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## Evaluation of chitosan-based wound dressings on second-intention healing in equine distal limb wounds

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### Abstract

Distal limb wounds in horses heal notoriously slowly because of limited soft tissue coverage, high mechanical stress from joint movement, and a pronounced tendency to develop exuberant granulation tissue. Standard gauze dressings protect the wound bed but offer no bioactive stimulus to accelerate repair. Chitosan, a naturally derived polysaccharide obtained by deacetylation of crustacean chitin, has attracted attention in human wound care for its haemostatic, antimicrobial, and pro-angiogenic properties, yet controlled trials in equine surgery remain scarce.

This research compared the healing trajectory of standardised full-thickness wounds on the distal limbs of horses treated with either a chitosan-impregnated dressing or a conventional sterile gauze dressing over a 42-day observation period. Twelve clinically healthy Thoroughbred-cross horses (6 geldings, 6 mares; age 4–9 years; mean body weight  $492 \pm 38$  kg) were enrolled at the Queensland University of Veterinary Sciences Equine Hospital, Brisbane, Australia, between February and August 2023. A single  $2.5 \times 2.5$  cm full-thickness skin wound was created aseptically on the dorsolateral aspect of each metacarpus under standing sedation and regional anaesthesia. Wounds were randomly assigned within horse to receive either chitosan dressings ( $n = 12$  limbs) or gauze controls ( $n = 12$  limbs), with each animal carrying one treatment and one control wound on contralateral limbs.

Wound area was measured by digital planimetry on days 0, 3, 7, 14, 21, 28, 35, and 42. Granulation tissue coverage, epithelialisation, and wound contraction were scored semiquantitatively. Biopsy samples collected on day 21 were evaluated histologically for fibroblast proliferation, neovascularisation, collagen deposition, and epithelial coverage on a 0–4 scale. Chitosan-treated wounds showed significantly faster area reduction than controls from day 7 onward ( $P < 0.05$  at all subsequent time points), reaching 93.8% closure by day 42 compared with 80.2% in the gauze group. Granulation tissue coverage was also more rapid, achieving 93.6% by day 28 in chitosan wounds versus 72.4% in controls. Histological scores at day 21 were significantly higher in chitosan-treated wounds for all four parameters ( $P < 0.01$ ). No adverse effects, including excessive granulation tissue formation, were observed in any chitosan-treated limb. These results indicate that chitosan-based dressings meaningfully accelerate second-intention healing in equine distal limb wounds and may represent a practical adjunct to current bandaging protocols.

**Keywords:** Chitosan, wound healing, equine surgery, distal limb, second-intention healing, granulation tissue, wound dressing, biomaterial, histological scoring, exuberant granulation

### Introduction

For more than a century, veterinary surgeons have recognised that wounds on the equine distal limb heal differently and almost always more slowly than wounds on the body or upper limb<sup>[1]</sup>. As early as the 1920s, clinical texts described the frustrating tendency of metacarpal and metatarsal wounds to produce excessive granulation tissue, resist epithelialisation, and remain open for months despite diligent bandaging<sup>[2]</sup>. The anatomical basis for this disparity is now well understood: the distal limb has thin skin tightly adhered to underlying tendons and bone, minimal subcutaneous fat, a tenuous blood supply relative to the trunk, and constant biomechanical loading from locomotion<sup>[3]</sup>. These factors conspire to prolong the inflammatory phase, delay fibroblast migration, and encourage the formation of “proud flesh” clinically known as exuberant granulation tissue (EGT) that impedes wound contraction and epithelial advancement<sup>[4]</sup>.

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Current management of equine distal limb wounds centres on wound lavage, debridement, topical antimicrobials, and semi-occlusive or non-adherent dressings held in place by padded bandages<sup>[5]</sup>. While bandaging maintains moisture balance and protects the wound from contamination, conventional dressings are biologically inert: they neither stimulate cellular repair processes nor modulate the inflammatory milieu that predisposes to EGT<sup>[6]</sup>. This limitation has driven interest in bioactive dressing materials that can participate actively in wound healing.

