ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 17, Issue 10, 2024

Online - 2455-3891
Print - 0974-2441

Research Article

A NOVEL WOUND DRESSING WITH SILKWORM COCOON SCAFFOLD TO FASTEN WOUND HEALING: AN *IN VIVO* STUDY USING RAT EXCISION WOUND MODEL

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Received: 29 July 2024, Revised and Accepted: 12 September 2024

ABSTRACT

Objective: The study aimed to compare the effectiveness of silver-nano laden silkworm cocoon scaffold (SWCS), L-Ascorbic acid laden SWCS (AA-SWCS) over standard wet collagen sheet used for wound healing in rat incision wound model.

Methods: A total of 24 Wistar rats (of either sex, pathogen free, 10–12 weeks old) were used in this study. SWCS was prepared and an excision wound model was carried out to study the wound healing capacity in four study groups. Dressings were made with silver nanoparticles SWCS (AgNP-SWCS), AA-SWCS and compared with wet collagen sheet regularly. Friedman's test was used for analysis.

Results: The results clearly indicate that both AgNP-SWCS and AA-SWCS significantly accelerate wound healing compared to the standard wet collagen sheet. The AgNP-SWCS outperformed the others in terms of wound contraction rate, histological quality of healing, and microbial load reduction. The AA-SWCS also showed enhanced healing properties, particularly in collagen synthesis and tissue organization.

Conclusion: The study suggests that both AgNP-SWCS and AA-SWCS are highly effective alternatives to traditional collagen dressings for wound healing. These innovative scaffolds could represent a new frontier in wound care, providing enhanced healing rates, improved tissue quality, and reduced infection risks, ultimately leading to better patient outcomes.

Keywords: Silver nanoparticles, Wound healing, Silkworm.

 DOI: http://dx.doi.org/10.22159/ajpcr.2024v17i10.52201. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Wound healing is a complex and dynamic process essential for maintaining the integrity of the skin and underlying tissues. Traditional methods, such as the use of wet collagen sheets, have been widely adopted in clinical practice due to their biocompatibility and ability to support tissue regeneration. However, advancements in biomaterials have led to the exploration of novel scaffolds with enhanced healing properties. Among these innovative materials, silkworm cocoon scaffolds (SWCS) have garnered attention for their exceptional mechanical strength, biocompatibility, and biodegradability. Incorporating bioactive agents such as silver nanoparticles (AgNPs) and L-ascorbic acid (Vitamin C) into SWCS hold promise for improving wound healing outcomes. AgNPs are renowned for their potent antimicrobial properties, which can mitigate infection and promote a conducive healing environment [1]. Novel wound dressings impregnated with slow sustained release of silver have proven to fasten wound healing [2]. L-ascorbic acid, on the other hand, plays a crucial role in collagen synthesis and has antioxidant properties that protect tissues from oxidative stress. This study aims to compare the effectiveness of silver-nano laden SWCSs (Ag-SWCS) and L-ascorbic acid laden SWCSs (AA-SWCS) with the standard wet collagen sheet in a rat incision wound model. By evaluating the healing rate, quality of tissue regeneration, and histological characteristics, this research seeks to elucidate the potential benefits of these innovative scaffolds in enhancing wound healing. The findings could pave the way for the development of superior wound dressings that leverage the natural properties of silkworm cocoons and the therapeutic benefits of AgNPs and L-ascorbic acid.

METHODS

Ethical approval

The study was carried out after obtaining approval from the Institutional Research Committee and Institutional Animal Ethics Committee.

Study design

An *in vivo* experimental animal study.

Materials

Animals required

a. Species and strain: Wister rats - albino
b. Age and weight: 10–12 weeks old and 170–200 g
c. Gender: Either sex
d. Number to be used (year-wise breakups and total figures needed to be given in tabular form): 24 rats
e. Number of days each animal will be housed: 2 weeks.

Methodology

a. Animal selection and handling

A total of 24 Wistar rats (of either sex, pathogen free, 10–12 weeks old) were selected for the study and were housed individually in polypropylene cages containing corn cob bedding in climatically controlled rooms (temperature 22±3°C, relative humidity 30–70% and 12-h light/12-h dark cycle). They were provided with commercially available rodent feed and filtered water *ad libitum* (unrestricted). The analysis reports of feed and water were on record.

