



(CASE REPORT)



PLATELET-RICH PLASMA'S IMPACT ON RABBIT WOUND HEALING: A HISTOPATHOLOGICAL INVESTIGATION

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ABSTRACT

The purpose of the study was to assess the positive benefits of platelet-rich plasma (PRP) on rabbit wound healing. A control group and a treatment group, each with nine rabbits, were randomly selected from among eighteen healthy adult male rabbits. A knife was used to make a 3 cm skin incision on each animal's back using aseptic methods. After that, the incision was promptly sutured using a straightforward interrupted approach and silk suture material (0.2 USP). Following the procedure, 1 milliliter of PRP was injected into the wound site in the treated group, whereas no therapy was administered to the control group. Histopathological analysis was used to track the skin's healing process at one, two, and three weeks after the procedure. One week after the procedure, observations in the treated group showed granulation tissue production together with epithelium regeneration. In addition to tissue desquamation, granulation tissue growth, and the creation of new blood vessels, there was a noticeable, severe inflammatory infiltration by the second week. The findings showed collagen deposition and fibroblast proliferation by the third week, indicating more progress in the healing process. Histopathological analysis of the control group during the first week shows a significant inflammatory infiltration along with bleeding and desquamated tissue. There is proof of a significant acute inflammatory infiltration two weeks after surgery. Findings show a persistent inflammatory infiltration with tissue debris and large cells by the third week following surgery. It is noticed that the treatment group's healing process outperforms the control group's.

Keywords: Rabbits, Wound Healing, Platelet Rich Plasma.

1 INTRODUCTION

The largest and most noticeable organ is the skin, which makes about 15–20% of the total weight. It gets more than one-third of the body's blood supply at a flow rate of 300 milliliters per minute. Healthy skin is composed of two layers: the dermis and the epidermis. The loose connective tissue layer known as the hypodermis, is located underneath the dermis. Protection, temperature control, waste product removal, vitamin D production, feeling, and communication are the six main roles of the skin. (1).

If a wound is not treated right away, it might deteriorate owing to subsequent bacterial infections and become lethal. Wound problems may also result from improper angioblast, fibroblast, and epithelial tissue growth. Numerous variables can interfere with one or more stages of wound healing, resulting in improper or inadequate tissue restoration. Impaired healing wounds, such as chronic and delayed acute wounds, frequently do not go thru the typical stages of healing. A delayed, insufficient, or disorganized healing process often causes these wounds to go into a condition of pathological inflammation(2). Ulcers caused by pressure, ischemia, diabetes, or venous stasis account for most chronic wounds.

Sometimes wounds take longer to heal, which puts additional strain on homeowners. By promoting growth, fibroblast activity, and the epithelialization of wounds, a range of biomaterials, proteins, antibiotics, vitamins, and minerals seem as ways to expedite wound healing. (2,3,4,5). Platelets are unquestionably a natural source of several growth hormones and cytokines that are essential for bone mineralization, tissue healing, and blood clotting. (6,7,8,9). The next economical source of proteins that significantly improve cellular regeneration and hemostasis is platelet-rich plasma (PRP)(11,12,13). Using PRP (Platelet-Rich Plasma), especially in gel form with a range of bioactive ingredients, offers a unique approach to tissue regeneration. (10,14). This PRP gel enhances the organization of dermal collagen in new wounds and encourages the quick differentiation of connective tissue(15). Remodeling growth factor- β 1 (TGF- β 1), platelet-derived growth factor (PDGF), fibrinogen, epidermal growth factor (EGF), histamine, and hydrolytic enzymes are all released when platelets degranulate. (16,17). These elements contribute to the angiogenic cascade, which promotes the healing of both soft and hard tissue wounds(7,9).

2 MATERIALS AND METHOD

2.1 Experimental Animals

A cohort of 18 adult male rabbits of a local breed, all exhibiting apparent health, was selected for this investigation. Prior to commencing the experimental protocols, each animal underwent a comprehensive clinical evaluation through physical examination. Throughout the duration of the study, the rabbits were housed in metal enclosures measuring 30x70x60 cm within an air-conditioned facility at the animal house. They were provided with unrestricted access to both water and food. A two-week acclimatization period was observed for the subjects, during which a prophylactic medication was administered. Subsequently, the animals were stratified into two equal groups: a control group and a treatment group. The control group did not receive any form of treatment. For histopathological analysis aimed at assessing wound healing progression, tissue specimens were collected from three animals' damaged skin from each group at intervals of 1, 2, and 3 weeks postoperatively.

