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Combining platelet rich fibrin with different bone graft materials: An experimental study on the histopathological and immunohistochemical aspects of bone healing

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ABSTRACT

Aim: The current study was based on the hypothesis that the use of PRF with bone graft materials might increase bone regeneration and focus on the histopathological and immunohistochemical aspects following application of PRF with autogenous graft, xenograft and B-TCP in a rabbit model.
Material and methods: This study was performed on the twenty-eight male New Zealand divided into four group. Two defects with a diameter 10 mm were opened in calvarium. After PRF preparation, right defects were evaluated as empty defect or graft group, and left defects were evaluated as PRF test group. All animals were sacrificed at the end of 8 weeks and specimens were examined histopathologically and immunohistochemically.
Results: The most superior histopathological results were obtained in the autograft group. The combination of β-TCP-PRF could not provide superiority over the β-TCP group. The immunohistochemical results showed that, in the PRF/BTCP group, the expression of osteopontin and osteonectin was relatively higher compared to the only-BTCP group.
Conclusion: In terms of new bone formation, autograft combined with PRF yielded superior results but the combination of β-TCP-PRF had no effect compared to the only-BTCP group. However, further experimental and clinical studies might be beneficial to clarify the exact mechanism and results of combining PRF with bone grafts on bone healing process.

1. Introduction

Lack of sufficient bone volume at the dental implant recipient sites has become one of the main problems in daily dental practice. In order to overcome the above mentioned challenge, several graft materials such as autografts, allografts, xenografts and alloplastic grafts are widely used. Although autogenous bone grafts are still accepted as the gold standard, their limited volume and necessity of creating a second operational zone were the main proclaimed problems related to their use (Pistilli et al., 2014). On the other hand, xenografts have a potential effect on long-term preservation of bone volume in large defects such as maxillary sinus elevation (John and Wenz, 2004; Simion et al., 2007). However; owing to their osteoconductivity effect, their combined use with extracellular matrix proteins or cell adhesion peptides has been studied with various results. Beta tricalcium phosphate (β-TCP) is a biologically compatible graft material which has only osteoconductive effects Jensen et al. (2006), and therefore does not show osteoinductive and osteogenic effects like autogenous grafts (Erbe et al., 2001).

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Owing to positive effects of growth factors on bone regeneration, platelet-rich fibrin (PRF), which contains a significant amount of platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGF-β), leukocytes, and cytokines, has gained popularity in the field of dentistry in the last few decades (Peterson et al., 2010). It has been reported that PRF contains cytokines, such as interleukin (IL) 1β IL-6, IL-4, and tumour necrosis factor, which are critical in the immune system and therefore may play supporting a role (Dohan et al., 2006a). In addition, they were also characterized as an autogenous fibrin matrix used as a scaffold for a variety of procedures, including serving the function of a barrier membrane in guided bone regeneration and guided tissue regeneration procedures (Toffler, 2014; Lekovic et al., 2012; Shivashankar et al., 2013). PRF, which promotes healing and immunity, is defined by Choukroun as a second-generation platelet concentrate (Choukroun et al., 2006).

Nowadays, PRF is used effectively in the treatment of intrabony defects, sinus elevation, gingival recession, socket preservation and to accelerate wound healing in the field of dentistry (Shivashankar et al., 2013; Hoaglin and Lines, 2013; Agarwal et al., 2016; Elgendy and Abo Shady, 2015; Gassling et al., 2009). It has been also suggested that the use of PRF solely or in combination with autogenous grafts, xenografts and β-TCP increases new bone formation and stimulates bone regeneration (Oliveira et al., 2015; Galav et al., 2016; Nacopoulos et al., 2014). On the other hand, PRF also has controversial aspects. Biodegradability and toughness problematical may create negativeiyy in guided tissue regeneration. Biodegradability time of PRF generally begins within ten days after insertion (Hartshorne and Gluckman, 2016; Tsukioka et al., 2018).

The preparation of PRF is quite simple, as suggested by Dohan et al. A 10 ml venous blood sample is drawn and centrifuged at 3000 rpm for 10 min. Then, the red blood cells are obtained in the lower part, PRP in the middle, and cell-free plasma (thrombocyte-poor plasma) in the upper part (Dohan et al., 2006b).

The current study was based on the hypothesis that the use of PRF with bone graft materials increases bone regeneration and focuses on the histopathological and immunohistochemical aspects following application of PRF with various bone graft materials in a rabbit model.

2. Material and method

2.1. Study design

The ethics committee report required for the current study was taken from the Local Ethics Committee of Animal Experiments at the University of Dicle (2013-30). The study was carried out in Dicle University Health Sciences and Research Center and Faculty of Medicine Histology and Embryology Department.

In the present study, a total of twenty-eight healthy, 6-8-month-old, male, New Zealand rabbits, weighing (2.6–3.9) kg were used as experimental subjects. The rabbits were housed in single cages at room temperature with a humidity content of 55–70% and ventilated every 15 min (Torres et al., 2007). Possible weight changes that could occur in the rabbits were re-measured at the time of sacrifice. Four equal groups were randomly selected, and each group was divided into two subgroups (Table 1).

2.2. Preparation of PRF

The blood required for PRF protocol was taken from the central ear artery of the rabbits. The hair on the ear was shaved with a sterile scalpel in order to increase the field of view and avoid damage to this artery which is very sensitive and thin. The area was cleaned with alcohol to make the artery observable. 8 ml blood was transferred from the central ear artery and collected into tubes free from anticoagulant (Aricioglu et al., 2017). Centrifugation was carried out at 3000 rpm for 10 min as recommended by Dohan et al. (2006b). Three layers emerged within the anticoagulant-free tube removed from the centrifuge. PRF was precisely removed from the tube by means of a forceps. Acellular plasma was poured and the red blood cell attached to the PRF was removed with a scissors.