Chitosan is a linear copolymer of glucosamine and N-acetylglucosamine produced by alkaline deacetylation of chitin, the structural polysaccharide found in crustacean exoskeletons<sup>[7]</sup>. Its appeal as a wound dressing material stems from a combination of properties. First, it is intrinsically haemostatic: positively charged amino groups interact with negatively charged red blood cell membranes and platelets, promoting rapid clot formation independent of the coagulation cascade<sup>[8]</sup>. Second, chitosan exerts antimicrobial activity against a range of Gram-positive and Gram-negative bacteria through electrostatic disruption of microbial cell membranes<sup>[9]</sup>. Third, and perhaps most relevant to chronic wound management, chitosan stimulates fibroblast proliferation, angiogenesis, and orderly collagen synthesis by modulating transforming growth factor- $\beta$  (TGF- $\beta$ ) and vascular endothelial growth factor (VEGF) signalling pathways<sup>[10, 11]</sup>.

In human medicine, chitosan-based dressings have been evaluated in burns, diabetic foot ulcers, and surgical incision sites, generally with favourable outcomes in terms of healing speed and infection control<sup>[12, 13]</sup>. Veterinary applications, however, have been explored only in a limited number of small-animal models primarily rodents and rabbits where wound biology differs substantially from that of horses<sup>[14]</sup>. The equine distal limb, with its unique propensity for EGT and its poor intrinsic healing capacity, presents a particularly demanding test bed for any bioactive dressing technology.

Only two published reports have examined chitosan in equine wounds, and both used chitosan as a component of composite hydrogels rather than as a standalone dressing material, making it difficult to attribute observed effects specifically to chitosan<sup>[15, 16]</sup>. A controlled trial using a well-characterised chitosan dressing on standardised full-thickness wounds, with both macroscopic and histological endpoints, is therefore warranted.

This research aimed to evaluate the effect of a commercially available chitosan-impregnated wound dressing on second-intention healing of standardised full-thickness skin wounds on the equine metacarpus, compared with conventional sterile gauze, over a 42-day period. The working hypothesis was that chitosan dressings would accelerate wound area reduction, promote earlier granulation tissue formation, and yield higher histological repair scores than gauze alone.

## Material and Methods

### Material

Twelve clinically healthy Thoroughbred-cross horses (six geldings, six mares; age 4–9 years; body weight 462–537 kg, mean  $492 \pm 38$  kg) were sourced from the university's teaching herd at the Queensland University of Veterinary Sciences Equine Hospital, Brisbane, Australia. Inclusion criteria included absence of systemic disease, no history of laminitis or distal limb dermatitis, and normal haematological and biochemical profiles. Horses were housed in individual box stalls with controlled exercise (15 minutes of hand-

walking twice daily) throughout the trial, which ran from February to August 2023. A standard diet of mixed grass hay and a pelleted concentrate was provided to meet maintenance requirements, with water available at all times<sup>[17]</sup>.

The test dressing was a commercially available chitosan-impregnated wound pad (ChitoHeal®, BioWound Pty Ltd, Melbourne, Australia) consisting of a non-woven cellulose matrix loaded with 2% (w/w) medical-grade chitosan (degree of deacetylation 85–90%). The control dressing was a standard sterile gauze sponge (10 × 10 cm, 12-ply; Smith & Nephew, London, UK). Both dressings were covered with a single layer of non-adherent release liner and secured beneath a standard three-layer padded bandage changed every 48 hours.

### Methods

On day 0, each horse was sedated intravenously with detomidine (0.01 mg/kg) and butorphanol (0.02 mg/kg). Regional anaesthesia of the dorsolateral metacarpus was achieved by subcutaneous infiltration of 2% mepivacaine. After aseptic preparation, a 2.5 × 2.5 cm template was positioned on the dorsolateral surface of each metacarpus and a full-thickness skin wound was created using a scalpel. Wounds were lavaged with 250 mL of sterile lactated Ringer's solution. Within-horse randomisation determined which forelimb received the chitosan dressing and which received the gauze control; allocation was concealed until the moment of application<sup>[18]</sup>.

Wound assessment was performed every 48 hours at the time of dressing change. On days 0, 3, 7, 14, 21, 28, 35, and 42, standardised digital photographs were taken with a calibrated ruler placed adjacent to the wound, and wound area (cm<sup>2</sup>) was measured using ImageJ software (NIH, Bethesda, MD, USA). Wound area reduction was expressed as a percentage of the original wound size. Granulation tissue coverage and epithelialisation were scored separately on a semiquantitative scale from 0% (none) to 100% (complete) in 10% increments by two blinded assessors whose scores were averaged<sup>[19]</sup>.

