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Research Article

Evaluation of the Accelerator Effect of Coral and Platelet Rich Fibrin on Bone Healing [1]

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Abstract

This experiment was conducted to investigate the accelerator effect of coral and platelet rich fibrin (PRF) on bone healing in rabbits (n=12) with clinically, radiologically, and histologically. The rabbits were divided randomly into two groups. There were two bone defects (3.5 mm diameter) created bilaterally on the proximal part of the tibia of rabbits. In control group, the defects were left empty. The other defects (twelve defects in each group) were filled with coral, PRF and coral plus PRF. Postoperatively, on the 30th and 60th days, clinical, radiographic and histologic examinations were performed. On the radiological examinations, bone healing was better seen in the grafted groups than in the control group (P<0.01). During the histological examinations on the 30th day, differences between groups were not important. On the 60th day, bone healing was found to be better in the coral, PRF and coral plus PRF groups than in the control group (P<0.01). However, the best bone healing was observed in the PRF group (P<0.01). In conclusion, applications of coral, PRF and coral plus PRF (especially PRF) are significantly effective for bone healing.

Keywords: *Coral, Platelet rich fibrin, Bone graft, Rabbit*

Mercan ve Trombositten Zengin Fibrinin Kemik İyileşmesi Üzerindeki Hızlandırıcı Etkisinin Değerlendirilmesi

Öz

Bu deney, tavşanlarda (n=12) mercan ve trombosit açısından zengin fibrinin (TZF) kemik iyileşmesi üzerindeki hızlandırıcı etkisini klinik, radyolojik ve histolojik olarak araştırmak amacıyla yapıldı. Tavşanlar rastgele iki gruba ayrıldı. Tavşanların tibialarının proksimalinde bilateral olarak iki kemik defekti (3.5 mm çapında) oluşturuldu. Kontrol grubundaki defektler boş bırakıldı. Diğer defektler (her grupta oniki defekt) mercan, TZF ve mercan ve TZF ile dolduruldu. Operasyon sonrası, 30 ve 60. günlerde klinik, radyografik ve histolojik muayeneler yapıldı. Radyolojik incelemelerde greftli gruplarda kemik iyileşmesi kontrol grubuna göre daha iyi olduğu gözlendi (P<0.01). 30. günde yapılan histolojik incelemelerde gruplar arasındaki farklar önemli değildi. 60. günde, kemik iyileşmesinin, kontrol grubuna göre mercan, TZF ve mercan artı TZF gruplarında daha iyi olduğu bulundu (P<0.01). Bununla birlikte, en iyi kemik iyileşmesi TZF grubunda gözlendi (P<0.01). Sonuç olarak, mercan, TZF ve mercan artı TZF (özellikle TZF) uygulamaları kemik iyileşmesinde önemli derecede etkilidir..

Anahtar sözcükler: *Mercan, Trombositten zengin fibrin, Kemik grefti, Tavşan*

INTRODUCTION

Bone defects due to trauma, resection of bone cysts and bone tumours, congenital defects or corrective osteotomies are a worldwide problem. Autogenous, allogeneic, xenogeneic and alloplastic bone graft materials are usually used to treat bone defects [1,2]. Autogenous bone grafts have been widely used as the gold standard for accelerating bone healing. However, it is known that there is donor site morbidity, and limited sources of autogenous bone grafts are an important problem. Disadvantages of using allografts include the risk of disease transfer and non-union [3,4]. Alternative options are attractive and continue to be sought [5,6].

Coral, as marine invertebrates, have been widely used

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for orthopaedics [4]. It is reported that the exoskeleton of corals is a good biomaterial that exhibits porosity very similar to that of human cancellous bone, with suitable mechanical, natural resorbable and osteoconductive properties. Many investigators have reported that coral has a high compressive breaking stress, interconnected porous structure, and good resorbability and biocompatibility [1,5].

Many factors, such as hormones and growth factors, are effective on increasing bone development and on accelerating bone healing. Platelet rich fibrin (PRF) is an autogenous fibrin matrix used to improve bone formation [7-12]. Platelets produce cytokine-like transforming growth factor beta, platelet-derived growth factor, insulin-like growth factor and vascular endothelial growth factor. The cytokines and growth factors in the fibrin network are released slowly [13,14]. Kang et al.[15] reported the influences of PRF in bone healing, either alone or in combination with other graft materials.