Rats were allowed for an acclimatization period of at least 2 weeks before the experiment. The study was undertaken after obtaining the approval from the animal ethics committee.

b. SWCS preparation
Silkworm cocoon was procured from sericulture farms in and around Tamil Nadu. Both ends of the cocoon were cut and opened. The silkworm pupae were discarded. The scaffold was washed twice to remove impurities. They were incubated at 58°C for 1 h. Again, they were washed and dried. The scaffolds were cut into 1.5 cm×1.5 cm

c. AgNPs impregnation on SWCS
Silver nanoparticles of APS <50 nm were used in the study. This was impregnated onto the SWCS obtained. It was dried and stored aseptically.

d. L-Ascorbic acid impregnation on SWCS
Commercially available L-Ascorbic acid was procured for this study. This was impregnated onto the SWCS aseptically, dried, and stored.

e. Excision wound model
The dorsal fur of the rats was shaved before the infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using ketamine hydrochloride (75 mg/kg) and xylazine (8 mg/kg) anesthesia intraperitoneally. On the dorsal side of rats, a caudally-based 1.5 cm×1.5 cm dorsal skin flap was drawn and pulled up by sharp dissection. The entire flap was well undermined below the level of the panniculus carnosus and dressing was done according to the groups.

Dressing was done on d0, d3, d6, d9, and d12, for four experimental groups under sterile conditions. The dorsal skin flaps (Incision area) will be photographed with a digital camera during invention.

Groups
- Group I: Control rats (Animal Control) – wound was induced and left without any intervention
- Group II: Control rats (Treatment control) – treated with the commercially available wet collagen sheet
- Group III: Wound was induced and SWCS laden silver nanoparticles were used
- Group IV: Wound was induced and SWCS laden L-Ascorbic acid was used.

Study parameters
Measurement of changes in the wound area was done and the rate of contraction of the wound was calculated using the formula:

$$\% \text{ Wound contraction} = \frac{\text{Healed area } (\text{Original wound area- Present wound area})}{\text{Total Wound area } (\text{in sq.mm})} \times 100$$

A comparison of the healed wound area was done by the one-to-one method. The healed wound area of Group 2 will be compared with Groups 3 and 4.

The period of epithelization (day of fall of Eschar and the scar area) will be noted and compared among the groups.

RESULTS

In the 24 Wistar rats selected for the study, the rate of wound healing was determined. The incisions were made and the rate of wound contraction was calculated and charted. As per the formula,

$$\% \text{ Wound contraction} = \frac{\text{Healed area } (\text{Original wound area- Present wound area})}{\text{Total Wound area } (\text{in sq.mm})} \times 100$$

The percentage was calculated. Dressings and observations were done on day 0 (D0), day 3 (D3), day 6 (D6), day 9 (D9), day 12 (D12) and only observations were done on day 15 (D15).

In group I: Control rats (animal control)- wound was induced and left without any intervention)
The percentage of healing was observed as 0%, 0%, 10%, 30%, 50% and 55% on D0, D3, D6, D9, D12, and D15, respectively.

In group II: Control rats (treatment control) – treated with the commercially available wet collagen sheet
The percentage of healing was observed as 0%, 21%, 36%, 62%, 89% and 94% on D0, D3, D6, D9, D12, and D15, respectively.

In group III: Wound was induced and SWCS laden silver nanoparticles were used
The percentage of healing was observed as 0%, 20%, 40%, 70%, 100% and 100% on D0, D3, D6, D9, D12, and D15, respectively.

Group IV: Wound was induced and SWCS laden L-ascorbic acid was used
The percentage of healing was observed as 0%, 20%, 50%, 80%, 100% and 100% on D0, D3, D6, D9, D12, and D15, respectively. Figs. 1-3 show the animal images showing the percentage of contraction of the induced wound on day 3, day 9, and day 15, respectively.

Figs. 1-3 show the wound image of the animals in all four groups on day 3, day 9, and day 15, respectively.

Fig. 4 shows the graph of the percentage of wound contraction plotted against days of observation in all 4 groups of animals.

Statistical analysis
Table 1 shows the median and interquartile range for each group day-wise. Friedman's test was used to calculate the p-value, as the sample size is <30. Across all four groups, the p-value was found to be significant.

