cells in *in vivo* models but also for its significant role in facilitating tissue repair processes [27]. In addition to its demonstrated impact on wound healing, silk fibroin's structural adaptability further expands its potential for broader biomedical applications. Easy processability of SF offers an additional advantage, enabling it to be cast into various structural formats such as thin films, hydrogels, injectable systems, porous scaffolds, 3D printed grafts, and nanofibrous mats [28, 29]. Among these formats, silk-based thin films and nanofibrous mats are particularly prominent in wound dressing applications due to their occlusive or semi-occlusive properties. These matrices effectively retain moisture and provide protective barriers that support the healing process [28, 30]. Hence, our study demonstrated that both VASF and KLSF are safe and effective biomaterials for skin repair and regeneration. Their biocompatibility, combined with their ability to enhance wound healing rates and improve re-epithelialization, supports their potential as promising candidates for clinical application in treating skin defects.

5. Conclusion

Silk fibroin is a highly promising biomaterial for skin repair and regeneration. In this study, the regenerated silk films effectively accelerated wound healing, promoted angiogenesis, and supported early re-epithelialization, indicating successful tissue repair. To the best of our knowledge, this is the first investigation using silk fibroin from *Kunugia latipennis* (KLSF) and *Vespa affinis* (VASF) in wound healing applications. The positive *in vivo* outcomes suggest a strong potential for these materials in future therapeutic use. While the outcomes are promising, further research is needed to better understand the underlying mechanisms and evaluate the performance of these silk variants across different wound models and experimental conditions.

Compliance with ethical standards

Acknowledgments

The authors would like to express gratitude to the Department of Environmental Studies, North-Eastern Hill University, Shillong, India, for providing the required facilities. The first author would also like to thank the Maulana Azad Fellowship for providing financial assistance for this research.

Disclosure of conflict of interest

The authors declare no conflicts of interest.

Statement of ethical approval

All experiments were approved by the institutional ethics committee (Animal model), North-Eastern Hill University, Shillong, India (Date: 28.07.2021) and were carried out in adherence with the same.

References

[1] Chouhan D, Chakraborty B, Nandi SK, Mandal BB. Role of non-mulberry silk fibroin in deposition and regulation of extracellular matrix towards accelerated wound healing. Acta biomaterialia. 2017 Jan 15;48:157-74.

[2] Ersel M, Uyanikgil Y, Akarca FK, Ozcete E, Altunci YA, Karabey F, Cavusoglu T, Meral A, Yigitturk G, Cetin EO. Effects of silk sericin on incision wound healing in a dorsal skin flap wound healing rat model. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research. 2016 Apr 1;22:1064.

[3] Chouhan D, Das P, Thatikonda N, Nandi SK, Hedhammar M, Mandal BB. Silkworm silk matrices coated with functionalized spider silk accelerate healing of diabetic wounds. ACS Biomaterials Science & Engineering. 2019 Jun 6;5(7):3537-48.

[4] Farokhi M, Mottaghitalab F, Fatahi Y, Khademhosseini A, Kaplan DL. Overview of silk fibroin use in wound dressings. Trends in biotechnology. 2018 Sep 1;36(9):907-22.

[5] Kundu B, Rajkhowa R, Kundu SC, Wang X. Silk fibroin biomaterials for tissue regenerations. Advanced drug delivery reviews. 2013 Apr 1;65(4):457-70.

[6] Andiappan M, Kumari T, Sundaramoorthy S, Meiyazhagan G, Manoharan P, Venkataraman G. Comparison of eri and tasar silk fibroin scaffolds for biomedical applications. Progress in biomaterials. 2016 Jul;5:81-91.

[7] Zubir N, Pushpanathan K. Silk in biomedical engineering: a review. Int J Eng Invention (IJEI). 2016;5:18-29.

[8] Zhang W, Chen L, Chen J, Wang L, Gui X, Ran J, Xu G, Zhao H, Zeng M, Ji J, Qian L. Silk fibroin biomaterial shows safe and effective wound healing in animal models and a randomized controlled clinical trial. Advanced healthcare materials. 2017 May;6(10):1700121.

[9] Gil ES, Panilaitis B, Bellas E, Kaplan DL. Functionalized silk biomaterials for wound healing. Advanced healthcare materials. 2013 Jan;2(1):206-17.

[10] Rockwood DN, Preda RC, Yücel T, Wang X, Lovett ML, Kaplan DL. Materials fabrication from Bombyx mori silk fibroin. Nature protocols. 2011 Oct;6(10):1612-31.

[11] Chawla S, Seit S, Murab S, Ghosh S. Silk from Indian paper wasp: Structure prediction and secondary conformational analysis. Polymer. 2020 Nov 3;208:122967.

