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An Experimental Study on the Effect of Platelet-Rich Plasma Application on Skin Graft Healing

Trombositten Zengin Plazma Uygulamasının Deri Grefti İyileşmesi Üzerine Etkisinin Deneysel Olarak Değerlendirilmesi

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ABSTRACT Objective: There is new information increasingly added about wound healing, which is one of the most complex physiological processes. In this study, it is aimed to evaluate the effect of platelet-rich plasma on the wound healing process in pursuit of creation of skin defect on the back skin of the rat and application of graft to it. **Material and Methods:** The study was planned on 30 randomly selected inbred Sprague-Dawley rats. In the rats in the experimental group, 3x2 cm full-thickness skin defect was created after the back area was shaved. The skin taken from the defected area was thinned and turned into a full-thickness skin graft and sutured again to the area where it was taken. Platelet-rich plasma was applied under the graft to the rats in the 1st group, and physiological saline was applied to the 2nd group. The 3rd group did not undergo any surgical procedure, and it was created as the control group. **Results:** Significant differences were observed in glucose, lactate, glycerol, and pyruvate values in the tissue in Group 1, compared to other groups. In the histopathological evaluation, a significant decrease was detected in the rate of inflammation and edema in the Group 1, in comparison with the other groups. **Conclusion:** Based on the data obtained from this study, it was taken into consideration by us that platelet-rich plasma application is a method that can be applied in addition to the classical wound care, especially in the cases with wound healing problems.

Keywords: Wound healing; platelet-rich plasma; microdialysis; skin grafting; angiogenesis

ÖZET Amaç: Oldukça karmaşık fizyolojik süreçlerden biri olan yara iyileşmesi ile ilgili hâlen yeni bilgilere ulaşılmaktadır. Bu çalışmada, sıçan sırt derisinde deri defekti oluşturulması ve sonrasında greft uygulamasını takiben trombositten zengin plazmanın yara iyileşme sürecine etkisinin değerlendirilmesi amaçlanmıştır. **Gereç ve Yöntemler:** Çalışma, randomize olarak seçilen 30 adet inbred Sprague-Dawley sıçan üzerinde planlandı. Deney grubundaki sıçanlarda, anestezi sonrası sırt bölgesi tıraşlandıktan sonra 3x2 cm boyutlu tam kat deri defekti oluşturuldu. Defekt oluşturulan alandan alınan deri inceltilerek tam kalınlıkta deri grefti hâline getirildi ve alınan yere tekrar sütüre edildi. Birinci gruptaki sıçanlara greft altına trombositten zengin plazma, 2. gruba ise serum fizyolojik uygulandı. Üçüncü gruba ise herhangi bir cerrahi işlem uygulanmadı ve kontrol grup olarak oluşturuldu. **Bulgular:** Mikrodiyaliz verileri karşılaştırıldığında Grup 1'de, diğer gruplara kıyasla dokudaki glukoz, laktat, gliserol ve pirüvat değerlerinde anlamlı farklar saptandı. Histopatolojik değerlendirmede ise Grup 1'de, diğer gruplara kıyasla inflamasyon ve ödem oranında belirgin azalma saptandı. **Sonuç:** Bu çalışmadan elde edilen verilere dayanarak, trombositten zengin plazma uygulamasının, özellikle yara iyileşme problemi bulunan durumlarda, klasik yara bakımına ek olarak uygulanabilecek bir yöntem olduğunu düşünmekteyiz.

Anahtar Kelimeler: Yara iyileşmesi; trombositten zengin plazma; mikrodiyaliz; deri grefti; anjiyogenezis

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Wound healing is a process organized by many intracellular and extracellular factors. Molecular control of cell interaction during the wound healing process is carried out by some growth factors and cytokines. Some factors can change the wound healing process by affecting the release of these cytokines and growth factors. Therefore, it is possible to provide positive effects in the treatment with the use of agents that accelerate the wound healing process. There are many different agents used for this purpose.[1]

Platelet-rich plasma (PRP) is an autogenous compound thought to accelerate wound healing. One of the most important advantages of starting the PRP application, which has attracted a lot of attention in the medical literature due to this effect, is that it contributes to the normal wound healing process and prevents the wound from turning into a chronic or problematic state.[2] In the literature, it is observed that various methods are used to measure wound healing parameters. Thanks to the microdialysis measurement method, which is one of these methods, vascular blood flow in the tissues, ischemic changes, fluid distribution and density in the cell compartments can be evaluated. The findings obtained provide useful data to follow the response of the tissues to the treatment during the treatment process.[3] In this study, it was aimed to investigate the effects of PRP in rats with tissue defects by microdialysis method and in a histopathological way.

