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Clinical study

Platelet-rich fibrin: An effective chronic wound healing accelerator

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ABSTRACT

Background: Platelets are cells that play a central role in wound healing, and they are the main source of the growth factor complex that plays the main role in natural wound healing. It is aimed to present the beneficial effects of topical application of PRF on chronic ulcers that do not respond to standard wound care in cases of chronic ulcers that require a long treatment process and high costs. Materials and Methods: The study included 16 patients between the dates of January 2017 and September 2019. The study was approved by the local ethics board and planned retrospectively.

Results: The mean number of PRF applications in the patients was 4.37 (range: 1-8), while the wounds of 10 patients were completely closed up to the mean number of applications, and at least 50% of the wounds of 4 patients were closed up to the mean number of applications. Discussion: Choukroun's platelet-rich fibrin may be considered as a 2nd-generation platelet concentration. Its preparation protocol is reported to be highly simple and low-cost.

Conclusion: PRF is a safe, practical, easy to use adjuvant treatment method which has a potential for closing chronic wounds.

1. Introduction

Wound healing is a complicated process that consists of 3 intertwined stages that follow each other as hemostatic and inflammatory reactions, the proliferative phase and the remodelling and maturation phase that follows it. The wound healing process is strictly regulated by various cytokines and growth factors that are secreted to the wound area [1]. Platelets are cells that play a central role in wound healing, and they are the main source of the growth factor complex that plays the main role in natural wound healing. They reach the wound region very fast and start coagulation [1,2]. Platelets not only facilitate clot formation and stop local loss of lymph and blood, but they also trigger angiogenesis in wound healing, and by stimulating mesenchymal cells, they play a highly significant role as providing more than 20 growth factors that are required for tissue regeneration. Some proteins such as thrombin achieve secretion of these factors by platelet degranulation [2–4].

Wound healing may be accepted as the oldest and most important issue of medicine and physicians. It is being aimed to develop new methods for the purpose of speeding up wound healing especially in cases where wound healing is a problem such as diabetic foot wounds, pressure sores and venous ulcer wounds [5]. Among these, clinical and experimental studies have been conducted on the effects of platelet-rich biomaterials, platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) on wound healing, but clinical studies on the effects of PRP on wound healing are still ongoing [4–6].

Platelet-rich fibrin is a fibrin matrix that is obtained by centrifuging fresh whole blood, and it is a second-generation platelet concentration that were defined for the first time by Choukroun [7]. The α granules of the platelets found abundantly in PRF include several growth factors such as the platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), epidermal growth f actor (EGF), vascular endothelial growth factor (VEGF), insulin-like growth factor I (IGF-I), platelet-derived epidermal growth factor (PDEGF), platelet-derived angiogenesis factor (PDAF) and platelet factor 4 (PF-4). The growth factors (GF) that are especially secreted from the granules of platelets after trauma stimulate cell reproduction, differentiation, chemotaxis, new blood vessel formation and wound healing [4,8,9].

There are 2 types of PRF: a solid and a liquid. The solid PRF is the initial form of PRF made by Choukroun and colleagues. PRF solid, an exudate, rich in plasma proteins such as fibronectin, vitronectin, and thrombospondin-1 can be also obtained. These plasma proteins have an important role in cell adhesion and migration into the fibrin clot and can further improve the early stages of wound healing. Liquid-PRF builds on this slow centrifugal force concept The result is a suspension without

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anticoagulation that can be manipulated like PRP but retains the ability to form a slow release matrix once applied to tissue. This form of PRF can be injected into deep tissue spaces, onto open wounds, and mixed with other graft materials, such as bone-grafting particles, to produce "sticky bone," which has a puttylike consistency [10].

The preparation procedure of PRF is simple and inexpensive, and without anticoagulants, the coagulation cascade is started immediately after the contact of the blood with the tube. After starting the coagulation stage, the thrombin inthe circulation converted from prothrombin transforms fibrinogen into fibrin during centrifuging. A fibrin coagulate is obtained in the middle of the tube where concentrated platelets are held. The compressed thrombocytes are naturally activated, and they slowly release GFs. The three-dimensional structure of fibrin protects GFs from proteolysis and allows them to be slowly secreted and show their effects long-term. The concentration of GFs reaches its peak between the 7th and 14th days and continues to stay on a high level for about 28 days [5,6,10,11].

In this study, it is aimed to present the beneficial effects of topical application of PRF on chronic ulcers that do not respond to standard wound care in cases of chronic ulcers that require a long treatment process and high costs.

