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Research Article

Effect of Platelet-Rich Fibrin (PRF) from Different Sources on Avulsion Wound Healing in a Rabbit Model

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Abstract | This study aimed to evaluate the effectiveness of platelet-rich fibrin (PRF) derived from different sources in promoting the healing of surgically induced circular skin wounds in rabbits. Ten adult rabbits were used, and four full-thickness circular wounds (1 cm in diameter) were created on both sides of the dorsal region of each rabbit. The wounds were assigned to four treatment groups: autogenic PRF, allogeneic PRF, xenogeneic PRF, and a control group treated with normal saline. Each rabbit received all four treatments. Wound contraction was assessed clinically on days 3, 7, 14, and 21 postoperatively, while histopathological evaluations were conducted on days 7, 14, and 21. No significant differences in wound diameter were observed among the groups on day 3. However, by day 14, the autogenic PRF group showed significantly greater wound contraction compared to the other groups. By the third week, wounds in the autogenic and allogeneic PRF groups were notably smaller and nearly closed, with the autogenic PRF group achieving complete wound closure and minimal scar formation. Histopathological findings supported these clinical observations, showing that the autogenic PRF group had enhanced healing characterized by early scab detachment, angiogenesis, and re-epithelialization. In conclusion, this study highlights the beneficial role of PRF, particularly autogenic PRF in accelerating avulsion wound healing in a rabbit model.

Keywords | Goat, Platelet rich fibrin, Rabbits, Skin, Wounds

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INTRODUCTION

The skin is the largest organ in the body, composed of two main layers: The epidermis and the dermis. The epidermis consists of various specialized cells including melanocytes, keratinocytes, Langerhans cells, dendritic cells, other immune cells, sensory axons, and the epidermal–dermal basement membrane (Lotfollahi, 2024). Wound healing is a complex and dynamic biological process involving a well-coordinated series of cellular and molecular events aimed at restoring the integrity of damaged tissue. This process occurs in three overlapping stages: inflammation, proliferation, and maturation. Each year, advances are made in understanding the cellular responses and inflammatory mediators involved, as well as the identification of novel mediators that contribute to wound repair (Sorg *et al.*, 2017).

These days, platelet-derived products have become important and are among the most often used biological products for wound healing. Although the use of platelet derivatives for the treatment of skin wounds has a five-

decade history with various names (Ozer and Colak, 2019; Pinto *et al*., 2018).

An avulsion wound is defined as the forcible tearing away of tissue from its original anatomical location. These injuries commonly occur during traumatic events such as high-impact vehicle accidents, gunshots, or violent physical assaults. Avulsion wounds are often heavily contaminated due to the nature of the trauma, particularly in cases involving road traffic accidents, which increases the risk of infection. Prolonged hospitalization further elevates the chance of secondary infections from the environment. If not properly managed, such wounds may lead to deep tissue infections, resulting in reduced animal productivity, increased morbidity, and even mortality. Therefore, appropriate wound care is critical to accelerate healing and minimize complications (Boettcher-Haberzeth and Schiestl, 2013; Sadiq *et al*., 2015).

The subject of platelet-rich fibrin (PRF) has been quite popular in recent years and is utilized for soft tissue regeneration in a variety of medical specialties. PRF is the second generation of platelet-rich plasma, which acts as scaffold and accelerates the healing of wounds in both soft and hard tissues by trapping autologous leukocytes and platelets in a fibrin matrix network. PRF functions as a fibrin matrix, trapping growth factors, platelets, cytokines, and cells that can eventually be released. It may also work as a membrane that degrades gradually (Abd Al-Hussein *et al*., 2023; Naik *et al*., 2013).

One of the primary limitations of platelet-rich fibrin (PRF) treatment lies in its applicability to large wounds or tissue defects. According to standard protocols, approximately 5 milliliters of autologous blood is required to produce a single PRF clot, which is typically sufficient to treat only about 2–2.5 cm^3 of wound area. Consequently, treating large lesions would necessitate the collection of a substantial volume of autologous blood, which may not be feasible or safe in all cases. To overcome this limitation, non-autologous sources of PRF such as allogeneic and xenogeneic preparations have been explored for treating extensive wounds. However, when using non-autologous PRF, clinicians must thoroughly screen donor animals to prevent the transmission of infectious agents (Soares *et al*., 2024).

The aim of the present study was to investigate and evaluate the potential of PRF derived from different sources autogenic, allogenic, and xenogenic to accelerate the healing of surgically induced circular wounds on the dorsal skin of rabbits, using both clinical (wound contraction) and histopathological assessments.