MATERIAL AND METHODS

RESEARCH DESIGN AND SUBJECT SELECTION

All experiments were performed according to the protocols approved by Local Ethics Committee of the Dicle University Selahattin Payzin Experimental Research Centre (date of approval: July 6, 2013, protocol no: 2013/37), and complied with the Guide for the Care and Use of Experimental Animals. Also, this study was conducted in accordance with the principles of the Declaration of Helsinki. Subjects were obtained from Prof. Dr. Selahattin Payzın Experimental Research Center of Dicle University. Thirty male isogeneic (inbred) Sprague-Dawley albino rats weighing 250-300 g, 9 months-old, were included in the study. Rats were housed in standard collective cages. Feeds were given in standard pellet form (TAVAS Inc, Adana, Turkey) and water needs were met through the standard methods. The room temperature was kept constant at about 21 °C. Laboratory lighting was set to be 12 hours on the daytime and 12 hours in the nighttime. The humidity level of the room was adjusted to remain at the level of 45±10%. All procedures were performed by a single surgeon.

SUBJECT GROUPS

Rats were selected according to the randomization list (computer generated randomization program) and 3 groups were formed with 8 rats in each group. All subjects were marked and numbered according to the coding system. Group 1 was divided into the group that underwent skin grafting by creating a full-thickness tissue defect and underwent PRP, Group 2 was divided into the group in which skin grafting was performed by creating a full-thickness tissue defect and applied saline, and Group 3 was divided into the reference cage control group without any surgical or medical procedures (Figure 1). The other 6 rats were donated to obtain PRP.

SURGICAL PROCEDURE

One hour before the operation, the subjects were given cefazolin sodium 0.25 g/kg (Sefazol® vial, Mustafa Nevzat İlaç Sanayii, İstanbul, Turkey) intramuscularly, 1.5 mL of saline was injected intraperitoneally to replace the fluid deficit due to surgery. General anesthesia was achieved by intraperitoneally administering a mixture of ketamine sodium (Ketalar® vial; Pfizer Ltd Şti, İstanbul, Turkey) 90 mg/kg and xylazine hydrochloride (Rompun® vial, Bayer Inc, Germany) 10 mg/kg to the experimental groups before the surgery. Afterwards, the back of the rats

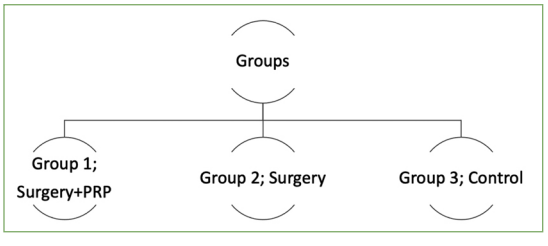

FIGURE 1: Schematic view of the experimental groups.

was shaved and disinfected with 10% povidone iodine solution (Batticon®, Adeka İlaç Ltd Şti, Samsun, Turkey).

PRP PREPARATION

5-8 mL of blood was collected via intracardiac route from the donor rats sacrificed by administering high dose intraperitoneal pentobarbital 1 g vial (Pentothal Sodium®, Abbott İlaç Sanayii, İstanbul, Turkey). Blood sample taken at 25°C room temperature was taken into Eppendorf tube containing 1.26 mL 10% sodium citrate anticoagulant. It was then centrifuged at 2,400 rpm for 10 minutes. At the end of centrifugation, platelet-poor plasma, gray colored PRP layer in the middle, and erythrocyte and leukocyte supernatant at the bottom of the tube were separated. The PRP portion was separated with the help of a pipette. The PRP portion was aspirated and centrifuged again at 3,600 rpm for 15 minutes. Thus, thrombocyte collapse was achieved. As a result, approximately 0.8-1.2 mL of PRP was obtained. The obtained PRP was mixed with 0.25 mL of 10% calcium chloride solution and 0.125 mL of 300 IU thrombin solution (Fibriquick® Thrombin, BioMerieux Inc, Durham, NC, USA) before the surgical application, and the PRP was activated.[4] Platelet level was measured by taking 0.3 mL sample from the supernatant obtained after the preparation.