2.3. Surgical procedure

Before operation, rabbits were sedated with 25–35 mg/kg ketamine (Ketalar, Parke Davis, Germany) and 6–8 mg/kg xylazine hydrochloride (Rompun, Bayer, Germany) intramuscularly. To ensure aseptic conditions, the operation site was shaved and washed with 10% povidone-iodine solution (Batikon, Adeka, Turkey). Anesthesia of the calvarial region was achieved with a local anesthetic solution containing 1.8 ml of 2% lidocaine HCl epinephrine (Jetosel 2 ml, Osel, Turkey). Mid-sagittal incision was made from the nasal region to the occipital region.

After the incision was made, bleeding was controlled, the subperiosteal elevation was performed, and the bone was reached. Two critical size bicortical defects were created with a physiodispenser on both sides of the sagittal suture via a trephine bur with a diameter of 10 mm and 1 mm depth. The distance between the defects was 4 mm and the distance from the sagittal suture was 2 mm. After the defects were prepared, the bleeding was controlled and the defect was filled according to the study plan (Fig. 1).

For group I, the right defect was left empty and only the PRF was placed in the left defect. In group II, the autogenous bone graft with block obtained in the calvarium using a trephine bur was particulated with a bone mill and was applied to the right defect, and for the left defect, PRF and autogenous graft were used together. In group III, only xenograft (Bio-oss®, Geistlich, Switzerland, 250 μm to 1 mm) was applied to the right defect, and for the left defect PRF and xenograft were mixed in equal volumes. Lastly, in group IV, β-TCP (R.T.R. syringe, Septodont, USA, 500 μm to 1 mm) was applied to the right defect, and for the left defect PRF and β-TCP were mixed in equal volumes (Xuan et al., 2014). The graft granules were carefully distributed over the PRF (Fig. 2).

After the defects were filled, the operation area was closed with 3/0 silk sutures (Ruschmed, Ipek, Turkey). To prevent post-op infection, cefazolin (25 mg/kg im, Cefamezin, Eczacıbaşı, Turkey) was given for 4 days. After surgery, the animals were housed in single cages. During this time, infection and side effects were not observed.

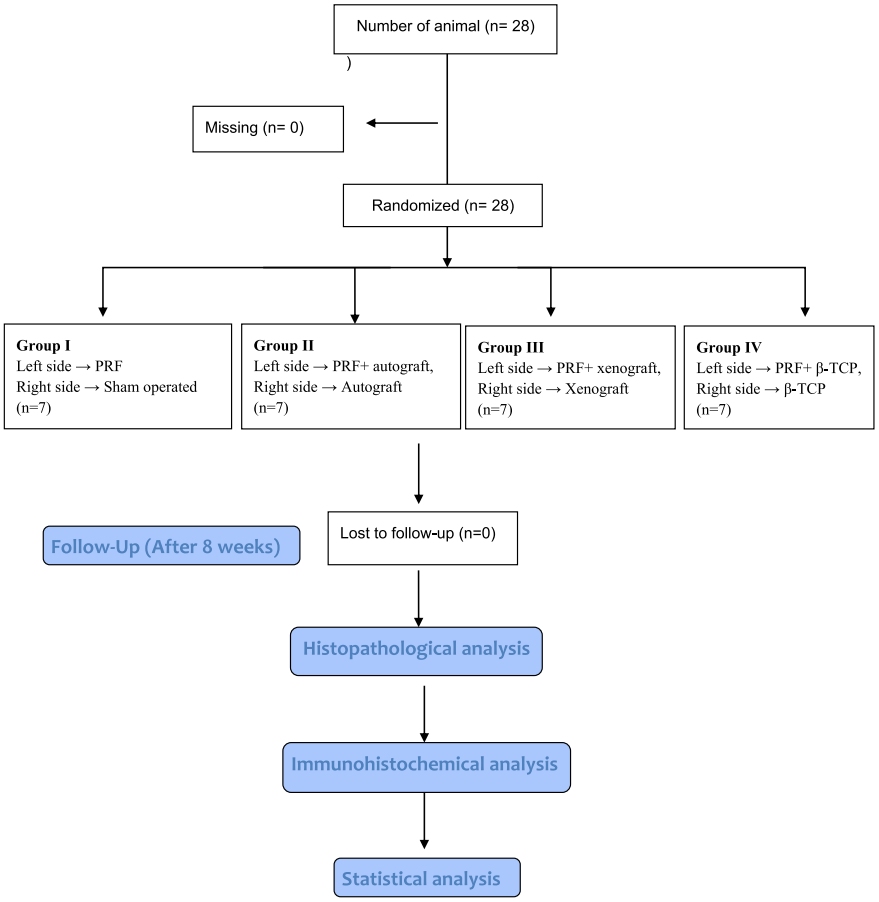
All animals were sacrificed at the end of 8 weeks by direct injection of 10 cc of 7.5 % Potassium Chloride (Potassium Clorur, Drogsan, Turkey).

2.4. Histopathologic Analysis

Calvarium specimens were carefully dissected, transported with 10% neutral formalin and decalcified with 5% nitric acid. They were then dehydrated by being passed through various concentrations of alcohol and blocked in paraffin. Sections with a thickness of 5 μm were taken from paraffin blocks. The paraffin sections were stained in hematoxylin-eosin (HE) and Masson trichrome. The stained tissue was covered with a lamella. The preparations were evaluated by Nikon Eclipse 80i microscope and micrographs were taken.

Fibrosis, inflammation, necrosis, foreign body reaction, remaining graft amount, and new bone formation criteria were evaluated in the prepared sections. For histopathological examination, scores of 1–4 were given according to the area covered by ×500 magnification. For the evaluated criteria, according to the area covered, from 1% to 25% of the total area coverage area was score 1, from 26% to 50% of the coverage area was score 2, from 51%

Table 1
Consort flow diagram of the study.