In light of the above information, we hypothesised that the PRF (osteoinductive graft material, containing cytokines and growth factors), and coral (osteoconductive graft material) would be accelerate bone healing alone or in combination. The establishment of therapeutic condition of PRF and coral would aid greatly in guiding clinicians in treatments of bone healing. Ideally these would be under animal studies that would help the analysis of bone regeneration capacity. The findings obtained from this study will be useful in the treatment of similar cases in domestic animals. For this purpose, clinical, radiological and histological comparisons of the effects of coral, PRF and coral plus PRF on bone healing were investigated. The purpose of this experimental study was to appraise the efficiency of coral and PRF (alone and in combination) on bone healing together in terms of clinical, radiological, and histological results.

MATERIAL and METHODS

The experiment was performed on twelve male New Zealand rabbits (5-6 months old, weighing 2.5-3 kg) in the Firat University Experimental Research Centre. The rabbits were fed standard rabbit food and allowed to move freely. This study was confirmed by The Animal Experiment Ethics Committee of the Firat University (Number: 102, 14.10.2010 dated).

Madreporaria sp. coral was used in this study. A coral branch was divided into small granules, and the granules were sterilized by gamma irradiation.

PRF was prepared using a previously described technique [16]. Venous blood samples (8 mL) were collected from vena jugularis (into a tube without an anticoagulant) and then centrifuged at 400 *g* for 10 min. The centrifuged product had three layers: platelet poor plasma at the upper of the tube, PRF in the middle and a red blood cell layer at the lower part. The PRF clot (2-3 mL) was removed from the tube, and it was isolated from the residual blood components. The PRF clots were kept in sterile petri dishes until they were grafted.

For coral plus PRF, the PRF clot was cut into small pieces, and then equal amounts of PRF and coral granules were mixed.

Surgical Procedure

Anaesthesia was performed using xylazine HCl (5 mg/kg, IM, Rompun, 23.32 mg/mL, Bayer) and ketamine HCl (35 mg/kg, IM, Ketalar 50 mg/mL, Parke-Davis). Both hind limbs of the rabbits were shaved. Operation regions were prepared with 1% povidone iodine. A skin incision (2 cm length) was made on the medial surface of the tibia. Totally forty-eight unicortical bone defects (3.5 mm diameter, two defects in the proximal part of each tibia,1 cm distance between two defects) were created on rabbits. Twelve defects of the 48 bone defects were left empty (Control group) *(Fig. 1A)*. The other defects were filled with coral granules *(Fig. 1B)*, PRF *(Fig. 1C)* or coral granules plus PRF *(Fig. 1D)* (12 defects in each group). After the bone defects were filled with grafts, the subcutaneous connective tissue was sutured with 3/0 chromic catgut, and the skin was sutured with 2/0 silk. The rabbits were administered intramuscularly with procaine penicillin (400.000 IU, IM, Iecilline, I.E. Ulagay) and metamizol sodium (25 mg/kg, IM, Novalgine 1000 mg/mL, Sanofi) for postoperative 5 days.

Radiographic Evaluations

Radiographic images were taken to evaluate new bone formation in the defects (after surgery, on the 30th and 60th days). Radiographic images were evaluated according to the modified Lane and Sandhu [17] scoring method. Each radiograph was given a score of 0-4: 0, no sign of bone formation; 1, 25% bone formation filling the defect; 2, 50% bone formation filling the defect; 3, 75% bone formation filling the defect; and 4, 100% bone formation filling the defect.

Histological Examinations

Rabbits were sacrificed 30 (n=6) and 60 days (n=6) after the surgery. Bone samples including grafts were resected, and bone samples were fixed in 10% buffered formalin. These samples were decalcified in nitric acid solution and processed for histological examination. Histological sections (5 µm thick) were prepared from the centre of each sample, were stained with haematoxylin and eosin, and were evaluated using a light microscope.

The histological scores were assigned in a blinded manner, as described previously [18]. Each specimen was given a score of 1-7: 1, only fibrous tissue; 2, more fibrous tissue than cartilage tissue; 3, more cartilage tissue than fibrous tissue; 4, more cartilage tissue than trabecular bone tissue;

