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ARTICLE

Comparison of the effect of the autogenic and xenogenic use of platelet-rich plasma on rabbit chondrocutaneous composite graft survival

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ABSTRACT

The platelet-rich plasma (PRP) has become popular in the medical world due to its content of growth factors and numerous studies are experimental. In experimental studies, the preparation and application of PRP are problematic and allogenic PRP transfers have been preffered, because of the difficulties in preparation of autogenic PRP in animal experiments. Xenogenic transfers and their effects have not been studied in this topic. This study aimed to investigate the effect of autogenic and xenogenic use of PRP on composite graft viability.

Methods: Two composite grafts are prepared for each ear of nine rabbits. Each ear was randomly divided into three groups. After the procedure, the wound edges and base were injected with 1 cc serum physiologic, autogenic PRP or 1 cc human-derived xenogenic PRP. At 3 weeks, samples were taken, photographic and histopathological evaluations were made.

Results: The graft viability was better in autogenic and xenogenic group compared to the control group. In comprasion of autogenic and xenogenic groups, although the macroscopic evaluation revealed better graft viability and less necrosis in the group which had been treated with autogenic PRP, the difference was not statistically significant. The three groups did not significantly differ in terms of inflammation. Vascularization examined histopathologically. CD31 staining, which was used to evaluate angiogenesis, was significantly higher in the autogenic PRP group than the remaining two groups.

Conclusion: Although autogenic PRP has better results histopathologically, the xenogenic use of PRP may be an alternative for studies, when macroscopic evaluation is necessary.

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Platelet rich-plasma; animal model; graft survival; xenotransfusion

Introduction

The use of platelet-rich plasma (PRP) obtained from human blood has gained the attention of many reconstructive surgeons. It is now known that platelets obtained by separating the plasma of autologous blood, which has a higher platelet concentration, contain a high amount of growth factor. These growth factors serve as basic building blocks, especially in wound healing, tissue regeneration and neovascularization stages. PRP has been used to accelerate the healing of many wounds and increase the viability of many tissues by increasing growth factors in the environment, and it has been shown to be beneficial in many clinical and experimental studies [1,2]. In addition to its use in clinically problematic wound healing such as diabetic foot and chronic wounds such as venous ulcers, PRP also has a wide area of application, including cosmetics, orthopedics and periodontics, as well as many other health problems [3].

Composite graft is a valuable technique for minor defect with multiple-layer deficiency. Composite graft is widely used in alar reconstruction, because of the easy 3D reconstruction with a good aesthetic result. The main obstacle is graft survival for defects larger than 1 cm in diameter. In literature, numerous methods have been investigated for overcome this problem. Recent studies have shown that PRP is one of these applications that allow the use of composite graft in larger sizes.

The greatest problem in animal studies with PRP is the preparation the blood product. The limited amount of blood that can be taken from healthy subjects. The appropriate blood volume that can be taken without stressing animals is limited. Considering that 5–8 cc whole blood is required to obtain 1 cc PRP in studies on PRP, the amount of blood that can be considered insignificant in humans. If the use of autogenic PRP is requested in small animals, such as mice, rats, and guinea pigs, this amount can cause stress-induced death [4]. The use of allogenic PRP is an option in small animals. Yet al. logenic PRP preparation is a tedious and costly process, since it would require sacrificing some of the animals before the study. Another option may be prefer of advanced animal species for experiment. This may be also costly and makes the study more difficult than necessary. The Xenogenic PRP may be the answer to this problem. The use of human-derived PRP solves the problems caused by stress-induced death in small animals, sacrificing some animals before study or necessity of selection advanced animal species.

Although autogenous materials taken from the patient are highly preferred in tissue transfer due to their high tissue compatibility and not affecting immune mechanisms, allogenic or

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xenogenic transfer is in practice for a long time in some cases to meet greater graft requirement or reducing donor site morbidity [5]. In light of these findings, in animal experiments involving PRP, the xenogenic use of this concentrate has also been considered as an option. This study aimed to investigate the effect of PRP on composite graft viability and determine whether the autogenic and xenogenic use of platelets resulted in any difference in PRP preparation.

Method

Selection of study groups

All the procedures were approved by the Ankara Training and Research Hospital Animal Research Ethics Committee. Each of the ear of the nine rabbits was individually numbered on the day of the operation. The skin and cartilage were removed in a composite manner, creating two defects in each ear. Each ear was randomly divided into three groups. Both ears of the rabbits were separated to be included in different groups. To prevent cross-interaction, two defects in the same ear were adjusted to be in the same group. Six ears and twelve defects in each group, resulting in a total of 36 defects in nine rabbits. The first group was designated as the control group, The second group was formed as the autologous PRP transfusion group and lastly, the third group was selected as the xenogenic PRP transfusion group.