2. Materials and methods

The study included 16 patients between the dates of January 2017 and September 2019. The study was planned retrospectively. Permission certificate was obtained from all patients included in the study. The patients were evaluated based on their demographic characteristics (age, sex) systemic diseases, wound etiology, wound localizations and dimensions, wound formation time and time of recovery after PRF application. Additionally, their wound erythema, edema, necrosis and discharge statuses were evaluated. Patients with uncontrolled infection in the wound, cellulitis or osteomyelitis and those with vascular deficiency in the wound region were excluded.

Regular photographs of the wound were taken before starting initial treatment and during the treatment process. Hemogram, biochemistry, CRP and ESR examinations were made for all patients before starting the treatment. Deep tissue cultures were taken. Antibiotics therapies suitable for their infection values and culture results were started. Consultations were made to the relevant departments for underlying systemic diseases (diabetes, hypertension, etc.), and treatments were organized. In the presence of necrotic tissue in the wound, debridement was provided with the appropriate debridement method. A suitable environment was created on the wound bed for PRF application.

2.1. PRF preparation

10 cc of venous blood was obtained from each patient. The collected blood was put into a glass tube that did not contain anticoagulants. As platelet activation and fibrin polymerization would be immediately triggered in the absence of an anticoagulant, without wasting any time, the tube was put into a centrifuge (35^0 rotor angulation, 80 mm radius at maximum, Medwelt, 800D, China) that was preset at 3000 RPM (RCFmax = 805 G) and centrifuged for 10 min. After centrifugation, a red blood cells layer at the bottom of the tube, a platelet-deficient acellular plasma layer at the top and PRF coagulation in the middle are formed. The PRF forms a complex, three-dimensional fibrin matrix. In this fibrin matrix, the majority of the platelets and leukocytes of the collected blood are gathered. The coagulate that forms is gently taken from the tube by forceps, placed between two sterile gauze bandages, compressed without pressure, turned into a membrane and applied onto the wound surface (Fig. 1).

After PRF application, the wound bed was closed for 72 h with a nonadherent dressing in a way that would not apply excessive pressure. The wound was monitored throughout the treatment period of shrinking in the area of the wound, granulation tissue formation, wound bed cleanliness and infection presence or reduction.

2.2. Statistical analysis

The data obtained in the study were analyzed by using the SPSS (Statistical Package for the Social Sciences) for Windows 25.0 program. Descriptive statistics were used to analyze the data. The minimum, maximum, mean and SD values were calculated.

3. Results

The study included 16 patients with cutaneous ulcers that did not respond to conventional treatment in the period of 2017–2019. The mean age of the patients was 63 years (range: 36–82 years), while 9 were male, and 7 were female (Table 1).

In terms of ulcer etiologies, it was observed that there were diabetic ulcers in 8 patients, trauma in 2 patients, vascular deficiency in 3 patients and formation due to neuropathy-related pressure in 3 patients. Most of the ulcers were in the lower extremities. They were in the lower extremities in 11 patients, in the hand in 2 patients and in the sacral region in 3 patients (Figs. 2 and 3).

The mean pre-application wound dimensions were determined as a length of 8 cm (range: 1–11 cm) and a width of 6 cm (range: 1–10 cm). The durations of ulcers before PRF application were in a very wide range of 1 month–15 years (mean: 1.2 years).

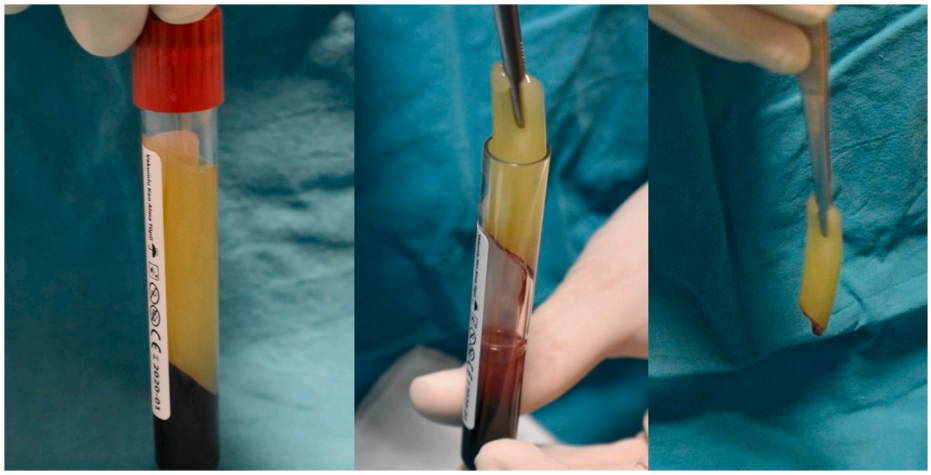

Fig. 1. PRF preparation.

