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Original Article / *Özgün Makale*

Is platelet-rich plasma treatment effective in healing of oral cavity wounds? Experimental animal study

Trombositten zengin plazma tedavisi oral kavite yaralarının iyileşmesinde etkili mi? Deneysel hayvan çalışması

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ABSTRACT

Objectives: This study aims to evaluate the macroscopic and histopathological effects of platelet-rich plasma (PRP) on healing of oral cavity wounds that were created in rats for experimental purposes.

Materials and Methods: Forty-two female Wistar albino rats (age 2.5-3 months; weighing 250 g - 350 g) were included in the study. Six rats were selected for the preparation of PRP from their blood. The remaining 36 rats were anesthetized and a wound was opened in their palate. Rats were randomized into six groups. Each group contained six rats (3 for the PRP, 3 for the control groups). Platelet-rich plasma was injected into the margins of the wounds in the PRP group. Control group did not receive any application. Six rats from the PRP and control groups were sacrificed in the first, second and third weeks, their hard palates were photographed macroscopically, and histopathological examination was performed regarding the severity of the inflammation, neovascularization, fibroblast proliferation and collagen concentration. Each parameter was statistically analyzed after scoring.

Results: Although the macroscopic wound area was significantly smaller in the PRP group compared to the control group in the first and second weeks ($p<0.05$), this significance disappeared in the third week ($p>0.05$). There was no statistically significant difference in the first and second weeks regarding the severity of inflammation, while in the third week, the severity of inflammation significantly improved in the PRP group compared to the control group ($p<0.05$). In respect of neovascularization, fibroblast proliferation, and collagen concentration, there was no significant difference between the PRP and control groups, but they showed a statistically significant increase in the PRP group compared to the control group in the second and third weeks ($p<0.005$).

Conclusion: Platelet-rich plasma application increased epithelization as a result of faster wound healing compared to regular wound healing in the wound area. It can be concluded that PRP application has positive histological and macroscopic effects on intraoral wound healing.

Keywords: Oral cavity; platelet-rich plasma; rat; wound healing.

ÖZ

Amaç: Bu çalışmada trombositten zengin plazmanın (TZP) sıçanlarda deneysel amaçlarla oluşturulan oral kavite yaralarının iyileşmesi üzerine makroskopik ve histopatolojik etkileri değerlendirildi.

Gereç ve Yöntemler: Çalışmaya 42 dişi Wistar albino sıçanı (yaş 2.5-3 ay; ağırlık, 250 g - 350 g) dahil edildi. Altı sıçan kanlarından TZP hazırlanması için seçildi. Geriye kalan 36 sıçana anestezi yapıldı ve damaklarında yara açıldı. Sıçanlar altı gruba randomize edildi. Her grup altı sıçan içerdi (TZP grubu için 3, kontrol grubu için 3). TZP grubunda yaraların marjinlerine TZP enjekte edildi. Kontrol grubuna herhangi bir uygulama yapılmadı. TZP ve kontrol gruplarından altı sıçan birinci, ikinci ve üçüncü haftalarda sakrifiye edildi, sert damakları makroskopik olarak fotoğraflandı ve enflamasyon şiddeti, neovaskülarizasyon, fibroblast proliferasyonu ve kolajen konsantrasyonu hakkında histopatolojik inceleme yapıldı. Her parametre skorlamadan sonra istatistiksel olarak analiz edildi.

Bulgular: Makroskopik yara alanı birinci ve ikinci haftalarda TZP grubunda kontrol grubuna kıyasla anlamlı şekilde küçük iken ($p<0.05$) bu anlamlılık üçüncü haftada kayboldu ($p>0.05$). Enflamasyon şiddetinde birinci ve ikinci haftalarda istatistiksel olarak anlamlı farklılık yoktu, fakat üçüncü haftada enflamasyon şiddeti TZP grubunda kontrol grubuna kıyasla anlamlı şekilde iyileşti ($p<0.05$). Neovaskülarizasyon, fibroblast proliferasyonu ve kollajen konsantrasyonu açısından TZP ve kontrol grupları arasında anlamlı farklılık yoktu, fakat bunlar ikinci ve üçüncü haftalarda TZP grubunda kontrol grubuna kıyasla istatistiksel olarak anlamlı artış gösterdi ($p<0.005$).