MATERIALS AND METHODS

Animals

The study was approved by the Ethical Committee of the College of Veterinary Medicine, University of Basrah, in accordance with the BCVM criteria (Approval No. 80-37/2025). The experiment was conducted on ten healthy mature rabbits of both sexes (5 males and 5 females), aged between 6–10 months and weighing approximately 1.5–2.0 kg. Prior to the start of the experiment, all animals were acclimatized for five days in a well-ventilated, temperature-controlled animal facility. Each rabbit was housed individually in a 50 × 50 cm^2 cage lined with soft hay bedding, which was replaced every two days to maintain hygiene. The rabbits were provided ad libitum access to clean drinking water and fed a diet consisting of high-quality green grasses, supplemented with limited quantities of energy- and protein-rich concentrate (Clauss and Hatt, 2017; Alrafas *et al*., 2023; Al-Alywi *et al*., 2025).

Preparation of platelet-rich fibrin

A 5 ml of fresh rabbit blood was collected from the auricular vein and also samples of the goat blood were collected via jugular vein. Sterile 6-milliliter vacum tubes devoid of anticoagulants are used to collect blood. After ten minutes of centrifuging at 3000 revolutions per minute (rpm), the tubes separate into three layers: the middle fraction, which contains the fibrin clot, the top straw-colored cellular plasma, and the red bottom fraction, which contains red blood cells. After removing the top straw-colored layer, the PRF the intermediate fraction is gathered (Figure 1). This method involves centrifuging fibrinogen, which is initially concentrated in the tube's top section, to combine with the thrombin in circulation to form fibrin. Following that, a fibrin clot forms in the middle of the tube, between the red corpuscle at the bottom and the cellular plasma at the top (Al-Hussein *et al*., 2023). We collected autogenic PRF from the same rabbit blood, allogenic PRF from other rabbit blood and xenogeneic PRF from a goat blood.

Surgical procedure

The dorsum area of the rabbits was prepared surgically by clipping and shaving and disinfectant application. The operation is done under aseptic technique and general anesthesia by using intramuscular injection of a mixture of xylazine-ketamine hydrochloride (Xylazine: 5 mg/kg B.W and Ketamine: 13 mg/kg B.W. (Jassim *et al*., 2023).

Four skin marks (1 cm diameter) were created in each side of the rabbit dorsum by a tube end rinsed with methylene blue dye (the tube diameter is 1 cm) then 4 full thickness of the marked skin was removed, These wounds were divided into four groups; Normal saline treated group (as control group), autogenic, allogeneic and xenogeneic PRF groups

(Figure 2). These groups were represented in each rabbit.

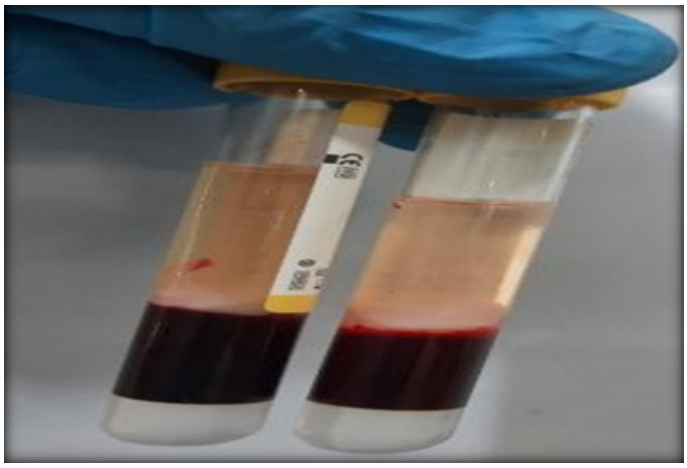

Figure 1: Platelet Rich Fibrin (PRF).