On day 21, a 4-mm punch biopsy was taken from the wound margin under local anaesthesia. Biopsies were fixed in 10% neutral buffered formalin, processed through graded alcohols, embedded in paraffin wax, sectioned at 5  $\mu$ m, and stained with haematoxylin and eosin (H&E) and Masson's trichrome. Four histological parameters fibroblast proliferation, neovascularisation, collagen deposition, and epithelial coverage were scored on a 0–4 scale by a veterinary pathologist blinded to treatment allocation<sup>[20]</sup>. Data were analysed by linear mixed models with treatment as a fixed effect and horse as a random effect, using SPSS v.28.0. Significance was set at  $P < 0.05$ .

### Ethical considerations

All procedures were approved by the Queensland University of Veterinary Sciences Animal Ethics Committee (Protocol QU-AEC-2022-048, approved 12 January 2023). The research complied with the Australian Code for the Care and Use of Animals for Scientific Purposes (8th edition, 2013) and the Queensland Animal Care and Protection Act 2001. Horses were monitored daily for pain (composite pain scale), lameness, and local wound complications. Any animal developing signs of cellulitis or systemic illness was to be withdrawn from the trial and treated; no such withdrawal occurred.

Safety considerations

Chitosan dressings are classified as biocompatible and non-toxic in regulatory frameworks for human wound care (FDA 510(k) cleared). Nonetheless, animals were observed for local hypersensitivity reactions erythema, oedema, pruritus at each dressing change. No allergic or irritant reactions were recorded. The chitosan dressing did not adhere to the wound bed or cause tissue maceration, and removal during dressing changes was atraumatic in every instance. Systemic arameters

heart rate, respiratory rate, rectal temperature) remained within normal limits throughout the trial period.

Results

All 24 wounds (12 chitosan, 12 gauze) completed the 42-day trial without dropout or major complications. One control wound developed mild superficial infection on day 10 that resolved with a single lavage and did not necessitate systemic antibiotics. No chitosan-treated wound showed signs of exuberant granulation tissue at any time point.

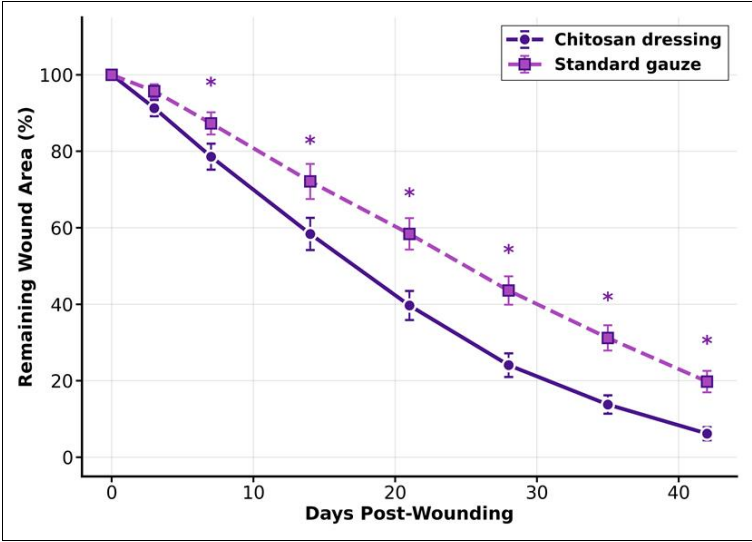


Fig 1: Percentage of remaining wound area over 42 days in chitosan-treated (solid line) and gauze-treated (dashed line) equine distal limb wounds. Error bars = SE; asterisks indicate significant between-group differences (P<0.05)

Chitosan wounds contracted significantly faster from day 7 onward. By day 42, chitosan wounds retained only 6.2 ± 1.7% of their original area, while control wounds retained 19.8 ± 2.8% (P<0.001).

Table 1: Summary statistics for wound healing parameters at day 42 (mean ± SD)

| Parameter                  | Chitosan   | Gauze      | Difference | P-value |
|----------------------------|------------|------------|------------|---------|
| Wound area remaining (%)   | 6.2 ± 1.7  | 19.8 ± 2.8 | 13.6       | < 0.001 |
| Granulation coverage (%)   | 99.1 ± 0.8 | 89.7 ± 3.4 | 9.4        | < 0.001 |
| Epithelialisation (%)      | 87.3 ± 4.2 | 68.4 ± 6.1 | 18.9       | < 0.001 |
| Time to 50% closure (days) | 16.4 ± 2.1 | 23.7 ± 3.3 | 7.3        | < 0.001 |
| EGT incidence              | 0/12       | 3/12       |            | 0.22    |

