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Original Article

Effectiveness of platelet-rich fibrin on tendon healing: experimental study in a rat model

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Abstract: Platelet Rich Fibrin (PRF) is a new biological augmentation material which is remarkable with its rich cellular components and growth factors. The aim of this study was to evaluate the effect of PRF on tendon healing in a rat model. PRF and control groups included eight rats. PRF was obtained with centrifuging blood collected from the rats and applied on the tendon repair site in Achilles tendon injury model created in rats. Effectiveness of PRF was evaluated with histological and immuno-histochemical methods. PRF improves formation of regular collagen structures ($P<0.01$) and also increases vascularity ($P<0.05$) of the tendon repair site. Cartilage formation occurred both in PRF and control groups. Gap formation was observed only in the control group. PRF is an autogenous biomaterial which enhances and support tendon healing.

Keywords: Platelet rich fibrin, Achilles tendon, platelet rich plasma, tendon injury, experimental model

Introduction

Tendon injuries are common in daily clinical practice. Sharp injuries, tendinitis and spontaneous ruptures of the Achilles tendon affect the person's quality of life adversely. Length of the healing period varies depending on the treatment methods. Surgical and conservative methods of treatment each have its own advantages and disadvantages. Increased treatment costs and the loss of labor occurring in this period are other disadvantages. Tendon recovery is a difficult and prolonged process due to lack of primary vascular elements. Early rehabilitation is indisputably important. Undesirable consequences contain re-rupture or gap formation during rehabilitation [1-3]. The incidence of re-rupture following surgical treatment is relatively low (1.7%-5.4%) [4, 5]. Although motion prevents adhesions after repair, unless movement is restricted, gap formation may occur at the repair site, which impairs the healing capacity. In recent years, tendon repair techniques, rehabilitation protocols and splints have been used in combination as supportive methods in order to prevent complications. Adhesion of the healing tendon to surrounding tissues is one of the most important problems after repair. Scarring and adhesion formation result in impaired gliding of the tendon, with loss of joint motion and function and reduced quality of life [2]. Forces acting on the tendon repair site are the active muscle contractions [3]. Surgical treatment results in lower re-rupture rates compared to non-operative methods. Surgery carries an increased risk of complications (neurovascular injuries, infection and wound healing problems) [4, 5]. In last decades, alternative treatment methods have been developed to resolve all these problems. One of the most popular current complementary methods is the use of Platelet-rich plasma (PRP). Platelet-rich fibrin (PRF) is currently considered a new generation of platelet concentrate. It comprises a matrix of autologous fibrin. Moreover, it has several advantages over PRP, including an easier and fully autologous preparation, not requiring the use of anticoagulants, chemical manipulation of the blood [6, 7]. The PRF involves pro-inflammatory cytokines (IL-1β, IL-6, TNFα), anti-inflammatory cytokines (IL-4, IL-10) and growth factors (Transforming Growth Factor-beta (TGF-B), basic Fibroblast Growth Factor (b-FGF), Platelet Derived Growth Factor (PDGF),

Table 1. Histological assessment scale [12]

Collagen grade	Degree of angiogenesis	Cartilage formation
0: Normal collagen oriented tangentially	0: Moderate infiltration of tissue with arterioles	0: No cartilage formation
1: Mild changes with collagen fibers less than 25% disorganized	1: Presence of capillaries	1: Isolated hyaline cartilage nodules
		2: Moderate cartilage formation of 25% to 50%
2: Moderate changes with collagen fibers between 25% and 50% disorganized	2: No vasculature infiltration	3: Extensive cartilage formation, more than 50% of the field involved

Table 2. Histological assessment scores for two study groups

	PRF (n=8)	Control (n=8)	P
Collagen Grade	0.875 ± 0.834	1.375 ± 0.744	P<0.01
Degree of Angiogenesis	0.25 ± 0.462	1.5 ± 0.755	P<0.05
Cartilage Formation	1 ± 0.755	1.375 ± 0.517	P>0.05

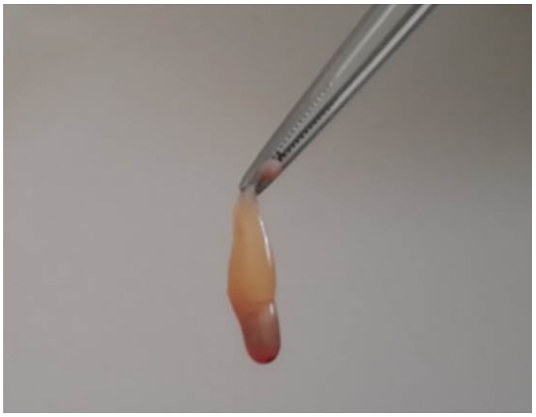

Figure 1. Standard PRF.