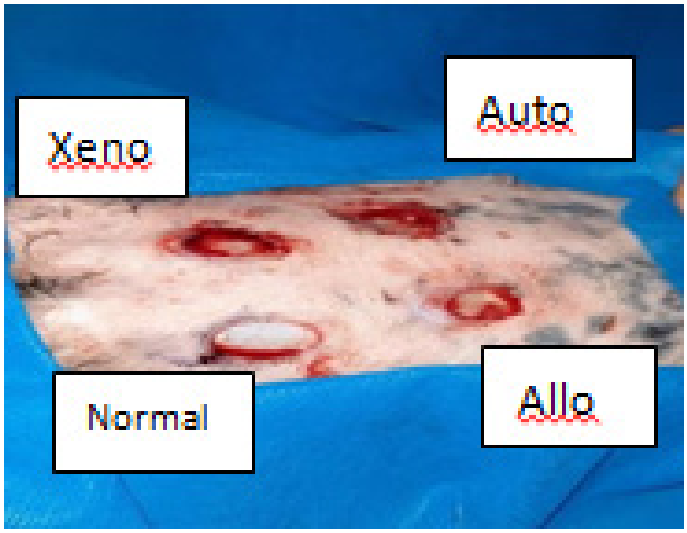

Figure 2: Wounds treated with Autogenic, Allogeneic, and Xenogeneic PRF, and normal saline.

Clinical evaluation

Wound diameters (mm) for all groups were measured on days 3, 7, 14, and 21 post-injury to assess the progression of wound healing through wound contraction analysis.

Histopathological evaluation

Skin tissue samples were collected from the wound area on days 7, 14, and 21 post-surgery. The samples were immediately fixed in 10% neutral-buffered formalin and submitted to the histopathology laboratory for processing and evaluation. Sections were stained with hematoxylin and eosin (H and E) for histopathological examination (Mohsin *et al.*, 2025).

Statistical analysis

The results were expressed as mean values ± standard errors (SE). Statistical analysis was performed using SPSS for Windows (version 22.0, IBM Corp., USA). One-way analysis of variance (ANOVA) followed by post hoc multiple comparison tests was used to determine differences among groups. A significance level of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Issue engineering aims to repair or regenerate damaged tissues resulting from disease, trauma, or aging by replacing, enhancing, or promoting the growth of new functional tissue. In recent years, platelet-rich fibrin (PRF) has gained significant attention for its potential in soft tissue regeneration across various medical fields, due to its autologous origin, ease of preparation, and ability to release growth factors that support healing (Miron et al., 2017).

The aim of the study was to evaluate healing process of surgically induced avulsion skin wounds in rabbit treated locally with a different sources of platelet rich fibrin, clinically (wound contraction) and histopathologically.

Clinical observation softhewound healing process in this study based on wound diameter, which is influence the level of wound contraction and healing process. At the 3rd and 7th day post injury, there were no significant differences between all treated groups and control normal saline group (Figure 3A). However, there is slight reduction in wound in autogenic group because the myofibroblast cells are responsible for the wound's constriction. After more than seven days following full-thickness avulsion incision, the fibroblasts that grow in the granulation tissue (at the conclusion of the proliferation stage) give rise to these cells (Jasim *et al.*, 2025; Bai and Zeng, 2023).

At 7, 14, and 30 days of follow-up, Sousa *et al.* (2020) examined the healing impact of advanced platelet-rich fibrin (A-PRF) clot membranes in palatal wounds and discovered that the A-PRF group had a greater reduction in palatal wounds than the control group. According to the findings of their investigation, A-PRF membranes speed up the healing process, significantly shorten the recovery period, and minimize discomfort following surgery.

At 14 days post-surgery, the wounds treated with autogenic PRF exhibited greater wound contraction compared to those in the allogenic, xenogenic, and normal saline groups (Figure 3B). By day 21, the autogenic PRF group demonstrated significantly enhanced wound contraction, characterized by complete wound closure and minimal scar tissue formation, as compared to the other treatment groups (Figure 3C; Table 1). These findings are consistent with those reported by Pitzurra et al. (2020), who observed that leukocyte-platelet-rich fibrin (L-PRF) and advanced platelet-rich fibrin (A-PRF) promote periodontal fibroblast migration and proliferation. Furthermore, A-PRF+ showed a more sustained wound closure effect than L-PRF.

Table 1: Wound Contraction and Diameter in Auto PRF, Allo PRF, Xeno PRF, and Normal Saline Groups at Days 3, 7, 14, and 21 post-wounding.

Post wounding day	Auto PRF	Allo PRF	Xeno PRF	Normal saline
Day 3	7.96±0.06^{Aa}	8.10±0.08^{Aa}	8.46±0.25^{Aa}	8.40±0.15^{Aa}
Day 7	7.38±0.14^{Aa}	7.56±0.07^{Aa}	8.08±0.33^{Aa}	8.02±0.26^{Aa}
Day 14	3.04±0.09^{Bb}	4.78±0.22^{Ab}	5.52±0.24^{Ab}	5.24±0.21^{Ab}
Day 21	0.00±0.00^{Bc}	2.84±0.13^{Ac}	2.86±0.27^{Ac}	2.94±0.16^{Ac}

A, B: Different uppercase letters indicate significant differences between groups (P < 0.05). a,b,c: Different lowercase letters indicate significant differences within a group (P < 0.05).