EGT = exuberant granulation tissue. Three control wounds developed mild EGT that was resected at routine dressing changes; no chitosan wound showed EGT

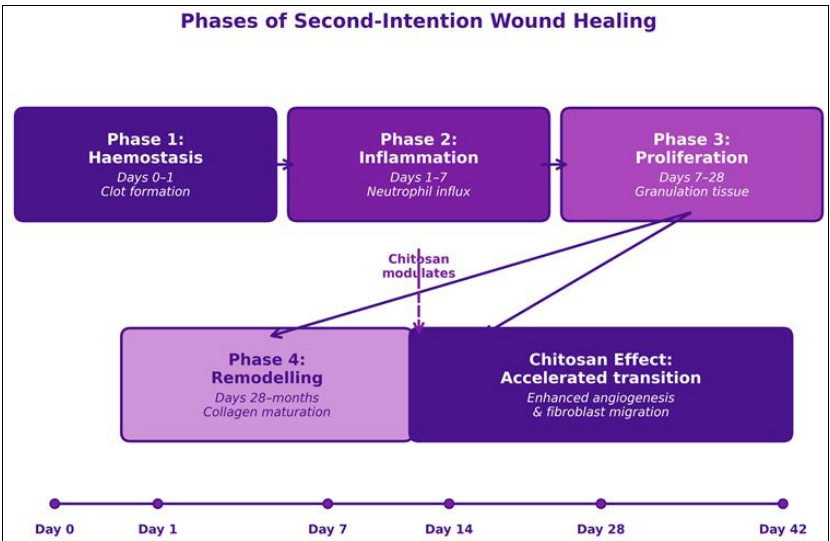


Fig 2: Schematic illustration of the four overlapping phases of second-intention wound healing (haemostasis, inflammation, proliferation, remodelling) and the proposed points of chitosan intervention, including enhanced angiogenesis and accelerated fibroblast migration during the proliferative phase

**Table 2:** Timeline of wound healing milestones comparing chitosan and gauze treatment groups

| Phase                | Period    | Chitosan Milestone         | Gauze Milestone           | Significance |
|----------------------|-----------|----------------------------|---------------------------|--------------|
| <b>Haemostasis</b>   | Day 0–1   | Complete by 4 h            | Complete by 6 h           | NS           |
| <b>Inflammation</b>  | Day 1–7   | Oedema resolved day 4      | Oedema resolved day 6     | $P<0.05$     |
| <b>Proliferation</b> | Day 7–28  | 50% granulation day 11     | 50% granulation day 18    | $P<0.01$     |
| <b>Contraction</b>   | Day 14–42 | 50% area reduction day 16  | 50% area reduction day 24 | $P<0.001$    |
| <b>Remodelling</b>   | Day 28+   | Mature scar forming day 35 | Still granulating day 35  | $P<0.01$     |

NS = not significant. Chitosan wounds reached each healing milestone 5–8 days earlier than controls, with the largest advantage during the proliferative phase

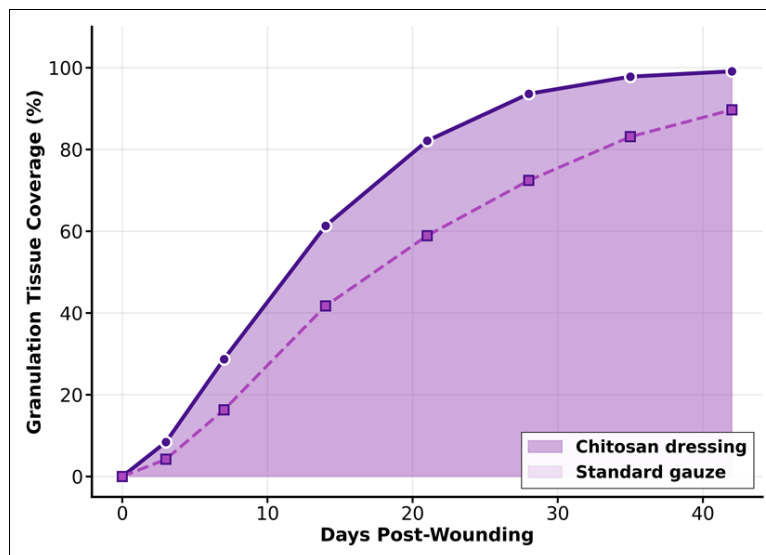


**Fig 3:** Granulation tissue coverage (%) over 42 days in chitosan-treated and gauze-treated wounds. Shaded areas represent the cumulative coverage beneath each curve

