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THE EFFECTS OF PLATELET RICH FIBRIN AND FIBRIN ADHESIVE ON WOUND HEALING: IMMUNOHISTOCHEMICAL RESULTS

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Abstract. This study was conducted to determine the effects of platelet rich fibrin (PRF) and fibrin adhesive (FA) on wound healing. Fourteen New Zealand rabbits were divided into 2 groups for 7 and 14 day postoperative follow-ups. It was created four full-thickness wounds which 5 mm in diameter on the dorsal back skin of all rabbits. Saline solution were instilled to the control group, autogenous PRF, FA and PRF plus FA were used in other wounds, respectively (n = 14). Measurements of wound were performed every day. On days 7th and 14th after the operation, seven rabbits were euthanized and skin specimens were obtained for histological examinations. As a result of the measurements of the wound areas, it was determined that the healing of the experimental groups was better than the control group. Histological evaluations showed that the granulation tissue and collagen accumulation were in the best PRF group on the seventh day and inflammatory cell infiltration was observed in at least PRF group. In conclusion, it was concluded that PRF and FA accelerate wound healing, the best wound healing is observed in PRF plus FA group and PRF and FA can be used confidently in open wound treatment.

Keywords: *Platelet rich fibrin, fibrin adhesive, wound healing.*

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1. Introduction

Platelets are necessary for clot formation and are responsible for the release of growth factors to trigger and support wound healing. After injury, growth factors increase tissue formation and healing (Choukroun *et al.*, 2006; Rozman & Bolta, 2007; Can & Durmuş, 2015).

From platelets in the Platelet Rich Fibrin (PRF) is released cytokines such as Transforming Growth Factor-β (TGF-β), Platelet-induced Growth Factor (PDGF) and Insulin-Like Growth Factor-I (IGF-I) (Dohan *et al.*, 2006a, 2006b, 2006c; Su *et al.*, 2009). Also cytokines is released by leukocytes in PRF are IL-1β (Interleukin-1β), IL-6 (Interleukin-6), TNF-α (Tumor Necrosis Factor α), Interleukin 4 (IL-4) and Vascular Endothelial Growth Factor (VEGF) (Anitua *et al.*, 2005; Dohan *et al.*, 2006a; Lee *et al.*, 2010).

PRF decreases bleeding, accelerates hard and soft tissue healing, accelerates vascularization of tissues through growth factors (Choukroun *et al.*, 2006; Dohan *et al.*, 2006b; Can & Durmuş, 2015).

It is reported that Fibrin Adhesive (FA) is a natural hemostatic agent (Kram *et al.*, 1988; Taha *et al.*, 2006). FA can be used in regions with difficult sewing application, in nerve and vascular anastomoses, porous vascular prostheses, parenchymatous

hemorrhages, coagulation defects, hemophilic patients, and skin transplantations (Garza & Rumsey 1990; Demirel *et al.*, 2008).

The aim of this report was investigate the efficacy of PRF and FA as compared with the control group on wound healing via the immunohistochemical investigation.

2. Material and methods

Fourteen, 5-6-months old, New Zealand male rabbits were used in this study. This study was approved by the institutional animal ethics committee (no. 177, 04.11.2015). This study was supported by Fırat University Scientific Research Projects Coordination Unit (FUBAP) (grant number: VF.16.07).

Rabbits were divided into two groups for postoperative 7 and 14 days follow-up (n=7). For anaesthetization, intramuscularly 35 mg/kg ketamine hydrochlorure (Ketalar, Parke-Davis, 50 mg/ml) and 5 mg/kg xylazine hydrochloride (Rompun, Bayer, 23.32 mg/ml) were given.

PRF was prepared by centrifuging of 6 ml of blood (400 g, 10 minutes). In this study, the combi set of FA (Beriplast P Combi-Set, 1 mL, CSL Behring, Marburg, Germany) was used.

Four full-thickness wounds were created the back skin of the rabbits with a 5 mm diameter skin biopsy instrument. Saline solution were instilled to the control group, autogenous PRF, FA and PRF plus FA were used in the other wounds, respectively (n=14).

In FA group, fibrinogen solution and thrombin solution were performed separately according to the manufacturer instructions. The fibrinogen solution was applied to the wound area and a solution containing thrombin spread just above it. In PRF plus FA group, after placing PRF in the wound area, a thrombin solution was spread on it by applying a fibrinogen solution. Applications were performed once after postoperatively in all groups.

