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PLATELET-RICH PLASMA AS AN ADJUVANT IN FULL-THICKNESS SKIN WOUND HEALING IN RABBITS

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ABSTRACT

Platelet-rich plasma (PRP) is an autologous concentration of platelets containing various growth factors that play a crucial role in tissue repair and regeneration. It has been widely investigated in various fields, including orthopedics, dentistry, and plastic surgery, for its potential to enhance healing processes. However, its efficacy in accelerating full-thickness skin wound healing remains a topic of ongoing research. This study aimed to evaluate the impact of PRP on wound closure in a rabbit model. Two full-thickness skin wounds (2-cm-diameter) were induced on the backs of 12 rabbits. Each animal received 0.5 ml of PRP on one wound, whereas the other wound served as a control. Wound healing was assessed grossly, morphometrically by digital imaging and histopathologically for 4 weeks. PRP significantly accelerated wound healing, with the PRP-treated group showing 58.17% closure by week 2 compared to 30.17% in controls. By week 4, both groups achieved near-complete closure (97.87% for PRP vs. 97.83% for control), but PRP demonstrated faster healing initially. Histopathological analysis at 2 weeks showed complete re-epithelialization and reduced inflammation in the PRP group, whereas the control group exhibited granulation tissue with infiltration of inflammatory cells and proliferation of fine capillaries (neo-vascularization). At 4 weeks of wound induction, the PRP-treated group showed increased epithelial coverage accompanied by hair follicle proliferation, while controls had an epithelial layer but no hair follicle proliferation. In conclusion, PRP significantly accelerates wound healing, particularly during the early phases, highlighting its potential in improving skin wound repair.

Keywords: Platelet-rich plasma, Skin wounds, Re-epithelialization, Wound healing.

INTRODUCTION

Worldwide, skin wounds pose a serious medical and societal burden (Laurano *et al.*, 2022). For example, wounds are thought to cost the United States more than $25 billion a year, which greatly raises the expense of healthcare (Veith *et al.*, 2019). Furthermore, skin wounds and their subsequent care present a substantial cost issue in the veterinary sector, particularly given that nearly three quarters of wounds in various animal species heal by secondary intention (de Macêdo *et al.*, 2018; Kožár *et al.*, 2018; Owen *et al.*, 2012). Common causes of cutaneous wounds include car accidents,

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flame or heat burns, surgical injuries, exposure to sharp objects, animal attacks, and wounds from firearms (Pavletic, 2018). One of the main causes of delayed wound healing is bacterial contamination, often resulting from delayed wound care (Kožár *et al.,* 2018). Many topical medicinal substances, including phenytoin (Qunaibi *et al.,* 2009), honey (Bergman *et al.,* 1983), and yeast cell derivatives (Crowe *et al.,* 1999), have been used to treat wounds.

Platelet-rich plasma (PRP) is an adhesive autologous substance that concentrates a lot of platelets in a small amount of plasma (Marx, 2004). By mimicking the final stage of the coagulation cascade, this product creates a fibrin clot that quickly solidifies and adheres to the application site (Luengo *et al.,* 2006). Because of its biocompatible and biodegradable characteristics, PRP cannot cause severe fibrosis, tissue necrosis, or foreign body reactions (Dijkstra-Tiekstra *et al.,* 2005). Human PRP has been utilized in a variety of treatments in fields including orthopedics (Oyama *et al.,* 2004), dentistry (Carlson & Roach Jr, 2002), ophthalmology (Duchesne *et al.,* 2001), and maxillofacial surgery (Moghe *et al.,* 2012). So, the aim of the current study is to evaluate the effects of local application of platelet-rich plasma on the healing of full-thickness skin wounds in rabbits.

MATERIALS AND METHODS

Ethical approval

The study was approved by the Assiut Veterinary Medicine Research Ethics Committee (No. 06/2024/0254) in compliance with OIE guidelines on animal welfare and Egyptian laws regarding the care and use of animals in research and teaching.