Sonuç: Trombositten zengin plazma uygulaması yara alanında sıradan yara iyileşmesine göre daha hızlı yara iyileşmesi sonucunda epitelizasyonu artırdı. TZP uygulamasının intraoral yaraların iyileşmesinde pozitif histolojik ve makroskopik etkileri olduğu sonucuna varılabilir.

Anahtar sözcükler: Oral kavite; trombositten zengin plazma; sıçan; yara iyileşmesi.

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Wound healing is a complex process, which is controlled by molecular signals and mediators and cellular events that interact with each other.[1] Although platelets are the primary cells of coagulation and hemostasis steps, which consist the first phase of wound healing, they also play an essential role in the whole phases of wound healing, as they affect other phases (inflammation, proliferation, remodeling, and formation of the scar tissue) as a result of the increase of matrix synthesis, differentiation, proliferation and cellular migration caused by growth factors, which are released following platelet activation.[2,3] Today, there are several treatment modalities defined for wound healing. A popular one among them is the platelet-rich plasma (PRP), which was investigated in several trials.[3] Platelet-rich plasma is the autologous component of the plasma and contains higher concentrations of platelets, growth factors, and cytokines than the basal levels.[3,4] It was suggested that the acceleration and increasing of wound healing by PRP depended on the synergistic effects of these growth factors.[5] In this study, we aimed to evaluate the macroscopic and histopathological effects of PRP on healing of oral cavity wounds that were created in rats for experimental purposes.

MATERIALS AND METHODS

Forty-two female Wistar albino rats (age 2.5 - 3 months; weighing 250 g - 350 g) were enrolled in this study conducted at Bağcılar Training and Research Hospital between October 2016 and December 2016. During the study, the rats were kept in standard cages in a room that was artificially illuminated between 08.00 and 20.00, had a relative humidity of 50±10% and a temperature of 23±4°C. They were distributed into seven cages, each containing six rats. The rats were allowed to access food and water freely (Figure 1). General anesthesia was administered on the operation table with intraperitoneal injections of 50 mg/kg ketamine hydrochloride (Ketalar®, Pfizer Warner-Lambert, Istanbul, Turkey) and 5 mg/kg xylazine hydrochloride (Rompun®, Bayer, Istanbul, Turkey). The surgical interventions, follow-up procedures, and sacrifications were carried out in the Bağcılar Experimental Studies Center after the study was approved by the Bağcılar Animal Experiments Local Ethics Committee (2016/78).

The blood necessary for the preparation of PRP was obtained from six rats. Approximately 10 mL intracardiac blood was drawn from each rat into

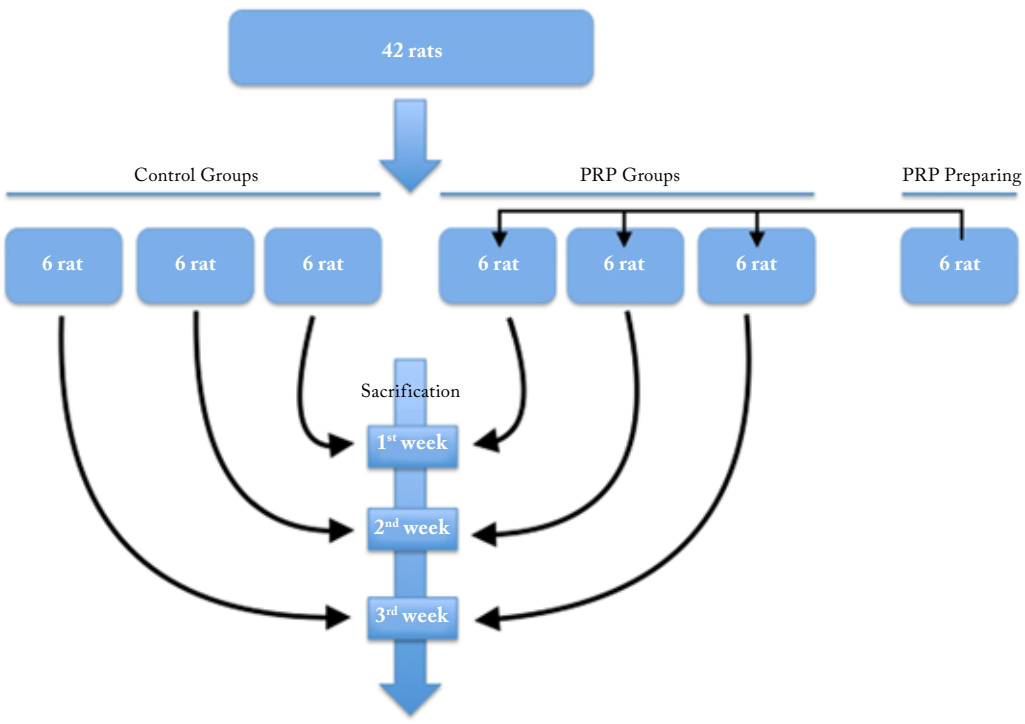

Figure 1. Schema of groups. PRP: Platelet-rich plasma.