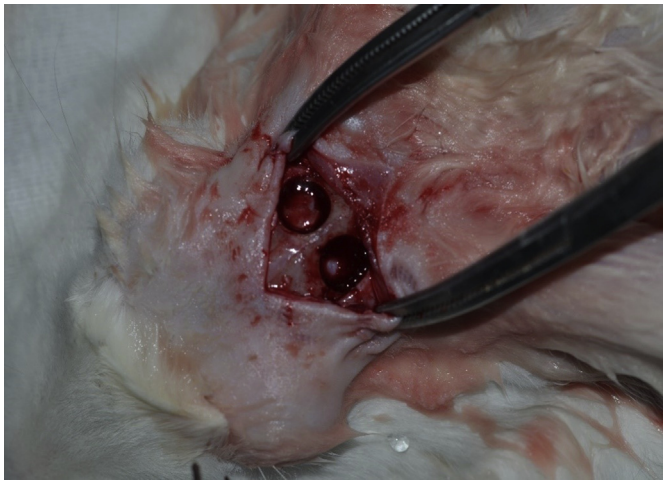


Fig. 1. Preparing of defect sites.

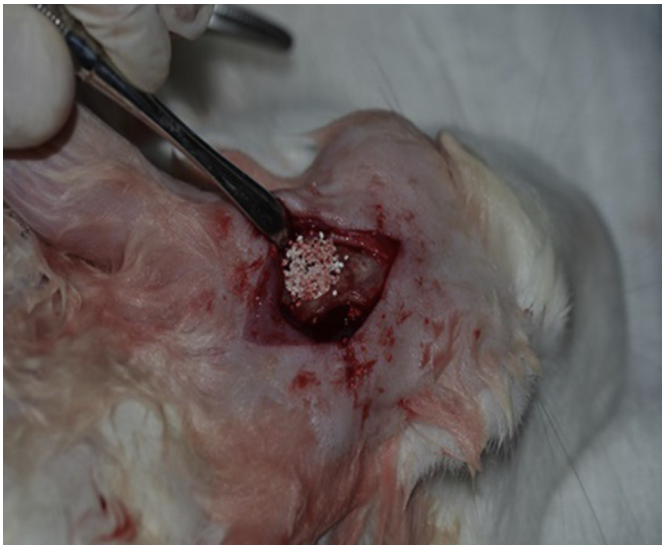


Fig. 2. The view of graft in the defect area.

to 75% coverage area was score 3, from 76% to 100% coverage area was score 4 (Bosch et al., 1995; Develioglu et al., 2009).

2.5. Immunohistochemical Procedure

The avidin-biotin peroxidase method was performed for the immunohistochemical studies. After deparaffinization, passing the antigen retrieval procedure, sections were rinsed with TBST (Twin Buffer Solution Tampon) (Ph 7.4) and exposed to 3% hydrogen peroxide (Cat: TA-015-HP, Lot: HP15754, Lab Vision, Thermo Scientific, Fremont, USA) for 15 min then rinsed with TBST. Ultra-V block (Cat: TA-015-UB, Lot: AUB90917, Lab Vision, Thermo

Scientific) was applied for 20 min to prevent nonspecific binding. Following the blocking stage, sections were washed with primary antibodies: osteonectin (SPARC (H-90): sc-25574 Santa Cruz, Biotechnology) and osteopontin (OPN (AKm2A1): sc-21742, Santa Cruz, Biotechnology) were diluted 1:100 and tissue sections were incubated with primary antibody at +4 °C overnight. Sections were then incubated with biotinylated secondary antibody (Cat: TP-015-BN, Lot: PBN90807, Lab Vision, Thermo Scientific) for 20 min at room temperature and reacted for 20 min with streptavidin peroxidase enzyme (Cat: TS-015-HR, Lot: SHR90722, Lab Vision, Thermo Scientific). Sections were rinsed with TBST before all incubation. Finally, color reaction was developed by incubation with chromogen DAB (Cat: TA-015-HSX, Lot: 90824B, Lab Vision, Thermo Scientific), prepared with the substrate containing diaminobenzidine (DAB) (Cat: DABC-004, Lot: HSX17609, Lab Vision, Thermo Scientific) for approximately 5–10 min to ensure an immune reaction at room temperature. Mayer's hematoxylin (Cat: TA-125-MH, Lot: MH21121, Lab Vision, Thermo Scientific) was used as a counterstain. Immunoreactivity was evaluated using with imaging system.

The efficacy of PRF was assessed by scoring from 0 to 3 according to the area of immunological involvement of osteonectin and osteopontin antibodies in the cytoplasm of osteocytes for immunohistochemical examination of the prepared sections (Eadon et al., 2017).

Scoring was done according to area covered by ×500 magnification, as follows:

0- No immunostaining
1- Minimal immunological staining
2- Mild level of immunostaining
3- Maximal immunological staining

2.6. Statistical Analysis

For statistical evaluation, mean, standard deviation, minimum and maximum values were given, and parametric and non-parametric statistical test methods were used. In the analyzing of in-group and inter-group variables, one-way analysis of variance (ANOVA), Kruskal–Wallis, Dunnett, Mann Whitney U, Wilcoxon and Paired-t tests were used for analysis. In all of the tests in this study, 95% confidence interval was applied and for $p < 0.05$, the results were considered statistically significant.

3. Results

3.1. Histopathologic Evaluation

All experimental animals tolerated surgical procedures without any complications. According to eight weeks follow-up, evaluated criteria were shown in the graphic (Figs. 3–5).

3.1.1. Fibrosis

For fibrosis, statistically significant results were observed between control group and autograft group in non-PRF groups ($p = 0.007$). In addition, there was a statistically significant difference between the xenograft group and autograft groups ($p = 0.014$). The results between the β-TCP group and autograft group were similar ($p = 0.015$). However; there were no statistically significant results between xenograft and β-TCP groups, xenograft group and control group, β-TCP group and control group in non-PRF groups.

In PRF groups, there were statistically significant results between autograft group and only PRF group ($p = 0.006$), autograft group and xenograft group ($p = 0.014$), xenograft group and only PRF group ($p = 0.032$) and lastly autograft group and β-TCP group ($p = 0.014$).

In intra-group comparison where the efficacy of PRF was assessed, there was no significant difference for fibrosis.

3.1.2. Inflammation and Necrosis

No significant results were observed for necrosis and inflammation parameters.

3.1.3. Foreign Body Reaction

In all samples of control and autograft groups, foreign body reaction was not observed. However, although foreign body reaction was seen in the xenograft and β-TCP groups, there is no statistical significance.