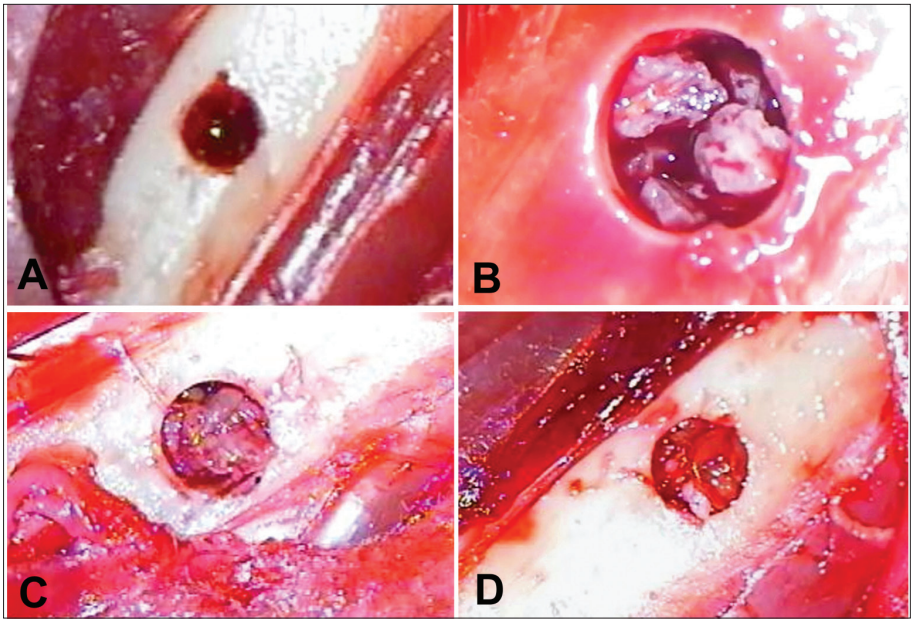

Fig 1. Postoperative appearance of the bone defects. **A-** Control, **B-** Coral, **C-** Platelet rich fibrin, **D-** Coral plus platelet rich fibrin

5, same amount of immature bone tissue and cartilage tissue; 6, more trabecular bone tissue than cartilage tissue; and 7, compact bone tissue.

Statistical Analysis

SPSS (22.0 version) was used for statistical evaluations. The non-parametric Kruskal-Wallis H test were used to identify differences among the groups. The non-parametric Mann-Whitney-U test was used to compare significant differences between the results on the 30th and 60th days within each group. Values are presented as the means±SEM. Differences were considered statistically significant when $P<0.05$.

RESULTS

The surgical procedures were well tolerated by all rabbits. Surgical regions healed uneventfully. The rabbits completed the study without death or surgical complications. During the study, adverse effects related to graft materials on health were not observed.

The radiological appearances of the bone defects are given in *Fig. 2*. Radiologic evaluation of the bone formation according to the modified Lane and Sandhu [17] radiologic scoring method is given in *Table 1*.

In postoperative radiographs, bone defects and coral granules were observed clearly. The PRF showed decreasing radiolucent contrast. On the 30th and 60th days, in the coral and coral plus PRF groups, non-resorbed small coral particles were found. Migration of placed graft materials was not observed. When the comparison between groups was made, bone healing was found to be worse in the control group compared to in the other groups ($P<0.01$).

Microscopic images are shown in *Fig. 3*. According to the modified histological scoring criteria [18], the evaluations of bone healing on the 30th and 60th days are summarised in *Table 2*.

On the 30th day, in the control group, the defects were filled with neoformed granulation tissue and capillary vessels. Minor cartilage tissue formation was observed. The defects were partially filled with newly formed granulation tissue in the coral group. New bone formation was started between coral grafts and the host bone. In the PRF group, the defects were completely filled with a collagen-rich fibrous callus. In addition to the formation of fibrous tissue and cartilage tissue, osteoblastic activity was evident. Spicular primary bone areas incorporated with each other, and some of the structures transformed to trabecular structures. In addition, bone marrow formation began within defects. In the coral plus PRF group, defects were partially filled with fibrous callus, but the edges of the defects did not have union. Areas of cartilage tissue separate from connective tissue were evident. New trabecular bone formation around the coral grafts was observed. However, differences between groups on the 30th day were not important.