Animals

The study was carried out on 12 adult healthy rabbits (6 males and 6 females) aged 8 – 12 months and weighing 1.5 - 2 Kg. The animals were housed in standard cages, provided with a balanced ration, and given *ad libitum* access to water.

PRP preparation

Autologous blood samples (5 mL) were collected from the jugular vein into two tubes containing sodium citrate. The samples were centrifuged for 5 minutes at 1,100 rpm. The plasma and buffy coat layers were transferred into a plain tube and centrifuged again for five minutes at 2,500 rpm. The PRP layer was collected, and the remaining plasma was discarded.

Wound induction

Food and water were withheld for 12 hours before surgery. General anesthesia was induced and maintained by isoflurane 5% (Anahal®; PHARCO, Egypt) in 100% oxygen (2 L/min) using non-rebreathing system. The absence of jaw tone and ear-pinch reflexes confirmed anesthesia within 1–3 minutes. Rabbit back was prepared for aseptic surgery; clipped, shaved, disinfected several times with alcohol and Povidone iodine (BETADINE, El-Nile Co. for Pharmaceutical and Chemical Industries, Egypt). Two circular 2-cm-diameter full thickness skin wounds were inflicted on the back with a minimum distance of 2 cm between each wound.

Wound management

The right wounds were treated subcutaneously with PRP (0.5ml) using a syringe, whereas the left wounds were treated with normal saline as a control. After the procedure, the rabbits were placed in a recovery box for 10 minutes, before being transferred back to their cages for monitoring.

Gross examination

Wounds were grossly inspected for the presence of hemorrhage, edema, inflammatory exudate, granulation tissue formation, scar formation, epithelialization, and wound healing time. To monitor the healing process, digital photographs of the wounds

were taken using a digital camera with a uniform ruler incorporated into every image to facilitate digital calibration. The camera was positioned at least 30 cm away from the wound, which was centered in the frame. Photos were analyzed by (ImageJ program 4.48v, National Institutes of Health, USA).

Histopathological examination

After euthanasia of the rabbits (6 rabbits at each time point) by exsanguination, full thickness wound samples, including surrounding tissue, were obtained 2- and 4-weeks post-wound creation. The collected wound samples were placed in neutral buffered formalin solution (10%) for fixation, dehydrated in a sequence of increasing ethyl alcohols, cleared with xylene, and implanted in paraffin blocks. Hematoxylin and eosin (H&E) staining was applied to 5-µm-thick tissue slices for histopathological analysis (Marques *et al.*, 2004). Each stained section was examined using a leitz Dialux 20 microscope, and images were captured using a digital camera (Cannon powershot A95) attached to the microscope.

Statistical analysis

The data of wound closure percentages are expressed as means ± standard deviation (SD) and were analyzed using Student's t-test to compare the differences between the PRP-treated and control groups. A significance level of $P<0.05$ was considered statistically significant.

RESULTS

Gross appearance

At the baseline (time of wound induction), the wounds appear fresh with clear exposure of underlying tissues. Minimal signs of clot formation were visible, indicating the immediate impact of the wound creation process.

After 2 weeks, significant differences in the healing were observed between the groups. In the treated group (PRP), granulation tissue formation was prominent, characterized by a bright red appearance and visible vascularization, suggesting active tissue repair and angiogenesis. The control group exhibited slower healing with incomplete coverage of the wound bed and less pronounced granulation tissue, indicating delayed re-epithelialization and wound contraction (Figure 1).

After 4 weeks, the PRP group showed nearly complete closure of the wound with minimal scarring; reflecting accelerated healing and advanced tissue remodeling. The skin surface appears smoother with reduced redness, indicating a transition to the maturation phase. In contrast, the wounds remained partially closed, with residual redness and incomplete epithelial coverage, highlighting a slower healing process (Figure 1).