3.1.4. Remaining Graft Amount

In both PRF and non-PRF groups, the most positive results were observed in the autograft group. Additionally, xenograft and β-TCP groups were significant compared to control and autograft groups in both PRF and non-PRF groups for remaining graft amount.

3.1.5. New Bone Formation

In terms of new bone formation, in intra-group comparison of the effect of PRF on grafts, the best results were obtained in the autograft group ($p = 0.015$), on the other hand in comparison, the xenograft-PRF combination and xenograft alone, the combination showed superiority but the results were not significant. The most negative results were seen in the combination of β-TCP-PRF. The combination of β-TCP-PRF did not provide superiority to the only-β-TCP group.

In comparisons between non-PRF groups, all graft groups showed significant results compared to control group ($p = 0.006$, $p = 0.015$, $p = 0.040$), and the graft groups did not show statistically significant results compared with each other. In comparisons between PRF groups, the combination of autograft-PRF and xenograft-PRF showed significant results compared to the group with only PRF ($p = 0.026$, $p = 0.046$). Although the β-TCP-PRF combination had a supremacy to only-PRF group, there was no significant difference compared to this group ($p = 0.266$) (Fig. 6).

3.2. Immunohistochemical Evaluation

Immunohistochemical analysis evaluating osteonectin involvement in osteocytes showed a crucial boost in all PRF-combined groups as compared to non-PRF groups in intra group comparison; however, only the PRF combined autogenous graft ($p = 0.046$) group was statistically significant compared with the only-autogenous graft group. In non-PRF groups, autogenous group was dominant to other graft groups. The remaining groups were not superior to each other or to the control group. In PRF groups, while PRF combined autogenous graft was statistically significant compared with the only-PRF group ($p = 0.006$), similarly, the graft groups were not statistically different (Fig. 7).

Assessment of another osteopontin antibody of involvement in osteocytes, as in osteonectin antibody, in terms of PRF effectiveness, showed a positive impression. Intra group comparison, PRF group compared to sham operated group ($p = 0.019$) and PRF combined autogenous graft group compared to only autogenous graft group ($p = 0.013$) were statistically significant. On the other hand, both

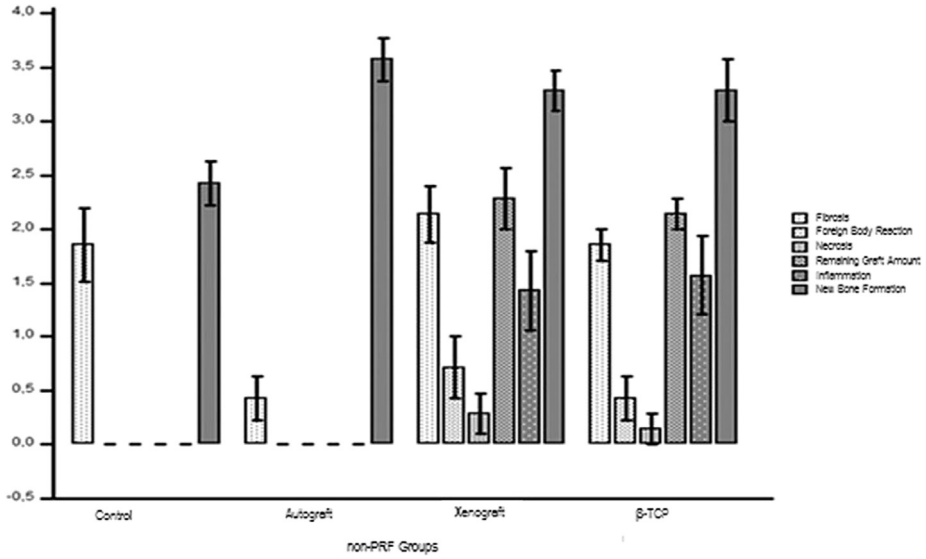


Fig. 3. Histopathologic parameters in non-PRF groups. The autograft group was statistically significant for fibrosis between control group ($p = 0.007$), xenograft group ($p = 0.014$) and β-TCP group ($p = 0.015$). When new bone formation was assessed, all graft groups showed significant results compared to the control group ($p = 0.006$, $p = 0.015$, $p = 0.040$), the graft groups did not show statistically significant results compared with each other.

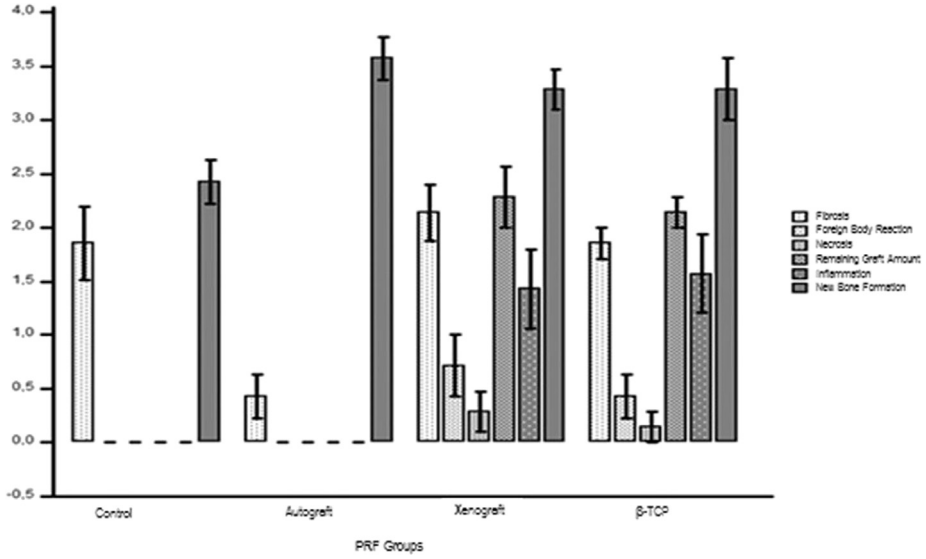


Fig. 4. Histopathologic parameters in PRF groups. For fibrosis, the results between autograft group and the only PRF group ($p = 0.006$), autograft group and xenograft group ($p = 0.014$), xenograft group and only PRF group ($p = 0.032$) and autograft group and β-TCP group ($p = 0.014$) were statistically significant. The combination of autograft-PRF and xenograft-PRF showed significant results compared to the group with only PRF ($p = 0.026$, $p = 0.046$) for new bone formation.

