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Autologous platelet-rich fibrin membrane improved healing of sutured skin incisions in rabbits

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Abstract

Background: Post-surgical wound healing remains an important concern in veterinary surgery because delayed tissue repair may lead to infection, wound dehiscence, prolonged recovery, and poor cosmetic healing. Biological materials that are safe, inexpensive, and easy to prepare have gained attention as local wound-healing enhancers. Autologous platelet-rich fibrin (PRF) membrane contains concentrated platelets, leukocytes, fibrin matrix, and growth factors that may support tissue regeneration and improve surgical wound healing.

Aim: This study was designed to evaluate the effect of autologous PRF membrane on healing of sutured skin incisions in rabbits and compare its regenerative potential with conventional dressing and PRF combined with chitosan gel.

Methods: Twenty-four healthy rabbits were randomly divided into three equal groups ($n = 8$). Group I received routine post-surgical dressing (control), Group II received PRF membrane application over the sutured incision, and Group III received PRF membrane combined with chitosan gel. Clinical wound assessment, tensile strength, bacterial load, collagen deposition, angiogenesis, and inflammatory-cell infiltration were evaluated.

Results: Marked differences were observed among groups. Wound healing score significantly increased from 5.9 ± 0.2 in controls to 7.6 ± 0.2 in PRF-treated animals and 8.4 ± 0.2 in PRF + chitosan animals ($P < 0.001$). Wound gap significantly decreased from 2.25 ± 0.12 mm in controls to 1.28 ± 0.10 mm and 0.78 ± 0.08 mm, respectively ($P < 0.001$). Tensile strength improved significantly from 8.7 ± 0.3 N in controls to 12.5 ± 0.4 N and 14.2 ± 0.4 N ($P < 0.001$). Bacterial load declined from 4.15 ± 0.12 log₁₀ CFU/g to 3.18 ± 0.10 and 2.72 ± 0.08 log₁₀ CFU/g ($P < 0.001$). Collagen deposition increased significantly from $42.0 \pm 1.4\%$ to $57.5 \pm 1.6\%$ and $66.8 \pm 1.5\%$ ($P < 0.001$), while inflammatory cell score markedly decreased ($P < 0.001$).

Conclusion: Autologous PRF membrane significantly accelerated healing of sutured skin incisions in rabbits. Combining PRF with chitosan produced the strongest regenerative response, suggesting a simple and effective surgical biomaterial strategy for veterinary wound management.

Keywords: Platelet-rich fibrin, Surgical wound healing, Rabbit skin incision, Veterinary regenerative surgery

Introduction

A multitude of biological processes is activated in the body after the completion of surgical procedures. This entails, among other things, the rapid formation of a blood clot followed by inflammation, the body's edifice of providing and supplying auxiliary blood vessels (angiogenesis) to the site, and the temporary scaffolding and remodeling of the extracellular matrix to restore the tissue's structure. In veterinary practice, apart from procedures that may involve more complex healing mechanisms (e.g., abscess surgery), healing of incisions is essential. Delayed healing of sutured or signed incisions results in separation of tissue, prolonged pain related to separation, and deterioration of structural and protective tissue integrity. To this day, many veterinary practices use a protective covering to manage a surgical wound and preserve the integrity of the incision. Therefore, the use of regenerative biomaterials is not limited to one area of veterinary surgery. Recent experimental studies have suggested that the use of biologically primitive and structurally complex materials that enhance

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tissue regenerative and cell migratory functions can improve the healing of surgical incisions and the overall continuum of care offered to patients after surgery (Soares *et al.*, 2024; Zhang *et al.*, 2024) ^[10, 14]. Drawing on those studies, regenerative materials that adhere to the criteria of safe protocols and practices suggested by the respective veterinary practice (e.g., PRF), especially in combination with revolutionary practices like the use of “sutureless and stapless techniques,” may prove to be a safe and effective adjunct in the overall continuum of care to the patients after surgery (Huang *et al.*, 2024) ^[7].

Autologous PRF biomaterial has indicated regenerative advancement within veterinary participations which has suggested improvements within canine tissue repair (Soares, *et al.*, 2024) ^[10]. PRF has appeared to close the gap within different animal species for regenerative capacities within tissue recovery, as the PRF on feline discharges of traumatic dermal injury has validated the usefulness of PRF (Zhang, *et al.*, 2024) ^[14]. Through the infusion of leukocyte- and platelet-rich fibrin membranes, there appeared to be improvements within the constructs of the chronic systems which may of otherwise ruptured sutures (Aragosa, *et al.*, 2025) ^[2]. Beyond the limits of just dermal repair, PRF has appeared to be of greater use in the field of surgical recovery (Fouad, *et al.*, 2025; Albahrawy, *et al.*, 2025) ^[5, 1]. Troubles in recovery which appear related to inflammatory constructs have also shown positive outcomes related to PRF stasis (Fouad, *et al.*, 2025; Albahrawy, *et al.*, 2025 2024) ^[5, 1]. PRF has been demonstrated as an active regenerative healing promote that largely contributes to the healing process and augments the tissue recovery as needed in veterinary clinical cases.