Surgical Technique

Anesthesia was subcutaneously administered with 40 mg/kg ketamine hydrochloride (Ketalar® 50 mg/ml vial, Pfizer, New York, NY) and 5 mg/kg xylazine (Rompun® vial, Bayer, Leverkusen, Germany). After the induction of anesthesia, each ear was shaved, and the skin was cleaned.

For both ears of the rabbits in each group, two composite grafts from each ear were planned, approximately 4 cm from the external meatus and 5 mm from each side of the central ear vein (Figure 1). Following local anesthesia with a dental injector, the subchondral composite graft containing the marked 2 cm circular skin and cartilage removed. Then, the harvested graft was replaced with the other composite graft in the same ear and sutured to the defect area with 4.0 Prolene sutures (Figure 2). After suturing, 1 cc injection was made into the outer periphery of the grafts in all the three groups (Figure 3).

PRP preparation and application

Before the surgical procedure, a total of 10 cc of blood was taken from each animal and placed in tubes containing 10% sodium citrate (BD Vacutainer® ACD-A, UK). The tubes were separately numbered for each rabbit to prevent any mix-up, and autogenic PRP prepared from their own blood was applied to each rabbit in autogenic PRP group.

For xenogenic PRP group, 20 cc of blood was collected from three volunteer human donors, and PRP was prepared for 12 defects.

PRP in both groups was prepared in the same technique to ensure standardization. Whole blood was centrifuged at 400 g for 15 min at room temperature and transferred to a new centrifuge tube including the buffy coat. The second centrifuge was centrifuged at 800 g for 15 min, and 1.5 cc PRP was obtained from each sample by including the buffy coat. In the first group, 1 cc of serum physiologique was injected into the wound edges after suturing. In the second group, 1 cc PRP prepared from the rabbits' own blood was injected into the wound edges after suturing. In the third group, 1 cc of xenogenic PRP prepared from the volunteer human donors was injected into the wound edges after suturing. Once the procedures were completed, wound dressing was applied. Antibiotic ointment is applied as daily dressing. Oral antibiotics and analgesic drugs were used for enfection control and pain management.

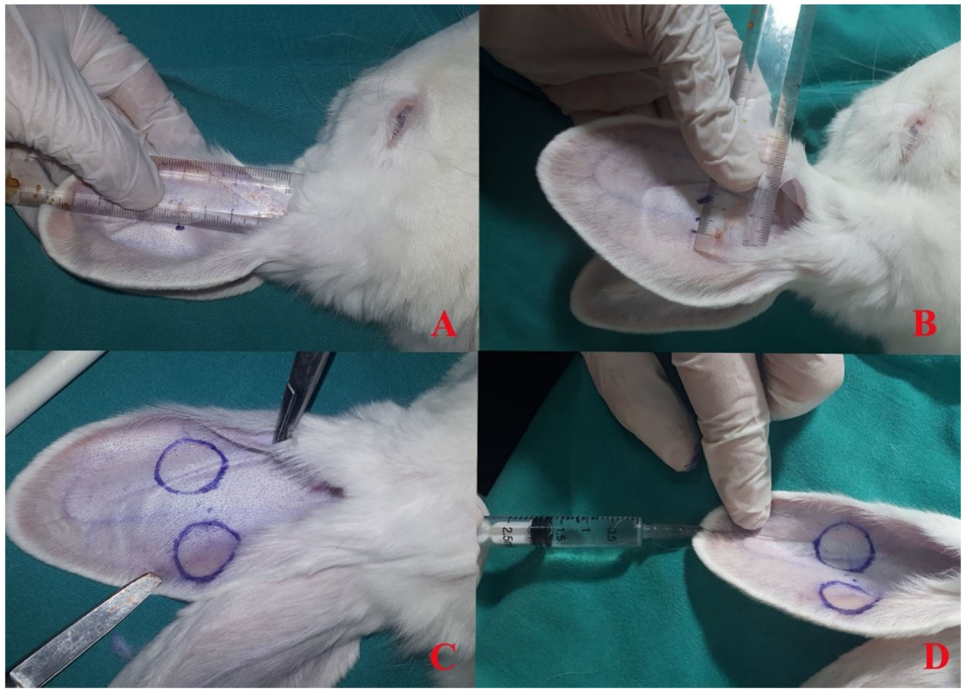

Figure 1. (A) Measurement is performed approximately 4 cm from the external meatus. (B) Measurement of 5 mm distance on both sides of the ear vein. (C) Marking the 2 cm circular graft. (D) Application of local anesthesia.