Table 1
Wound etiology, demographic characteristics of the patients and PRF application outcomes.

Patients	M/F	Age (Year)	Location of Ulcer	Size (cm)	Ulcer time before PRF application	Cause of Ulcer	Number of PRF applications	Recovery time
1	M	54	Left lower extremity	7 × 6	6 months	Diabetes	5	2 months
2	M	46	Right upper extremity	3 × 3	3 months	Trauma	2	1 month
3	F	75	Left lower extremity	5 × 4	9 months	Diabetes mellitus	6	3 months
4	M	78	sacrum	10 × 5	15 years	Pressure sore	8	4 months
5	F	57	Left lower extremity	7 × 8	2 years	Diabetes mellitus	6	3 months
6	M	82	Right lower extremity	6 × 7	1 year	Venous insufficiency	5	2.5 months
7	M	72	Left lower extremity	4 × 3	5 months	Diabetes mellitus	4	2 months
8	M	45	Right upper extremity	3 × 2	1.5 months	Trauma	1	10 days
9	F	66	Left lower extremity	3 × 2	8 months	Diabetes mellitus	3	1.5 months
10	F	68	Left lower extremity	1 × 1	6 months	Diabetes mellitus	1	20 days
11	M	36	sacrum	6 × 10	1 year	Pressure sore	6	3 months
12	M	52	Left lower extremity	6 × 4	1.5 years	Peripheral arterial disease	4	2 months
13	M	57	Left lower extremity	11 × 8	1.2 years	Venous insufficiency	5	2.5 months
14	F	77	Right lower extremity	5 × 6	6 months	Diabetes mellitus	3	1.5 months
15	F	74	Sacrum	7 × 6	6 years	Pressure sore	8	4 months
16	F	69	Right lower extremity	2 × 2	2 months	Diabetes mellitus	2	1 month

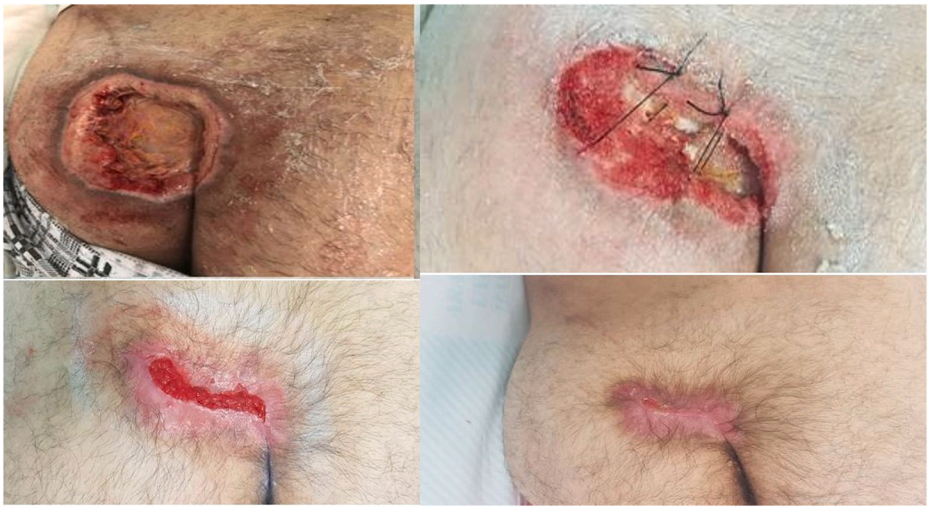

Fig. 2. Image of the decubitus patient in the sacral region, 3rd PRF after debridement, after application of the 5th PRF and the healed image of the wound in the 3rd month.

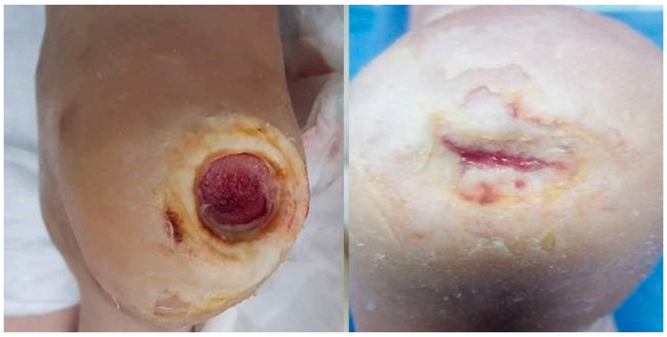

Fig. 3. Diabetic ulcer in the heel region before treatment and in the 2 nd month after 4th application of PRF.