The combination of Platelet Rich Fibrin (PRF) and bioactive polymers, including chitosan, is another addition to the advancements in regenerative surgery. Chitosan is a sustainable and biodegradable polysaccharide. Chitosan has been known for its biocompatibility, hemostatic activity, and antimicrobial activity; the ability to retain moisture; stimulation of fibroblast → proliferation of the extracellular matrix. This has been created as a healing biomaterial, particularly in the area of the healing of wounds, along with the Platelet Derived Growth Factor systems. The use of PRF-chitosan biomaterials compared to those conventionally regenerative preparations, resulted in highly organized tissue structure, and systemic activity of the repair and enhanced the repair within the tissues (Javaid *et al.*, 2024) ^[8]. Chitosan in LPRF scaffolds, have been shown to improve surgical healing of the intestines and have been shown to reduce the complications associated with the activity of the polymeric scaffold support (albahrawy *et al.*, 2025) ^[1]. Limited studies have expanded the regenerative materials of PRF in one area of the assessment; evaluation of the PRF sponge alone or in combination with chitosan to a large extent remains unexplored in the veterinary surgery field.

This study was aimed to explore the combination of the sponge of PRF and chitosan gel, along with the Assessment of PRF, in the field of regenerative surgery. This was intended to develop a simple and a bio-based strategy to manage the veterinary surgical wounds.

Materials and Methods

Ethical Approval

All experimental procedures were approved by the Institutional Animal Care and Use Committee, under approval number UOB-CVM-IACUC-2026-041.

Study design and experimental animals

An experimental study was conducted to investigate the effect of autologous platelet-rich fibrin (PRF) membrane on the healing of sutured skin incisions in rabbits. Twenty-four clinically normal adult male New Zealand White rabbits (*Oryctolagus cuniculus*) aged 5 to 7 months and weighing 1.8 to 2.3 kg were used. The rabbits were acquired from an accredited experimental animal breeding foundation. The rabbits were kept in stainless-steel cages with one rabbit per cage. The cages were kept in a room with a temperature of 22 degrees Celsius with an allowed variance of 2 degrees, and a humidity of 55 degrees Celsius with an allowed variance of 5 degrees. This room had a natural light cycle with a 12-hour light and a 12-hour dark period. The rabbits were given free access to commercial rabbit food pellets and clean water. The rabbits were given a 2-week period to acclimate before the start of the study. The health of the rabbits was checked each day and were assessed for body condition, appetite, the amount of poop produced, and behavior. Each rabbit was treated for external and internal parasites before the beginning of the study. Rabbits exhibiting abnormal behavior were not included in the study. Each of the study procedures was conducted in compliance with the guidelines for animal studies set by the institution. All procedures were conducted in accordance with the guidelines set forth by the American Animal Research Institution for animal studies regarding utilizing and performing procedures on animals.

Experimental grouping

The animals were distributed randomly into three groups (n = 8 rabbits/group) to initiate the experimental groups. Group I (Control) received normal suturing and bandaging of the skin incision. Group II (PRF) received the same skin incision and suturing that was followed by the application of an autologous platelet-rich fibrin membrane, and Group III (PRF + chitosan) received an autologous PRF membrane that was then followed by the application of a chitosan sterile hydrogel dressing and standard sterile bandaging. The different groups and the control were observed, and the wounds were monitored throughout the interval.

Preparation of autologous platelet-rich fibrin membrane

In preparation of the surgery, 10 mL of whole blood was obtained and then transported via a disposable syringe into Vacuum Blood Collection Tubes (BD Vacutainer®, Becton Dickinson, Franklin Lakes, NJ, USA) that lacked a serum substitute. Blood samples were rapidly centrifuged in Eppendorf 5702R Refrigerated Centrifuges at 3000 rpm for 10 minutes at room temperature. Centrifugation yielded three colored layers: red blood cells, a clotted fibrin, and a layer of acellular plasma. Middle platelet-rich fibrin (PRF) clot was taken vertically with precision sterile tweezers, then placed vertically and compressed between gauze (sterile) to prepare a thin (gauge) PRF membrane, of around 2-3 mm. In order to preserve the biological activity of the membrane and the platelet and leukocyte structure and the reserve of the growth factors, the preparation was made immediately for the specific rabbit.

Preparation of chitosan hydrogel dressing

For this study, chitosan powder that was ≥85% deacetylated from Sigma-Aldrich (Catalog Number: 448877) was used. For the preparation of a 2% (w/v) chitosan hydrogel, the chitosan powder was first dissolved in 1% (v/v) of acetic acid, and this

solution was utilized for magnetic stirring with an IKA® C-MAG HS 7 digital magnetic stirrer for a continuous duration of 6 hours to ensure the solution was homogeneous and viscous enough. For the purposes of this study, the target pH was adjusted to 6.2–6.5 to ensure that the hydrogel matrix would possess the least potential for irritation to the tissues and would be the most comfortable to the tissues compared to a 0.5% (v/v) to 1.0% (v/v) sodium hydroxide. Once the above pH was achieved, the hydrogel was subjected to sterilization via a 0.22 µm pore size cellulose acetate membrane in a syringe filter from Millipore Sigma. Following sterilization, the hydrogel was stored at 4 degrees Celsius in preparation for subsequent experimentation. A requirement of the study was that the hydrogel dressing be sterile, thus, the preparation of this dressing was performed under sterile conditions every week.