DEFECT MODEL

The rat was fixed on the experimental table in the prone position. A 3×2 cm defect was planned in the dorsal region of the rat. The skin and subcutaneous tissue were passed through the incision. Dissection was performed up to the fascia. The tissue was completely excised, by preserving the fascia (Figure 2). Bleeding foci were coagulated with bipolar cautery. The excised tissue was thinned with the help of dissection scissors. The dermis and epidermis were preserved and turned into a full-thickness skin graft. The prepared tissue was inserted in the appropriate position and sutured with 4/0 polyglycaprone (Monocryl®, Ethicon, Norderstedt, Germany) (Figure 3). In Group 1, PRP was administered under the graft from all 4 quadrants in the form of subdermal injection, and in Group 2, physiological saline was applied (Figure 4). Approximately 2 dzm was applied for each quadrant. Then, the graft site was closed by tie-over dressing with chlorhexidine gauze (Bactigras®, Smith & Nephew, Massachusetts, USA).

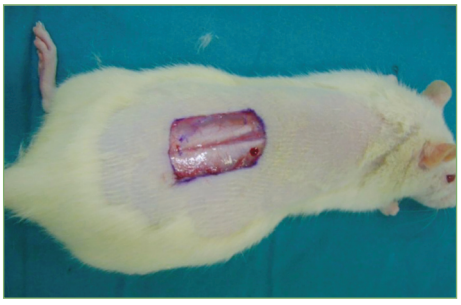

FIGURE 2: Full-thickness tissue damage in the dorsal region of the rat.

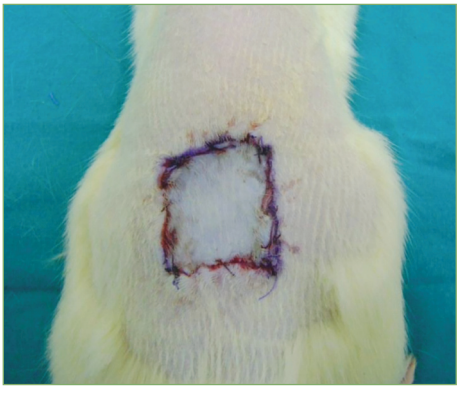

FIGURE 3: Application of full thickness skin graft.

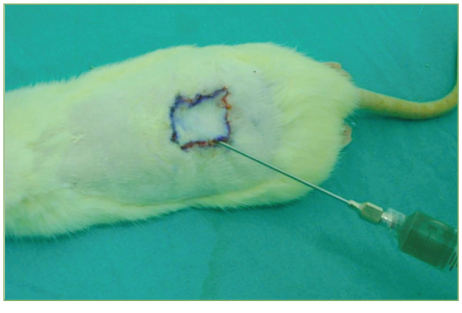

FIGURE 4: Making the platelet-rich plasma injection.

At the end of the surgical procedure, intraperitoneal saline solution (10 mL/kg/surgery time-hour) was injected into the intraperitoneal region. Later, analgesia was provided by administering 0.25 mL of metamizole sodium (Novalgin® 1 g/2 mL ampoule, Sanofi-Aventis Ltd Sti, İstanbul, Turkey) intramuscularly. The analgesic dose was repeated at 12, 24, and 48 hours after the operation. Intraperitoneal

saline injection was repeated on the first day after the surgery. Antibiotic prophylaxis was continued until the 3rd postoperative day.

The dressing was opened on the 5th day and then covered with an antiseptic dressing containing chlorhexidine daily. After each dressing, rats were housed in the cages each of which was reserved for a single rat.