The mean number of PRF applications in the patients was 4.37 (range: 1–8), while the wounds of 10 patients were completely closed up to the mean number of applications, and at least 50% of the wounds of 4 patients were closed up to the mean number of applications. The mean recovery time was 2.12 months (range: 10 days –4 months). When local infection findings were observed, and there was reproduction in their deep tissue cultures during the treatment, antibiotic treatments appropriate for their antibiograms were started.

No side effect was observed during the applications. It was observed that there was an increase in healthy granulation tissue formation and decrease in wound dimensions in all patients starting with the first application. However, it was seen that wound healing time and number of applications increased based on the ulcer size and the time of ulcer formation. Relapse was not observed in the mean follow up times of 6 months (Figs. 4–6).

4. Discussion

PRF was developed in 2001 in France by Choukroun et al. [11] Choukroun's platelet-rich fibrin may be considered as a 2nd-generation platelet concentration [11,12]. Its preparation protocol is reported to be highly simple and low-cost. Venous blood is taken into 10-ml dry glass

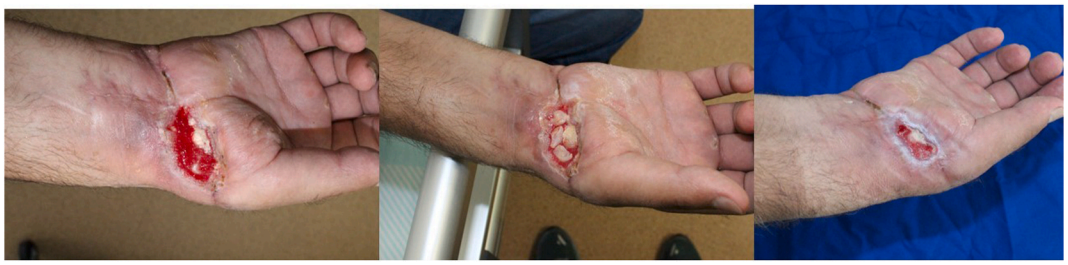

Fig. 4. Open wrist wound after trauma, single-session PRF application and 5th day image.

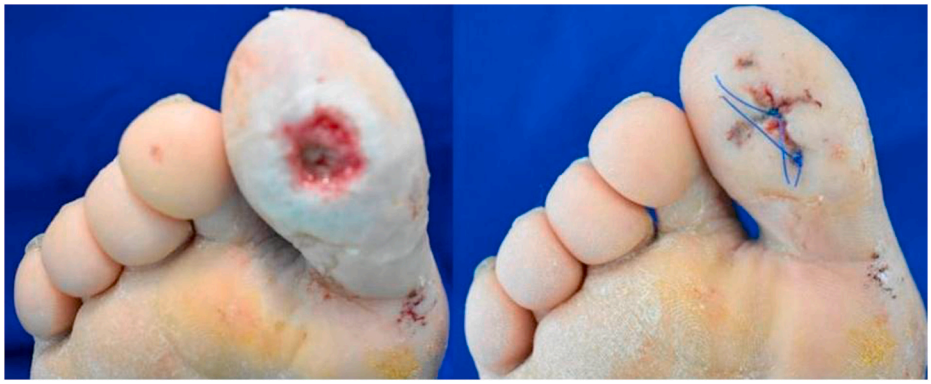

Fig. 5. Diabetic foot pre-treatment and 10th day image after single-session PRF application.