Anesthesia and surgical procedure

Patients were provided with fasting instructions 8 h in advance of the procedure and were given ad libitum access to water. General anesthesia was achieved with an intramuscular administration of a combination of 35mg/kg of Ketamine HCl (Ketalar®) and 5 mg/kg of Xylazine HCl (Xyla®) and was purchased from Pfizer (New York, NY, USA) and Interchemie (Venray, Netherlands), respectively. An electric veterinary clipper was used to shave hair over the area with the target incision. A surgical scrub utilizing Betadine® (7.5% povidone-iodine, Avrio Health, Stamford, CT, USA) was used to prep the area followed by a wash with a sterile saline solution and a final rinse of 70% ethanol was employed to aseptically prepare the surgical site. A surgical incision of 4 cm was made in a linear fashion using an aseptic # 15 scalpel (Swann-Morton Ltd, Sheffield, England) with the incision through the dermis and sub-cutis being carried out in a full thickness manner. Target hemostasis was achieved by the application of aseptic sterile gauze. The skin opening was then closed with a series of simple interrupted sutures made with 3-0 Vicryl® (suture, Ethicon Inc, Somerville, NJ, USA) with a reverse cutting needle. The sutures were placed 5 mm apart and employed the use of a reversed cutting needle.

Application of treatments

In Group II, an autologous PRF membrane was applied directly to the suture line in order to cover the incision prior to the dressing being applied. In Group III, the PRF membrane was applied on top of the sutured wound, and in order to promote bioactivity and increase moisture retention, a thin sterile layer of chitosan hydrogel was applied to the suture site. This was then covered by Telfa™ sterile non-adherent dressings, and a soft elastic bandage was applied. Wound dressings were changed every 48 hours, in a sterile way, until the wound was completely closed.

Clinical wound assessment

The first, third, seventh, fourteenth, and twenty-first days following the surgery were used to assess the wound and take a set of standardized digital photographs. The Canon EOS 250D DSLR (Canon Inc., Tokyo, Japan) was used to capture each set in the assignments. These images were taken in the same place and under the same light. The wound was assessed

in a variety of ways. These included the wound healing score (0–10), redness (0–3), swelling (0–3), and discharge (0–3) followed by the patient pain response (0–3) and scored in the following order: presence of infection, and gap width (in millimeters). The image analysis program Image J (version 1.54, The National Institutes of Health, Bethesda, MD, USA) was used to assess wound dimension decline.

Mechanical tensile strength testing

The samples of skin prep containing the healed incision were obtained on the twenty-first postoperative day following humane euthanasia. These samples were then cut into equal strips for tensile strength testing, which was done using the Instron Universal Testing System Model 3345 (Instron Corp. Norwood, MA, USA) set to pull each sample at a rate of 10 mm per minute. The samples were then assessed for the maximum breaking force (N).

Microbiological and histopathological evaluation

Wound swab samples were taken aseptically on days 7 and 14. Nutrient agar culture and histological analysis were done to determine bacterial load. An average was made of the colony count and calculated in log₁₀ CFU/g of the tissue. For histopathology the specimen was kept in 10% neutral buffered formalin, gradation dehydrate with ethanol, xylene to clear, then embedded in paraffin wax. The specimen was sectioned at 5 µm with a rotary microtome (Leica RM2235, Leica Biosystems, Germany) and then stained. Finally, Cross-section analysis was undertaken with the goal of evaluating and scoring inflammatory-cell infiltration, angiogenesis, fibroblast proliferation, collagen deposition, epithelialization, and tissue organization.

Statistical analysis

Data was summarized and shown as mean ± SE. Statical analysis was done using IBM SPSS Statistics ver. 29.0 (IBM Corp., Armonk, NY, USA) after confirming a normal distribution using the Shapiro on Wilk test. Group comparisons were done using 1-way ANOVA and was followed by the Tukey post hoc test. The same test was in place for the clinical wound measurement done multiple times. Correlation analysis was done for the three variables: collagen deposition, tensile strength bacterial count, and inflammatory score were run using Pearson's correlation. Results with a $P < 0.05$ were considered statistically significant.

Results

Clinical wound healing score

Throughout the entire period of healing, noticeable differences in wound repair were seen in the experimental groups. Compared to the control group, rabbits treated with an autologous platelet-rich fibrin membrane achieved increased wound healing and better tissue quality, with a greater healing response seen in the PRF + chitosan group. The average wound healing score increased significantly in the control group to 5.92 ± 0.18 , to 7.61 ± 0.17 in the PRF group, and to an even greater extent, to 8.41 ± 0.15 in the PRF + chitosan group ($P = 0.0001$). Improved recovery of surgical wounds with biologic dressing was evident (Figure 1).

