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Original Research Article

The Effects of Platelets Rich Fibrin on Cutaneous Wounds: Macroscopic Observations Study in Male Rabbits Model

Ali Ataie Khadim[1], Salah Hasan Alwan Al-Ameri[1]*

[1]Surgery and Obstetrics Department, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq

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Abstract: This study aimed to assess the effects of topical platelet-rich fibrin (PRF) matrix application on full-thickness cutaneous wound healing. The present experiment was carried out on 16 adults, clinically healthy, and male rabbits. A circular, full-thickness skin wound measuring 2 centimeter diameter was created on the thoracic-dorsal region for each rabbit. Rabbits were randomly divided into two groups equally: control n=8, and treatment, n=8. The wounds in the control group were left untreated, whereas, in the treatment group, the wounds were topically treated with platelet-rich fibrin matrix. The results of this experiment demonstrated that the wounds treated with topical applications of platelet-rich fibrin matrix healed better than the untreated ones. The animals' wounds, which were clinically examined for re-epithelialization, contraction, and total-percent of wound healing from day 7 until the end of the study, reached significantly higher values for all of three parameters than those in the control group ($P < 0.005$). Therefore, it can be concluded that topical application of platelet-rich fibrin matrix improved the macroscopic healing outcome of full-thickness cutaneous wounds in rabbits. Based on this experiment, it is rational to claim that platelet-rich fibrin matrix could be beneficial in wound healing due to its ability to promote the regeneration of epidermis and dermis, as well as inhibit tissue proliferation. further experimental and histopathological studies are required to evaluate the potential of this biological material that could improve the healing outcome of damaged tissues.

Keywords: Platelets Rich Fibrin, Cutaneous Wounds, Rabbits, Acute Wounds healing, PRF.

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INTRODUCTION

Cutaneous full-thickness wounds are serious injuries represent the complete loss of the epidermis and dermis, often extending into the subcutaneous tissue, fascia, muscle, or bone [1]. These wounds commonly result from trauma, burns, surgical procedures, diabetic ulcers, pressure sores, and severe infections [2]. Due to extensive tissue destruction and vascular impairment, full-thickness cutaneous wounds are associated with delayed healing, microbial contamination, chronic inflammation, and excessive scar formation [3]. Chronic and non-healing wounds have become a major global healthcare burden because of increasing rates of diabetes mellitus, obesity, vascular disease, and aging populations [4]. The development of advanced therapeutic techniques for wound management has become an important focus in regenerative medicine and surgery [5]. Wound healing is a highly arranged biological process designed to restore tissue integrity and function after injury [6]. The healing process occurs by four interfere phases: hemostasis, inflammation, proliferation, and remodeling [1]. The first hemostatic phase starts with platelet aggregation after vasoconstriction to form a fibrin clot which prevents blood loss temporarily by sealing the wound [2]. Platelets trapped within the clot release numerous cytokines and growth factors that regulate subsequent cellular responses [7]. During the second phase, macrophages and neutrophils migrate to the site of wound to remove bacteria, necrotic tissue, and foreign materials [3]. Growth factors and inflammatory mediators also secreted by Macrophages which stimulate angiogenesis and fibroblast activation [2]. Although inflammation is essential for normal repair, long time inflammation may weaken healing and contribute formation the chronic wound [6]. The phase of proliferation is characterized by fibroblast migration, collagen deposition, angiogenesis, granulation tissue formation, and epithelialization [1]. Fibroblasts synthesize extracellular matrix proteins that provide structural support for newly formed tissue [6]. Endothelial cells promote neovascularization to restore oxygen and nutrient supply to the wound area [3].

***Corresponding Author:** Salah Hasan Alwan Al-Ameri
Surgery and Obstetrics Department, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq

Keratinocytes emigrate across the wound bed to restore the epidermal barrier and achieve wound closure [6]. During the remodeling phase, collagen fibers are reorganized and cross-linked to improve tissue strength and stability [1]. However, healed tissue rarely regains the full biomechanical strength of uninjured skin [2]. Impairment of any healing stage may lead to delayed closure, infection, fibrosis, or chronic ulceration [3].