2.2 Surgical Procedure

An intramuscular injection of a xylazine-ketamine mixture (50 mg/kg body weight of ketamine and 10 mg/kg body weight of xylazine)(11) was used to perform surgical operations under general anesthesia. Aseptic preparation was done at the operative site. Using a knife, a 3 cm skin incision was created on the dorsal side. A straightforward interrupted approach was used to quickly close the wound using silk suture material, size 0.2 USP.

Following surgery, one milliliter of PRP was injected directly into the wound site of the treated group, while the control group got no therapy at all.

2.3 PRP Preparation

Using 3 mL disposable syringes, three milliliters of blood were drawn from each rabbit. After that, the samples were put into anticoagulant tubes with 0.35 mL of a 10% sodium citrate solution. The blood samples were first centrifuged for 10 minutes at room temperature at a speed of 160 rpm. Each sample showed two different strata after this first centrifugation procedure. Packed red blood cells made up the bottom, red-colored layer, while the plasma component was seen in the higher, straw-yellow layer. The buffy coat, the coating on top of the densely packed red blood cells, was rich in leukocytes and platelets. Both the plasma and buffy coat were transferred into new sterile tubes. In order to achieve a higher concentration of platelets, the remaining blood components underwent a second round of centrifugation at 160 rpm for an additional two minutes.

A second centrifugation was performed on the plasma and Buffy coat for 15 minutes at 400 rpm. As seen in Figure 5, this technique produced two layers: the lower third of the sample retained platelet-rich plasma (PRP), while the upper two-thirds were discarded as platelet-poor plasma (PPP). Furthermore, according to Maghsoudi et al. (2015), the platelets were activated by adding 0.05 ml of a 10% calcium chloride solution for every 1 ml of PRP.

3 RESULTS

3.1 Control group

When a piece of skin from the control creatures that are positive. is inspected one week following surgical incision, it shows a region with hemorrhage (H), desquamated tissue (DE), and severe inflammatory infiltrate (INF). Two weeks later, The skin of the control positive group had a region of significant acute inflammatory infiltration (INF) at the site of incision. The same group exhibits tissue debris (TD) and an area of severe chronic inflammation (CIN) at the wound site by the third week. An region of severe chronic inflammation (CIN) with giant cells (GS) is also highlighted in another part from the third week.

3.2 PRP treated group

Figure (1) illustrates how granulation tissue developed at the skin incision site in a slice of skin from Granulation tissue was seen in the PRP-treated group after a week. This finding is demonstrated in Figure 2. with epithelial regeneration at the wound site in another portion from the same group after one week. As shown in figure (3), skin slices of PRP-treated mice two weeks after therapy show the surgical incision site with a notable inflammatory infiltration, desquamated tissue, granulation tissue, and newly created blood vessels. Figure (4) illustrates fibroblast proliferation with collagen deposition at the wound site by the three-week mark. Figure (5) shows comparable fibroblast proliferation with collagen deposition on another slide

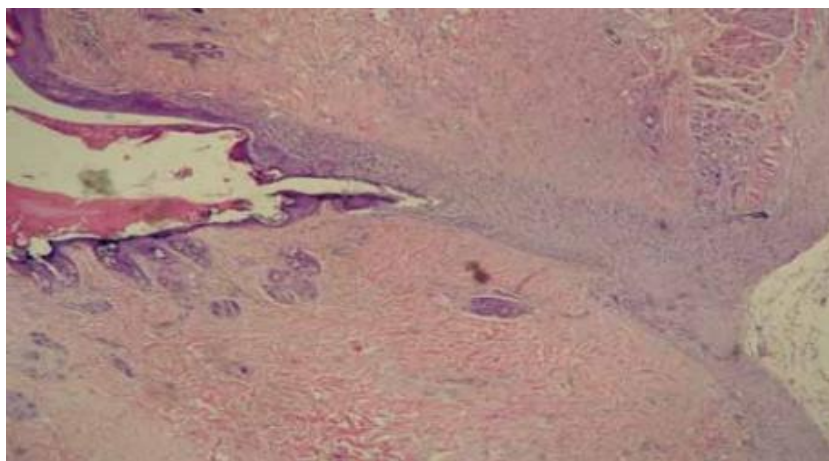


Figure 1: PRP: At 50X magnification with H&E staining, Figure (1) illustrates the formation of granulation tissue at the skin wound site in the group treated with PRP after a week.