the coagulation tube after two rats at a time were anesthetized with 75 mg/kg ketamine and 10 mg/kg xylazine hydrochloride in the first, third and fifth days (Figure 1). The obtained blood was centrifuged with a centrifuge device (220 rpm at a temperature of 22˚C) for 20 minutes. This initial centrifuging process enabled the accumulation of the erythrocytes at the bottom and separated platelets and leukocytes from the plasma, which accumulated at the top of the tube. After the initial centrifuging process, a narrow intermediate layer consisting of platelets and white cells (buffy coat) was observed between the erythrocyte layer at the bottom and the straw-colored plasma layer at the top. Plasma at the top of the tube was transferred into another tube with a micropipette, and the intermediate layer was added to this plasma. During this separation process, a few erythrocytes were allowed to mix up with the intermediate layer. This tube containing plasma and platelets was centrifuged for 20 minutes (480 rpm). The second centrifuging process enabled the separation of the remaining erythrocytes, the formation of a thin layer by platelets over these settled erythrocytes and the accumulation of the straw-colored platelet-poor plasma (PPP) at the top. The platelets concentrate and PPP, which were formed after the second centrifuge, were separated with micropipettes and transferred into two different tubes. Platelet-rich plasma was prepared with the addition of PPP to the platelet concentrate. According to the literature, platelet concentrate obtained with this method contains platelets with a concentration of approximately $1.5x10^{12}$ platelets/L after the dilution with PPP. In our study, we carried out the same dilution process as described in the literature.[3] Eventually, the platelets count in PRP was circa 2.5 times higher than the normal blood ($0.2\text{-}1x10^{12}$ platelets/L). The white cells in the prepared PRP were inactivated with 25 gray radiotherapy in order to prevent the graft-versus-host reaction. Bovine thrombin preparation (Thrombin, Sigma-Aldrich, Deisenhofen, Germany) was used for the activation of PRP. The powder, which contained 1000 units/mL thrombin, was diluted with 5 mL 10% calcium chloride, and a thrombin solution with a concentration of 200 unit/mL was prepared. Total 0.1 mL PRP and 5 µmL (1 unit) of the prepared PRP and thrombin solution, respectively, were injected into each wound in the PRP group. Thus, the interaction of the PRP and thrombin solutions in the operation field and so the activation of the platelets in the presence of the calcium ions were facilitated.

Under general anesthesia, the hard palates of all rats were incised from mucosa to the periosteum with a dermatological punch biopsy device. The diameter of these circular incisions was 3 mm. This palatal wound model was created with a blunt dissection from the mucosa (the centrum of the incision) to the periosteum (Figure 2).[6]

Three cages were randomized as PRP groups and three cages as control groups. In the first, second and third weeks, all cage members from each group were sacrificed, and their hard palates were excised (Figures 1). In the first, second, third and fifth days, to each of the 18 rats in the groups randomized to PRP administration,