Recent advances in regenerative medicine have encouraged the use of autologous biomaterials to enhance tissue repair and accelerate wound healing [8]. Among these biomaterials, platelet-rich fibrin arises as a hopeful therapeutic option for the treatment of full-thickness wounds [9]. PRF is considered a second-generation concentrate of platelet reoproduced from blood autologous with no need to use anticoagulants or biochemical additives [7]. The centrifugation process produces a fibrin as matrix which is rich in platelets, leukocytes, cytokines, and growth factors [10]. Not like the platelet-rich plasma, PRF produces a thick matrix of fibrin that controls the progressive release of platelets and other biologically active molecules [9[. The release of growth factors in slow way ensures the effective regeneration and healing of damaged tissues [8]. The growth factors in PRF include platelet-derived growth factor, transforming growth factor-beta, vascular endothelial growth factor, epidermal growth factor, and insulin-like growth factor [7]. These components promote healing and regeneration by boosting the proliferation of fibroblasts, angiogenesis, and epithelium production [9]. Moreover, activated leukocytes in PRF play an essential role in the process by reducing the risk of infection and controlling the inflammatory response [8]. The structural integrity of the fibrin clot also contributes to the enhanced wound-healing abilities of PRF by providing an appropriate environment for cells to adhere and migrate [11]. For instance, research has established the ability of PRF to facilitate oral surgery, skin grafting, orthopedic surgery, plastic surgery, and chronic wound healing [12]. Moreover, the growth factors present in PRF membranes are particularly useful in the treatment of full-thickness wounds since the membranes can be directly placed on the affected area [13]. Generally, the presence of abundant growth factors in PRF promotes faster healing, reduces the risk of infection, and enhances the functional and cosmetic outcomes of the affected tissues [14]. Indeed, treatment with PRF has been shown to increase formation of granulation tissue, accelerate wound closure, promote angiogenesis, and reduce the healing time of acute and chronic wounds [15]. Additionally, in comparison to conventional therapies, PRF reduces post-surgical pain and inflammatory response [15]. New variations of PRF such as leukocyte-rich PRF and advanced platelet-rich fibrin have also been shown to possess improved wound-healing properties over conventional platelet concentrates [10]. A good wound dressing should provide a conducive environment for healing while also supporting tissue regeneration. It should also be cost-effective and easily available. Overall, PRF has several advantages over conventional wound dressings, and platelet concentrates [5]. Firstly, it is a biocompatible material that reduces the risks associated with foreign substances and disease transmission [7]. Secondly, PRF is cost-effective and can be easily prepared in a short time (9). Despite promising findings, variations in centrifugation protocols and application methods continue to limit standardization among studies [13]. Therefore, further controlled clinical trials are necessary to establish standardized protocols and confirm the long-term efficacy of PRF in full-thickness wound healing [14].

MATERIAL AND METHOD

Experimental Animals Design

The study was approved by the Animal Use and Care Committee of the College of Veterinary Medicine, Al-Qasim Green University. Sixteen clinically healthy adult male rabbits were included in the experiment and randomly allocated into two equal groups. Animals in the control group received no treatment, whereas those in the treated group received topical application of platelet-rich fibrin (PRF) to the wounds. Macroscopic observations of wound healing were performed on postoperative days 0, 3, 7, 14, 21, and 28.

Preparation of Platelets Rich Fibrin Matrix

The PRF matrix was prepared at the surgery and obstetrics laboratory of the College of Veterinary Medicine, Al-Qasim Green University. Preparation protocol with some modifications as described by [16]. As illustrated in (Fig. 1). The blood was collected from the same rabbit will be treated. The collected blood was carefully cleaned of blood clots by washing with normal saline solution. Then the blood introduced to plane tube. The tube with blood was introduced into the centrifuge. The blood was centrifuged 2000 rpm for 15 minutes that made the blood to separate in to PRP, PRF and RBCS. This was followed by extraction of PRF with slim clean wood sticks. The PRF was then placed on a sterile clean slide. After that, the PRF was pressed by placing another slide over the PRF to prepare the flat matrix. The produced PRF matrix was ready to place over the wound.