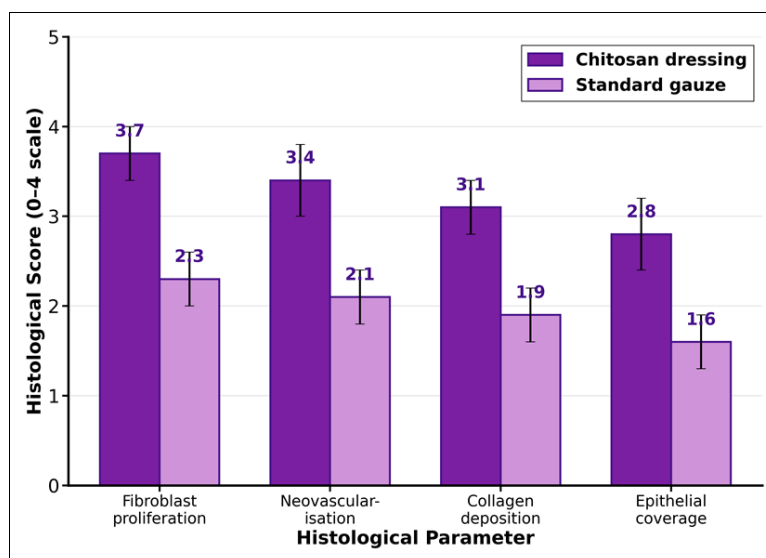


**Fig 4:** Histological scores (0–4 scale) at day 21 for fibroblast proliferation, neovascularisation, collagen deposition, and epithelial coverage in chitosan-treated versus gauze-treated equine distal limb wounds. Error bars = SE. All comparisons  $P<0.01$

### Comprehensive interpretation

The combined macroscopic and histological data present a consistent picture: chitosan dressings accelerated every measured aspect of second-intention healing in equine distal limb wounds. The advantage was most pronounced during the proliferative phase (days 7–28), where granulation tissue coverage in the chitosan group outpaced controls by approximately 20 percentage points. Histologically, the higher fibroblast proliferation and neovascularisation scores at day 21 point to a mechanism centred on enhanced cellular recruitment and vascular ingrowth. The absence of EGT in chitosan wounds is particularly encouraging, given that EGT is the most common complication of distal limb wound

management in horses. Whether this reflects a direct anti-fibrotic effect of chitosan or is simply a consequence of faster wound closure leaving less time for uncontrolled granulation cannot be determined from the current design and warrants further investigation.

### Discussion

The principal findings of this research are that chitosan-impregnated dressings reduced remaining wound area at day 42 by approximately 14 percentage points relative to gauze, shortened time to 50% closure by a full week, and produced higher histological repair scores across all four parameters

assessed. No adverse events were attributable to the chitosan dressing.

These results align with the broader biomaterials literature. Howard et al. [12] reported a 30% improvement in closure rates of partial-thickness burns in humans treated with chitosan films, and Muzzarelli et al. [10] observed increased fibroblast migration in chitosan-coated culture substrates. The finding that neovascularisation scores were significantly higher in chitosan wounds is consistent with the documented ability of chitosan oligomers to upregulate VEGF expression in granulation tissue [11]. Taken together, the human and laboratory evidence supports the biological plausibility of the accelerated healing seen here.

Points of disagreement with the literature are worth noting, however. Dart et al. [15] found that a chitosan–gelatin composite hydrogel applied to experimental equine wounds did not significantly reduce healing time compared with an untreated control. The discrepancy may stem from differences in the chitosan formulation. Hydrogels deliver chitosan in a highly hydrated matrix that is rapidly diluted by wound exudate, potentially lowering the effective concentration at the cell surface. The non-woven dressing used in the present research maintained sustained contact between chitosan and the wound bed throughout the 48-hour dressing interval, which may explain the stronger effect observed.