PRF and non-PRF groups did not show statistical significance in inter group comparison (Fig. 8).

4. Discussion

Platelet-rich fibrin, which acts as a fibrin scaffold, is the concentration of platelets as constituents on the blood sample to support healing (Toffler et al., 2009) and comprises many growth factors such as PDGF, TGF-β, IGF, EGF, fibroblast growth factor, and bone morphogenic protein (Dohan et al., 2006c). This experimental study aimed to investigate the effects of PRF on bone formation and maturation following its application, combined with autogenous graft, xenograft and BTCP into intrabony critical size defects.

Autogenous bone grafts have been assumed as gold standard owing to their osteogenic and osteoinductive features (Coradazzi et al., 2007). On the other hand, there are some restrictions such as the need for another surgical procedure, limited availability, and donor site morbidity. It is well known that Bio-Oss would have a positive effect on the volume of bone formation particularly in large defects (Simion et al., 2007). β-TCP has osteoconductive features and supports attachment, proliferation and differentiation of osteoblasts and mesenchymal cells and bone formation (Kiliç and Güngörmüş, 2016).

It is obvious that comparing the biology of an animal model and human biology will create some drawbacks. Although this study was well evaluated and ethically accepted during planning stages, these drawbacks were considered. However, animal

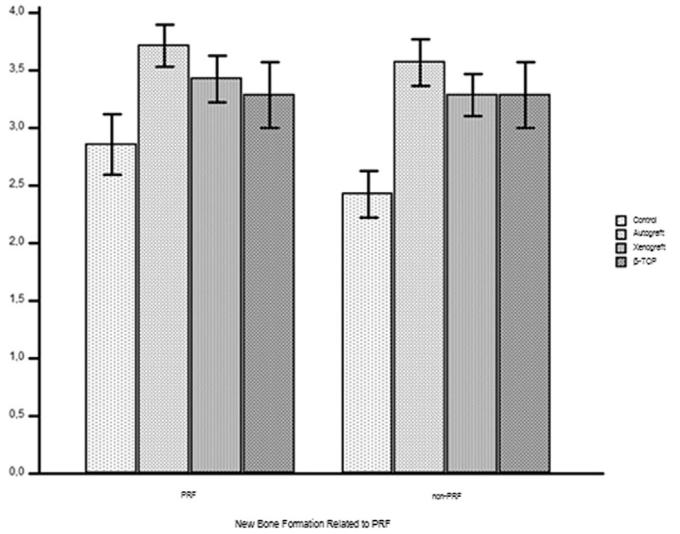


Fig. 5. New bone formation related to PRF. In intra-groups comparison of the effect of PRF on grafts, the autograft group was significant ($p = 0.015$). Xenograft-PRF group was positive in comparison to xenograft group but the results were not significant. β-TCP-PRF group was not superior to β-TCP group.

studies are frequently used as a "proof-of principle" (Thoma et al., 2017). It is a fact that the number of animals used in this study has been limited to twenty-eight due to ethical problems and existing possibilities lead to the limitation of this study. In previous studies the stages of bone healing were evaluated week by week. According to these, no significant difference was found between 8- and 12-week bone healing period (Pripatnanont et al., 2013; Sohn et al., 2010). Nevertheless Bodde et al. defined 12 weeks as the bone healing period for critical size defect (Bodde et al., 2008). Therefore, it might be suggested that a study duration of 8 weeks could be short. However, in the literature, Durmuşlar et al. turned to account PRF for bone regeneration in diabetic rabbits and 8 weeks was chosen in order to designate the duration of the experiment (Durmuşlar et al., 2016). Lee et al. also examined the effects of PRF against experimental peri-implant defects in rabbits and the test time was 8 weeks (Lee et al., 2012). Unlike the selected duration of experiment in these two studies, Faot et al. carried out research aimed at the effect of L-PRF in order to evaluate bone regeneration in rabbit tibiae and the duration of experiment was selected as 28 days. However, L-PRF had no effect on bone regeneration for 28 days (Faot et al., 2017). In this study, the experiment duration was limited to eight weeks and at the end of eight weeks, adequate bone maturation, formation and bone islets were observed.

In the literature, there are various studies focusing on the histological aspects of the efficacy of PRF used in combination with different graft materials. Kim et al. stated that platelet concentrates containing growth factors such as PRF, PRP, CGF enable new bone formation in early stages of bone graft healing (Kim et al., 2014). To support this statement, Kökdere et al. noted that PRF accelerated the healing of the bone defects for both PRF used alone and used in conjunction with autogenous bone graft (Kökdere et al., 2015). Additionally, Kang et al. expressed that PRF is a powerful element in maintaining tissue regeneration (Kang et al., 2011). According to our findings, although the results are favorable in terms of new bone formation between the PRF-administered group and the control group, the difference was statistically insignificant.

There are also numerous studies on the combination of PRF and xenografts with various results. In an animal study performed by Pripatnanont et al., PRF showed a positive effect on bone formation when used alone or combined with autogenous bone, however PRF has been shown to have no positive effects in the xenograft group (Pripatnanont et al., 2013). In another study which examined the relationship between PRF and Bio-Os in the rat calvarium, PRF has been shown to have a synergistic effect with Bio-Os (Oliveira et al., 2015). According to our results, the autogenous graft-PRF combination was more effective than other combinations, however, xenograft-PRF combination showed superiority compared to the only-xenograft group, but the results were statistically not significant.