Figure 1: Photographic illustration of the steps involved in platelet-rich fibrin (PRF) preparation, as described by reference [16]

Utilization of PRF to the Intended Wound

The animals were initially tranquilized with diazepam at a dose of 1 mg/kg. General anesthesia was then induced by intramuscular administration of a combination of xylazine hydrochloride (5 mg/kg) and ketamine hydrochloride (35 mg/kg) Prior all surgical procedures. The back of the animal was prepared according to ideal aseptic techniques; A (2X2) cm of square full thickness critical size defects by using of modified aluminum plate was used as a standard template to define the lesions with a number blade 15 in the dorsolateral cutaneous [16], male rabbits, one defect in each side. The back of each rabbit was divided into two sides; PRF was covered of wound bed as PRF group (right side). while, 2 ml normal saline was applied instead of PRF in the control group (left side). For each lesion and sheets fixed to the wound margin with 2.0 vicryl sutures at the midpoints of the four angles and at the four sides for a total of four fixation points.

Post-Operative Care

All wounds were dressed, during first five days post-surgery, the animals were administrated daily intramuscularly with a combination of penicillin and streptomycin in a dose of 10.000 IU and 10mg/kg B.W., respectively.

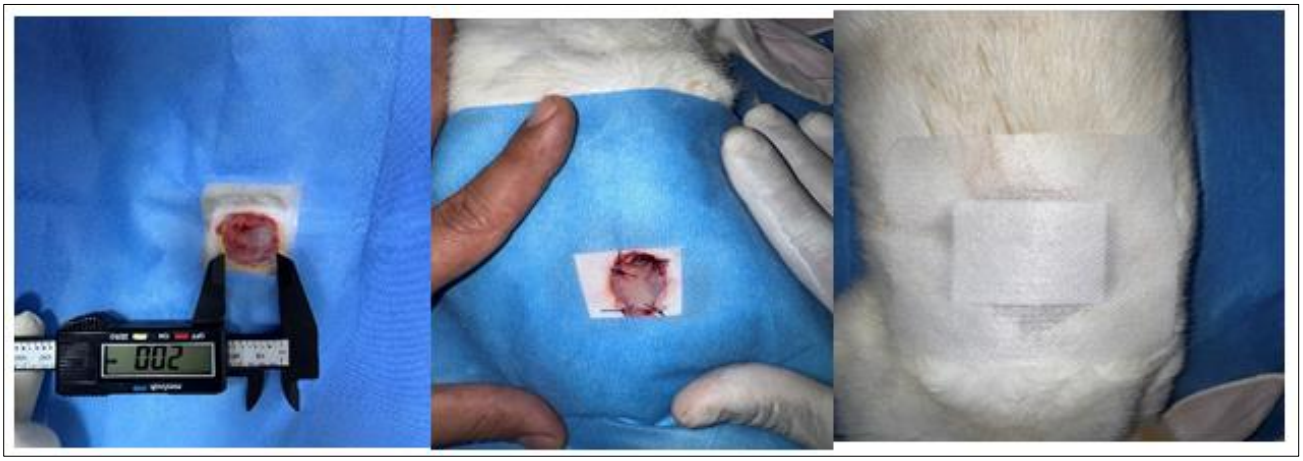

Figure 2: Representative photographs showing wound healing progression in the control and platelet-rich fibrin (PRF) groups throughout the experimental period

Evaluation of Wounds Healing

Healing Follow-Up

A general clinical examination was conducted for all experimental animals, including the assessment of behavior, cardiorespiratory activity, and body temperature. Digital photographs of the wounds were taken on postoperative days 0, 3, 7, 14, 21, and 28 to monitor the healing process. A standardized ruler was placed alongside each wound during photography to allow accurate calibration and measurement of wound dimensions.

The percentages of epithelization, wound contraction and total wound healing was calculated for

each wound, depending on the parameters mentioned by [18], as the following;

1. **Epithelization** was calculated by % Epithelization (day n) = Area of epithelium (day n) / Total wound area (day 0) x 100.
2. **Wound contraction**, was calculated by: Total wound size on (day n) as % of the original wound = Total wound area (day n) / Original wound area (day 0) x 100% Wound contraction (day n) = 100 – Total wound size on (day n) as % of the original wound.
3. **Total wound healing**, can be calculated by: Open wound size (day n) as % of the original = Open wound area (day n) / Original wound area (day 0) x 100% Total wound healing (day n) = 100 – Open wound size (day n) as % of the original wound.