On the 60th day, in the control group, it was observed that a small amount of cartilage tissue formed within the capillary vessels-rich fibrous callus. In addition, spicular primary bone tissue formation was present. In the coral group, the defects were fully filled with cartilage and primer bone tissue. Trabecular bone formation was detected, and osteoblastic activity was evident. Osteoclasts in contact with new bone formation were observed. It was determined that the defects were fully filled by fine primary and secondary bone formations in the PRF group. It was also determined that haversian canals were formed. Cartilage and fibrous tissue formations were not found. Osteoblastic activity was on the bone marrow defect side. In the coral plus PRF

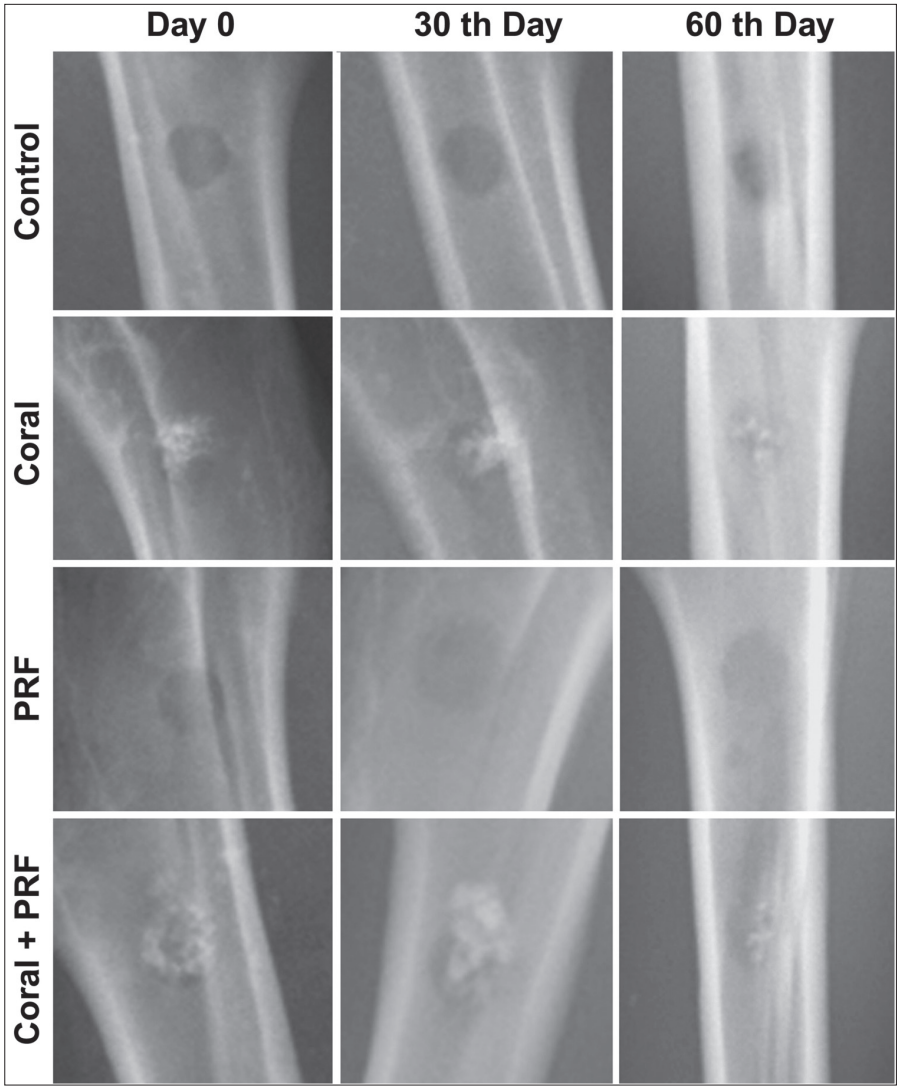

Fig 2. Radiological appearances of the bone defects. PRF: platelet rich fibrin

Table 1. *Radiological evaluation of the defects on 30th and 60th days according to the modified Lane and Sandhu [17] scoring system*

Groups (n=12)	Days	
	30	60
Control	0.50 ± 0.22^{aA}	1.50 ± 0.22^{aB}
Coral	1.83 ± 0.17^{bA}	3.17 ± 0.17^{bB}
PRF	2.67 ± 0.21^{bA}	3.83 ± 0.17^{bB}
Coral+PRF	2.50 ± 0.22^{bC}	3.50 ± 0.22^{bD}

The values are given as mean±SEM; Different superscripts (a,b) in same column represents is a significant difference (P<0.01); Different superscripts (A,B) in same row represents is a significant difference (P<0.01); Different superscripts (C,D) in same row represents is a significant difference (P<0.05); SEM: standard error of the mean

group, the defect was partially filled with bone trabeculae. Havers channel formation and lacuna formation were noted. There was significant bone marrow formation. On the 60th day, bone healing was found to be better in the coral, PRF and coral plus PRF groups than in the control group (P<0.01). The best bone healing was observed in the PRF group (P<0.01).