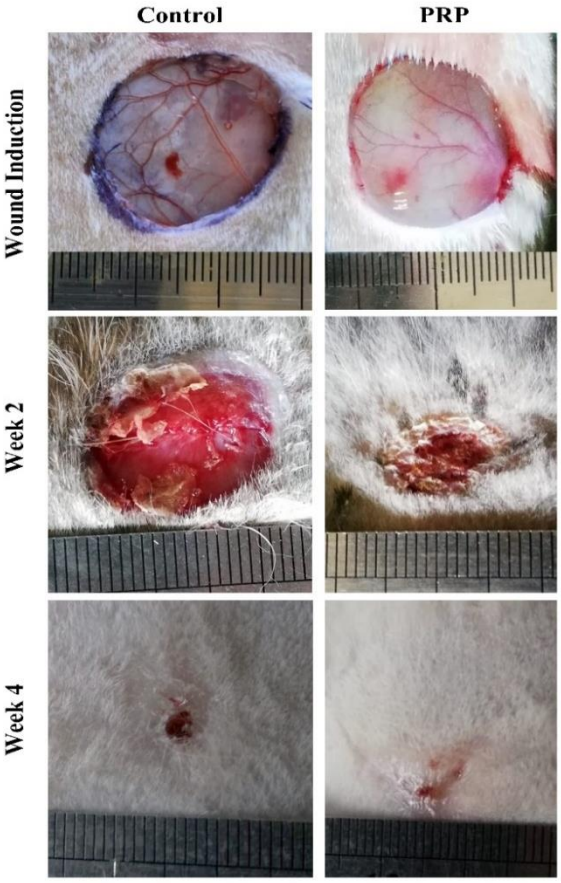

Figure 1: Gross appearance of skin wounds in two groups (Control vs. PRP) at two time points (2 and 4 weeks), illustrating the effect of PRP on skin wound healing.

Over the course of the two-week treatment period, there was a noticeable increase in the percentage of wound closure in rabbits treated with PRP compared to the control group. On week 2, the PRP-treated group showed significantly ($P<0.05$) greater wound closure (58.17 ± 2.82%) compared to the control group (30.17 ± 3.9%). By week 4, both groups had a substantial improvement in wound healing, with nearly complete closure observed in the control group (97.83 ± 1.9%) and the PRP-treated group (97.87 ± 1.7%).

Histopathological findings

After 4 weeks of wound induction, the PRP-treated group showed increased epithelial thickness and proliferation of hair follicles extending into the dermis. Fibroblast activity was also evident, with increased collagen fiber deposition in the dermis.

Our results demonstrated that after 2 weeks of wound induction, the PRP-treated wounds were completely covered by a single layer of epithelium, topped with a keratin layer. Additionally, the inflammatory reaction was reduced, and irregular collagen fiber deposition was observed in the dermis (Figure 2).

On the other hand, at two weeks post-wound induction, the control group exhibited granulation tissue formation characterized by the proliferation of fine capillaries (neo-vascularization) and infiltration of inflammatory cells.