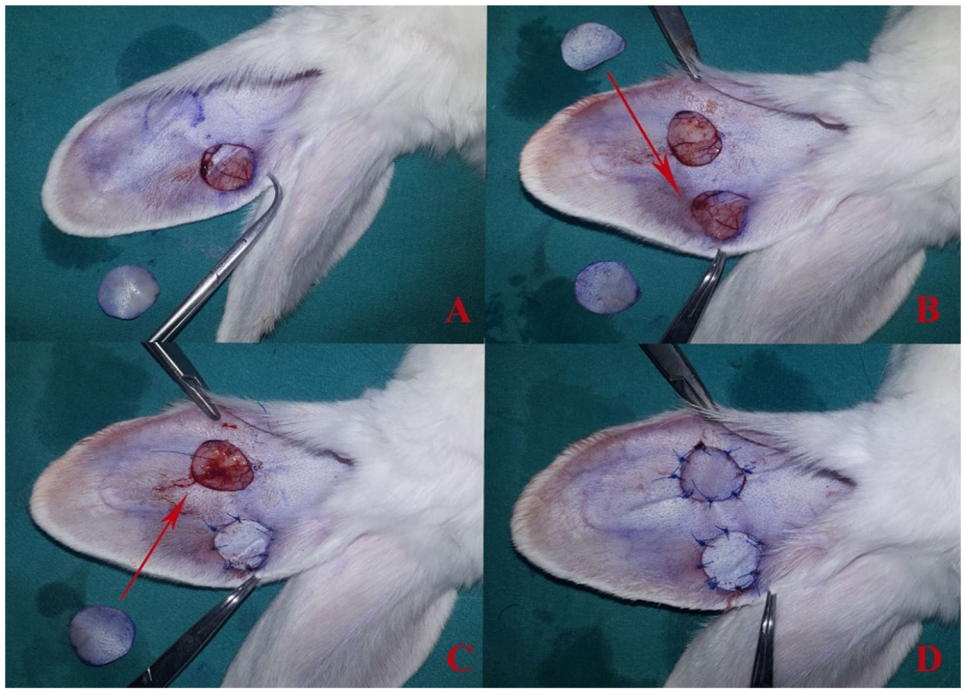

Figure 2. Harvesting and adaptation of the grafts marked on the left ear. (A) Harvesting of the medial and lateral graft from the left ear. (B) Harvesting of the lateral graft from the left ear. (C) Adaptation of the lateral graft to the medial defect. (D) Adaptation of the medial lateral graft to the lateral defect.