In the literature, there are controversies regarding the use of β-TCP and PRF for bone regeneration. Nacopoulos et al. compared PRF and the combination of PRF and ceramic synthetic material composed of hydroxyapatite (HA) and β-TCP in a rabbit femur and reported that PRF and HA/β-TCP combination may be useful to gain new bone formation (Nacopoulos et al., 2014). Similarly, Acar et al. investigated the efficiency of PRF on bone formation when used alone or in combination with HA-βTCP and suggested that PRF has a positive effect on bone formation when used alone and in combination with HA/βTCP (Acar et al., 2015). In another study, Bölükbaşı et al. showed an increase in bone formation with the addition of PRF to biphasic calcium phosphate in sheep tibia (Bölükbaşı et al., 2013).

Despite positive results, the slow degradation property of the β-TCP has been also suggested (Cömert Kılıç et al., 2017) to jeopardize the actual bone-regeneration capacity of the PRF owing to the slow resorption property which may retard the replacement of new bone formation (Zhang et al., 2012). Additionally, it has been proclaimed that bone cells are required for PRF to have a positive effect on bone regeneration (Marx et al., 1998; Roldan et al., 2004). It is obvious that β-TCP does not contain any living bone cells. The insufficient bony regeneration obtained from the PRF-BTCP group could be attributed to the fact that the release of growth factors would provide a stimulus for only viable cells. Another reason supporting this result may be that PRF cannot release growth factor as much as PRP (Gassling et al., 2009).

Current immunohistochemical findings were also in agreement with the results of the study performed by Jun and Yun, which have stated that combined use of autologous grafts with platelet products could increase bone formation related biomarkers (Jun and Yun, 2016). Similarly, Cerci et al. have suggested that platelet products alter the biological characteristic of the autograft by increasing the IHH + bone cells which could be observed by the increase in the immunohistochemical expression (Cerci et al., 2015). Nagata et al. have evaluated the influence of the proportion of particulate autogenous bone graft/platelet-rich plasma on bone healing in surgically created critical-size defects in rat calvaria via immunohistochemical analysis and reported a significant higher expression of osteopontin, which was also in agreement with the findings of the present study (Nagata et al., 2009).

In the literature, the number of studies focusing on the immunohistochemical evaluation of the combined use of synthetic bone graft materials with platelet products is limited. Moreover, the effects of the use of PRF on BTCP in defects have not been immunohistochemically analyzed until now. The immunohistochemical results expressed herein show that, in the PRF/BTCP group, the expression of osteopontin and osteonectin was relatively higher compared to the only-BTCP group. Similarly, Nair et al. have studied the efficacy performance of a triphasic ceramic (calcium silicate, hydroxyapatite and tricalcium phosphate)-coated hydroxyapatite