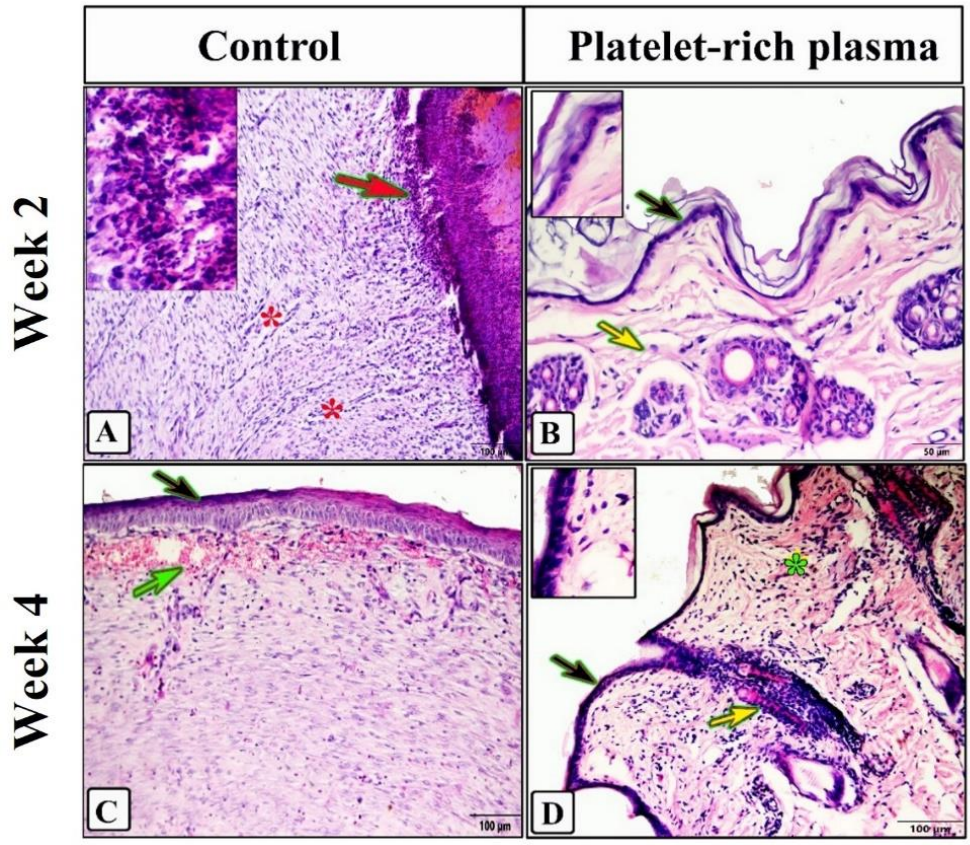

Figure 2: Paraffin sections from both groups stained by H&E. A: Control group after 2 weeks of wound induction showing granulation tissue formation (red arrow), with leucocytic infiltration (inset) and neovascularization (star). B: PRP group after 2 weeks of wound induction showing re-epithelization of the wound (black arrow and box inset) and deposition of irregular collagen fibers in the dermis (yellow arrow). C: Control group after 4 weeks of wound induction showing an epithelial layer covering the wound (black arrow) and hemorrhage beneath the epidermis (green arrow). D: PRP group after 4 weeks of wound induction showing increased epidermal thickness (black arrow and box inset), proliferation of hair follicles (yellow arrow), and enhanced collagen fibers (star).

In the control group, although the wound was covered by an epithelial layer at four weeks, there were no signs of hair follicle proliferation. Hemorrhage was observed beneath the epidermis, indicating incomplete or less effective healing compared to the PRP-treated group. As, the use of PRP improved skin wound healing and enhancement of re-epithelialization.

DISCUSSION

This study aimed to evaluate the efficacy of PRP on epithelization and promoting effective wound healing in rabbits. The findings demonstrated that PRP significantly improved skin wound healing and accelerated re-epithelialization. These results align with those (Badis *et al.,* 2018), who reported that PRP facilitated effective re-epithelialization in sheep skin wounds (Anitua, 1999; Swaim *et al.,* 2001).

Re-epithelialization is required for efficient skin wound closure. Several researchers have reported that PRP treatment accelerates the re-epithelialization process and epidermal differentiation in acute wounds in dogs (Farghali *et al.,* 2017; Jee *et al.,* 2016). In the current study, PRP significantly increased the thickness and length of neo-epidermal tissue, leading to faster wound closure. By the second week, the PRP-treated group exhibited a significantly higher wound closure rate than the control group. Comparatively, Badis and Omar (2018) reported a low rate of wound contraction in sheep over the same period (Badis & Omar, 2018). PRP's efficacy in promoting wound healing is likely due to its ability to induce the production of key growth factors, such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth factor-beta (TGF-β), and insulin-like growth factor-1 (IGF-1). Xu *et al.* (2020) demonstrated that PRP enhances IGF-1 production in wound tissues, contributing to accelerated re-epithelialization (Xu *et al.,* 2020).

In addition to promoting wound closure, the present study observed significant hair follicle development in the PRP-treated group. This aligns with findings from Xu *et al.,* who noted that PRP promotes the regeneration of skin appendages (Xu *et al.,* 2020). Other studies have shown that PRP positively affects hair growth cycles, aiding in hair follicle reconstruction (Gentile & Garcovich, 2020; Paththinige *et al.,* 2020; Zhu *et al.,* 2020).