Wound size measurements were performed every day. The day wound contraction started, wound contraction rate, expansion rate, day of epithelialization begins and the number of days in which healing was fully complete were evaluated.

Postoperative on the 7th and 14th days 7 rabbits euthanized and the skin samples taken were fixed in 10% buffered neutral formalin solution for 48 hours. Paraffin blocks were prepared from samples undergoing tissue follow-up.

In this study, with streptavidin-biotin peroxidase method (using Ultravision kit, Labvision, Cat No: TP-125-HL), immunohistochemically were compared TGF-β_1, bFGF releasing in normal, PRF, FA and PRF plus FA treated wounds.

To evaluate TGF β_1, 10 different areas were randomly selected at a cross section of each animal at × 40 magnification. Scoring was performed as 0: no positive cells, 1: 1-25% positive cells, 2: 26-50% positive cells, 3: 51-75% positive cells, 4: 76-100% positive cells. In the study, fibroblast growth factor 2 (bFGF) was considered as an indicator of angiogenesis. New angiogenesis indicators in 10 different areas were counted with ×40 magnification under light microscope.

SPSS (22.0 version) program was used for the statistical evaluation of wound measurement values and histological scoring. On the 7th day, comparisons among control, PRF, FA and PRF plus FA groups on the same animal were made with the non-parametric Kruskal-WallisH test. In-goup comparisons were made with the Mann-

Whitney-U test. The same tests were used in comparisons between groups on the 14th day.

Intra-group comparisons of each group on different animals on the 7th and 14th days were made with the non-parametric Mann-Whitney-U test. Data were presented as ± SEM value. P <0.05 value was accepted as significantly.

3. Results

In the present study wound contraction percentages were given in Table 1.

Table 1. Wound contraction percentages (%)

		Groups			
	Days	**Control**	**PRF**	**FA**	**PRF + FA**
WA (%)	7	40.05±0.17^{a}	20.38±0.16^{b}	18.04±0.23^{b}	14.93±0.22^{c}
	14	9.48±0.09^{a}	2.19±0.13^{b}	2.19±0.17^{b}	1.43±0.10^{b}
WC (%)	7	59.95±0.17^{a}	79.62±0.16^{b}	81.96±0.23^{b}	85.07±0.22^{c}
	14	90.52±0.09^{a}	97.81±0.13^{b}	97.81±0.17^{b}	98.57±0.10^{b}

Wound area (WA) (%) = (Wound area on day X / Wound area on day 0) × 100. Percentage of wound contraction on day X (WC) (%) = 100 – Percentage of wound area on day X. Control: Untreated group. **PRF:** Platelet rich fibrin group. **FA:** Fibrin adhesive group. **PRF+FA:** Platelet rich fibrin plus fibrin adhesive group. **^{a,b,c}:** The differences between the groups in the same row are significant. P < 0.001.

No significant difference was observed between the groups on the 7th day of wound healing in terms of new angiogenesis formations showing FGF positivity (Figure 1). On the 14th day, FGF positivity was found to be more marked in the control and FA groups than in the other groups (Figure 2). On the 7th and 14th days FGF scoring results are summarized in Table 2.