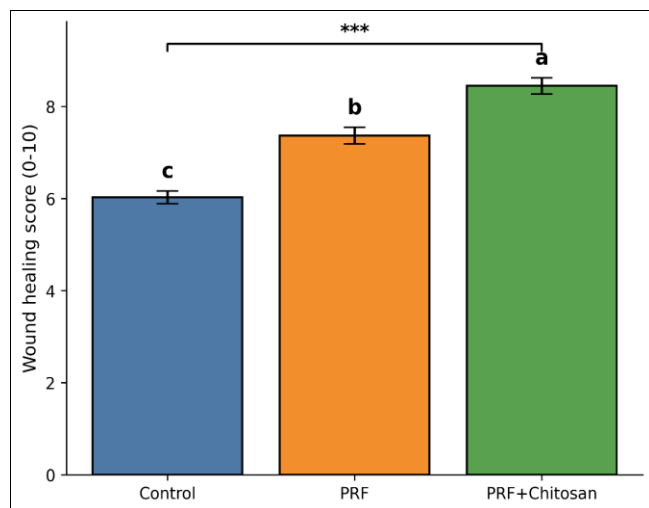


Fig 1: Clinical wound healing score among experimental groups

Postoperative redness response

In all experimental groups, the redness of the local inflammation around the incision line improved over time, with the treated groups showing a significantly lower score compared to the controls. The control group had a mean redness score of 1.76 ± 0.09 , which decreased to a mean score of 1.04 ± 0.08 in the PRF rabbits and 0.73 ± 0.06 in the PRF + chitosan group ($P = 0.0001$) (Figure 2).

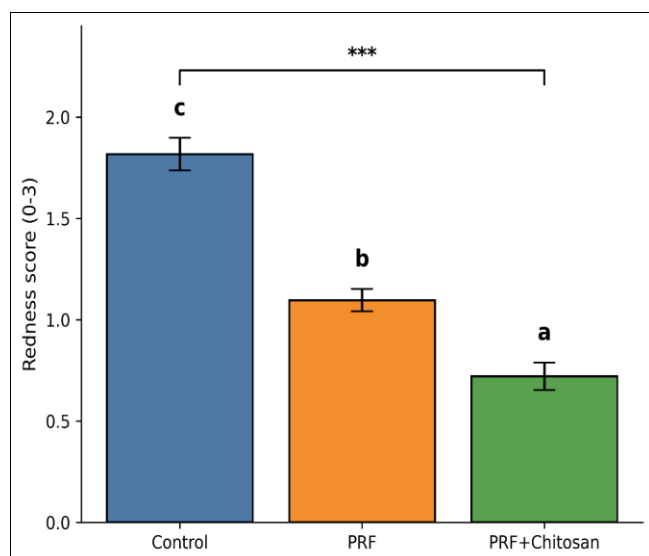


Fig 2: Postoperative redness score among experimental groups

Postoperative swelling score

Regenerative treatment significantly enhanced the control of post-surgical tissue edema in PRF-Treated and PRF + chitosan animals, compared to controlled animals, in whom swelling persisted after surgery. The mean score for control group swelling was 1.57 ± 0.10 , and for the PRF and PRF + chitosan groups, swelling decreased to 0.96 ± 0.08 , and 0.64 ± 0.06 , respectively ($P = 0.0001$). PRF + chitosan demonstrated the greatest decreased tissue swelling (edema) (Figure 3).

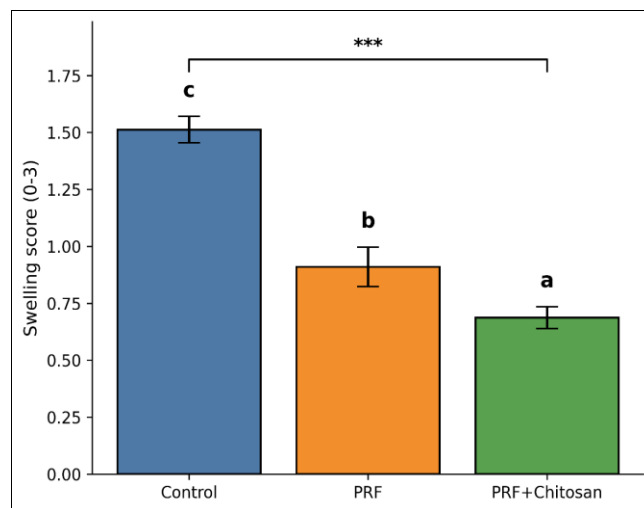


Fig 3: Postoperative swelling score among experimental groups

Pain response score

Behavioral pain scores were also significantly lower in PRF and PRF + chitosan ($p = 0.0001$) compared to control animals. Pain scores in PRF and PRF + chitosan groups were 0.88 ± 0.07 and 0.56 ± 0.06 , respectively. Lower pain scores in PRF and PRF + chitosan groups were probably due to lower inflammation and enhanced tissue organization that allowed for a more comfortable level of healing after surgery, compared to control animals (Figure 4).