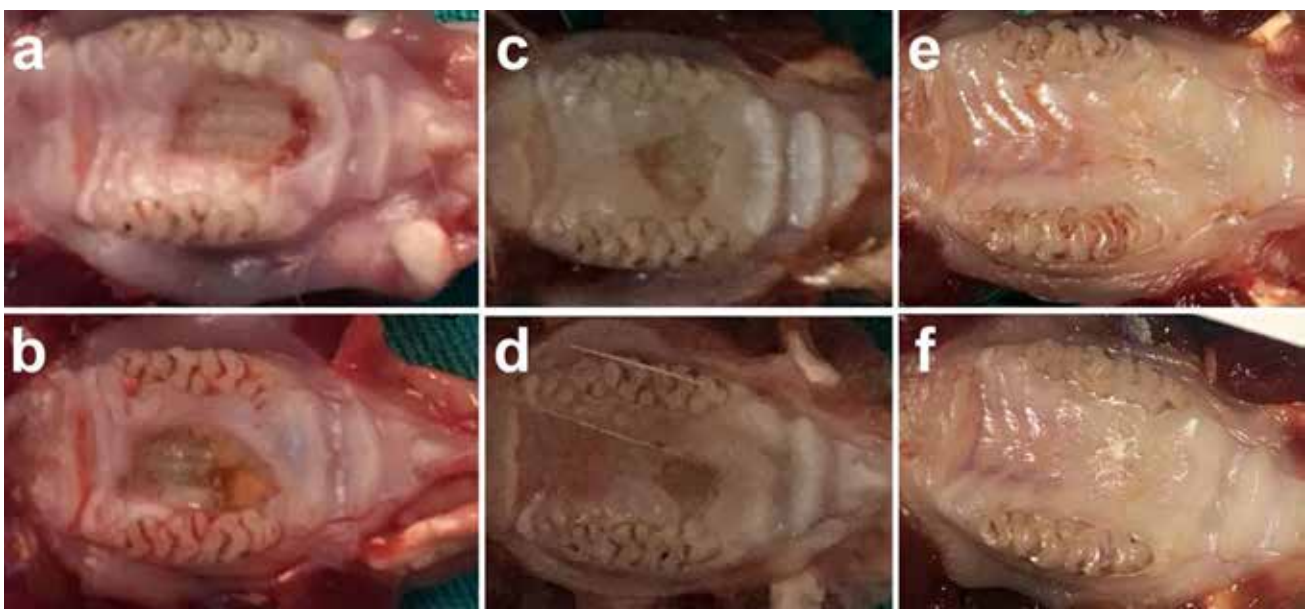

Figure 2. Macroscopic pictures of palatal wound model. **(a, c, e)** Control group; first, second and third week, respectively. **(b, d, f)** Platelet-rich plasma group; first, second and third week, respectively.