MICRODIALYSIS METHOD

Microdialysis measurement method using CMA/ microdialysis (Harvard Apparatus SN T18258-1, US) analyzer was applied to the subjects from all 3 groups on postoperative 1st, 7th, and 14th days. Microdialysis was performed for 10 minutes at a rate of 2.0 mmol/L and 1 µL of dialysate sample was taken (Figure 5). In our study, a polyethersulfano CMA-11 microdialysis probe with an internal diameter of 0.12 mm, an internal volume of 1.2 µL and a length of 100 mm, was used. The shaft material used was of steel structure, with a diameter of 0.64 mm, a length of 14 mm, a membrane diameter of 0.5 mm and an intraoral volume of 3 µL. CMA 402 syringe and CMA 470 refrigerated fraction collector were used in the system.

Isotonic, sterile and perfusion fluids suitable for peripheral tissues were used for T1 microdialysis applications. Reagent kits were used to analyze the dialysate samples taken from the interstitial area, and to measure glucose, lactate, glycerol, and pyruvate in the product in cassette form. Before the application, calibration was performed with the calibration liquid within the scope of the kit.

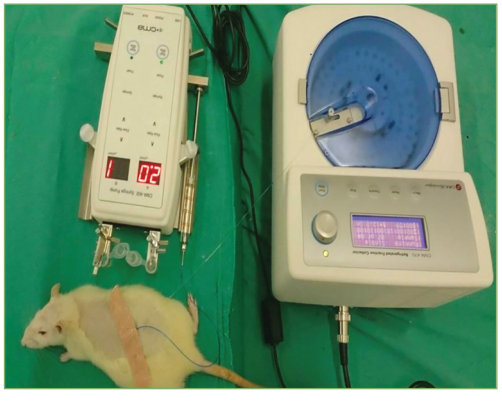

FIGURE 5: Appearance of microdialysis application under anesthesia.

HISTOPATHOLOGICAL EVALUATION

On the 21st day, the rats were sacrificed and biopsies including normal skin were taken from the dorsal region of the rat. After the skin samples were fixed in 10% formaldehyde solution for one-week, routine histological follow-up (consisting of alcohol, xylene, and paraffin series) was performed with an automatic tissue tracking device (Leica® TP1020, Nussloch, Germany), and embedded in paraffin blocks. 7 µm thick sections were taken from the obtained paraffin blocks using a Thermo Shandon Finesse ME microtome (Thermo Fisher Scientific®, Cheshire, UK). Sections were stained with hematoxylin-eosin and Masson trichrome stain for histopathological examination. Histopathological evaluations were performed with a Axiocam ERc5 (Carl Zeiss® Microscopy GmbH 07745 Jena, Germany) microscope. Each parameter was independently and blindly evaluated by an expert histopathologist.

Scoring was done by evaluating the foci of inflammation, edema, ulceration, fibrosis, and necrosis in the microscopic examination. Findings were evaluated semi-quantitatively using Abramov's histological scoring system.[5] Groups were classified between 0-4 according to the severity of pathological findings. Accordingly, "0" indicates no pathology or minor injury, "1" indicates limited injury, "2" indicates intermediate injury, "3" indicates extensive injury, and "4" indicates very severe injury.

STATISTICAL EVALUATION

One-way ANOVA test was used for parametric evaluations between groups and Tukey-HSD test was used for post-hoc evaluation. Statistical evaluations were made using the Kruskal-Wallis test for the histopathological parameters between the groups.

RESULTS

In the process of interpretation of the findings, the researchers unknowingly evaluated the experimental groups as double-blind.

During the follow-up period, partial necrosis was observed at the graft site in one rat in Group 2. The necrotic area was debrided, and it was observed that the defect was closed with secondary healing on the 12th postoperative day.

Platelet levels obtained from samples taken after PRP preparation are demonstrated in Table 1. The mean and standard deviation values of the obtained platelet levels were observed to be 780.3±34.53.

A significant difference was detected between groups 1 and 3 in terms of glucose values on the 1st and 7th days (p=0.021) when the microdialysis data are compared. A significant difference was evaluated between Group 1 and 2 on the 7th day (p=0.026). A significant difference was observed between Group 2 and 3 on the 1st and 7th days (p=0.028) (Table 2).