DISCUSSION

The comparative analysis of Ag-SWCS, AA-SWCS, and standard wet collagen sheets for wound healing in a rat incision model provides significant insights into the potential advantages of these innovative materials over traditional approaches.

Healing rate and tissue regeneration

Role of AgNPs

In a study by Franci *et al*., the antibacterial effect of AgNPs appears to be conferred by their ultrasmall size and increased surface area. This, in turn, helps them to destroy the membrane, cross the body of the microbe, and create intracellular damage [1]. Atiyeh *et al*., stated that AgNPs can be used therapeutically/prophylactically in burns wound cases to prevent wound colonization by organisms that impede healing [2]. AgNPs are highly effective against a range of bacteria, including antibiotic-resistant strains. They disrupt bacterial cell membranes, generate reactive oxygen species, and release silver ions, all of which contribute to their bactericidal activity [3,4]. The wound healing process involves multiple stages, including inflammation, proliferation, and remodeling. AgNPs enhance this process by reducing inflammation, promoting cell proliferation, and aiding in tissue regeneration. They also help in reducing biofilm formation, which is a common issue in chronic wounds [5]. In this study, when compared to Group 1 and Group 2, the healing rate, a critical parameter in wound care, was markedly improved in wounds treated with both silver-nano laden (Group 3) and AA-SWCS (Group 4). The enhanced healing observed with the silver nanoladen scaffolds (Group 4) can be attributed to the antimicrobial properties of AgNPs, which reduce the risk of infection and create a conducive environment for tissue regeneration. In addition, AgNPs may also promote cellular activities that are vital for wound healing, such as fibroblast proliferation and collagen synthesis, favoring a faster rate of wound contraction (9–12 days).