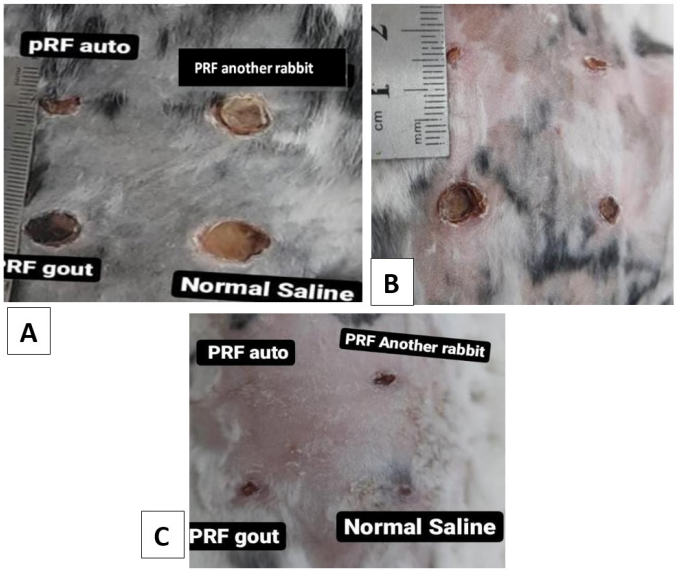

Figure 3: Wound healing and contraction at 7, 14, and 21 days postoperatively. (A) Wound healing and contraction in treated and control groups at 7 days postoperatively. (B) At 14 days postoperatively, the autogenic PRF group showed greater wound contraction and smaller wound size compared to other groups. (C) By 21 days postoperatively, significant wound contraction was observed, characterized by complete wound closure and minimal scarring in the autogenic PRF group.

In the autogenic group, full wound contraction and good wound closure are clearly visible 21 days after surgery. Concentrated platelets' capacity to deliver 6–8 times supraphysiological amounts of growth factors is thought to be the fundamental mechanism that promotes wound healing (Miron *et al.*, 2017). Additionally, the release of growth factors from platelets promotes the development and need for mesenchymal stem cells as well as other target cells that aid in the healing process (Karimi and Rockwell, 2019).

The protocol used for PRF preparation does not need any anticoagulants, wound healing process is not depressed by anticoagulants, and formation of clot occurs normally. PRF has several advantages, which involved high leukocyte concentration which not play role in antibacterial and immune responses but also enhance the process of wound healing (Barbon *et al.*, 2019).

The PRF forms a network of dens fibrin that allows degradation in a slow rate and hence delay growth factor release the surrounding tissue during healing of wound. Growth factors has been reported to be release from PRF up to seven days from almost of them (Egle *et al.*, 2021; Dohan et al., 2009). In addition to this, PRF advantages also include standardization of production protocol, less expensive, and method of its preparation is very easy (Egle *et al.*, 2021).

Histopathological results of the normal saline group after the 1st week of skin wound induction showed formation of a profoundly eosinophilic scab over the wound area of skin and detached part of scab. Furthermore, there are areas of the wound exposed and not covered by scab (Figure 4A). At the same time, the allogenic PRF group revealed a thick scab over the wound area of skin, the formation of a thickened eosinophilic basal epidermal layer, which was rich in newly formed blood vessels, and a slight edema was also observed (Figure 4B). In comparison, in the auto PRF group at 1st week after wound induction, the affected skin indicates the formation of a deep eosinophilic scab slightly detached from the beneath dermis layer, and edema was observed in the dermis layer (Figure 4C). The fourth xeno PRF group, after 1st week, wounded skin showed the formation of a deep eosinophilic scab slightly detached from the beneath dermis layer, and edema was observed in the dermis layer (Figure 4D). Moreover, infiltration of inflammatory cells and extra vascular RBCs were also observed.

These results are in agreement with the findings of Wu *et al.* (2022), who reported that PRF, an autologous blood derivative, is safe, easy to prepare, and rich in growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-beta1 (TGF-β1), and insulin-like growth factor (IGF), all of which contribute to tissue regeneration and wound healing. These growth factors initiate downstream signaling cascades by binding to the proper cell membrane receptors. These signaling pathways then enter the nucleus and participate in a variety of processes, including inflammation, collagen formation, tissue granulation, and angiogenesis. Additionally, after being activated during centrifugation, the large numbers of leukocytes contained in PRF produce both inflammatory and anti-inflammatory substances. These substances exhibit the capacity to control inflammation and fend off infection by binding to the fibrin network and lowering the immunological response.