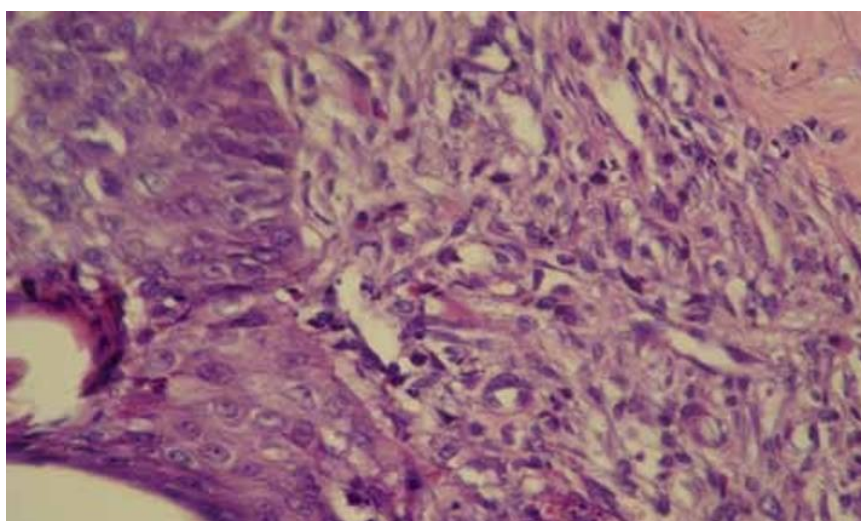


Figure 2 illustrates the formation of granulation tissue (GT) at the site of skin wound with epithelial regeneration (ER) H & E 500X in the skin of the PRP-treated group following one weak.

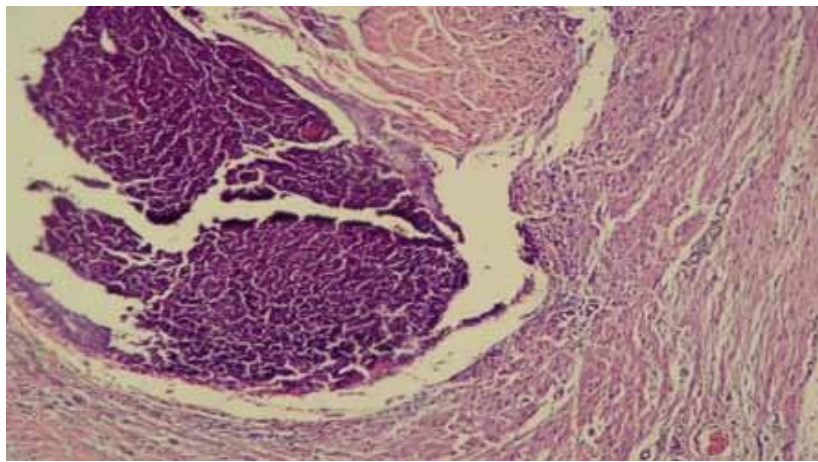


Figure 3: After two weeks, a section of the skin of animals treated with PRP shows the location of the surgical incision. It has a severe inflammatory infiltration (INF), A) 50 H & E, granulation tissue (Gr), and newly formed blood vessels (DE).

In the skin of the group treated with platelet-rich plasma after three weeks,

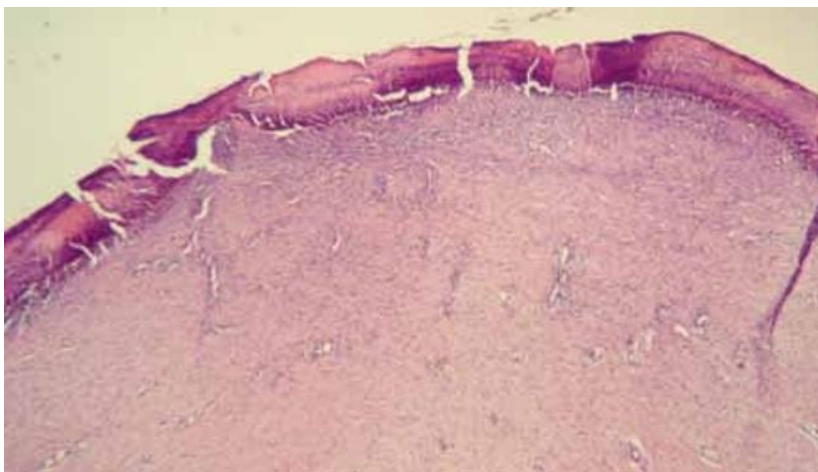


Figure 4: The growth of fibroblasts and the deposition of collagen at the site of a skin wound as seen by Hematoxylin and Eosin staining at 50X magnification.