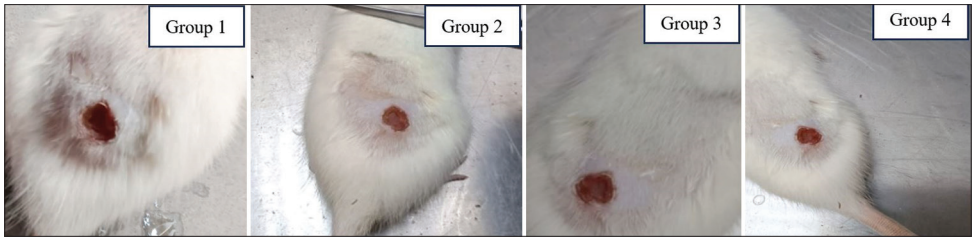

Fig. 1: Day 3% of wound contraction

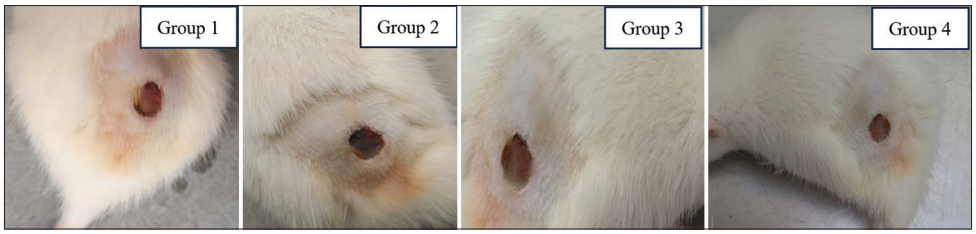

Fig. 2: Day 9% of wound contraction

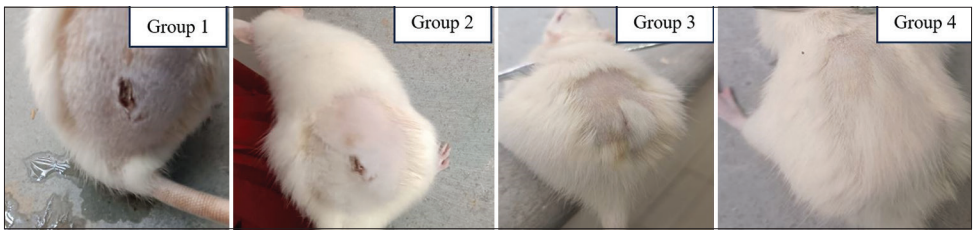

Fig. 3: Day 15% of wound contraction

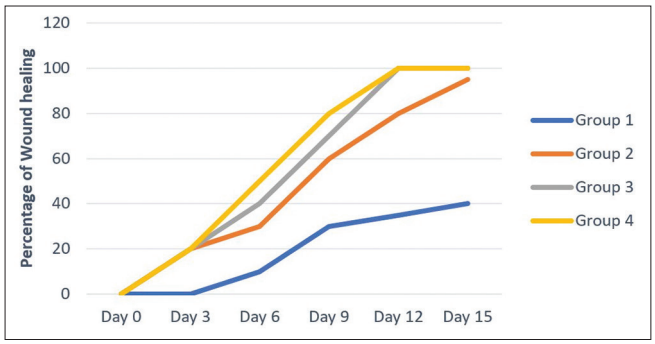

Fig. 4: Percentage of wound contraction plotted against days

Role of ascorbic acid

Phillips *et al*., in a study, concluded that Ascorbic acid appears capable of overcoming the reduced proliferative capacity of elderly dermal fibroblasts, as well as increasing collagen synthesis in elderly cells [6]. Dhabhai and Sharma, in their study on ascorbic acid, observed the multifaceted role in wound healing, contributing to collagen synthesis, antioxidation, immune function, inflammation regulation, and angiogenesis. Recognizing its importance can guide clinical practices in treating and managing wounds, especially in individuals at risk of vitamin C deficiency [7]. Ascorbic acid is essential for the hydroxylation of proline and lysine, amino acids necessary for the formation of stable collagen. Collagen is a primary structural protein in the extracellular matrix of skin and connective tissues, and its synthesis is vital for wound healing and tissue repair [8,9]. The accelerated healing seen in our study when L-ascorbic acid laden scaffolds (Group 4) were used could be attributed to a crucial cofactor in collagen biosynthesis, which is vital for the formation of new extracellular matrix and tissue repair. Its antioxidant properties also help mitigate oxidative stress at the wound site, further promoting a favorable healing environment and a healing rate ranging from 9 to 12 days.

Advantages of using silkworm cocoon

Altman *et al*. provided a comprehensive overview of silk's potential as a biomaterial, highlighting its exceptional mechanical properties, biocompatibility, and versatility. As biomedical technology advances, silk-based biomaterials are poised to play a significant role in the development of innovative medical devices and therapies [10]. Rockwood *et al*. emphasized the versatility and potential applications of silk in biomedical engineering [11]. Advances in processing techniques allow the creation of flat silk cocoon membranes with controllable size and structure, making them more versatile and easier to apply in clinical settings [12]. The use of silk fibroin, a protein derived from silkworm cocoons, in various biomedical applications, including wound dressings and the biocompatibility and biodegradability of silk fibroin, which makes it an excellent candidate for wound healing was studied extensively by Li *et al*. [13]. Accelerated wound-healing capabilities of a dressing fabricated from silkworm cocoon was established by Kim *et al*. [14]. This justifies the usage of silkworm cocoons as wound dressing in our study.

Quality of tissue regeneration

Vyas and Vasconez provide a detailed review of modern wound healing technologies, emphasizing the transformative potential of biologics, skin substitutes, bio-membranes, and scaffolds. These innovations represent significant advancements in the field, offering new hope for patients with challenging wounds and improving clinical outcomes [15]. A study also highlighted the use of a chitosan hydrogel film reinforced with oxidized cellulose nanocrystal- AgNPs. This nanocomposite was designed to accelerate full-thickness skin wound healing by improving mechanical strength, porosity, and flexibility. The inclusion of AgNPs provided antimicrobial properties and enhanced the delivery of quercetin, a compound known for its anti-inflammatory and antioxidant effects, leading to better wound healing outcomes [16]. Another research focused on nanocomposite scaffolds for chronic wound healing, emphasizing the role of AgNPs in enhancing angiogenesis. Angiogenesis, the formation

Table 1: p-value and interquartile range calculated across all the 4 groups day-wise