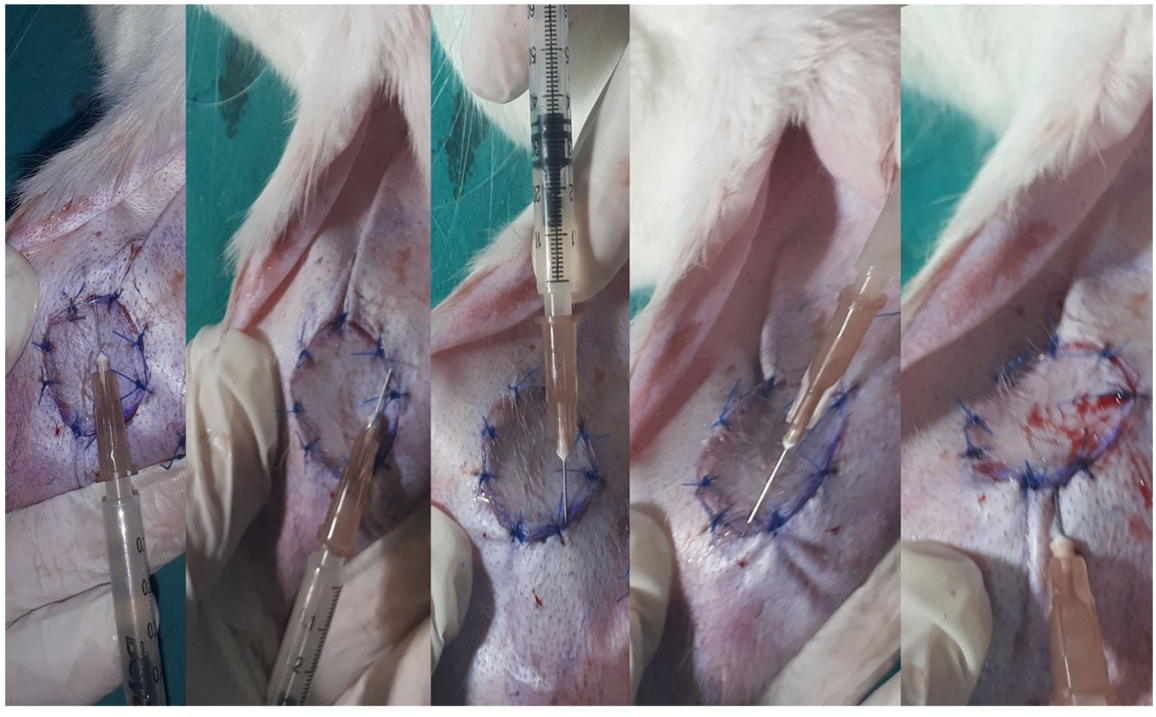

Figure 3. Application of the prepared PRP around and on the base of the graft.

Macroscopic and histopathological evaluation

The macroscopic evaluation was made at the end of the third week. Composite graft viability was photographed and analyzed using the computer system. Every graft area was calculated in pixels using Adobe® Photoshop CS2 and the ratio of viable area to necrosis area is found.

At the end of the third week, the study was terminated, and a full-thickness excision was made from the base of the ear. Inflammation and vascularization were evaluated in the histopathological examination and classified as mild and moderate. Vascularization was revealed by counting the vessels in hematoxylin-eosin and CD31 staining at 40 magnification. Vessel counts under 10 were considered mild, vessel counts between 10 and 20 was considered moderate vascularization. In addition, angiogenesis density was compared with CD31 immunostaining, which is an endothelial cell marker.

Statistical analysis

The obtained data were analyzed using SPSS version 15.0 (SPSS Inc., Chicago, IL). The statistical analysis of the macroscopic area measurement and CD31 evaluation was performed with the Kruskal–Wallis H-test. The chi-square test was used to analyze the scores of the histopathologically evaluated inflammation and vascularization. $p < .05$ was considered statistically significant.

Results

The mean of tissue survival ratio performed using a computer system was 46% in control group, 69% in autogenic group and 58% in xenogenic group (Table 1). Tissue viability was found statistically significant between control group and autogenşc PRP group ($p < .05$) Although viability in the autogenic PRP group appeared to be better than xenogenic group macroscopically (Figure 4), no statistically significant difference was observed in tissue viability between these two PRP groups.

In the histopathological examination, no significant difference was revealed between the control group and the autogenic and xenogenic PRP groups in relation to inflammation and vascularization. However, there was a significant difference in the results of CD31 staining between the control (mean: 5.1) and PRP groups (mean: 20.5/13.1). Comparing the PRP groups, CD 31 staining provided better results in the autogenic PRP group.

Discussion

Over the past few decades, platelet that is rich in plasma has attracted the attention of many researchers and clinicians. It is now known that platelets obtained by centrifuging autologous blood and separating the plasma from this blood with a higher platelet concentration contain a high amount of growth factor. It has been observed that these growth factors, which are released into the environment through the fragmentation of platelets, have a positive effect on many tissue responses, especially in wound healing, tissue regeneration and neovascularization. Many wounds with problematic healing such as diabetic foot, chronic wounds such as venous ulcers and some acute wounds such as burns have been successfully treated with PRP. PRP has also gained popularity in a wide range of treatment areas, including cosmetics, orthopedics, periodontics and other health problems [2,6].