A significant difference was detected between Group 1 and 3 in terms of lactate values on the 1st, 7th, and 14th days (p=0.032). A significant difference was observed between Group 1 and 2 on the 7th, and 14th days (p=0.036). A significant difference was determined between Group 2 and 3 on the 1st, 7th, and 14th days (p=0.038) (Table 3).

A significant difference was observed between Group 2 and 3 in terms of glycerol values on the 1st and 7th days (p=0.022). A significant difference was recognized between Group 1 and 3 on the 1st and 7th days (p=0.029). There was no significant difference between Group 1 and 2 on the 1st, 7th, and 14th days (p=0.086) (Table 4).

A significant difference was noticed between Group 2 and 3 in terms of pyruvate values on the 1st and 7th days (p=0.014). A significant difference was evaluated between Group 1 and 3 on the 1st day (p=0.017). A significant difference was detected between Group 1 and 2 on the 1st and 7th days (p=0.021) (Table 5).

When histopathological data are compared, a significant difference was discovered between Group 1 and 2 in terms of inflammation and edema (p=0.008) (Figure 6, Figure 7).

TABLE 1: PRP values obtained from donor rats.

	Donor experiment no					
	1	2	3	4	5	6
PRP values (K/μL)	726	794	827	786	805	744

PRP: Platelet-rich plasma.

TABLE 2: Descriptive statistics results of the groups in terms of glucose values on the 1st, 7th, and 14th days.

Groups	Days					
	1		7		14	
	Minimum-Maximum (mmol/lt)	Median±SD	Minimum-Maximum (mmol/lt)	Median±SD	Minimum-Maximum (mmol/L)	Median±SD
1	1.72-8.43	4.91±2.22*	6.22-9.55	7.90±1.03	8.13-16.31*	11.33±2.58
2	2.10-7.30	4.81±1.62*	4.24-7.31	5.84±0.91	6.79-14.16*	11.15±2.42
3	10.24-13.12	11.38±1.07*	9.30-14.63	12.44±1.85	10.36-14.37*	12.44±1.24

*Significant difference; SD: Standard deviation.

TABLE 3: Descriptive statistics results of the groups in terms of lactate values on the 1st, 7th, and 14th days.

Groups	Days					
	1		7		14	
	Minimum-Maximum (mmol/lt)	Median±SD	Minimum-Maximum (mmol/lt)	Median±SD	Minimum-Maximum (mmol/L)	Median±SD
1	11.90-23.44	17.39±4.09*	10.31-19.02	14.04±2.64*	3.15-8.20	6.00±1.72*
2	15.18-26.39	20.43±4.45*	11.60-23.20	17.40±3.39*	5.29-10.29	7.92±1.64*
3	1.80-6.13	3.70±1.52*	1.68-4.40	2.46±0.94*	1.97-5.27	3.33±1.24*

*Significant difference; SD: Standard deviation.

TABLE 4: Descriptive statistics results of the groups in terms of glycerol values on the 1st, 7th, and 14th days.

Groups	Days					
	1		7		14	
	Minimum-Maximum (mmol/lt)	Median±SD	Minimum-Maximum (mmol/lt)	Median±SD	Minimum-Maximum (mmol/L)	Median±SD
1	27.60-40.16*	32.49±4.31	26.50-32.70	28.65±2.13*	15.50-25.69	21.75±3.59
2	26.16-47.21*	36.77±7.11	28.00-37.62	31.03±3.03*	19.00-26.03	23.14±2.77
3	16.20-22.50*	19.13±2.18	15.61-25.61	19.89±3.46*	15.90-24.50	20.13±3.25

*Significant difference; SD: Standard deviation.

TABLE 5: Descriptive statistics results of the groups in terms of pyruvate values on the 1st, 7th, and 14th days.