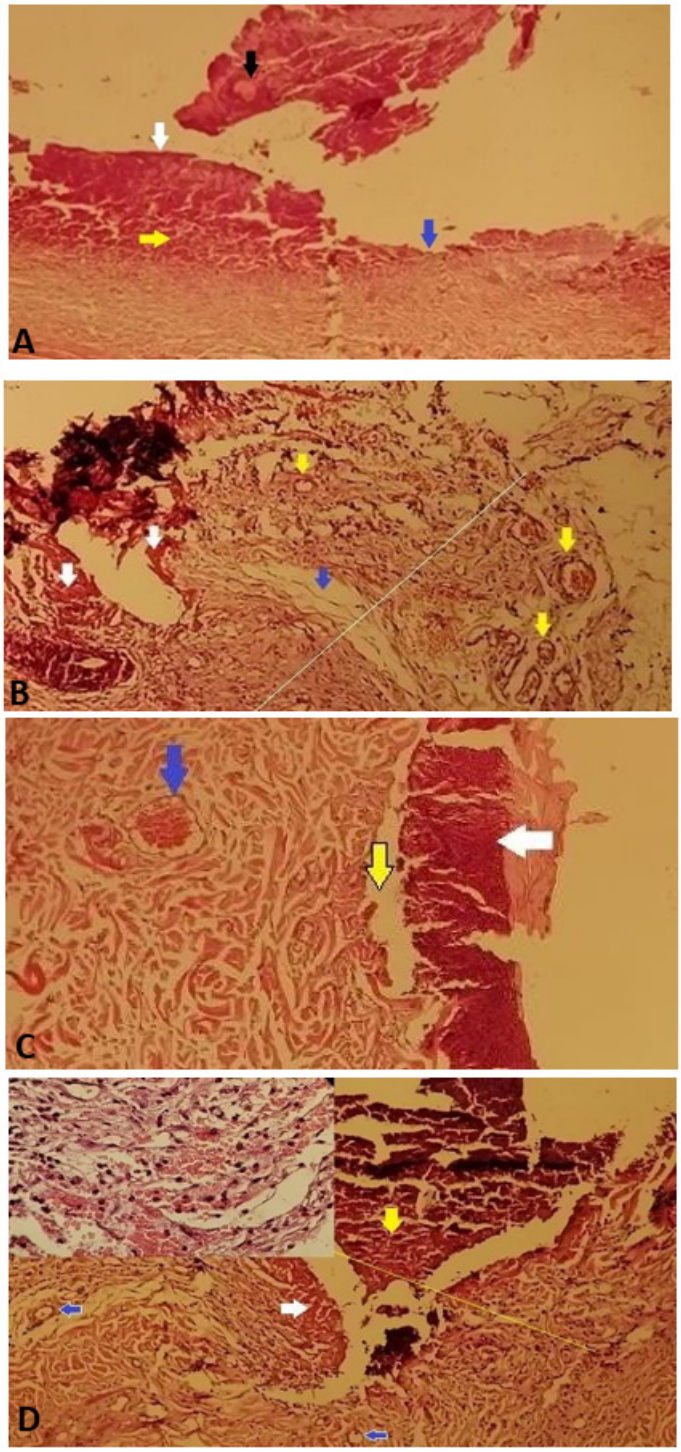

Figure 4: Histopathological sections of skin from different treatment groups at 1 week post-wounding (H and E Staining). (A) Normal Saline (NS) group: Section shows a deeply eosinophilic scab over the wound area (white arrow) with partial detachment (black arrow), and exposed wound areas not covered by scab (blue arrow). H and E, 40×. (B) Allogeneic PRF group: Section shows a thickened eosinophilic basal epidermal layer (white line), rich vascularization (yellow arrows), and slight dermal edema (blue arrow). H and E, 400×. (C) Autogenic PRF group: Section shows a deep eosinophilic scab (white line) slightly detached from the underlying dermis (yellow arrows), with noticeable dermal edema (blue arrow). H and E, 400×. (D) Xenogeneic PRF group: Section shows a deep eosinophilic scab (yellow arrow) partially detached from the epidermis (white arrow), along with dermal edema, newly formed blood vessels (blue arrow), infiltration of inflammatory cells, and extravasated red blood cells (yellow line). H and E, 100×.