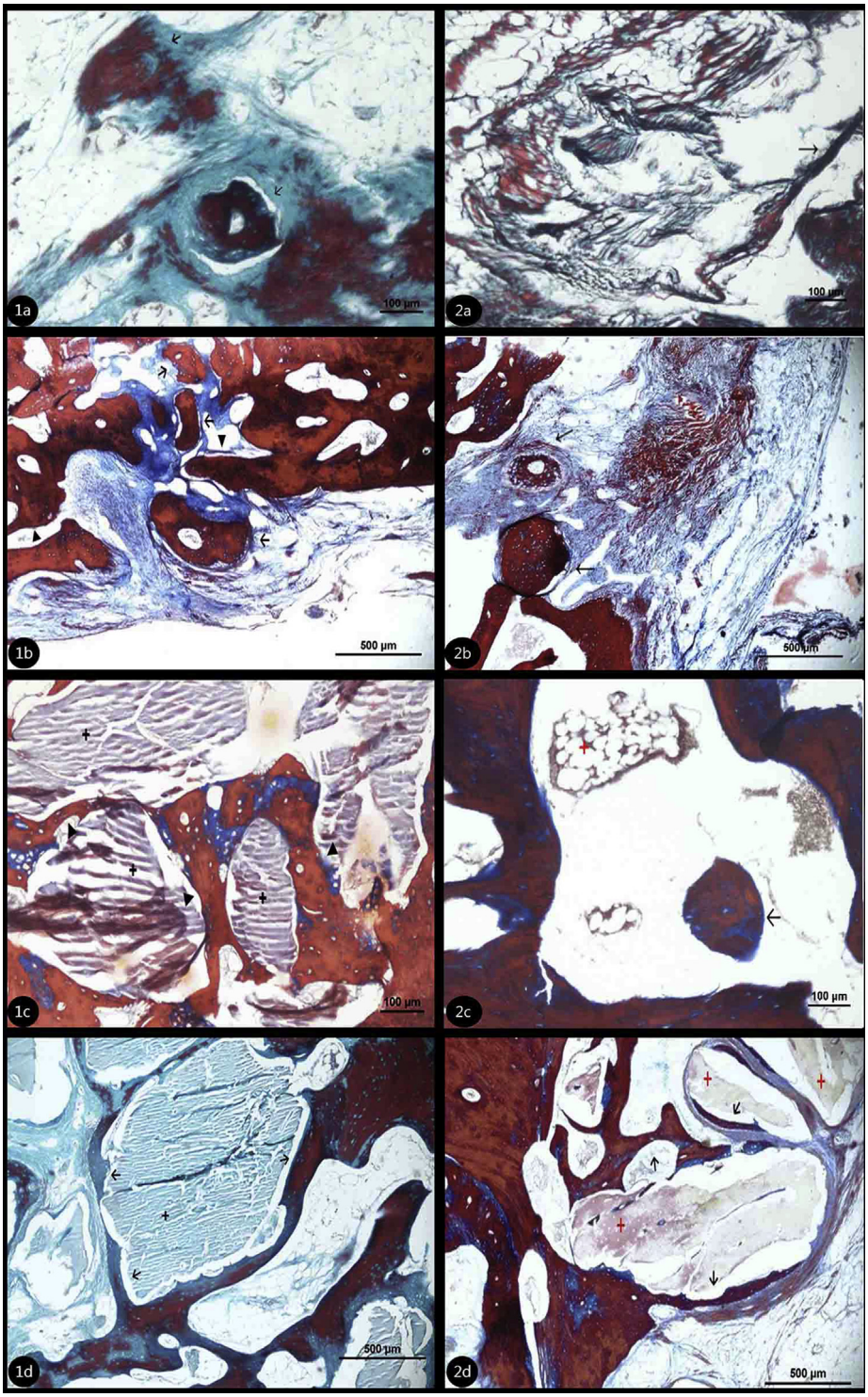


Fig. 6. PRF applied samples in left column and non PRF samples in right column are shown. 1a: Spongious bone tissue formed in loose connective tissue in defect region where only PRF applied (→). 2a: In Sham operated samples loose connective tissue filling the defect area was observed (→). 1b: In autograft-PRF group new bone mineralized trabecular structure (▼) and new bone islets (←) were seen. 2b: In autograft group, new bone-forming islets were observed within the vascular rich fibrous tissue (→). 1c: In xenograft-PRF group, new mineralized bone trabeculae (▼) surrounding the residual graft and gaps (+) between graft materials were seen. 2c: In xenograft group, new bone islands (←) were observed between the fatty bone marrow (+) in the defect site. 1d: Osteoid tissue surrounding the grafts (+) (←) in β-TCP-PRF group. 2d: New bone trabeculae (←) surrounding the graft material (+) in the defect area were clearly observed in β-TCP group.

bone graft material in healing the defects in a goat femur model and stated that a combination of triphasic ceramic coated hydroxyapatite with platelet products and autologous cells added an advantage that could promote the expression of many osteoinductive proteins, leading to faster bone regeneration and material degradation (Nair et al., 2009).

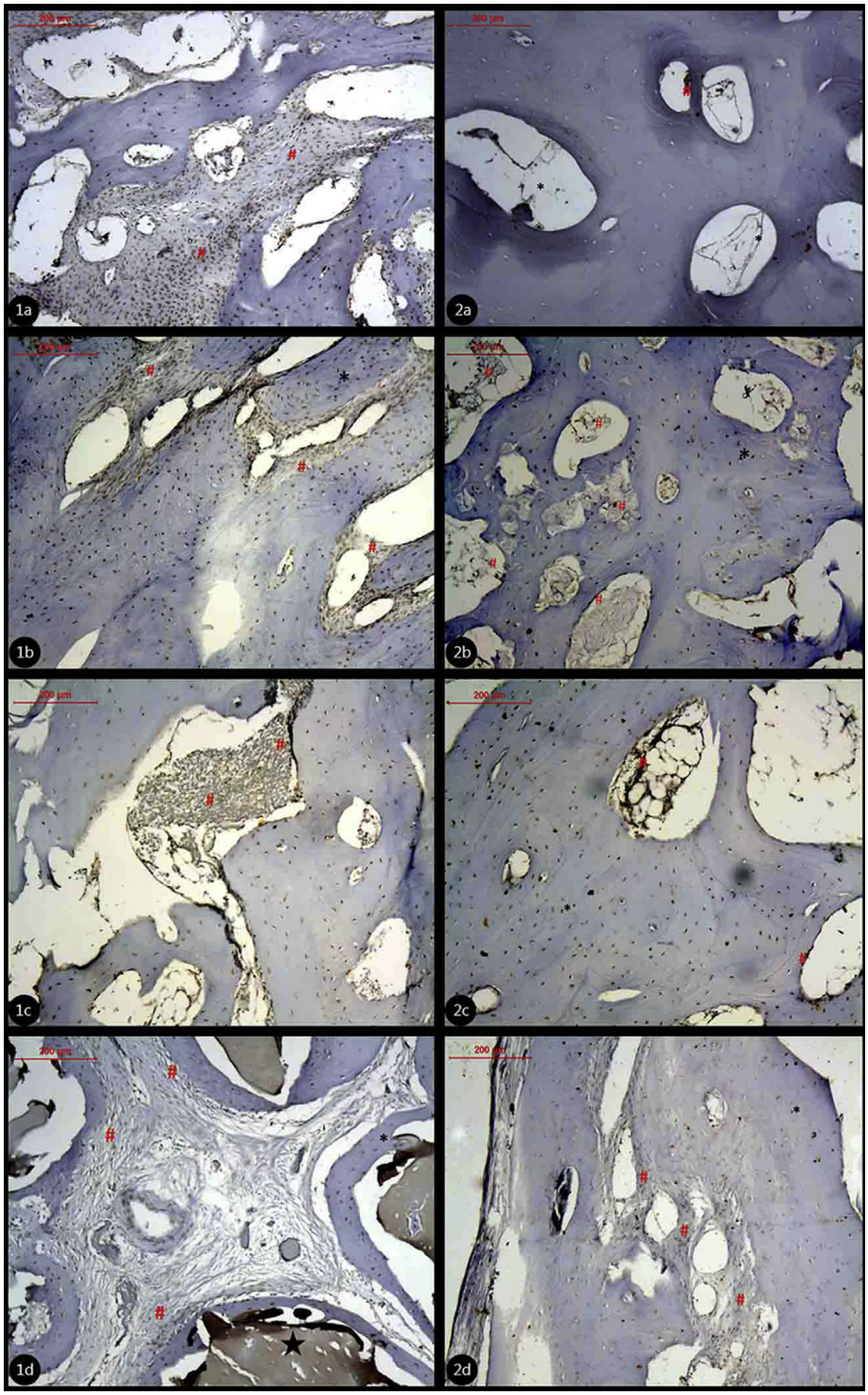


Fig. 7. PRF applied samples in left column and non PRF samples in right column are shown. 1a-2a: In PRF group, looser connective tissue developed (*) in the defect area and osteonectin (#) was more intense in terms of immunological involvement than the sham operated area. 1b-2b: In autograft-PRF group, new mineralized trabeculae (*) and new bone islets (#) were observed. Immune involvement was observed in osteocyte cytoplasm in bone trabeculae, while more intense osteonectin immunoreactivity was observed in islets than autograft group. 1c: In xenograft-PRF group, immune involvement was observed in non-calcified osteoid tissue (#). 2c: In xenograft group, osteonectin immunoreactivity was observed less in the new bone islets formed between osteoblasts and osteoblasts surrounding these islets than xenograft-PRF group (#). Involvement of osteonectin in cells of bone tissue was observed in osteocyte cytoplasm (*). 1d: In β-TCP-PRF group, more intense immunological involvement was observed in the new mineralized bone trabeculae (*) surrounding graft residues and in the non-calcified osteoid tissue surrounding the trabeculae than β-TCP group (#). 2d: the osteonectin immunoreactivity in the new bone tissue around the graft material was observed less intensely (#).