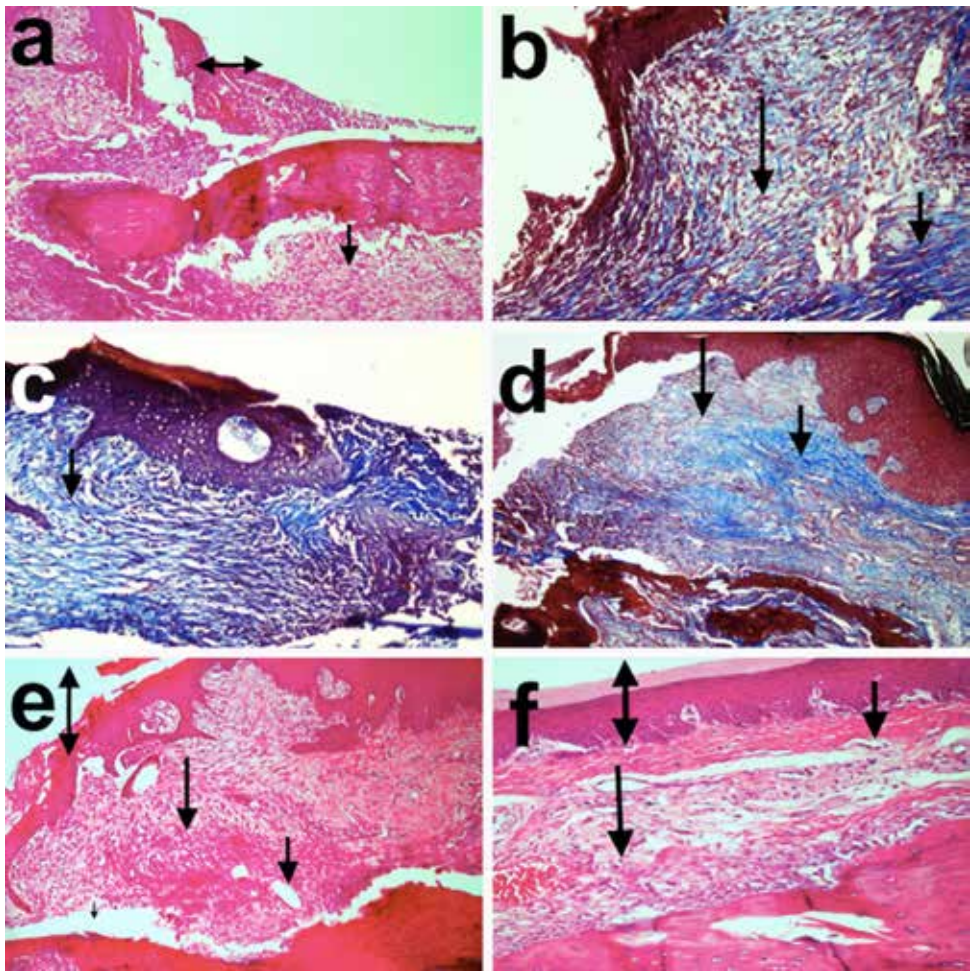

Figure 3. Microscopic appearance of palatal wound model: **(a)** Control group first week, intensive mixed type inflammation cells and start of epithelization. Inflammation cells (short arrow), wound area with minimal epithelization (double-point arrow) (H-E×100). **(b)** Control group second week fibroblast proliferation (long arrow), collagen concentration (long arrow) (MT×100). **(c)** Control group first week collagen concentration in lamina propria with slight blue staining and fibroblasts in between (arrow) (MT×100). **(d)** Platelet-rich plasma group first week, fibroblast concentration (short arrow), collagen concentration (long arrow) (MT×100). **(e)** Platelet-rich plasma group first week, mixed inflammation (long arrow), epithelization less than 1/3 (double-point arrow) and slight vascular density (short arrow (H-E×40). **(f)** Platelet-rich plasma third week, mixed inflammation (long arrow), completed epithelization (double-point arrow) and vascular density (short arrow) (H-E×40). HE: Hematoxylin-eosin; MT: Masson's trichrome.

total 0.1 mL/day PRP solution was injected to four equal segments of the wound margins with a dental syringe. Eighteen rats in the control groups did not receive any injection after the formation of the wounds in their hard palates. Sacrification process was implemented with surgical decapitation under general anesthesia, and the palatal bones of all animals were excised under microscopic examination (D.F. Vasconcellos MC-M22, DFV Company, Rio De Janeiro, Brazil).

Palatal specimens were photographed with fixed magnification and distance with a Samsung Galaxy camera (Samsung Inc., Seoul, South Korea) (Figure 2).[7,8] Magnification was adjusted to 16x9 and photographs were taken from a distance of 30 cm. Using AutoCAD software (Autodesk Corp., USA), the wound edges were marked on these photographs, and the wound area calculation was performed.

The hard palates of the sacrificed rats were excised en-bloc and fixated in a 10% buffered formaldehyde solution for one day. Following the routine tissue processing, the specimens were embedded in the paraffin blocks, and sections with a thickness of 4 μm

Table 1
Statistical analysis of histological parameters between groups

	PRP Groups		Control Groups		
	Mean±SD	Median	Mean±SD	Median	*p**
Wound area evaluation (mm²)					
First week	0.37±0.11	0.36	0.65±0.14	0.69	0.009
Second week	0.02±0.01	0.02	0.07±0.02	0.08	0.008
Third week	0±0	0	0±0	0	1.000
Severity of the inflammation					
First week	2.7±0.5	3	3.0±0	3	0.138
Second week	1.8±0.4	2	2.0±0.6	2	0.560
Third week	0.8±0.4	1	1.5±0.5	1.5	0.043
Neovascularization					
First week	1.8±0.4	2	2.3±0.5	2	0.092
Second week	2.4±0.5	2	1.3±0.5	1	0.018
Third week	2.8±0.4	3	1.8±0.8	2	0.023
Collagen concentration					
First week	1.0±0	1	1.0±0	1	1
Second week	2.4±0.5	2	1.3±0.5	1	0.018
Third week	3±0	3	2.2±0.4	2	0.005
Fibroblast proliferation					
First week	0±0	0	0±0	2.00	1
Second week	2.2±0.4	2	1.2±0.4	2.00	0.009
Third week	2.8±0.4	3	2.2±0.4	1.00	0.027

PRP: Platelet-rich plasma; SD: Standard deviation; * Mann-Whitney U test.

were obtained. The sections were stained with Masson's trichrome and hematoxylin-eosin and evaluated with 400× magnification (Olympus BX51, Olympus Optical Co., Ltd., Shinjuku, Tokyo, Japan) in order to detect the histopathological changes. The severity of the inflammation, neovascularization, fibroblast proliferation and collagen concentration were evaluated with histopathological examination. The evaluation was performed with the submucosal inflammatory tissue located under the two margins of the wound, and the mean value was calculated after scoring between 0 to 3+. The evaluation scores were: 0=not applicable, 1=mild, 2=moderate, 3=severe.[9] The histopathological examination of the tissue samples obtained from the rats was performed with a single-blind procedure (Figure 3).