Moreover, intradermal PRP injections have been reported to enhance hair follicle formation in goat skin wounds. This may be attributed to PRP's ability to stimulate the production of alkaline phosphatase and chondroitin sulfate proteoglycan, both of which promote the proliferation of dermal papilla cells, critical for hair follicle development (Ferdousy *et al.,* 2013; Wang *et al.,* 2017).

CONCLUSION

PRP therapy significantly enhances the healing of full-thickness wounds by reducing the rate of contraction, while promoting angiogenesis and accelerating epithelial migration. These findings underscore the potential of PRP as an effective treatment for improving wound healing. Future research with larger sample sizes is recommended to increase statistical power and further validate these results.

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تعتبر البلازما الغنية بالصفائح الدموية تركيزا ذاتيا للصفائح الدموية التي تحتوي على عوامل نمو مختلفة تلعب دورًا هاما في إصلاح الأنسجة وتجديدها والذي بدوره شجع العلماء باجراء البحوث عليه في مجالات مختلفة بما في ذلك جراحة العظام وطب الأسنان والجراحة التجميلية وذلك لقدرته على تعزيز عمليات الشفاء، ومع ذلك فإن فعاليته في تسريع شفاء جروح الجلد كاملة السُمك تظل موضوعًا للبحث المستمر، لذلك هدفت هذه الدراسة إلى تقييم تأثير البلازما الغنية بالصفائح الدموية على شفاء الجروح في الأرانب كنموذج لحيوانات التجارب. تم إحداث جرحين جلديين بسمك كامل (قطر 2 سم) على منطقة الظهر في اثني عشر أرنبًا، وحقن كل حيوان (0.5 مل) من البلازما الغنية بالصفائح الدموية تحت الجلد في جرح واحد، في حين مثل الجرح الآخر المجموعة الجروح الضابطة، وتم تقييم التئام الجروح ظاهريا وقياسيا عن طريق التصوير الرقمي وبالفحص النسيجي للجروح لمدة 4 أسابيع من إحداث الجروث، وتم الفحص النسيجي بعد الموت الرحيم لستة أرانب في أسبوعين والستة المتبقية في 4 أسابيع لتقييم تكون الظهارة وانقباض الجرح والشفاء وتواجد الأوعية الدموية. أظهرت النتائج ان العلاج بالبلازما الغنية بالصفائح الدموية ادى إلى تسريع عملية شفاء الجروح بشكل ملحوظ، حيث أظهرت المجموعة المعالجة بالبلازما الغنية بالصفائح الدموية إغلاقًا بنسبة 58.17% بحلول الأسبوع الثاني مقارنة بـ 30.17% في المجموعة الضابطة، و حققت كلا المجموعتين إغلاقًا شبه كامل (97.87% للبلازما الغنية بالصفائح الدموية مقابل 97.83% للضابطة) بحلول الأسبوع الرابع، لكن أظهرت البلازما الغنية بالصفائح الدموية شفاءا أسرع في البداية، وأظهر التحليل النسيجي المرضي بعد أسبوعين إعادة تكوين الظهارة بشكل كامل وانخفاض الالتهاب في مجموعة البلازما الغنية بالصفائح الدموية، في حين كان لدى المجموعة الضابطة أنسجة حبيبية مصابة بالالتهاب وعدم إعادة تكوين الظهارة. بحلول الأسبوع الرابع، أظهرت الجروح المعالجة بالبلازما الغنية بالصفائح الدموية زيادة في سمك الظهارة، وتكاثر بصيلات الشعر، وترسب الكولاجين المعزز، في حين كان لدى المجموعة الضابطة طبقة ظهارية ولكن لم يكن هناك تكاثر لبصيلات الشعر وعلامات النزف. خلصت الدراسة الى ان العلاج بالبلازما الغنية بالصفائح الدموية يؤدي الى تسريع عملية شفاء الجروح بشكل كبير، خاصة خلال المراحل المبكرة، مما يسلط الضوء على إمكاناته في تحسين شفاء جروح الجلد.