Epidermal Growth Factor (EGF), Vascular Endothelial Growth Factor (VEGF), Connective Tissue Growth Factor (CTGF). PRF involves high cellular content, especially platelets and leukocytes [16]. PRF allows for a great potential in bone and soft tissue regeneration, with lower incidence of inflammatory reactions [8-11].

The purpose of this experimental study was to evaluate the histopathological effect of PRF on an Achilles tendon injury model in rats.

Material and methods

Twenty adult Sprague Dawley male rats, weighing 200-300 g, were used in this study. The study was approved by the Institutional Ethical Committee of Laboratory Animals Care and Use. The animals were housed in Laboratory Animal Care-Augmentation facility of Dumlupinar University in a temperature-controlled room (20-22°C) on a twelve-hour light-dark cycle and were provided with rat pellets and water ad libitum. There were eight animals in per standard cage.

Preparation of platelet-rich fibrin

The blood to obtain standard PRF was collected from four rats without addition of anticoagulants and was centrifuged at 2700 rpm for 12 minutes. The PRF matrix was immediately withdrawn from the tube and separated from the remaining blood (**Figure 1**).

Surgical procedure

The remaining sixteen rats were randomly divided into two (PRF and control) groups. Following the induction of anesthesia with intraperitoneal injection of ketamine 50 mg/kg and xylazine hydrochloride 10 mg/kg, the right hind limb of each animal was shaved and prepared for aseptic surgery with chlorhexidine gluconate. With use of aseptic technique, a longitudinal incision was made on the posterior aspect of the right hind limb. The Achilles tendon was exposed, incised perpendicularly at its midpoint and repaired with use of a 6/0 Polypropylene suture. The rats in both groups were operated in the same way, but in PRF group; the pre-prepared standard PRF was placed around the tendon repair site and the skin was closed with 5/0 polypropylene. No infections or allergic reactions were observed. The rats were left free to move without immobilization for four weeks. After 4 weeks, the rats were killed and the tendons were removed and fixed in buffered formalin 10% solutions for 48 hours.

Histological and immunohistochemical study

The tendon specimens were dehydrated and embedded in paraffin. They were sectioned (4