The normal saline-treated group's histological alterations in the second week revealed the development of a highly eosinophilic scab over the skin wound region. A thin regenerated epidermis layer covered the wound area, and newly formed blood vessels in the dermis layer were seen. Also, infiltration of few inflammatory cells was there (Figure 5A). The autogenic RPF group at the same period showed a deeply eosinophilic scab over the wound area of skin, a thin regenerated stratum basal epidermis layer, and the dermis layer showed mild edema that was rich in newly formed congested blood vessels. The autogenic PRF group at the 2nd week revealed the continuous presence of the thick eosinophilic scab over the wound area of skin, a thin regenerated stratum basal epidermis layer was evident, and newly formed blood vessels in the dermis layer also were clear (Figure 5B). The allogenic RPF group at the 2nd week showed the continuous presence of the profoundly eosinophilic scab that covers the wound area of skin, and the thin regenerated stratum basale and stratum spinosum epidermis layer were evident. Also, mild edema was present between the regenerated stratum basal layer and dermis. However, the allogenic PRF-group indicated the formation of a thin eosinophilic scab partially detached from the beneath layer; the wound area was not covered by the epidermis, reflecting an incomplete wound healing process (Figure 5C). Additionally, a small number of inflammatory cells and fibroblasts were shown to accumulate. The second-week group of xenogenic RPF revealed a thin layer of regenerated stratum basal epidermis and a deeply eosinophilic scab over the wound region of skin. In addition, the development of the epithelial tongue with dermal edema was seen, the group of xenogenic PRF showed a thick eosinophilic scab covering all wound areas and a thick layer of stratum basal epidermal layer (Figure 5D).

The platelet distribution and fibrin properties of PRF are more akin to the body's reaction to the wound and bending macroscopic structures. The procedure creates a three-dimensional fibrin matrix, which is helpful for collecting growth factors (scaffolding) and platelet clot formation. Growth factors are localized with scaffolding to enhance tissue regeneration (Reksodiputro *et al.*, 2018).

By the third week post-surgery, the normal saline group exhibited a fully regenerated epidermal layer, with the dermis showing newly formed blood vessels and hair follicles (Figure 6A). In contrast, the allogenic PRF group