DISCUSSION

Concentrated platelets are used for bone healing. It is reported that PRF is equipped with many growth factors, and it is effective in bone healing. The growth factors are secreted from α granules 10 min after platelet activation. These factors are transmitted to the wound area in 1 hour, and the healing process begins [19-21]. Platelet-derived growth factor is one of these factors, and this factor accelerates cell proliferation, neovascularization and restructuring in the wound area [8-12,22-24]. It is reported that PRFs have significant advantages for haemostasis and wound healing. It is believed that interleukins in the PRF help wound healing by suppressing inflammation [13,14]. In this study, PRF was obtained according to Choukroun's technique [25,26]. Low speed centrifugation was performed, and we aimed to obtain PRF similar to the natural fibrin network.

To increase bone formation and promote the healing of bone defects, osteoinductive graft materials have been used alone or with osteoconductive graft materials [2,6,15,27,28].

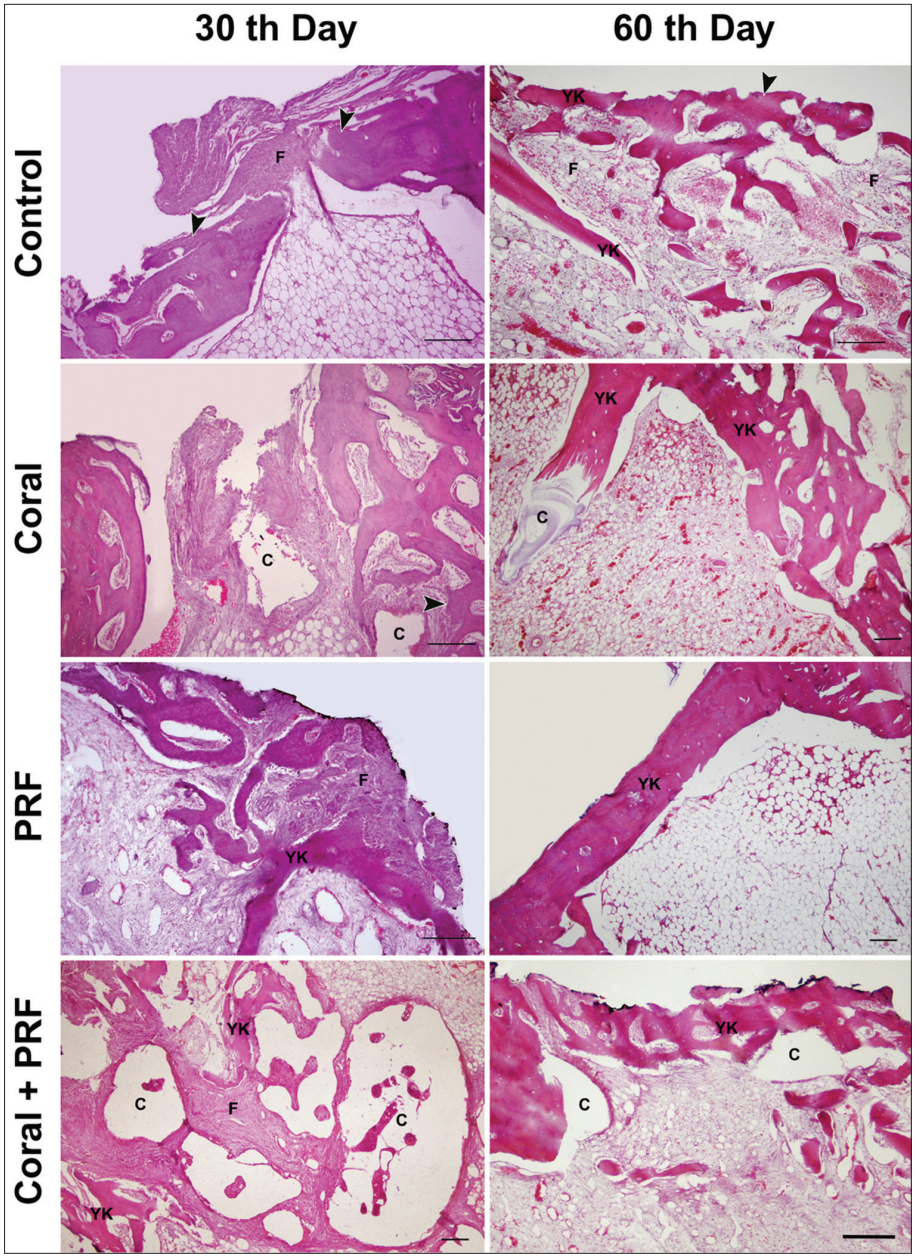

Fig 3. Appearance of the haematoxylin-eosin-stained samples. PRF: platelet rich fibrin, F: fibrous tissue, *Arrow heads:* cartilage tissue, C: coral remnant, YK: new bone, H-E, Bar: 200 µ