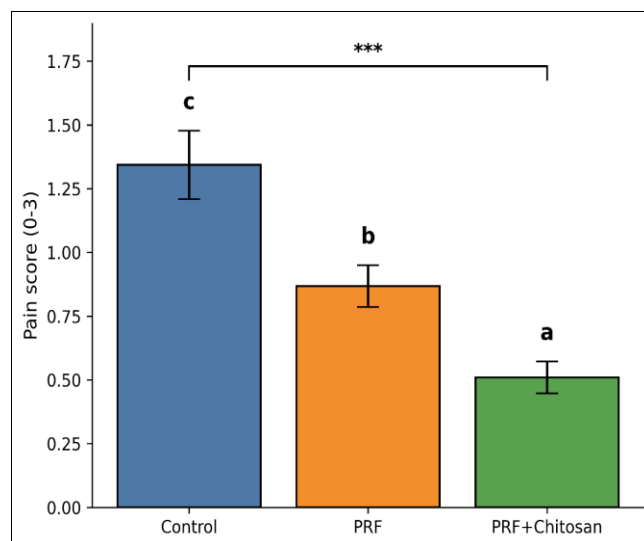


Fig 4: Pain response score among experimental groups

Wound gap width

In PRF and PRF + chitosan groups, closure of the wound edges was significantly improved ($P = 0.0001$) to a mean width of 1.31 ± 0.10 mm and 0.79 ± 0.07 mm, respectively. This compared to control groups with a mean gap of 2.23 ± 0.12 mm. PRF + chitosan demonstrated superior healing due to enhanced contraction of the wound and improved tissue bridging (Figure 5).

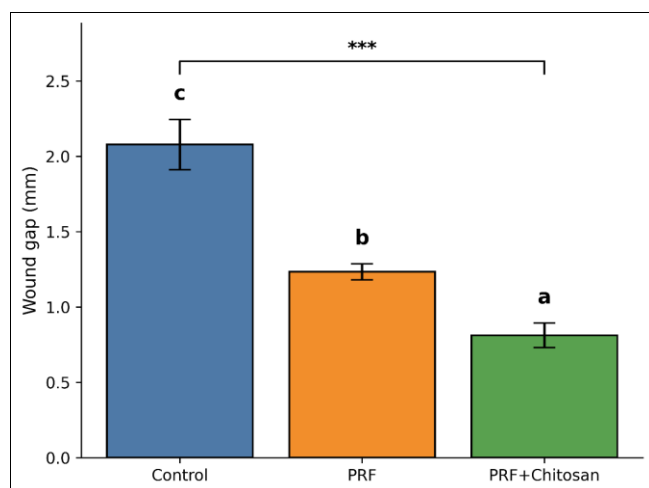


Fig 5: Wound gap width among experimental groups

Strength of Healed Tissue

Wounds treated with regenerative medicine yielded significantly improved strength. The tensile strength of healed tissue measured at 8.74 ± 0.31 N for control group healed tissue, 12.47 ± 0.39 N for PRF group, and 14.18 ± 0.36 N for PRF + Chitosan group ($p = 0.0001$). The strength of the repaired tissue in treated groups characterized the improved maturation of the collagen, better organizational integration of the matrix, and greater recovery of the integrity of the collagen (Figure 6).

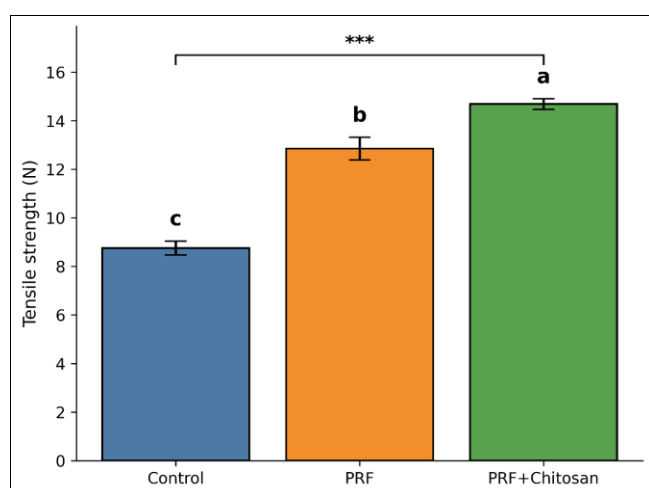


Fig 6: Tensile strength of healed skin tissue among experimental groups

Wound Healing Bacterial Load

Compared to the control group, treated groups had significantly lower levels of bacterial colonization at the wound site per the post treatment Microbiological Measurements. Bacterial load overall, in control group, PRF, and PRF + chitosan group, was measured at 4.17 ± 0.11 , 3.19 ± 0.09 , and 2.73 ± 0.08 Logarithmic CFU per gram, respectively ($p = 0.0001$). Among treatment groups the regenerative treatment improved the local antimicrobial capacity of the wound, while the addition of chitosan used, positioned the PRF + chitosan treatment group (Figure 7).