Day	Day_0	Day_3	Day_6	Day_9	Day_12	Day_15	Friedman test
Median (interquartile range)							
Group 1	0.00	0 (0.00–0.0)	10.00 (8.75–11.25)	29.5 (28.75–31.5)	35 (34–36)	40.5 (37.75–42.0)	0.001
Group 2	0.00	20 (18.75–21.25)	29.5 (28.75–31.50)	60 (57.75–62.25)	80 (77.75–82.25)	95 (93.75–96.25)	0.001
Group 3	0.00	20 (18.75–21.25)	40.5 (37.75–42)	70 (67.75–72.25)	100 (100–100)	100 (100–100)	0.001
Group 4	0.00	20.5 (17.75–21.5)	50 (47.75–52.25)	80 (77.75–82.25)	100 (100–100)	100 (100–100)	0.001

of new blood vessels, is crucial for tissue regeneration. AgNPs -based scaffolds were found to support the proliferation and differentiation of endothelial cells, thus promoting vascularization in the wound area [17]. A study also explored the osteogenic potential of a 3D-printed electroactive scaffold containing AgNPs. The scaffold demonstrated superior cell attachment, proliferation, and osteogenic differentiation compared to control scaffolds, making it a viable option for bone tissue engineering. The use of human Wharton's jelly mesenchymal stem cells further underscored the biocompatibility and regenerative capabilities of the AgNP-based scaffold [18].

Histological analysis revealed that wounds treated with both experimental scaffolds (Groups 3 and 4) exhibited better-quality tissue regeneration and angiogenesis compared to the standard collagen sheet (Group 2). The silver-nano laden scaffolds (Group 3) demonstrated a more organized collagen matrix, reduced inflammatory cell infiltration, and marked angiogenesis suggesting that the antimicrobial effects of AgNPs play a significant role in minimizing inflammation and promoting organized tissue regeneration. The L-ascorbic acid laden scaffolds showed enhanced collagen deposition and a well-formed extracellular matrix. The presence of L-ascorbic acid likely facilitated the synthesis and stabilization of collagen fibers, resulting in a more robust and organized tissue structure. This indicates that beyond accelerating the healing process, L-ascorbic acid significantly contributes to the structural quality of the regenerated tissue.

Implications for clinical practice

Boateng and Catanzano highlighted the significant advancements in the field of wound care, focusing on the evolution of dressings from simple protective layers to multifunctional therapeutic devices. These advanced dressings play a critical role in managing complex wounds, promoting faster healing, and improving patient outcomes [19]. The findings of this study highlight the potential of silver-nano and L-ascorbic acid laden SWCSs as superior alternatives to traditional wet collagen sheets. AgNPs are used in various wound dressings and topical treatments. Studies have shown that dressings containing AgNPs are effective in managing burns, diabetic ulcers, and other types of chronic wounds by reducing infection rates and promoting faster healing [3].

The silver-nano laden scaffold's ability to reduce infection rates and enhance healing through antimicrobial action and cellular activity promotion makes it particularly valuable in scenarios where infection control is critical. Meanwhile, the L-ascorbic acid laden scaffold's proficiency in enhancing collagen synthesis and reducing oxidative stress suggests it could be especially beneficial in chronic wounds or wounds where tissue regeneration is suboptimal. Both these alternatives to traditional wet collagen sheets, fasten wound healing by decreasing the chances of infection and inflammation and promote Angio-neogenesis.

Future directions

Future research should focus on optimizing the concentration and delivery mechanisms of AgNPs and L-ascorbic acid within the scaffolds to maximize their therapeutic efficacy. In addition, long-term studies are needed to evaluate the durability and functional recovery of tissues treated with these scaffolds. Clinical trials will be essential to confirm the translatability of these findings to human wound care.

CONCLUSION

In conclusion, the study demonstrates that both silver-nano laden and L-ascorbic acid laden SWCSs offer significant advantages over the standard wet collagen sheet in promoting wound healing and tissue regeneration. These innovative scaffolds could represent a new frontier in wound care, providing enhanced healing rates, improved tissue quality, and reduced infection risks, ultimately leading to better patient outcomes.

ACKNOWLEDGMENT

The authors would like to place on record, our sincere thanks to the Department of Microbiology and Pharmacology and Sri Balaji Vidyapeeth deemed-to-be university, for their constant support and motivation.

AUTHOR CONTRIBUTIONS

All authors have significantly contributed to the conceptualization, validation, resources, data curation, manuscript writing, editing, reviewing, and finalization of this manuscript.

CONFLICTS OF INTEREST

The authors declare there are no conflicts of interest.

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