Groups	Days					
	1		7		14	
	Minimum-Maximum (mmol/lt)	Median±SD	Minimum-Maximum (mmol/lt)	Median±SD	Minimum-Maximum (mmol/L)	Median±SD
1	1.20-2.21	1.87±0.40*	0.81-1.70	1.24±0.27*	0.64-1.40	1.01±0.24
2	1.90-3.80	2.89±0.69*	1.30-3.20	2.12±0.64*	0.80-1.90	1.29±0.40
3	0.40-1.30	0.87±0.28*	0.68-1.47	0.99±0.27*	0.77-1.84	1.13±0.37

*Significant difference; SD: Standard deviation.

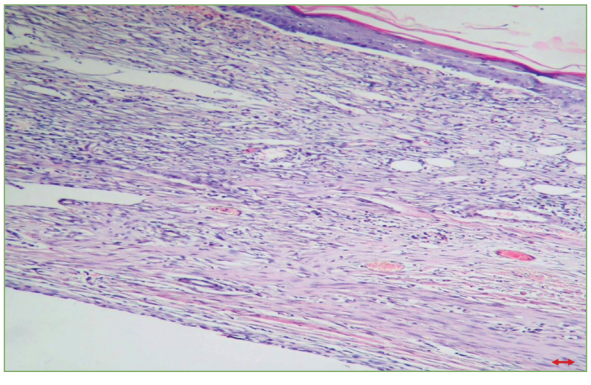

FIGURE 6: Mild signs of inflammation and edema in Group 1 (H&E, x100).

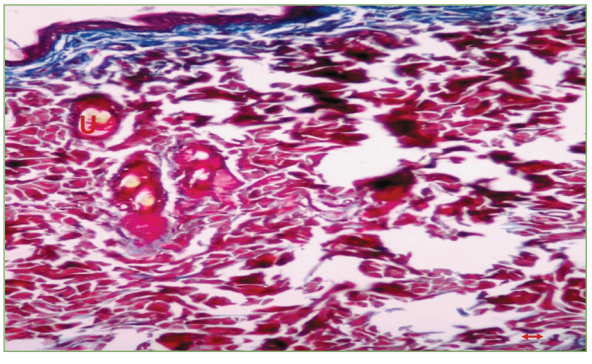

FIGURE 8: The appearance of mild ulceration and fibrosis in Group 3 (Masson Trichrome, x100) (U: Ulceration).

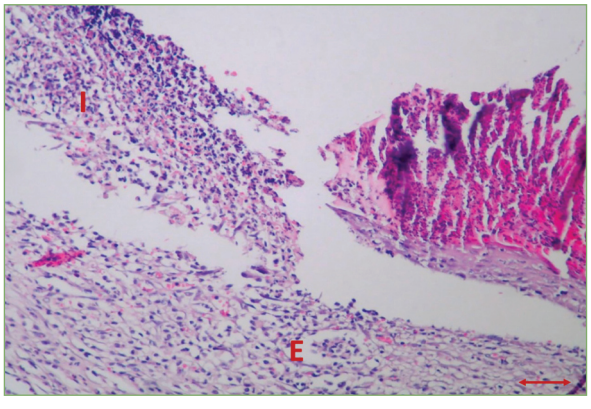

FIGURE 7: Intense inflammation and edema findings in Group 2 (H&E, x200) (I: Inflammation, M: Edema).

There was no significant difference between the groups in terms of ulceration, fibrosis, and necrosis (p=0.248) (Figure 8).

DISCUSSION

It is known that the wound healing process, which begins in response to a pathological event in any part of the body, is a complex process and is regulated by many growth factors and mediators.[6,7]

In most experimental studies, deficiency of growth factors has been shown in non-healing (atonic) wounds. It has been suggested that this fail-

ure may be caused by lack of production, excess destruction, inactivation, or combined mechanisms. For example, a lack of growth factors was detected in pressure sores compared to acute wounds. Growth factors have been indicated to become ineffective since they bind with the pericapillary fibrin layer. In this type of wounds, more growth factors and cytokines are needed than the normal wounds.[8] Therefore, in our study, it was planned to investigate the efficacy of PRP, which is a composition rich in cytokines and growth factors, in a sample wound model.