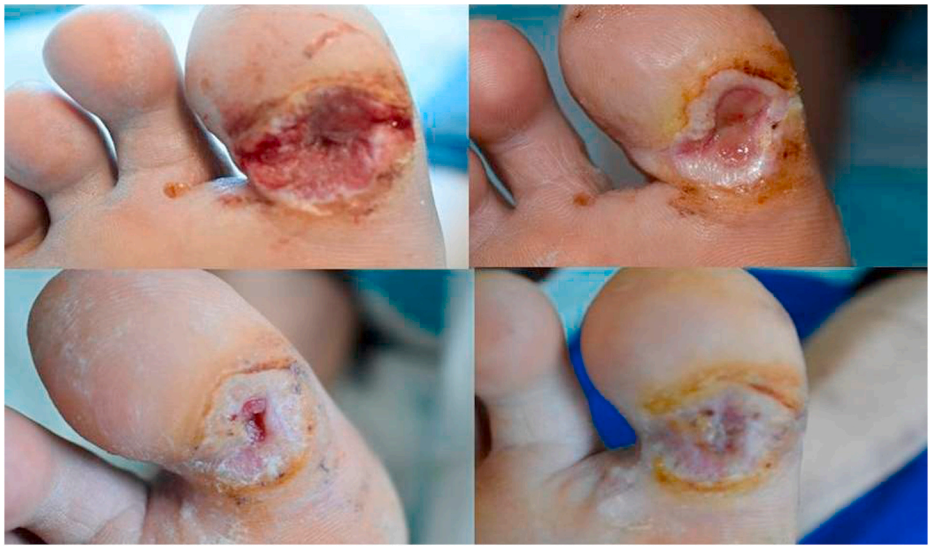

Fig. 6. Diabetic foot pre-treatment and 1st-session PRF, 2nd-session PRF and 1st month image.

tubes without using anticoagulants and centrifuged at 3000 RPM (approximately 400 G) for 10 min. Among the 3 layers that are formed after centrifugation, there are red blood cells at the bottom, PRF coagulate in the middle and acellular plasma at the top. As opposed to PRP, PRF does not immediately dissolve after application. It was shown that the fibrin matrix inside PRF increases the activity of the growth factors secreted from the platelets [12].

In the studies minimize confusion in the field and create more transparent research reporting RCF values in future studies. They described necessary parameters pivotal for the future report of RCF in studies related to PRF, which include dimensions of the rotor (radius at the clot and end of the tube), rotor angulation for the tube holder, revolutions per minute (RPM) and time, RCF value calculated at either the RCF-minimum, RCF-clot or RCF-maximum, composition and size of tubes used to produce PRF and centrifugation model used [13,14].

PRF produced via horizontal centrifugation accumulated a higher number and concentration of platelets/leukocytes when compared to either fixed-angle centrifugation [15].

PRF is a gel-like autologous biomaterial that contains an abundance of GF in its three-dimensional fibrin structure. PRF firstly started to be used in dentistry in the world as an inexpensive and easy to use method to increase recovery in oral surgery and implant practices and stimulate tissue regeneration [16,17]. The data obtained in clinical studies published on practices in the oral cavity (wound healing, bone regeneration) have been promising and have increasingly raised the method's usage in this field. Today, thanks to its strong fibrin structure and permanent

release of abundant amounts of GF, PRF has become attractive especially for plastic and reconstructive surgeons in terms of cosmetic usage, maxillofacial traumas, increasing postoperative recovery and chronic wound healing [17,18].

Pinto et al. [19] investigated the effects of PRF on lower extremity ulcers in 49 cases. After debriding the wound, a PRF membrane was placed onto the entire wound region, the wound was examined once a week, and new PRF membranes were applied when needed. At the end of the study, significant healing was observed in all wounds. Numbers of PRF applications and recovery times were positively related to the sizes of the wounds. There was no relapse in the first year following the treatment. It was found that the results we obtained in our study were similar to those obtained in Pinto et al.'s study.

Another study applied PRF on 33 patients with acute palm wounds. Consequently, in comparison to the control group, the PRF group was found to feel less pain, experienced less exudation and bleeding and required fewer dressing changes. More importantly, despite larger sizes, the wounds in the PRF group healed 5.4 days before those in the control group, and this showed that the 5.4-day improvement in the healing time could be better if the wound sizes were similar [20].

The humane and financial costs of cutaneous wounds are high, and considering the low cost, safety and effectiveness of PRF, it should be thought to be a suitable treatment option for such wounds and a promising alternative treatment method in cases where standard treatment fails. It has also been shown that PRF speeds up the healing of acute wounds and it heals larger wounds in a shorter time and less requirement of wound dressing change. Additionally, while the difference was not statistically significant, PRF was shown to also reduce findings such as pain, exudation and edema [21,22].

Consequently, PRF is a safe, practical, easy to use adjuvant treatment method which has a potential for closing chronic wounds. In addition to cases where standard treatment methods fail, PRF treatment also appears to be a promising alternative to advanced treatment methods. Additionally, lack of observing relapse in wound patients shows that PRF is not only a good promoter of wound coverage, but it also helps in reaching a better quality in the regenerated tissue. Considering the effectiveness, cost and safety of using such an autologous "optimized blood clot" for closing these wounds, this treatment option should be kept in mind as an alternative method for treatment of skin ulcers. There is a need for further clinical studies.

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