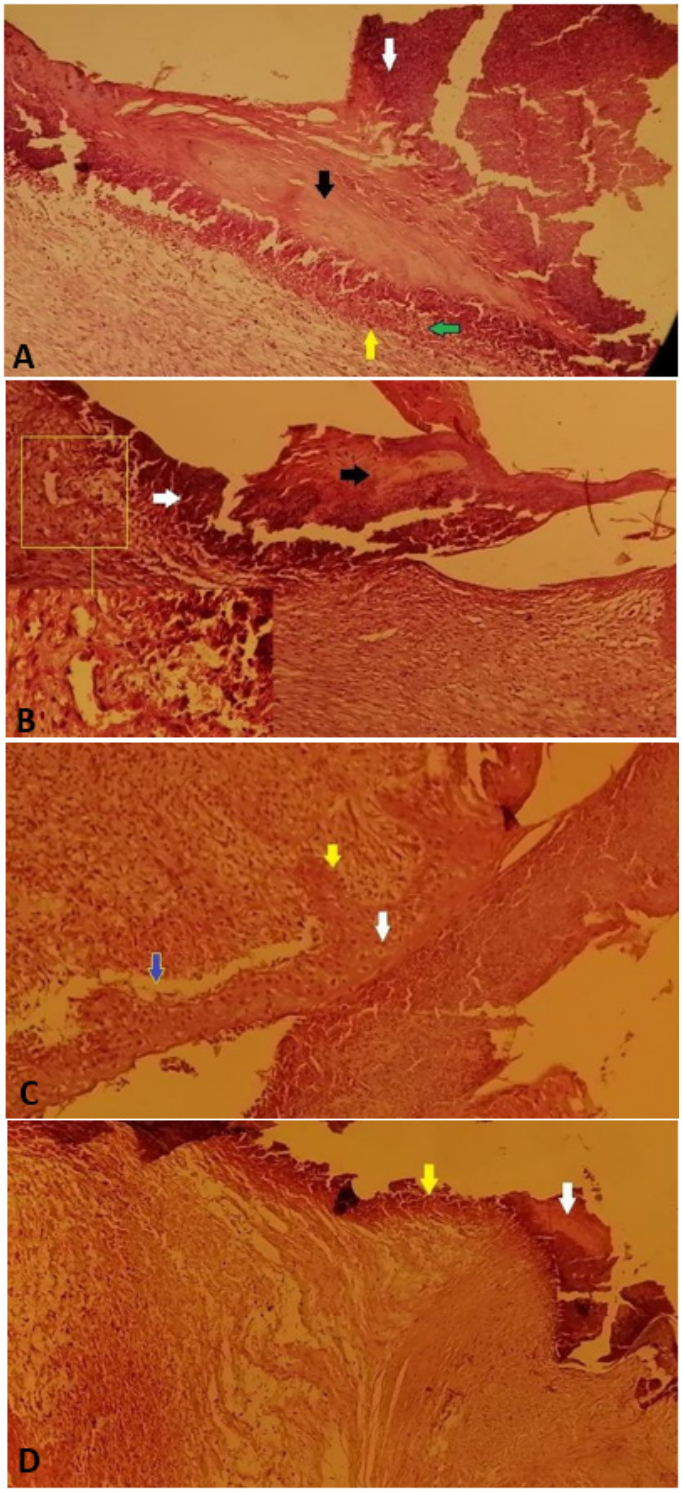

Figure 5: Histopathological Sections of Skin from Different Treatment Groups at 2 Weeks Post-Wounding (H and E Staining). (A) Normal Saline (NS) group: Section shows a profoundly eosinophilic scab covering the wound area (white arrow), a thick layer of granulation tissue (black arrow), and a thin regenerated epidermis with stratum basale (yellow arrow) and stratum spinosum (green arrow). The dermis exhibited mild edema. H and E, 100×. (B) Autogenic PRF group: Section reveals a deeply eosinophilic scab (black arrow) over the wound, a thin regenerated stratum basale (white arrow), and dermis showing mild edema with abundant newly formed congested blood vessels (yellow box). H and E, 400×. (C) Allogeneic PRF group: Section displays a profoundly eosinophilic scab and a thin regenerated epidermis comprising stratum basale (yellow arrow) and stratum spinosum (white arrow). Mild edema is observed between the regenerated stratum basale and dermis (blue arrow). H and E, 100×. (D) Xenogeneic PRF group: Section shows a deeply eosinophilic scab (white arrow), a regenerated stratum basale (yellow arrow), dermal edema, and formation of an epithelial tongue. H and E, 100×.

showed incomplete regeneration of the epidermal and dermal layers. This included a thin, regenerated stratum basale, a thick eosinophilic scab covering the wound area, signs of edema, and the formation of an epithelial tongue in the dermis. Additionally, there was a notable accumulation of collagen fibers and the presence of newly formed blood vessels (Figure 6B). The autogenic PRF group displayed a complete and structurally normal epithelial layer, along with newly developed hair follicles and blood vessels in the dermis, indicating that the healing process was nearly complete (Figure 6C). Similarly, the xenogenic PRF group showed full re-epithelialization, with the dermis containing fresh blood vessels and developing hair follicles (Figure 6D).

The remodeling phase starts around three weeks after an accident and lasts for over a year. An area rich in collagen and other extracellular matrix deposition (ECM) proteins remains after all activities that were triggered during the healing stages are halted and macrophages, isolated endothelial cells, and myofibroblasts either undergo apoptosis or are removed from the wound (Tottoli *et al.*, 2020).

CONCLUSION

Platelet-rich fibrin is considered more effective in tissue regeneration due to its simple, cost-effective preparation protocol that does not require the use of anticoagulants, unlike platelet-rich plasma (PRP). Among the different sources, autologous PRF has demonstrated superior efficacy in enhancing the wound healing process particularly in promoting scab detachment, angiogenesis, epidermal regeneration, and wound contraction compared to allogenic and xenogenic PRF.