Table 2. *Histological evaluation of the defects on 30th and 60th days according to the modified histological scoring system [18]*

Groups (n=12)	Days	
	30	60
Control	1.50±0.22^{A}	3.50±0.22^{Ba}
Coral	2.33±0.21^{A}	5.00±0.37^{Bb}
PRF	2.67±0.33^{A}	6.17±0.17^{Bc}
Coral+PRF	2.33±0.21^{A}	5.17±0.31^{Bb}

The values are given as mean±SEM; Different superscripts $^{(A,B)}$ in same row represents is a significant difference (P<0.01); Different superscripts $^{(a,b,c)}$ in same column represents is a significant difference (P<0.01); SEM: standard error of the mean

In the present study, a tibial defect model was used to evaluate the effects of coral (osteoconductive graft material) and PRF (osteoinductive graft material) on bone healing. We also aimed to create a resource for researchers regarding the acceleration of bone healing.

Healing in bone defects depends largely on the size of the defect. If a bone defect does not heal spontaneously during the experiment, it is a critically sized bone defect [29]. In this study, the 3.5 mm tibial defects in the rabbits were considered to be critically sized bone defects for a 2-month test period.

The coral skeleton has an open, interconnected porous structure, and this structure makes it an important scaffold for bone healing. The pore size of corals is very important. When the coral pores are very small, occlusion by cells will occur. Coral pore size should be at least 150 µm for satisfactory osteoblast and vascular invasion [1,5].

The properties of the bone graft determine the graft resorption rate. The particle size and porosity of the surface are important for graft resorption. Oversized grafts are resorbed more slowly, while porous grafts are resorbed more quickly [1]. The coral used in this study belongs to the *Madreporaria* genus. Its structure is similar to cancellous bone, and it has an aragonite crystal structure. The coral had a 400-800 µm wide interconnected porous structure. This structure had many advantages for vascularization and cell invasion.

It has been reported that in postoperative radiologic examinations, a grafted coral structure was clearly observed, and it was resorbed over time [1,5]. In this study, the coral and its porous structure were clear in radiologic examinations. At the 30th and 60th days postoperatively, coral grafts were partially resorbed, and the density was decreased depending on new bone formation. However, normal bone contrast was not seen. In the coral ($P<0.01$), PRF ($P<0.01$) and coral plus PRF groups ($P<0.05$), callus formation increased over time, and the defects were filled by newly formed bone tissue. Radiological results showed that the graft materials have a stimulating effect on bone healing ($P<0.01$).

Histological examination is necessary to determine bone healing at the cellular level. It is reported that ossification starts in 1-2 weeks, and defects are filled and reorganized in 6-8 weeks [4,16,28]. In this study, the histopathological investigation periods for bone healing were 30 and 60 days.

The histological consequences of this study are in agreement with the findings of other investigators [7,29]. These results showed that bone healing increased during the study period in the coral, PRF and coral plus PRF groups. At the same time, it was shown that bone grafting is required for increasing new bone formation. On the 60th day, in the grafted groups, new bone formation was significantly better than in the control group ($P<0.01$). But it was observed that coral was slightly reduced bone healing in coral plus PRF group compared to the PRF group on the 60th day ($P<0.01$). In addition, on the 60th day, the best bone healing was observed in the PRF group ($P<0.01$).

Bone grafts should be resorbed after the formation of new bone. Additionally, the grafts, which had high osteoinductive properties, should increase new bone formation in a short time and be replaced by new bone. During this study, in agreement with the reports of other investigators [5,28], the resorption of coral grafts continued, but complete resorption was not observed in any cases at the end of the 2 month period. At the same time, foreign body cells or inflammatory reactions were not observed in the coral group.

In conclusion, this study showed that coral, PRF, and coral plus PRF are significantly effective in bone healing. This may be of importance for raising the possibility that PRF may have potential in the treatment of bone defects.

Conflicts of Interest

The authors declare no conflict of interest.

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