Statistical analysis

IBM SPSS version 22.0 software (IBM Corp., Armonk, NY, USA) was used in the analysis of the data. Descriptive statistics of the data were evaluated with mean, standard deviation, and median values. The distribution of the variables was analyzed with the Kolmogorov-Smirnov test. Mann-Whitney U test was used for the analysis of the independent quantitative data.

RESULTS

In the third week, the macroscopic evaluation of wound field (0±0 mm, 0±0 mm) did not show any significant difference between the PRP and control groups ($p>0.005$). Macroscopic wound area was significantly smaller in PRP group in the first week (0.37±0.11 mm², 0.65±0.14 mm²) and second week (0.02±0.01 mm², 0.07±0.02 mm²) than in the control group ($p<0.05$) (Table 1).

In the PRP and control groups, there was no statistically significant difference regarding the severity of inflammation in the first week (2.7±0.5, 3.0±0) or second week (1.8±0.4, 2.0±0.6) ($p>0.05$). However,

in the third week (0.8±0.4, 1.5±0.5), the severity of inflammation was significantly lower in the PRP group compared to the control group ($p<0.05$) (Table 1).

Although no significant difference was observed considering neovascularization ($p>0.05$) in the PRP and the control groups in the first week (1.8±0.4, 2.3±0.5), there was a significant difference in the second week (2.4±0.5, 1.3±0.5) and vascular density was significantly higher in the PRP group than in the control group ($p<0.05$) in the third week (2.8±0.4, 1.8±0.8) (Table 1).

Although there was no significant difference between the PRP and control groups in the first week (1.0±0, 1.0±0) ($p>0.05$), second week (2.4±0.5, 1.3±0.5) or the third week (3±0, 2.2±0.4), collagen concentration in the PRP group was significantly higher than the control group ($p<0.05$) (Table 1).

Although there was no significant difference between the PRP and control groups regarding fibroblast proliferation (0±0, 0±0) ($p>0.05$), fibroblast proliferation was significantly higher in the PRP group compared to the control group ($p<0.05$) in the second week (2.2±0.4, 1.2±0.4) and third week (2.8±0.4, 2.2±0.4) (Table 1).

DISCUSSION

Wound healing is a repair and regeneration process facilitated by growth factors which regulate migration, proliferation, and differentiation of cells, production of proteins, enzymes and extracellular matrix and remodeling. Wound healing process in the oral cavity distinguishes itself with its relatively more warm and humid environment and the presence of abundant bacteria.[10] Bacteria can form a biofilm in the oral cavity after combining with the food. This biofilm may have a negative impact on wound healing and also may increase the risk of superinfection. Moreover, physiological functions such as speaking, chewing and swallowing cause tension and pressure on soft tissues. These forces may affect the separation of the wound margins and have a negative effect on the healing process.[11]

Several agents were tried in the chronic and delayed wound healing, and PRP is one of them. It was demonstrated in clinical and animal studies that PRP has positive effects on wound healing process.[12] Platelet-rich plasma is the autologous plasma component, which contains higher concentrations of platelets compared to basal levels.[1-4] Platelets, which have a low concentration in the plasma, are separated from other cellular elements, and thus higher concentrations are achieved. Platelets concentration of PRP is three to five times higher than the basal plasma concentration. The normal platelet count in the blood is 150.000-400.000/mm^3. Although the concentration may change depending on the preparation technique and used devices, the targeted standard platelets count of PRP is 1.000.000/mm^3.[1-3] Consequently, PRP also contains high concentrations of all coagulation and growth factors. According to several studies, the important factors in PRP which contribute to wound healing are platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF), epidermal growth factor, interleukin-8 and tumor necrosis factor.[2]