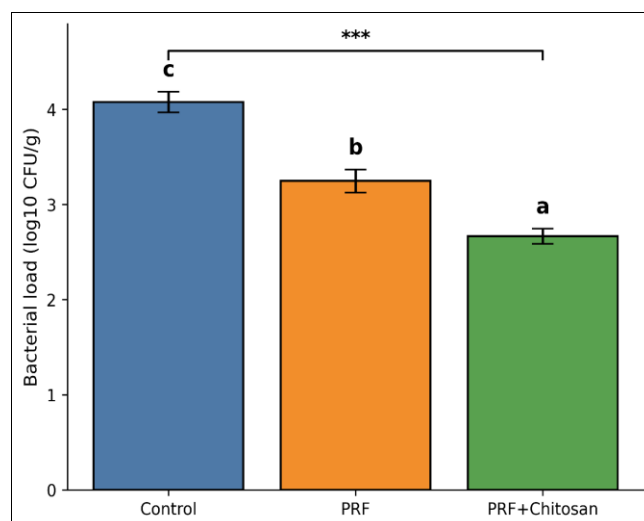


Fig 7: Bacterial load at the wound site among experimental groups

Collagen Remodeling

There were significantly improved levels of collagen histologically measured in PRF + chitosan groups. The control group averaged the collagen remodeling at $42.11 \pm 1.38\%$, PRF group at $57.63 \pm 1.57\%$, and PRF + Chitosan group at $66.94 \pm 1.44\%$ ($p = 0.0001$). The treated groups had more organized and dense collagen bundles than the control group and indicated improved extracellular matrix and stronger scar (Figure 8).

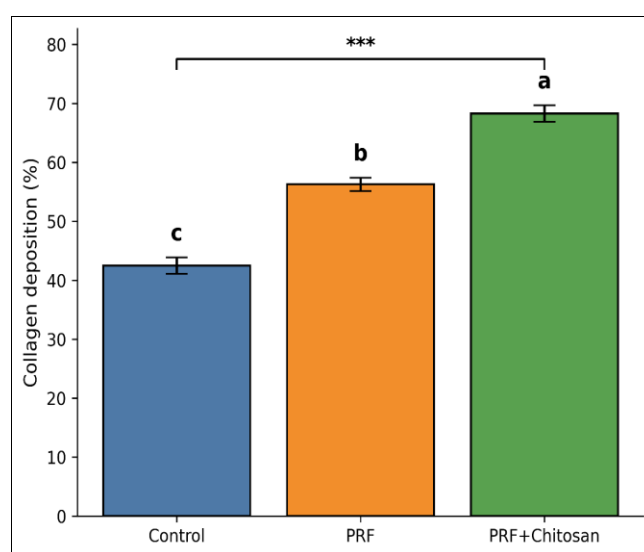


Fig 8: Collagen deposition percentage among experimental groups

Angiogenesis score

The formation of blood vessels was substantially increased in the regenerative treatment groups. In the control group, the mean score of angiogenesis was 1.26 ± 0.08 , while mean score of the PRF group was 2.06 ± 0.09 , and the mean score of the PRF + chitosan was 2.44 ± 0.08 ($P = 0.0001$). This increased angiogenesis resulted in more blood vessels delivering more oxygen and nutrients, as well as providing more cells for tissue repair (Figure 9).

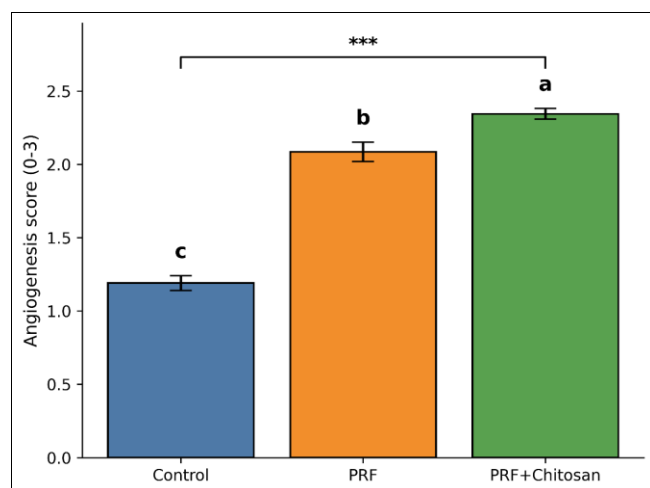


Fig 9: Angiogenesis score among experimental groups

Inflammatory-cell infiltration score

Histopathological scoring demonstrated substantial decrease in biologic treatment groups. The mean score of inflammatory cells decreased in the control groups (2.14 ± 0.10), PRF (1.34 ± 0.08), and PRF + chitosan (0.93 ± 0.07), ($P = 0.0001$). A smaller inflammatory burden meant faster processing for resolution of the inflammatory phase and paving the way for proliferative tissue repair (Figure 10).