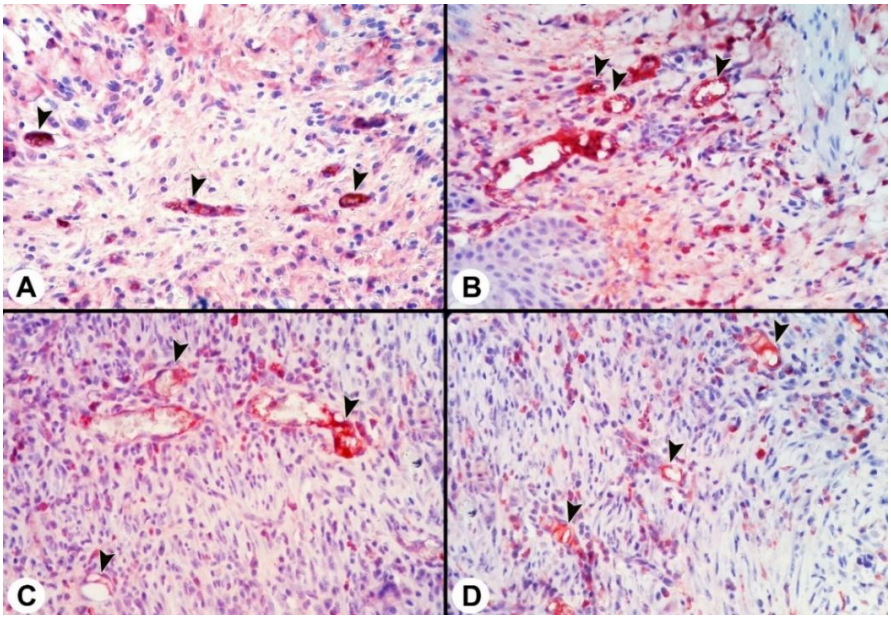

Fig. 1. A. Control, **B.** FA, **C.** PRF, **D.** PRF+FA. On the 7th day, capillar vessels formations in the wound area which is showing FGF positivity in all groups, × 200 MH

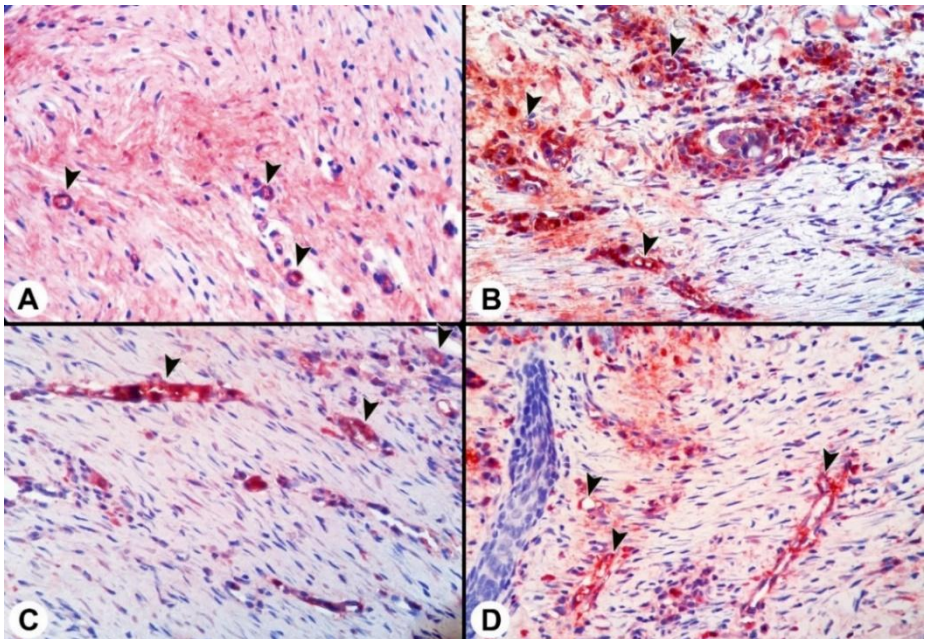

Fig. 2. A. Control, **B.** FA, **C.** PRF, **D.** PRF+FA. On the 14th day, capillar vessels formations in the wound area which is showing FGF positivity in all groups, × 200 MH

Table 2. Average values of new angiogenesis formations showing FGF positivity in the wound area

Day	Control	FA	PRF	PRF+FA	SE	P
7	63.00 ± 5.80	70.67 ± 9.17	61.67 ± 10.72	68.00 ± 4.49	3.78	NS
14	54.33 ± 5.42[a]	62.67 ± 5.49[a]	36.67 ± 2.23[b]	37.33 ± 2.01[b]	3.02	0.001

NS: There is no statistically significant difference between the groups. **[a,b]:** There is a statistical difference between the average values carrying different letters in the same row.

TGF positivity was detected in fibroblasts and fibrocytes, inflammatory cells, keratinocytes and vascular endothelial cells. It was found that TGF positivity was generally more severe on the 7th day compared to the 14th day (Figure 3). It was noteworthy that on the fourteenth day, the reduction in TGF releasing was more pronounced in the control and PRF groups than in the FA groups (Figure 4). No statistically significant difference was observed between the 7 and 14 day groups in terms of TGF score. Seven and 14 day TGF scoring results are presented in Table 3.

Table 3. Average scoring values of cells showing TGF-β_1 positivity in the wound area on the 7th and 14th days in the control and treatment groups

Day	Control	FA	PRF	PRF+FA	SE	P
7	3.43 ± 0.14	3.27 ± 0.17	3.90 ± 0.04	3.13 ± 0.42	0.13	NS
14	2.67 ± 0.33	3.10 ± 0.35	2.30 ± 0.30	2.93 ± 0.04	0.15	NS

NS: There is no statistically significant difference between the groups.