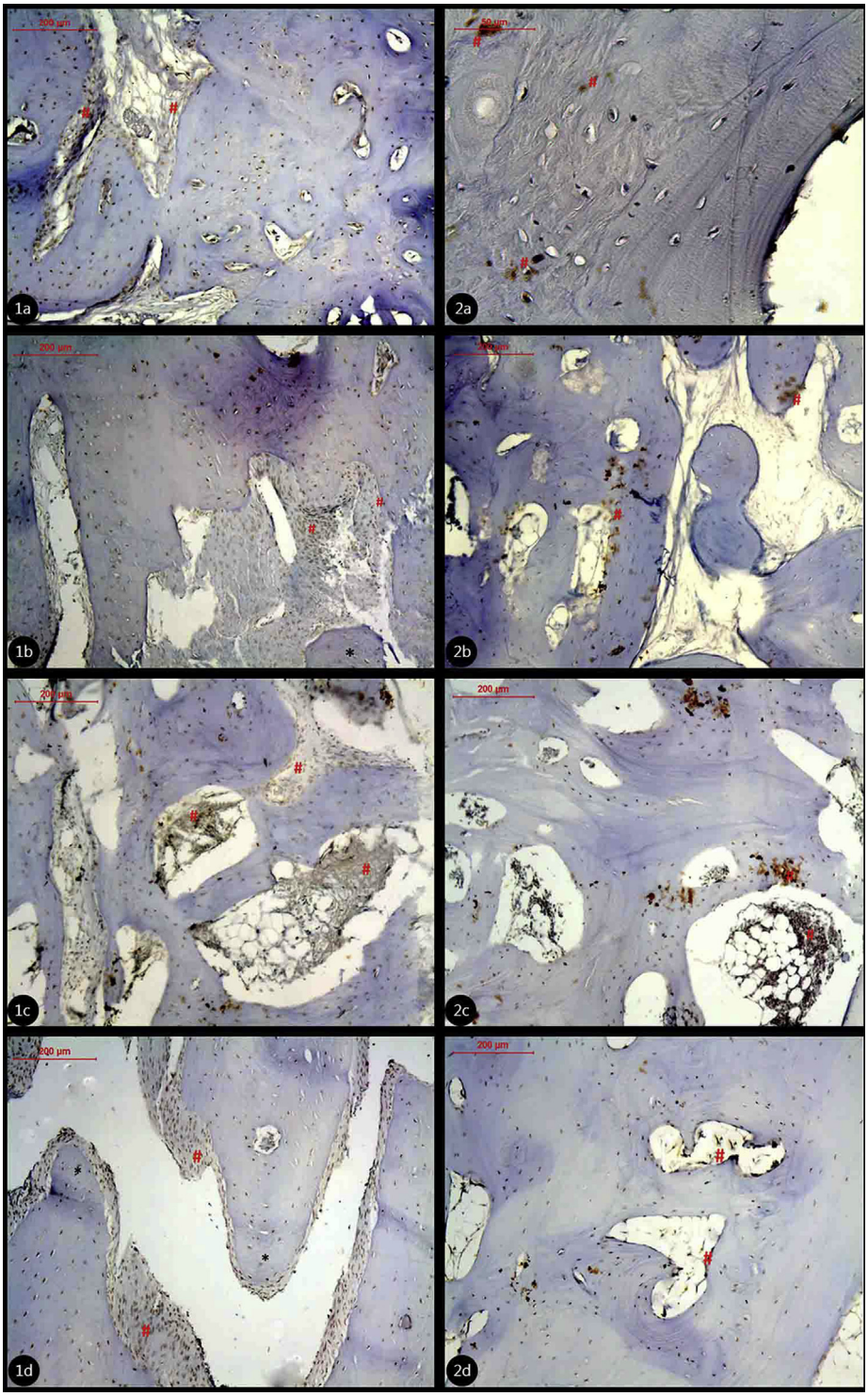


Fig. 8. PRF applied samples in left column and non PRF samples in right column are shown. 1a-2a: In only PRF applied group, fibrous connective tissue formation developed in the defect sites and more intense cytoplasmic and extracellular osteopontin involvement was observed in these regions compared to the sham operated side (#). 1b-2b: New mineralized bone trabeculae (*) and bone-making islands (#) are observed in the autograft-PRF group. More intense cytoplasmic and extracellular osteopontin immunologic involvement was observed in bone trabeculae and islets compared to autograft group. 1c: Intense osteopontin was observed in the non-calcified osteoid tissue (#) in the defect area for xenograft-PRF group. 2c: In xenograft group, less intense cytoplasmic and extracellular osteopontin immunologic involvement was observed in loose connective tissue and new bone islets formed between the fatty bone marrow (#). 1d: In β-TCP-PRF group, there was more intense immune involvement in the new mineralized bone trabeculae (*) and in the non-calcified osteoid tissue surrounding the trabeculae (#) compared to β-TCP group. 2d: β-TCP group showed less osteopontin immunoreactivity in loose connective tissue rich in fatty tissue around the graft material (#).

5. Conclusions

This study found that autograft combined with PRF yielded superior results in terms of new bone formation. In addition, xenograft- PRF combination also offered promising results. Since synthetic bone graft materials do not have a live structure, using them with biomaterials such as PRF may lead to a drawback for new bone formation. However, further experimental and clinical studies might be beneficial to clarify the exact mechanism and results of combining PRF with bone grafts on the bone healing process.

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Conflicts of interest

Authors have no potential conflict of interest regarding any relationships with other people or organizations that could inappropriately influence this study.

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The data of this study has been presented at the 46. Congress of the Turkish Society of Periodontology.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcms.2019.01.023.

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