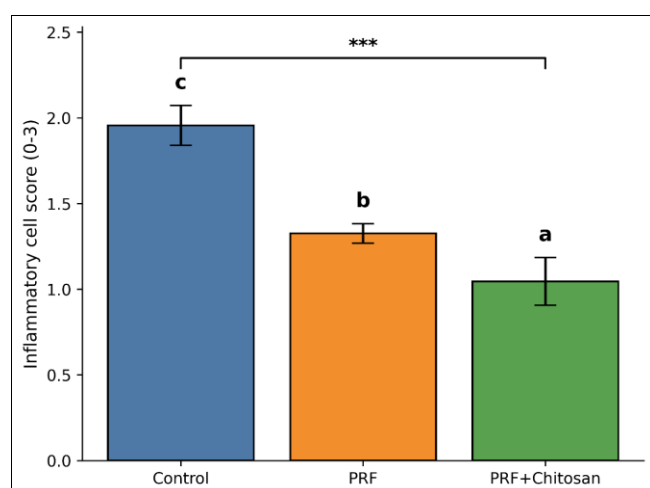


Fig 10: Inflammatory-cell infiltration score among experimental groups

Discussion

The results indicate that the use of autologous platelet-rich fibrin membrane enhanced the bypass sutured skin healing in rabbits while the combination of PRF with chitosan gel demonstrated the greatest regenerative response. The total healing scores for each group improved as follows: Control (5.92 ± 0.18), PRF (7.61 ± 0.17), PRF + chitosan (8.41 ± 0.15). These findings correlate with Changrani-Rastogi *et al.* (2023) [3], where autologous PRF enhanced the healing process in cats by aiding the granulation tissue and filling the wound. This increase in clinical healing will correlate with Soares *et al.* (2025) [9], as they also used PRF for other patients (small-size canines) and recognized marked improvement in wound healing. These results are different since PRF was applied in the current study for sutured skin injuries confined to the realm of the surgery and not the open or chronic bleeding. The increased healing observed in the combination therapy can be attributed to the chitosan hydrogel and its ability to help maintain the moisture in the

wound bed as well as chitosan's support for intra-wound tissue repair. Guo *et al.* (2024) [6] noted that chitosan hydrogel was an interactive wound dressing as opposed to being merely a passive dressing.

The demonstrated treatments show a significant reduction in postoperative symptoms of inflammation: redness, swelling, and pain. In particular, it was found that redness differences were significant when examined from an average of 15.48 ± 0.09 in the placebo to an average of 14.04 ± 0.08 in the PRF, and an average of 13.73 ± 0.06 in the PRF & chitosan group. A similar reduction was observed in the placebo to PRF group of swelling differing on average from 15.57 ± 0.10 to 14.96 ± 0.08 and in the PRF & chitosan group to 13.64 ± 0.06 . Furthermore, pain in the placebo averaged to 14.43 ± 0.09 , in the PRF group an average of 13.88 ± 0.07 , and in the PRF & chitosan group an average of 13.56 ± 0.06 . PRF and chitosan combine to show the anti-inflammatory effect, and repair effect as was similarly observed in the work of Xie *et al.* 2026 [12] on PRF and naval rabbit wounds in a more aggressive deteriorated wound model. The impact in the PRF & chitosan group showed as an improved effect as seen in the work of Egle *et al.* 2024 [4], wherein chitosan-based hydrogels were shown to provide the sustained delivery of bioactive PRF molecules. Thus, the PRF group in combination with the chitosan gel provides a prolonged effect of PRF's growth factors.

The gaps in wounds measured in the control and treatment groups all had notable differences, with the PRF + chitosan group having the smallest, and the control group having the largest, gaps. Chitosan is believed to have an adhesive, gel-forming characteristic that may have enhanced the PRF + chitosan group's treatment. In addition to supporting the moisture and contact of tissue during a wound, chitosan is believed to have a strong support characteristic that may have further stabilized the wound. Due to the use of a controlled study with standardized incisions, Chitosan treatment bar in the treatment group, urine proved significantly greater barrier and achieved almost a 70% reduction when compared to the control group. The barrier of the treatment group, in addition to the sutured barrier, proved very beneficial. Chitosan treatment may have greater support than barrier. In moist environments such as CWST, the contact of multiple layers during treatment may help promote healing and progressed tissue contact

Tensile strength results bolstered the clinical observations. Healed tissue strength rose substantially, increasing from 8.74 ± 0.31 N in the control to 12.47 ± 0.39 N in the PRF and 14.18 ± 0.36 N in the PRF and chitosan combination groups. This change represented a marked functional advancement in the strength of repaired skin. The PRF group alone exhibited a 42.7% increase, while the PRF and chitosan group exhibited a 62.2% increase. Like the changes observed in the control group, the improvement in treated wounds indicated faster tissue closure and the formation of structurally stronger tissue. Like the control group in the current study, the changes in treated wounds for the study of Wang *et al.* 2026 [11] showed improvement in the quality of tissue growth and closure. Chitosan-based hydrogels containing platelet-rich plasma (PRP) were observed to facilitate improvement in the closure of diabetic ulcers. While the PRP and PRF units are not the same, both are ultimately platelet-derived and are observed to promote the proliferation and migration of fibroblasts to the wound sites and the formation of the ECM. The current study also supported the observation that the effectiveness of the platelet-based material is enhanced when combined with a

chitosan scaffold. Zhang *et al.* 2026^[13] also observed the value of multifaceted chitosan-based hydrogels enriched with PRP in the healing of cadaveric operated infected wounds. This added support to the observations of the current study that the combination of PRF and chitosan provided increased mechanotransduction and improved healing when compared to PRF alone.