Although there are numerous clinical studies on PRP, most are experimental [7]. For ethical considerations, subject selection in animal experiments should be based on the lowest phylogenetic scale [8]. The use of PRP poses a problem due to the limited amount of blood that can be collected from animals. The amount of blood that can be obtained from a healthy animal should not exceed 20% of the total blood amount calculated on average or 1% of its total weight. The appropriate blood volume that can be taken without stressing the animals is known to be 0.2–0.3 cc in mice, 2–3 cc in rats, 4–8 cc in guinea pigs and 20–40 cc in rabbits. The maximum amount of blood is 1 cc for mice, 10–15 cc for rats, 1–25 cc for guinea pigs and 60–200 cc for rabbits. Considering that in studies on PRP, it is necessary to take an average of 5–8 cc whole blood to obtain 1 cc PRP. The amount of blood taken from small animals, such as mice, rats and guinea pigs is not sufficient for an evaluation, and the collection of an appropriate amount of blood may stress the animal [4]. Because of this problem, some researchers have prepared allogenic PRP obtained from different animals of the same breed by sacrificing a certain number of animals before the study or some researchers have prepared autogenic PRP, prepared from the animals' own blood by selecting a type of animal with a larger blood volume, such as rabbits. However, selection of advanced animal species for experiment to collecting autogenic PRP is costly and makes the study more difficult than necessary with larger animal group.

Autogenous materials taken from the patient are highly preferred in tissue transfer due to their high tissue compatibility and not affecting immune mechanisms. However allogenic or xenogenic transfers are in practice. The common purposes of using xenogenic material in plastic surgery are greater graft requirement and reducing donor site morbidity. In transfers made using this method, immunological tissue compatibility is important for the tissue to survive. Especially in vascularized organ studies, materials taken from primates cannot be used due to the risk of hyperacute rejection caused by anti-GAL antibodies generated in mammals against the α-1,3-galactosyl transferase (ABO blood group) enzyme, which is only present in primates [9]. When xenogenic material's tissue compatibility is required in animal experiments, athymic animals are selected to suppress immune reactions. Materials that do not require tissue compatibility can be xenogenically transferred from human to animal or from animal to human by researchers [5,10]. Although platelets are non-nucleated cells lacking a complex secretory apparatus with distinct Golgi/endoplasmic reticulum compartments, previous

Table 1. Graft viability percent and CD 31 staining results of the subjects.

	Control group	Autogenic PRP group	Xenogenic PRP group	
CD31 staining (Mean)	5.17 *2.6	20.50 *4.1	13.17 *4.9	**p:.001
Graft viability (Mean)	46.67 *20.3	69.80 *13.02	58.08 *12.6	p:.067

*Standard deviation.
**Statistically significant result.

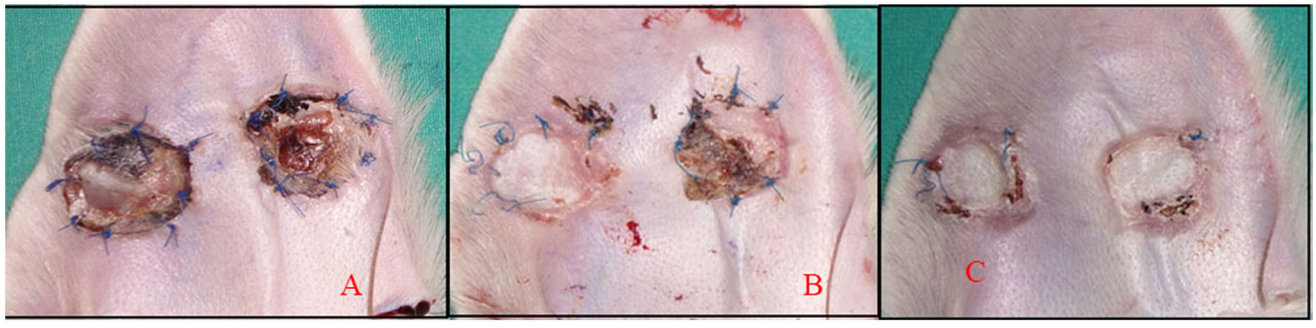

Figure 4. (A) Photograph of the composite graft in the control group at the end of the third week. (B) Photograph of the composite graft in the xenogenic PRP group at the end of the third week. (C) Photograph of the composite graft in the autogenic PRP group at the end of the third week.

studies have shown that they have glycosyltransferase activities [11]. However, in xenogenic transfer of PRP, growth factors rather than platelet function are prioritized. Tissue compatibility may not be important when performing xenogenic transfer since platelet destruction and growth factors are intended to be released during the use of PRP. This is also supported by our findings showing no significant difference between the autogenic and xenogenic PRP groups in terms of the vascular density rates measured by CD31 and the changes in inflammation and vascularization histopathologically examined with the hematoxylin-eosin and bluing reagent staining methods. Effect of xenogenic growth hormone transfer is not clear in literature, but it has been used in some experiments [12,13].