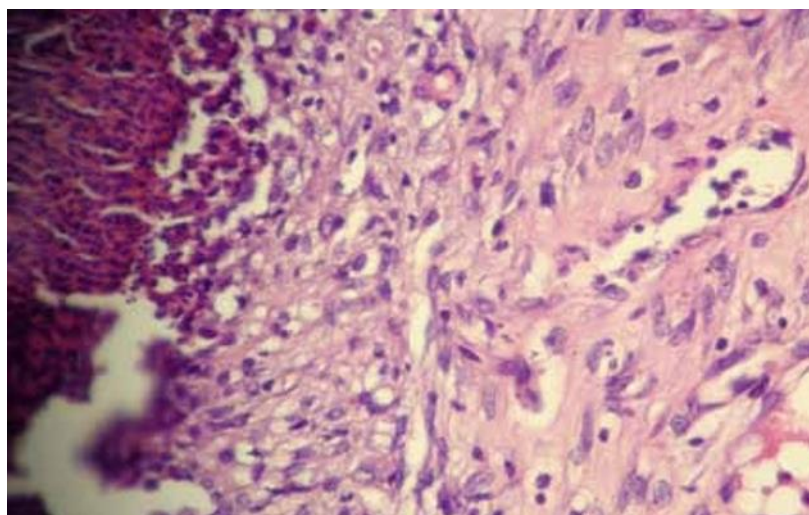


Figure 5: Demonstrates fibroblast (FB) proliferation and collagen deposition (CD) at the location of skin wound H & E 500X in the PRP-treated group's skin after three weeks.

4 DISCUSSION

A speedier and more effective recovery is made possible by platelets' activation of wound healing growth factors, which greatly improves Healing is a continuous process. These growth factors accelerate and enhance the quality of healing by promoting the recruitment and differentiation of cells involved in tissue repair at the wound site. Hemostasis(20), inflammation, proliferation, maturation, and remodeling are some of the steps that make up wound healing. Platelet-activated growth factors have a significant impact on accelerating this intricate series of phases, leading to a quicker and more flawless healing process.

These elements accelerate and improve the healing process when they are present at the wound site because They improve tissue repair-related cell recruitment and differentiation. A platelet concentration of at least 1,000,000 platelets per microliter within five milliliters of plasma is what defines platelet-rich plasma (PRP). Growth factors are increased by around three to five times at this concentration. Platelets include about 800 proteins, which undergo a variety of post-translational changes to produce over 1,500 bioactive protein-based factors, according to proteomic analyzes(21). In comparison to control groups, horses' wounds treated with PRP gel exhibited better dermal collagen organization and quicker epithelial differentiation(22), The experimental group showed granulation tissue production and epithelial regeneration at the incision site seven days after the surgical treatment. Intense inflammatory cells and granulation tissue were seen in the treated group in the current research two weeks after surgery, which is consistent with (23). in a study that was done on dogs. Granulation formation, collagen deposition, and re-epithelialization were all observed to benefit from PRP injection into the wound site. Ostvar et al.'s experiment on rabbits is consistent with a previous study that looked at the effect of platelet-rich plasma (PRP) on rabbits' acute wound healing. This study showed that, in comparison to the control group, the PRP-treated group had increased angiogenesis at the wound sites..(24), Our results consistently demonstrate that the PRP-treated lesions on the animals' backs exhibited considerably higher levels of angiogenesis, collagen deposition, and re-epithelialization than the control group. PRP has been shown in several studies to hasten the inflammatory process. (25). In line with our research, the treated group exhibited a significant inflammatory process two weeks after surgery due to an intensive inflammatory infiltration at the injury site. Although (26) There were no discernible differences between treated and untreated wounds in terms The study on the application of platelet-rich plasma (PRP) for skin regeneration and wound healing in dogs examined various histological parameters, including epithelialization, the presence of inflammatory cells, the formation of dermal granulation tissue, the arrangement and proliferation of fibroblasts, as well as collagen deposition.

5 CONCLUSION

This conclusion, however, is at odds with the findings of our investigation, which showed notable differences between the treated and control groups. Increased fibroblast growth, re-epithelialization, collagen accumulation, new blood vessel development, and inflammatory cell presence were all indicative of the treated group's superior wound healing.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Statement of ethical approval

Ethical approval was obtained from respective ethical committee.

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