Statistical Analysis

Statistical analysis was performed using the Statistical Analysis System (SAS) to evaluate the effects of treatment and postoperative assessment time on the measured study parameters, which were expressed as percentages. The least significant difference (LSD) test was used for multiple comparisons between group means. Differences were considered statistically significant when the calculated P-value was below the predetermined significance level.

RESULTS

Acute Wound Analysis

The wounds in both the treated and control groups showed a progressive reduction in size throughout the observation period. However, careful examination of the wound photographs revealed that wound closure progressed significantly faster in the treated group than in the untreated control group ($P \leq 0.05$) during the study period.

Total Wound Healing Percent

The mean ± SD of percentage total wound healing on day 7 of the study were in control group (17.18 ±0.16), in PRF group (25.27 ±0.22), By day 14, mean percentage total wound healing, in control group (22.57 ±0.22), in PRF group (35.57 ±0.21), By day 21, mean percentage total wound healing, in control group (32.57 ±0.21), in PRF group (65.95 ±0.29), By day 28, mean percentage total wound healing, in control group (76.07 ±0.42), in PRF group (93.30 ±0.49). Mean percentage total wound healing in AM groups healed faster than untreated wounds. Consequently, the healing percentage in treated group was significantly higher ($P \leq 0.05$) than that in the control group during (7th, 14th, 21st and 28th) days of the study (Tab 1).

Percent Epithelialization

On day 7, open wounds measurable epithelialization in control group (15.55 ±0.21), in PRF group (25.72 ±0.31), By day 14, mean percentage epithelialization in control group (26.12 ±0.28), PRF group (45.75 ±0.32), By day 21, mean percentage epithelialization in control group (55.52 ±1.94), PRF group (77.25 ±0.79) and AM group (83.57 ±2.08), By day 28, mean percentage epithelialization in in control group (84.05 ±1.60), PRF group (93.57 ±0.38) (Tab 2). Mean percentage epithelization were increased in treatment groups faster than in control group and revealed significantly greater ($P \leq 0.05$) during (7th, 14th, 21st, 28th) of the study between treatment and control group with preference of PRF- group with significant difference.

Wound Contraction Percent

Mean percentage contraction on day 7 of the study were in control group (16.55 ±0.68), in PRF group (26.87 ±0.44). By day 14, mean percentage contraction in control group (30.52 ±0.07), in PRF group (44.23 ±1.19). By day 21, mean percentage contraction in control group (40.50 ±0.08), in AM group (75.67 ±0.43). By day 28, mean percentage contraction in control group (75.12 ±0.84), and in PRF group (96.47 ±0.53) (Tab 3). Mean percentage contraction was noticed in both treatment groups significantly greater ($P \leq 0.05$) compare with control groups stay to the end of the study, with preference of PRF- group.

Table 1: Comparison of total wound healing percentages (WH%) (cm²) between the treated and control groups at different postoperative time points

Period	Mean ±SE		
	Control	PRF group	L.S.D.
0 day	E 0.00 ±0.00 a	E 0.00 ±0.00 a	0.00 NS
3 days	E 0.00 ±0.00 a	E 0.00 ±0.00 a	0.00 NS
7 days	D 17.18 ±0.16 c	D 25.27 ±0.22 b	1.073 *
14 days	C 22.57 ±0.22 c	C 35.57 ±0.21 b	1.268 *
21 days	B 32.57 ±0.21 c	B 65.95 ±0.29 b	1.694 *
28 days	A 76.07 ±0.42 c	A 93.30 ±0.49 b	2.056 *
L.S.D.	0.665 *	0.789 *	---

[a,b,c]Values (Mean ± SE) having with the different small letters in same row and big letters in same column differed significantly at $p \leq 0.05$.

Table 2: Comparison of wound epithelialization percentages (AE%) (cm²) between the treated and control groups at different postoperative time points

Period	Mean ±SE		
	Control	PRF group	L.S.D.
0 day	E 0.00 ±0.00 a	E 0.00 ±0.00 a	0.00 NS
3 days	E 0.00 ±0.00 a	E 0.00 ±0.00 a	0.00 NS
7 days	D 15.55 ±0.21 c	D 25.72 ±0.31 b	2.057 *
14 days	C 26.12 ±0.28 c	C 45.75 ±0.32 b	2.774 *
21 days	B 55.52 ±1.94 c	B 77.25 ±0.79 b	3.192 *
28 days	A 84.05 ±1.60 c	A 93.57 ±0.38 b	2.856 *
L.S.D.	3.090 *	1.196 *	---