A good number of studies showed that PDGF increased the granulation tissue, epithelization, and neovascularization and accelerated wound healing without changing the wound healing process.[13,14] Its efficacy was shown in chronic wounds, neuropathic and diabetic ulcers and it is the only growth factor which is approved by the Food and Drug Administration in this indication.[13] The positive effects of IGF were observed in diabetic experimental animals and animals with inflammatory response suppressed by steroid administration.[15] The combined use of PDGF and IGF has a synergistic effect and support wound healing.[16] Studies have shown that VEGF accelerated the accumulation of granulation tissue in dermal wounds in rabbit ears and rat skin.[15] Animal studies had shown that transforming growth factor beta (TGF-β) had positive effects on partial and full-thickness wound in lower extremities of horses, on eardrum perforations of rats, on chronic ulcers, hypertrophic scars and intestinal mucosa ulcers of rats.[14,15] Epidermal growth factor which was applied to full-thickness skin wounds created in rabbits enabled faster healing and almost normal histological appearance.[17,18]

Although PRP was used initially in oral surgery and bone healing, reconstructive and cosmetic surgery, spinal surgery, cardiopulmonary bypass surgery, ligament, and tendon healing, nerve healing and wound healing, investigators focused mainly on bone and wound healing in the following studies after Whitman et al.[19] used it for the first time in maxillofacial reconstruction surgery, which was performed with osseointegrated titanium implants. The objective of the PRP studies was the application of the growth factor concentrates, which are the triggers of wound healing, to the wound area in order to accelerate the healing process. In our study, we monitored the severity of the inflammation, neovascularization, fibroblast proliferation, collagen concentration and epithelization in the 7^{th}, 14^{th} and 21^{st} days on rats with experimental oral wound and scored each parameter individually for histopathological evaluation of the effect of PRP on wound healing. According to our findings, wound treated with PRP

healed faster than the control group. These features represent accelerated tissue degeneration, which is one of the known effects of PRP (Figures 2 and 3).[20]

El-Sharkawy et al.[21] determined the anti-inflammatory effects of PRP. This anti-inflammatory effect of PRP depends on the suppression of the release of inflammatory cytokines, caused by PRP. Although the severity of inflammation was not significantly different between the PRP and control groups in the first or second weeks, it was considerably lower in the PRP group compared to the control group in the third week. Considering that PRP is rich in angiogenetic factors such as FGF, PDGF, TGF-β and VEGF, the hypothesis about PPR's increasing effect on wound healing is a consistent proposition.

In our study, there was no significant difference between the PRP and control groups regarding neovascularization in the first week; however, it was significantly increased in the PRP group in the second and third weeks. We detected that these findings were in concordance with clinical studies.[18] Several clinical and animal studies reported that fibroblast proliferation and collagen concentration were stimulated by growth factors like TGF-β, IGF-1, and b-FGF, which were found in PRP.[13,18] In our study, there was no statistically significant difference between the PRP and control groups in the first week regarding the fibroblast proliferation and collagen concentration. However, in the second and third weeks, there was a statistically significant difference between the groups with and without PRP application regarding fibroblast proliferation and collagen concentration (Table 1). These findings are in concordance with the study of Kajikawa et al.,[22] who demonstrated that collagen fibers and count of fibroblasts were increased after PRP injection. Lee et al.[23] conducted an animal study and showed that PRP application accelerated epithelization. Moreover, Cervelli et al.[24] showed that PRP accelerated dermal re-epithelization in patients who underwent fat tissue graft excision during reconstructive surgery. Similar to these studies, we observed in our study with the help of the macroscopic photographs that the wound area epithelization was significantly accelerated in the group with PRP application in the first and second weeks (Figure 2).

To our knowledge, this is the first study in which PRP was applied to oral wounds in rats for the first time. Although in our study wound healing time was shorter in the PRP group compared to the control group, further clinical studies are needed to determine the role of PRP in the healing process of the oral cavity wounds.

This study had some limitations. First of all, we did not prepare homologous PRP but used heterologous PPR injections. In order to neutralize this limitation, we exposed PRP to radiation and tried to reduce the possible antigenic reactions. Secondly, our histopathological evaluation was based on our subjective assessment. In order to eliminate this limitation, we designed a single-blind evaluation process. More objective and clear information may be obtained in further studies designed to evaluate the physiopathological markers of wound healing with immunohistochemical staining.

In conclusion, regarding the findings of our study, the application of PRP to the wound area accelerated epithelization as a result of better healing process in both the wound area and wound histopathological parameters compared to normal wound healing. We may conclude that PRP application has positive effects on intraoral wound healing in terms of both histological and macroscopic aspects.

Declaration of conflicting interests

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