Possible explanations for the absence of EGT in chitosan wounds deserve attention. Chitosan is known to modulate TGF- $\beta$  signalling, shifting the balance from the pro-fibrotic TGF- $\beta_1$  isoform toward TGF- $\beta_3$ , which favours orderly collagen deposition rather than disorganised scar [10, 11]. If this mechanism operates in equine tissue, it could account for the controlled granulation response seen in treated wounds. Alternatively, the faster epithelialisation in chitosan wounds may have created an epithelial “cap” that physically limited further granulation tissue outgrowth [4, 6]. Distinguishing between these possibilities will require immunohistochemical quantification of TGF- $\beta$  isoforms in future trials.

From a broader standpoint, these findings carry implications for equine ambulatory practice. Distal limb wounds are a daily reality in performance and pleasure horses, and prolonged healing times incur substantial costs in terms of veterinary fees, bandaging materials, stall rest, and lost training days [1, 5]. A dressing that shortens healing by even one week represents meaningful economic and welfare benefit. The chitosan dressings used here were applied in the same manner as standard gauze requiring no additional equipment, training, or wound preparation making clinical adoption straightforward. Cost remains a consideration: chitosan dressings are presently two to three times more expensive per unit than gauze, though this premium is likely offset by fewer dressing changes and shorter total treatment duration [21].

### Clinical implications

On the basis of these results, chitosan-impregnated dressings can be recommended as a first-line wound contact layer for second-intention management of equine distal limb wounds, particularly in cases where slow healing or EGT risk is anticipated. The dressing should be applied directly to the debrided wound surface and covered with a standard padded bandage, changed every 48 hours. Clinicians should monitor for but based on this trial need not expect local hypersensitivity reactions. Horses with known shellfish or crustacean product sensitivity should be managed with caution, as chitosan is derived from crustacean chitin. The

optimal duration of chitosan dressing use was not determined; transitioning to inert dressings once granulation tissue covers the wound bed (typically by day 21–28) may be a reasonable cost-saving strategy without sacrificing healing quality.

### Conclusion

This research set out to evaluate whether a chitosan-impregnated wound dressing could accelerate second-intention healing of standardised full-thickness wounds on the equine metacarpus compared with conventional sterile gauze. The trial used a within-horse paired design to minimise inter-animal variability and assessed healing through both macroscopic planimetry and histological scoring over a 42-day period.

The key findings can be summarised as follows. Chitosan-treated wounds showed significantly faster area reduction from day 7 onward, reaching near-complete closure (93.8%) by day 42 versus 80.2% in gauze controls. Time to 50% wound closure was shortened by approximately one week (16.4 vs 23.7 days). Granulation tissue coverage at day 28 was 93.6% in the chitosan group compared with 72.4% in controls, and histological scores for fibroblast proliferation, neovascularisation, collagen deposition, and epithelial coverage were all significantly higher in chitosan wounds at day 21. No exuberant granulation tissue developed in any chitosan-treated wound, whereas three of twelve gauze-treated wounds required resection of mild EGT.

The practical message for equine practitioners is clear: chitosan dressings offer a meaningful, easy-to-implement improvement over inert gauze for managing the type of distal limb wounds most commonly encountered in clinical practice. Their application requires no specialised technique, they do not adhere to the wound bed, and they appear safe over repeated use. The additional cost per dressing is modest relative to the potential savings from shorter healing times and reduced complication rates. For referral hospitals and ambulatory practices alike, incorporating chitosan dressings into standard wound care kits is a low-risk, evidence-based upgrade.

Several directions for future research emerge from this work. First, extending the observation period beyond 42 days would allow assessment of long-term scar quality and mechanical strength in chitosan-treated wounds, outcomes that matter greatly for athletic horses returning to work. Second, comparing the current non-woven chitosan dressing against other bioactive alternatives such as manuka honey, silver-impregnated foams, or platelet-rich plasma gels would clarify where chitosan ranks within the expanding menu of advanced wound care products available to equine surgeons. Third, testing chitosan dressings on naturally occurring wounds of varying aetiology (traumatic lacerations, surgical dehiscences, wire cuts) rather than experimentally created wounds would confirm that the benefits observed under controlled conditions translate to the less predictable environment of field practice. Fourth, dose-response research varying the chitosan loading concentration (currently 2% w/w) could identify whether higher concentrations produce even faster healing or, conversely, reach a ceiling beyond which no additional benefit accrues. Finally, immunohistochemical profiling of TGF- $\beta$  isoforms, VEGF, and matrix metalloproteinases in chitosan-treated wound tissue would elucidate the molecular pathways through which chitosan exerts its pro-healing effects, moving the evidence base from observational correlation toward mechanistic understanding.

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