Table 3: Comparison of wound contraction percentages (WC%) (cm²) between the treated and control groups at different postoperative time points

Day	Mean ±SE		
	Control	PRF group	L.S.D.
0 day	E 0.00 ±0.00 a	E 0.00 ±0.00 a	0.00 NS
3 days	E 0.00 ±0.00 a	E 0.00 ±0.00 a	0.00 NS
7 days	D 16.55 ±0.68 c	D 26.87 ±0.44 b	1.702 *
14 days	C 30.52 ±0.07 c	C 44.23 ±1.19 b	2.255 *
21 days	B 40.50 ±0.08 c	B 70.12 ±0.84 b	2.165 *
28 days	A 75.12 ±0.84 b	A 96.47 ±0.53 a	2.378 *
L.S.D.	1.313 *	1.959 *	---

Figure 3

DISCUSSION

The study examined the effect of (PRF) on healing of cutaneous wound, comparing the rate of total wound healing, epithelialization at the wound site, and wound contraction. The results demonstrated that PRF may have a good effect on wound healing. The underlying reason for this might be the special structure of the PRF matrix with a three-dimensional fibrous network containing platelets and leukocytes. This structure serves as a temporary scaffolding. PRF assists cell movement while acting like a container storing various proteins released slowly. These proteins consist of platelet-derived growth factor, transforming growth factor-beta, vascular endothelial growth factor, plus fibroblast growth factors which perform roles across different stages of tissue recovery [17]. Experimental research verifies these regenerative properties associated with PRF. Miron and colleagues (2017) examined clinical evidence about soft-tissue repair finding that eleven peer-reviewed studies reported improved patient outcomes during usage of regenerative PRF treatment [18]. Investigators proposed how fibrin matrix composition holding these specific growth factors constructs suitable surroundings promoting healthy regeneration while speeding wound closure [19]. Consistent release involving proteins initiates active cellular proliferation, extracellular synthesis, vessel formation, plus movement throughout skin gaps. Active sequences advance natural healing transitions beginning from intense inflammation toward active reconstruction phases. Hence, observed improvements for successful

tissue mending identified here stem through interconnected biological activity spanning several distinct systems instead of relying upon just one specific path. Successful clinical recovery usually follows various metabolic pathways activated after application to patients during these challenging medical procedures focused toward restoring complex physiological structures after traumatic tissue trauma occurred inside body sites. The results from this study also correlate with the findings from clinical and studies experiments. a systematic review and meta-analysis of randomized controlled trials using PRF and/or concentrated growth factors in the treatment of chronic untreated skin ulcers, Chen *et al*., (2022) reported that the proportion of ulcers that entirely healed by 4 weeks and at the end of follow-up were highly significant in ulcers were treated with platelet concentrates compared to those in the control groups [20]. Experimental studies have also shown that wounds treated with PRF accelerate wound healing [21]. In addition, nearly complete wound closure was observed in all PRF-treated wounds by the end of the follow-up period, indicating that PRF can promote cutaneous tissue repair [21]. Recently, treatment of full-thickness contaminated wounds with PRF in combination with decellularized bovine pericardium resulted in significantly greater healing of wounds compared to the untreated wounds. The mean healing times for the treated and control wounds were approximately 61 days and 90 days, respectively [17]. Most recently, PRF-treated full-thickness wounds of a rabbit model showed substantially greater healing by day 14 post-wounding compared to the untreated wounds [22].

In this study, we also determined the rate of wound epithelialization following a PRF treatment. The process of re-epithelialization is critical to the healing process, since it will form a protective barrier on the surface of the newly healed skin. The process of re-epithelialization of a wound primarily depends on the migration and proliferation of keratinocytes from the wound edge as well as from hair follicles, sweat and sebaceous glands. A fibrin scaffold, as well as growth factors released from it, may stimulate these processes by facilitating the migration of cells and promoting their proliferation.

CONCLUSION

Platelet-rich fibrin (PRF) possesses several biological properties that make it a promising and cost-effective biological material for wound coverage. In addition to promoting wound closure, PRF may support tissue regeneration and improve the quality of the healed tissue, potentially resulting in reduced scar formation.

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