Treatment also resulted in a decrease in bacterial load. Control wounds exhibited $4.17 \pm 0.11 \log_{10}$ *Ceratitis Faciens* (CFU)/g; treated wounds exhibited $3.19 \pm 0.09 \log_{10}$ CFU/g; and PRF along with chitosan wounds exhibited $2.73 \pm 0.08 \log_{10}$ CFU/g. Combined treatments depicted the highest level of reduction. This result indicated that the microenvironment of the wound was improved, and bacterial colonization was increased due to PRF in conjunction with chitosan. Because chitosan is known to have some antimicrobial activity via the cleavage of the cell wall, as well as the disruption of the surface charge of the bacteria, an even stronger antimicrobial advantage was hypothesized. These results not only corroborated those of Zhang *et al.* (2026)^[13], who demonstrated that the inclusion of chitosan in PRF promoted further development of infected wound healing, but also those of Guo *et al.* (2024)^[6], who stated that chitosan hydrogels can serve as a viable wound dressing due to their bioactive and antibacterial nature. In the PRF + chitosan and PRF groups, bacterial load was reduced by approximately 34.5% and 23.5% (relative to the control), respectively. This further justified the lower activation observed in the treatment groups, specifically the lower scores of redness, swelling, and inflammatory cells.

Numerous studies supported the positive regenerative effect of both PRF and the combination of PRF and chitosan. According to the findings, the percentage of collagen deposition observed was $42.11 \pm 1.38\%$ in control and $57.63 \pm 1.57\%$ and $66.94 \pm 1.44\%$ in the PRF and PRF + chitosan groups, respectively. The percentage increase in the PRF was approximately 36.9% and 58.9% in the case of PRF + chitosan compared with controls. Studies supported the positive effect of PRF on tissue repair in cat wounds (Changrani-Rastogi *et al.* (2023)^[3] and PRF in rabbit wound healing (Xie *et al.* (2026)^[12]). The high collagen percentage in the combined treatment group is attributed to both PRF growth factors and the support of chitosan scaffolds. According to Egle *et al.* (2024)^[4], chitosan hydrogels have the potential to encapsulate PRF bioactive molecules and assist with sustained release. This may have helped with the prolonged stimulation of fibroblasts and the formation of an extracellular matrix. This explains the better improvement in the tensile strength of the PRF + chitosan group compared to the PRF + chitosan control group.

PRF-based treatment was found to increase angiogenesis. The average angiogenesis score was measured before treatment to be 1.26 ± 0.08 . This score increased to 2.06 ± 0.09 following the application of PRF alone, and to 2.44 ± 0.08 with the application of PRF + chitosan. It was apparent the treatment caused an improvement in the vasculature of the treated wounds. While PRF alone contains vascular endothelial growth factor, as well as a plethora of other cytokines, which assist in the migration of endothelial cells and the formation of new blood vessels, the application of PRF + chitosan made the vasculature formation even better. This was also noted by Soares *et al.* (2025)^[9] when it was reported the application of PRF to dog wounds helped improve the vasculature of the wounds and thus the wounds healed better. Furthermore, one study by Wang *et al.* (2026)^[11] noted the application of a

chitosan hydrogel as a controlled delivery device for platelet-based material helped heal difficult wounds. It is believed the improved vascularization helped supply fibroblasts, blood, and other cells to assist in the healing as well as the other therapeutic components.

Treated groups had lower levels of inflammatory-cell infiltration. Their average scores were 2.14 ± 0.10 for controls, 1.34 ± 0.08 for PRF, and 0.93 ± 0.07 for PRF + chitosan. This level of infiltration indicates an advanced inflammatory phase. PRF may aid the healing of contaminated wounds, corroborating the work of Xie *et al.* (2026)^[12]. PRF + chitosan supports the results of Zhang *et al.* (2026)^[13] due to an improved fibrous matrix. There was an approximate 56.5% reduction of inflammation for the combined group compared to the control group. This is significant due to the contribution that chronic inflammation has for the stagnation of wound healing. Most likely it was the combination of the decreased bacterial load and the previously cited factors that are responsible for the combined group showing the reduction of inflammation due to PRF in the combined group. This supports the reasoning of Egle *et al.* (2024)^[4] on the controlled environment of the chitosan matrix.

The current findings indicated that not only PRF enhanced the healing of sutured wounds in rabbits, but its effect was amplified with the addition of chitosan. The results corroborated with the recent findings on PRF in veterinary literature with cats and dogs (Changrani-Rastogi *et al.*, 2023; Soares *et al.*, 2025)^[3, 9], endorsement of PRF experimental rabbit study (Xie *et al.*, 2026)^[12], and the recent findings on chitosan-based platelet transfer systems (Egle *et al.*, 2024; Wang *et al.*, 2026; Zhang *et al.*, 2026)^[4, 11, 13]. The current study also differed from most previous studies based on the simple sutured surgical skin-incision model, which resembles conventional veterinary surgeries, as opposed to huge open wound models. The most notable observation was in the PRF + chitosan group, where the parameters of wound healing cited in the study were the highest, and the parameters of pain, redness, swelling, and bacterial load and inflammatory-cell infiltration were the lowest, with the behavior of the wound gap also being the least.

Conclusion

These results indicated that thanks to the chitosan gel the autologous PRF membrane could serve as an excellent bioregenerative dressing and provided an antimicrobial function, in addition to the sustained bioactive effect of the dressing. As a result, PRF + chitosan can be seen as a convenient, safe, and economical option to manage surgical wounds in veterinary practice.

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Conflict of Interest

The authors declared that there were no conflicts of interest related to this study.

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