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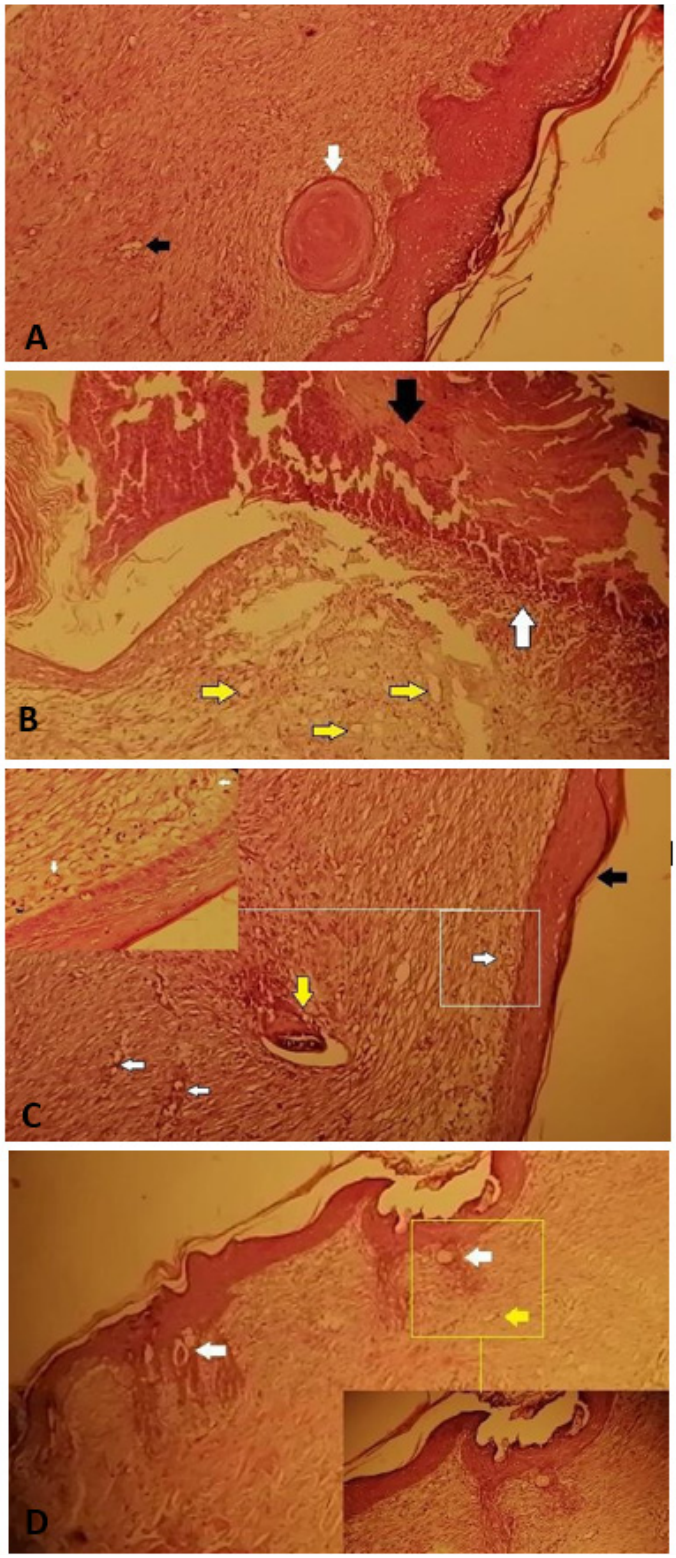

Figure 6: Histopathological Sections of Skin from Different Treatment Groups at 3 Weeks Post-Wounding (H and E Staining). (A) Normal Saline (NS) group: Section shows a completely regenerated epidermis with newly formed hair follicles (white arrow) and blood vessels (black arrow) in the dermis. H and E, 400×. (B) Allogeneic PRF group: A thin regenerated stratum basale (white arrow) is observed in the epidermis, along with newly developed blood vessels (yellow arrows), a thick eosinophilic scab covering the wound area (black arrow), and dermal edema. H and E, 400×. (C) Autogenic PRF group: Section shows a fully developed epithelium layer (black arrow); the dermis contains newly formed blood vessels (white arrows) and hair follicles (yellow arrow). H and E, 100×. (D) Xenogeneic PRF group: Section reveals a complete epithelial layer with the dermis displaying freshly formed hair follicles (white arrows) and blood vessels (yellow arrows). H and E, 100×.

NOVELTY STATEMENT

The novelty of this study lies in its direct comparative evaluation of platelet-rich fibrin (PRF) derived from autologous, allogeneic, and xenogeneic sources in a rabbit avulsion wound model. While PRF's therapeutic potential is well-documented, most studies focus on a single-source application. This research uniquely assesses differences in wound healing efficacy, angiogenesis, cellular regeneration, and inflammatory response among PRF sources. By identifying the most effective PRF type, the study addresses a critical gap and contributes to optimizing wound management strategies in both veterinary and human medicine.

AUTHOR'S CONTRIBUTION

All authors eqully contributed.

Generative AI and AI-assisted technology statement

The authors confirm that no Generative AI or AI-assisted technology was used in any aspect of this manuscript's preparation.

Conflict of interest

The authors have declared no conflict of interest.

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