PRP in regenerative medicine is used in the treatment of many clinical conditions such as the treatment of acute and chronic wounds, ulcers, burns, muscle repair, bone diseases and tissue healing after the surgery. It is thought that PRP plays an important role in wound healing, endothelial repair, epithelial and epidermal tissue regeneration, angiogenesis, cell migration and proliferation.[9-11] It achieves this effect with the growth factors it contains.[12,13] These are platelet-derived growth factor, fibroblast growth factor, vascular endothelial growth factor, and transforming growth factor. These growth factors play an important role in the control of mesenchymal cell formation, proliferation, and extracellular matrix synthesis in the healing process. Most studies suggest that this result is mediated by the action of platelets on the fibroblast. Indeed, PRP enhances the biosynthetic activity of dermal fibroblasts, inducing migration, proliferation, extracellular matrix restoration and differentiation.[12-14] In addition to the high biocompatibility of PRP, it is thought that it increases collagen synthesis and soft tissue healing, and reduces dermal scar formation.[15,16] In addition, the presence of leukocytes and interleukin in the content of PRP also mediates its antimicrobial effects.[17,18]

PRP preparation stands out as the most critical step of PRP research.[6,19] Different methods to be able to obtain it, have been described.[19,20] After the preparation, an average of 0.5 mL of PRP is gained.[19] The amount of platelet can be increased 4.1-6.5 times and the amount of platelet obtained varies between 450,000 and 800,000 K/μL, with the defined preparation techniques.[21,22] In addition, it is understood that higher platelet values are gained in the literature. However, the appropriate biologically active concentration of PRP is still unknown.[23-25] It is suggested that very high platelet values have an inhibitory effect, therefore, it is stated that there is no need for high concentrations.[23,26] If the mean and standard deviation values of the platelet levels we obtained with the preparation technique used in our study were compared with the values stated in the literature, they were at optimal levels, and we think that the preparation values we used could be easily obtained and used in clinical applications.

It is stated that platelet degranulation occurs, and growth factors are released into the environment because of the treatment of PRP with thrombin and calcium.[4,23] Although it is difficult to quantitatively determine the amount of growth factors contained in PRP, it is known that even growth factors at normal level are quite effective.[4] There is also no clear information about the duration and degree of activity of the mediators included in PRP.[6] However, it is suggested that PRP has significant effects on early regeneration.[20,26-28] In our study, a decrease in the rate of inflammation and edema is detected histopathologically in the early period of wound healing, and positive findings are observed in regeneration findings. Therefore, we believe that the earlier the PRP application is started in acute and chronic wounds, the faster the wound healing process will be.

PRP stimulates angiogenesis, increases collagen synthesis, endothelial, epithelial and epidermal regeneration, and accelerates soft tissue healing. In addition, PRP has the potential to induce early vascularization by increasing growth factor release. Other beneficial effects of it include reduction of hematoma and infection. It has been reported that PRP application increases the uptake rate of graft in the vascularization and inosculation phase of the wound bed in skin graft applications due to these features of it.[29] There are also some studies on this subject in the literature. In the study of Gibran et al., it was indicated that PRP application increases skin graft uptake and healing rates in burn cases.[30] Accordingly, it was stated in the study of Schade and Roukis that PRP accelerates wound healing and

shortens the healing process in the skin graft recipient site.[31] In the study of Kakudo et al., it was reported that PRP accelerates epithelialization and angiogenesis in the skin graft area.[32]

Unlike other regenerative treatments, the use of PRP does not require complex equipment and is an economical method. Being an autologous source of growth factor and minimally invasive easy application technique and low infection risk have recently increased the interest in the use of PRP.[20] Since it is of autologous origin, there is no risk of transmitting any disease or developing an immune reaction. It is non-toxic and has minimal side effects.[4]

In the literature, there are many techniques for measuring the concentration of a molecule in tissue. These include measurement of agent concentrations in skin bullae, biopsied tissue, fibrin clots, wound exudates, and lymphatic fluid. All these traditional methods are often not sufficient in terms of pathophysiological aspects and can provide limited information about drug concentrations in target tissues in humans. An alternative and promising method for determining the concentration of any agent at the site of action is the microdialysis technique. Microdialysis currently uses to measure drug concentration in soft tissue, tendinous tissue, heart muscle, brain, bone, lung and tumoral tissues.[33]

The microdialysis method is based on placing a semipermeable membrane into living tissue and collecting the sample consisting of particles that are freely located in the extracellular space. After its discovery in the 1970s, it has been used mainly in laboratory research. Since the 1990s, this method has been applied to the clinical field and has been used by many specialties today.[34] Microdialysis is a measurement-based sampling method and when connected to analytical devices, it helps to measure the substance concentration in selected tissues.