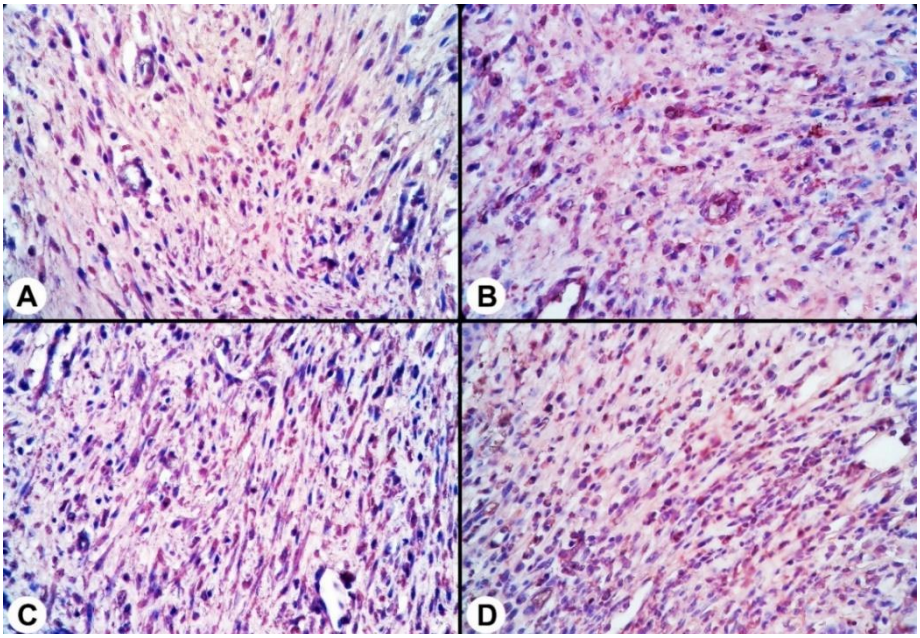

Fig. 3. A. Control, **B.** FA, **C.** PRF, **D.** PRF+FA. TGF positivity in the wound area on the 7th day, × 200 MH

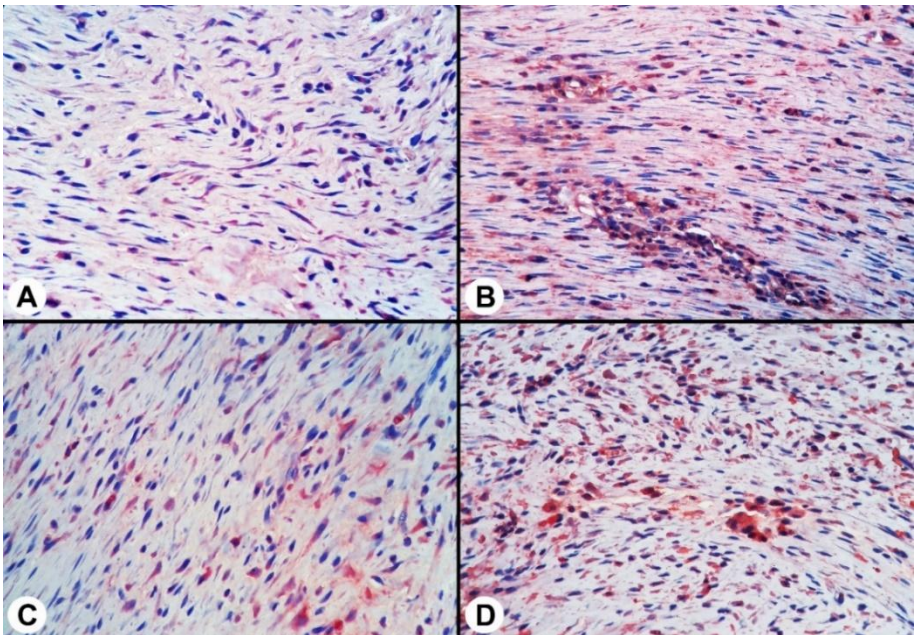

Fig. 4. A. Control, **B.** FA, **C.** PRF, **D.** PRF+FA. TGF positivity in the wound area on the 14th day, × 200 MH

4. Discussion

Extended healing process in the skin wounds decreases the quality of life of patients. Healing is wanted without infection, in a short time, without scarring (Durmus *et al.*, 2012; Durmus *et al.*, 2017).