[12] Li X, Zhang J, Feng Y, Yan S, Zhang Q, You R. Tuning the structure and performance of silk biomaterials by combining mulberry and non-mulberry silk fibroin. Polymer Degradation and Stability. 2018 Jan 1;147:57-63.

[13] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry. 1976 May 7;72(1-2):248-54.

[14] Ersel M, Uyanikgil Y, Akarca FK, Ozcete E, Altunci YA, Karabey F, Cavusoglu T, Meral A, Yigitturk G, Cetin EO. Effects of silk sericin on incision wound healing in a dorsal skin flap wound healing rat model. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research. 2016 Apr 1;22:1064.

[15] Chouhan D, Chakraborty B, Nandi SK, Mandal BB. Role of non-mulberry silk fibroin in deposition and regulation of extracellular matrix towards accelerated wound healing. Acta biomaterialia. 2017 Jan 15;48:157-74.

[16] Chouhan D, Das P, Thatikonda N, Nandi SK, Hedhammar M, Mandal BB. Silkworm silk matrices coated with functionalized spider silk accelerate healing of diabetic wounds. ACS Biomaterials Science & Engineering. 2019 Jun 6;5(7):3537-48.

[17] Chouhan D, Chakraborty B, Nandi SK, Mandal BB. Role of non-mulberry silk fibroin in deposition and regulation of extracellular matrix towards accelerated wound healing. Acta biomaterialia. 2017 Jan 15;48:157-74.

[18] Chouhan D, Mandal BB. Silk biomaterials in wound healing and skin regeneration therapeutics: From bench to bedside. Acta biomaterialia. 2020 Feb 1;103:24-51.

[19] Zhang W, Chen L, Chen J, Wang L, Gui X, Ran J, Xu G, Zhao H, Zeng M, Ji J, Qian L. Silk fibroin biomaterial shows safe and effective wound healing in animal models and a randomized controlled clinical trial. Advanced healthcare materials. 2017 May;6(10):1700121.

[20] Park YR, Sultan MT, Park HJ, Lee JM, Ju HW, Lee OJ, Lee DJ, Kaplan DL, Park CH. NF-κB signaling is key in the wound healing processes of silk fibroin. Acta biomaterialia. 2018 Feb 1;67:183-95.

[21] 21. Santin M, Motta A, Freddi G, Cannas M. In vitro evaluation of the inflammatory potential of the silk fibroin. Journal of Biomedical Materials Research: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials. 1999 Sep 5;46(3):382-9.

[22] Teuschl AH, Zipperle J, Huber-Gries C, Kaplan DL. Silk fibroin based carrier system for delivery of fibrinogen and thrombin as coagulant supplements. Journal of Biomedical Materials Research Part A. 2017 Mar;105(3):687-96.

[23] Thurber AE, Omenetto FG, Kaplan DL. In vivo bioresponses to silk proteins. Biomaterials. 2015 Dec 1;71:145-57.

[24] Chouhan D, Lohe TU, Samudrala PK, Mandal BB. In situ forming injectable silk fibroin hydrogel promotes skin regeneration in full thickness burn wounds. Advanced healthcare materials. 2018 Dec;7(24):1801092.

[25] Chouhan D, Lohe TU, Thatikonda N, Naidu VG, Hedhammar M, Mandal BB. Silkworm silk scaffolds functionalized with recombinant spider silk containing a fibronectin motif promotes healing of full-thickness burn wounds. ACS Biomaterials Science & Engineering. 2019 Aug 9;5(9):4634-45.

[26] Padol AR. Efficacy of the silk protein based biofilms as a novel wound healing agent. Group. 2011;1:18.

[27] Zubir N, Pushpanathan K. Silk in biomedical engineering: a review. Int J Eng Invention (IJEI). 2016;5:18-29.

[28] Zhang W, Chen L, Chen J, Wang L, Gui X, Ran J, Xu G, Zhao H, Zeng M, Ji J, Qian L. Silk fibroin biomaterial shows safe and effective wound healing in animal models and a randomized controlled clinical trial, Adv. Healthc. Mater. 6 (2017), 1700121 [Internet]. 2017

[29] Chouhan D, Thatikonda N, Nileback L, Widhe M, Hedhammar M, Mandal BB. Recombinant spider silk functionalized silkworm silk matrices as potential bioactive wound dressings and skin grafts. ACS applied materials & interfaces. 2018 Jun 25;10(28):23560-72.

[30] Chouhan D, Janani G, Chakraborty B, Nandi SK, Mandal BB. Functionalized PVA–silk blended nanofibrous mats promote diabetic wound healing via regulation of extracellular matrix and tissue remodelling. Journal of tissue engineering and regenerative medicine. 2018 Mar;12(3):e1559-70.