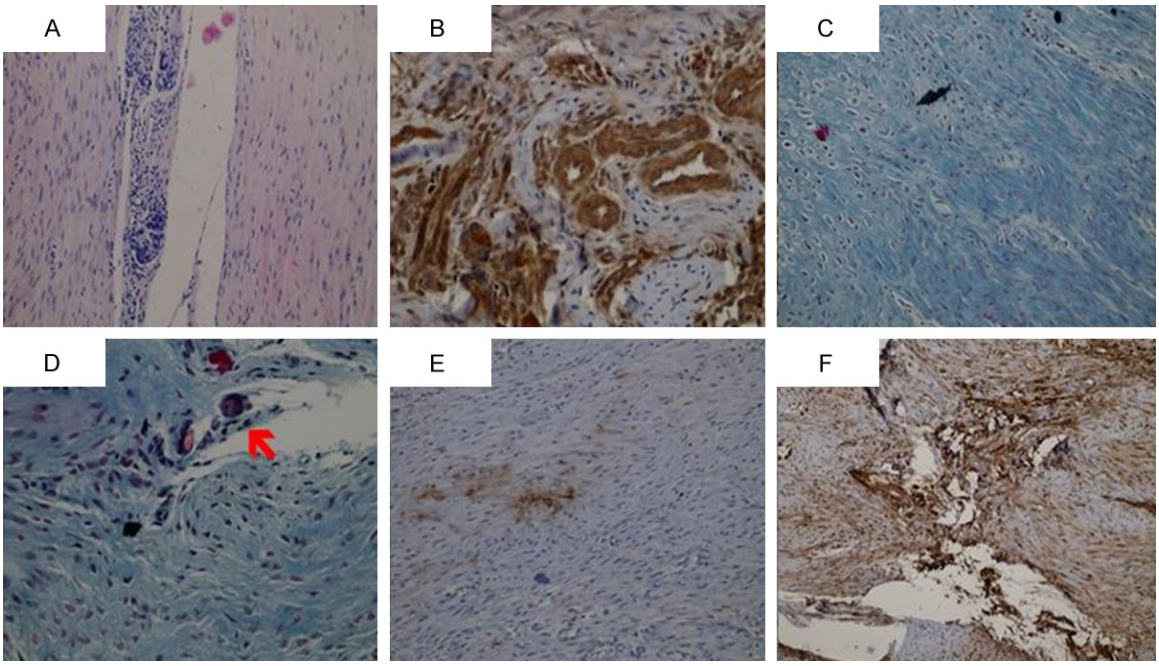

Figure 2. PRF group histological and immunohistochemical assessment: A. Normal Collagen orientation (Grade 0) with H&E 40X. B. Vascular proliferation in PRF-M group CD34 40X. C. Chondrocyte and Collagen fibers at the healing zone Masson's trichrome 40X. D. Giant cells due to polypropylene suture (red arrow) Masson's trichrome 40X. E. Collagen antibody 10X mild collagen changes. F. Healing zone with FGF antibody 40X.

μm) and placed on slides, and stained with hematoxylin & eosin (H&E) and Masson's trichrome. Collagen orientation was also assessed using collagen I, III and FGF antibodies. CD34 immunostaining was used for detecting angiogenesis. A scoring system for collagen organization, degree of angiogenesis and cartilage formation described by Rosenbaum (**Table 1**) was used [12]. Grades were determined based on the visual field. Slides were examined under a light microscope (Olympus BX51, Tokyo, Japan) by two pathologists who were blinded to the treatment and control groups. Each specimen was graded regarding the data obtained from ten sections. According to this scoring system, lower scores correlate with better histological results.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences SPSS® 21.0 (SPSS Inc, Chicago, Illinois, USA). Results were expressed as the median (min.-max). The non-parametric Mann-Whitney U test was used to evaluate the differences between the PRF and control groups. Significance was set at $P<0.05$.

Results

Tendon healing was complete in all rats in PRF group. Gap formation at the repair site was observed in four rats in the control group. Histological grading was done by H&E staining. A slight cartilage formation was observed both in the PRF and control groups. The mean histological score for the collagen grade ($P<0.01$) and angiogenesis ($P<0.05$) in PRF group was better than the control group (**Table 2**). However, there was no significant differences between the two groups regarding cartilage formation ($P>0.05$). According to histological scaling, normally oriented collagen fibers are shown in **Figure 2A**. Regular alignment of type I and type III collagen fibers was apparent in PRF group (**Figure 2A**, **2E**). Vascular proliferation was marked in PRF group with CD34 immunostaining (**Figure 2B**).

Collagen fibers in the tendons of the control group demonstrated irregular orientation ($P>0.05$). Gap formation was more prominent in the PRF group (**Figure 3A-C**). Cartilage formation at healing zone in control group is shown in **Figure 3B**, **3D**. Vascularity scores were signifi-