Acceleration of healing in wound treatment is among the main goals. For this purpose, alternative treatment methods are performed today (Jorgensen *et al.*, 2011;

Chignon-Sicard *et al.*, 2012; Ding *et al.*, 2017). In the present study, it was aimed to evaluate the findings obtained by applying PRF (which has many growth factors) and FA in skin defects in rabbit model. In this study it is aimed that to find efficient, fast, reliable, uncomplicated, easy alternative treatment method.

In some studies it was reported that the effects of systemic hormones and growth factors on soft and hard tissue metabolism. Growth factors plays important role in the regulation of cellular events such as proliferation, differentiation, chemotaxis and morphogenesis in the wound healing process (Chignon-Sicard *et al.*, 2012; Can & Durmuş, 2015; Fırat Öztopalan *et al.*, 2017).

PRF contains high platelet concentration, FGF, TGF-β, PDGF, IGF-1, VEGF, and connective tissue growth factor (Nurden *et al.*, 2008). These factors accelerate wound healing and tissue regeneration by promoting cell proliferation, enhancing collagen synthesis, encouraging vascularization, sitimulating cell differentiation, and leading to the destruction of necrotic tissues (Can & Durmuş, 2015). It is reported that in some studies (Jorgensen *et al.*, 2011; Chignon-Sicard *et al.*, 2012; Lundquist *et al.*, 2013), PRF supports wound healing in many pathological processes.

There are some authors reporting platelet rich plasma (PRP) use to increase tissue healing (Sampson *et al.*, 2008; Sakata *et al.*, 2012). However, since PRP, which is in liquid form, flows away from the wound area immediately after application to the wound area, the use of PRP is limited. PRF, on the other hand, has concentrated growth factors that accelerate wound healing. PRF clot can be fixed where it is applied and secretes growth factors continuously to the wound area. In the present study, it is aimed to benefit from growth factors in the PRF. Therefore, it was considered to use PRF, which has the property to stimulate wound healing for maximum time.

Wounds of several sizes were performed in studies investigating wound healing. Wound sizes is important in shortening wound healing time (Durmus *et al.*, 2012; Durmus *et al.*, 2017; Fırat Öztopalan *et al.*, 2017). For experimental studies, it is considered that it is adequate to create wound sizes that does not healing during the follow-up period in the untreated (control) groups. On the 14th day, the observing of unhealing wounds in the control group indicates that the excised skin wound of 5 mm in diameter was of adequate size for this study.

In the present study, it was observed that PRF is easy to carry out to the wound area, but the PRF is difficult to immobilization on the wound area. FA was easily applied to the wound area according to the manufacturer instructions. It was easy to apply PRF plus FA, and FA had a positive effect to the pasting of the PRF on the wound area.

When the data in table 1 are examined, on day 7 and 14, it is observed that the wound healing in the treatment groups was faster than the control group. Likewise, on the 7th day, in the PRF plus FA group was found the best wound healing compared with the other groups. Also, on the 14th day, although there was no statistical difference compared to the PRF and FA groups, the improvement in the PRF plus FA group was observed to be better.

On the 7th day, although there is no statistical difference between the PRF and FA groups, the quick decrease on the FA group's wound area can be explained by the positive contribution of FA to the wound contraction. It is considered that FA helps for uninterrupted release of growth factors from the PRF via pasting the PRF to the wound area for the faster healing in the PRF plus FA group.

Ding *et al.* (2017) reported that PRF accelerates skin wound healing by increasing blood vessel formation in diabetic mice models. It has been reported that PRF significantly contributes to the sciatic nerve healing (Metineren *et al.*, 2017).

Growth factors have an important role in all stages of wound healing. Growth factors play a role in cell division, migration, differentiation, and enzyme and protein production. Growth factors contributes to the wound healing by stimulating angiogenesis and cellular proliferation (Steefos *et al.*, 1994; Fırat Öztopalan *et al.*, 2017).

Although there was no significant difference in FGF positivity on the 7th day in this study, on the 14th day, it was more evident in the control and FA groups compared to the other groups. In terms of TGF score, no statistically significant difference was observed between the groups of 7 and 14 days.

5. Conclusion

As a result, it was concluded that PRF and FA can be used as a useful material in accelerating the healing of wounds. The use of PRF and FA together not only helps the fixation of PRF to the wound area, but also enhancement wound healing even if there is no statistical difference. It is thought that the data obtained in this study may be a reference source for wound treatment. However, it is thought that the contribution of PRF and the growth factors to wound healing can be better understood by further clinical studies.

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