The allogenic effect of PRP has been investigated in several clinical studies to increase PRP function. It has been shown that the effects of PRP may differ because the number of platelets and their functions vary from one person to another. Based on this idea, studies conducted in recent years have investigated the effect of allogenic PRP prepared from the blood of young and healthy individuals with various indications [14–16]. In these studies, the effect of allogenic PRP has been found to be significantly better than autogenic PRP in the treatment of venous ulcer, alopecia, joint disorders, and non-healing wounds. The allogenic use of PRP is preferred in animal studies, especially in studies using rats. However, allogenic PRP preparation with animal sacrifice is tedious and costly process in experimental animal studies.

Although there are studies using xenogeneic PRP, the comparasion of xenogenic of PRP with autogenic or allogenic PRP has not been previously investigated [17]. We used human-derived PRP conciseness easy to harvest and no animal sacrifice is needed. In recent studies, researchers have preffered human derivated growth factor products for the same reason [18–20]. In the literature, some researchers have investigated intra-articular effects of combined xenogenous serum rich in growth factors and vitamin C, and they have used human PRP in rat model. They claimed that xenogenic PRP with vitamin C group has showed significant effect over two groups histopathologically, however, they also saw that PRP-only group has better effect over sham-control group in histopathologic grading and staging [21]. In our study, when the autogenic PRP and xenogenic PRP groups were compared, it was observed that the autogenic PRP group performed macroscopically better in increasing tissue viability compared to the group in which PRP was xenogenically transferred from human to rabbit. However, this difference was not statistically significant. According to the CD31 staining, vascular density was better in the autogenic PRP group than in the xenogenic PRP group. The vascular density rates measured by CD31 staining and the ratio of viability to necrosis were significantly higher in both PRP groups compared to the control group, which is consistent with the literature. However, in the histopathological examination undertaken with the hematoxylin-eosin and bluing reagent staining methods, the changes in inflammation and vascularization were not statistically significant.

Many drugs and non-drug materials have been used to increase tissue viability after tissue transfer, and some have been introduced into daily clinical practice. The effects of products to increase tissue viability are primarily tested on graft or flap viability in animal experiments. Animal models are sufficient for the investigation of most drug and non-drug applications [22].

Although various methods have been tested to increase tissue survival, only few have been adapted in daily clinical practice. Studies have shown that the subcutaneous injection of PRP increases arteriogenesis in the rabbit skin flap and has positive effects on flap viability [3]. In light of this information, PRP has been shown to have a positive effect on composite graft viability. In a study published in 2014, Jeon et al. harvested one chondrocutaneous graft from each ear of 20 rabbits and placed it as a composite graft, and then performed the autogenic PRP application. In the preparations evaluated after 12 d, graft viability and blood flow based on laser Doppler flowmetry were observed to have increased in the PRP group when compared to the control group. In addition, the number of blood vessels and vascular endothelial growth factor expression were found to be significantly increased. Based on these findings, the authors concluded that PRP increased the viability of the composite graft by enhancing neovascularization [23]. In our study, graft vascularity is evaluated with CD 31 stanning after 21 days, and results were found to be significantly better in both PRP groups when compared with control groups, consistent with this study. In another study conducted in 2014, Hyun et al. created a composite graft model on rabbits and investigate the ideal PRP injection time by applying PRP to each group at different times. A total of 24 rabbits were divided into three PRP groups according to the time of application (3 d before autologous PRP grafting, during grafting and 3 d after grafting), and a control group was also formed. According to the evaluations performed after 21 d, although the most successful results were obtained from the group in which PRP had been applied before grafting, all the PRP groups had significantly increased viability compared to the control group. Microvessel density and revascularization were evaluated with CD31 staining, and significantly positive effects were observed in all the PRP groups compared to the control group [24]. In this study, the vascular density rates measured by CD31 and the ratio of viability to necrosis were found to be significantly higher in both PRP groups compared to the control group, which agrees with the literature. However, in the histopathological examination performed with the hematoxylin-eosin and bluing reagent staining methods, inflammation and vascularization did not statistically significantly differ between the groups. In our study, we also evaluate the specimens after 21 d and despite the viability of grafts were better macroscopically and in CD 31 stanning in PRP groups, inflammation was not significant in three groups, consistent with this study.

In light of the findings obtained from this study, the use of xenogenic PRP for the preparation of PRP in animal studies is an option that can be preferred as an alternative to autogenic or allogenic PRP, especially when macroscopic results are needed. But there is a need for comprehensive studies which evaluates xenogenic growth factor effect on this subject.

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