The inner tubing of the probe used in the microdialysis technique is connected to a precision pump. The microdialysis probe is constantly perfused with perfusion fluid, usually physiological Ringer's lactate, at a flow rate of ~1.5 µL/min. The flow rate of the perfusion fluid varies greatly with the time required for dissolution in the experiment and the sensitivity of the analytical experiment. Perfusion fluid enters from the proximal end of the inner cannula. Then the flow continues in the opposite direction and the fluid moves towards the proximal end of the outer cannula. Dialysis takes place between the perfusion fluid and the interstitium at the tip of the probe. There is no net fluid exchange between the two compartments. However, molecules switch in both directions. The difference in concentration on the two sides of the membrane determines which direction the change will take. The perfusion fluid (perfusate) partially equilibrates with the dissolved substances from the intercellular fluid and leaves the microdialysis probe through the outer tube and is called the dialysate. The dialysate is also sent for chemical analysis.[34]

The advantage of microdialysis compared to conventional methods is that it can continuously sample the free substance fraction in the intercellular fluid. Accordingly, our results can be used to monitor the effects of medical treatment and drug concentrations as well as to detect early markers of surgical complications.[35] For this reason, we preferred this method, which allows us to obtain highly sensitive and quantitative data and obtained objective numerical parameters in our study. We think that this method is a very sensitive and useful method for measuring metabolites in tissue.

Ischemia causes some changes in metabolism, by decreasing glucose value and increasing lactate level and lactate/pyruvate ratio. The lactate/pyruvate ratio reflects the cytoplasmic redox state, therefore, provides information about tissue oxygenation. A high lactate/pyruvate ratio (>40) is often interpreted as a sign of cerebral hypoxia or ischemia.[35] Cell damage causes the glycerol level to increase as well. Reagents used in microdialysis method can be used for the analysis of glucose, lactate, pyruvate, and glycerol. These reagents were also used in our study, and significant differences were observed in the interstitium glucose, lactate, and pyruvate values in the PRP group.

In this study, the effects of PRP on graft viability and wound healing are quantitatively evaluated in rats with a defect model. In this study, it is observed that PRP shortens the wound healing period and in-

creases regeneration. Therefore, it can be considered that this application can be included as a supportive treatment in the wound care protocol.

CONCLUSION

In skin graft applications, especially the basal layer of the skin has high proliferation rates. This potential is closely related to the released growth factors. Especially in chronic non-healing wounds, insufficient production, or release of these growth factors in the basal layer is detected, and a decrease in graft uptake rates is observed. PRP has a feature that normalizes the physiological process in these situations where the wound healing is delayed. PRP, which is increasingly used in wound healing and grafting applications, is a reliable, cost-effective, effective application that increases the uptake rate of the graft and reduces the rate of graft loss in graft applications.

In experimental studies, it has been observed that there are few studies examining the protective effect of PRP against skin damage after soft tissue injury. Therefore, we believe that this experimental study provides quantitative findings that will contribute to the literature.

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Conflict of Interest

No conflicts of interest between the authors and /or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

***Idea/Concept:** Niyazi Gülsün; **Design:** Mehmet Fatih Akkoç; **Control/Supervision:** Caferi Tayyar Selçuk; **Data Collection and/or Processing:** Niyazi Gülsün; **Analysis and/or Interpretation:** Emin Kapı, Ulaş Alabalık, Mehmet Bozkurt, Yusuf Çelik, İsmail Yıldız; **Literature Review:** Niyazi Gülsün; **Writing the Article:** Niyazi Gülsün, Emin Kapı; **Critical Review:** Veysi Akpolat; **References and Fundings:** Caferi Tayyar Selçuk; **Materials:** Niyazi Gülsün.*

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