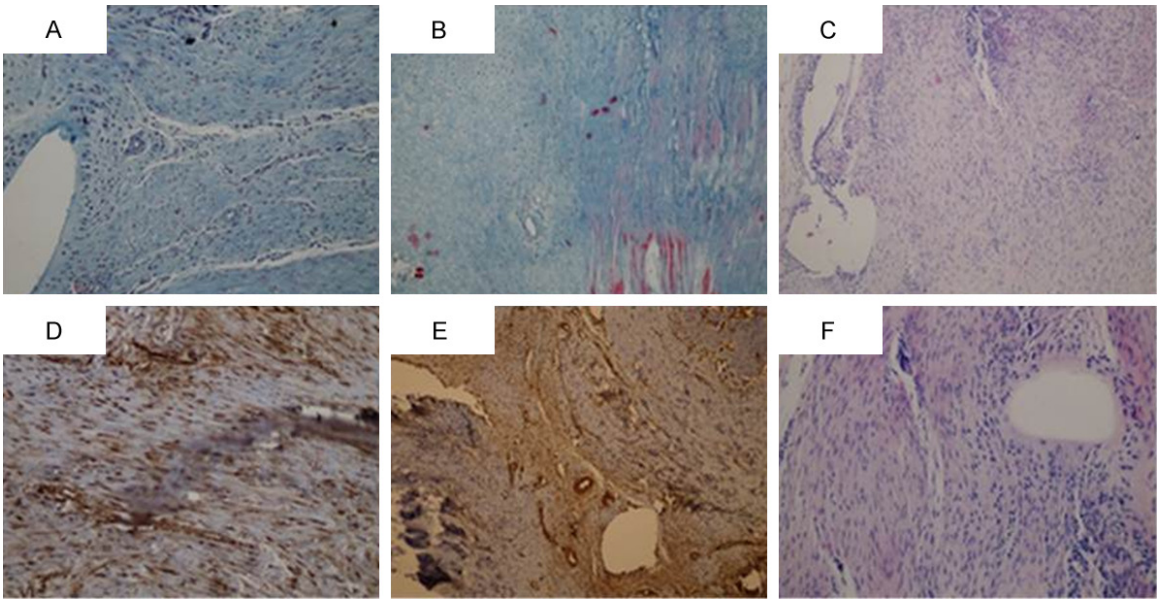

Figure 3. Control group histological and immunohistochemical assessment: A. Gap and chondrocyte formation at the healing zone Masson's trichrome 40X. B. Chondrocyte proliferation in control group Masson's trichrome 10X. C. Collagen orientation (Grade 2) H&E 40X. D. Chondrocytes with FGF. E. CD34 40X. F. Collagen orientation (Grade 1) H&E 40X.

cantly lower in the control group compared to the PRF group (**Figure 3E**) ($P<0.001$).

Discussion

In this experimental study, we examined the effects of PRF on Achilles tendon injury model in the rats. We found that PRF was significantly superior to the control group regarding the organization of collagen fibrils, tendon healing and angiogenesis. However, cartilage formation within the healing zone was present in both groups. Some studies have shown that the rats are prone to cartilage formation in their Achilles tendons following injury [25].

Today, increasing numbers and diversity of trauma require healing enhancer/accelerator biological augmentation techniques in addition to the conventional treatment. Two commonly applied methods are the use of PRP and PRF. There are large amounts of growth factors in the platelet granules. The growth factors (TGF, b-FGF, PDGF, VEGF) arising after degranulation are responsible for the results obtained in this study [13-15]. VEGF is considered to be one of the most potent vascular growth promoters and plays a direct role in the migration, proliferation, and differentiation and the stable matrix leads to a continuous slow release of VEGF for up to 28 days [17, 26]. However, the effect of PRF cannot be limited to its growth factor content [27].

In contrast, Rodeo et al found that PRF may have an adverse effect on healing [19]. Although some authors have expressed overdose of growth factor leads to undesirable effects on healing. Molloy et al reported that the use of growth factors increase the efficacy and efficiency of tendon and ligament healing [20].

Zumstein et al demonstrated that PRF increases vascularity of the tendon in rotator cuff repair [18, 26]. Currently, many materials for healing enhancement and acceleration are commercially available. Synthetic or animal-derived materials also cause numerous problems. Immune reaction, disease transmission, high costs, ethical problems and infections are some of them [21, 22]. The indisputable advantage of PRF is that the preparation method is all autologous. Thus, it does not cause allergic reaction.

The PRF production protocol attempts to accumulate platelets and release cytokines in a clot fibrin [23]. Dietrich et al claim that the basal platelet count must be diminished to 89.04% to 95.10% of the basal concentrations in whole

blood [24]. This technique has recently been developed with different centrifuge methods [28]. Predictably, platelet aggregation disruptive drugs (acetyl salicylate) may complicate preparation of PRF. In elderly patients using anticoagulants it may be difficult to obtain PRF. There are no studies about this subject yet. Further studies to investigate the effect of PRF on bone, cartilage and nerve tissues are needed.

In conclusion; Platelet-rich fibrin seems to be a promising minimally invasive method with low risks and favorable results on tendon healing.

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Disclosure of conflict of interest

None.

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