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A HISTOMORPHOMETRIC EVALUATION OF THE EFFECTS OF PLATELET-RICH FIBRIN AND RIFAMYCIN IN COMBINATION WITH AN ALLOGRAFT ON BONE AUGMENTATION WITH SIMULTANEOUS IMPLANT PLACEMENT IN RABBIT TIBIA

Trombosit Zengin Fibrin ve Rifamisinin Bir Allogreft ile Kombinasyonda Kemik Büyütme Üzerindeki Etkilerinin Tavşan Tibiasında Eşzamanlı İmplant Yerleştirmesiyle Histomorfometrik Olarak Değerlendirilmesi

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ABSTRACT

Objective: To evaluate the potential of platelet-rich fibrin (PRF) and rifamycin to enhance guided bone augmentation with simultaneous high-profile dental implant placement in a rabbit tibia model.

Materials and Methods: Dental implants protruding 2 mm were covered with dome-shaped stiff occlusive titanium barriers filled with demineralized freeze-dried bone allograft (DFDBA)+saline (7 rabbits), DFDBA + rifamycin (8 rabbits), or DFDB +PRF (8 rabbits). After 4 weeks, the animals were sacrificed, and undecalcified histomorphometric examination with toluidine blue staining was performed.

Results: The bone-to-implant contact (BIC) was 58.43 ± 1.92% in the saline group, 68.3 ±20.37% in the rifamycin group, and 80.70±2.55% in the PRF group, and the percentage of new bone formation was 36.90 ± 0.94%, 45.26±0.60%, and 51.82±0.82%, respectively. Conclusions: Both PRF and rifamycin have potential to enhance GBA, and using DFDBA + PRF or DFDBA+rifamycin beneath a stiff occlusive titanium barrier next to a high-profile implant may enhance both BIC and new bone formation.

Keywords: Guided bone augmentation, platelet-rich fibrin, rifamycin, allograft, high-profile dental implants

ÖZ

Amaç: Yüksek profilli dental implant yerleştirilmesinde yönlendirilmiş doku augmentasyonunu geliştirmek için plateletten zengin fibrin (PRF) ve rifamisin uygulamasının potansiyelinin değerlendirilmesi.

Materyal ve metod: İki mm'lik koronal kısmı kemik dışında kalan dental implantlar kubbe şeklinde sert kaplayıcı titanyum membran ile kapatılmış ve membranın içi demineralize dondurularak kurutulmuş kemik allogrefti (DFDBA)+salin (7 tavşan), DFDBA+rifamisin (8 tavşan) veya DFDBA+PRF (8 tavşan) ile doldurulmuştur.

Bulgular: Kemik implant kontağı (KİK) salin grubunda 58,43±1,92%, rifamisin grubunda 68,34±20,37% ve PRF grubunda 80,70±2,55% olarak tespit edilmiştir. Sırasıyla yeni kemik oluşum yüzdesi de 36,90±0,94, 45,26±0,60 ve 51,82±0,82 olarak bulunmuştur.

Sonuç: Hem PRF hem de rifamisinin yönlendirilmiş doku augmentasyonunu geliştirici potansiyeli olduğu ve yüksek profilli dental implant uygulamalarında sert kaplayıcı titanyum membran altında DFDBA + PRF veya DFDBA + rifamisin kullanılmasının hem KİK değerini hem de yeni kemik oluşum yüzdesini artırabileceği görülmüştür.

Anahtar Kelimeler: Kemik Takviyesi, Trombosit bakımından zengin fibrin, rifamisin, Yüksek profilli diş implantları

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INTRODUCTION

Vertical bone insufficiency is still a challenging problem in oral surgery for dental implant placement.[1] Conventionally, a therapeutic method known as guided bone regeneration/ augmentation (GBR/GBA) is used to solve this problem. The basic principle of GBA involves placement of a mechanical barrier to protect the blood clot and to isolate the bone defect from the surrounding connective tissue. This allows osteoprogenitor cells to proliferate and differentiate without competition from overlying soft tissue cells.[2-4]

For vertical ridge augmentation with GBA, barriers have been used alone or in combination with bone grafts. It may be possible to enhance the bone healing obtained with grafts by combining regenerative therapies.[5] Platelet-rich fibrin (PRF) is a second-generation autologous platelet concentrate (APC) that is easy and inexpensive to prepare. It is a fibrin meshwork in which platelets, leukocytes, cytokines, and growth factors are trapped during centrifugation.[6,7] Clinically, PRF enhances bone healing in dentistry.[8,9] In this study, PRF was combined with graft material based on the hypothesis that two distinct wound-healing processes would occur together and promote bone regeneration.

Antibiotics combined with bone autografts and allografts have been used clinically to treat infections.[10] Bone grafts can be soaked in an antibiotic solution or combined with antibiotic powder. Rifamycin is a semisynthetic macrocyclic antibiotic derived from natural rifamycin B that has been combined with bone grafts and has positive effects on bone healing and graft incorporation. Kaya *et al.*[11] showed that topical rifampin can accelerate the bone repair process. Taşdemir *et al.*[12] combined rifamycin with autogenous bone grafts and found earlier revascularization and osteogenesis in the experimental group; there was also significantly more BMP-2 in the experimental group than in the controls. Therefore, we combined rifamycin with grafts to enhance bone healing.

Investigators continue to search for optimal bone augmentation strategies to achieve predictable results and precise bone formation. This study used tissue engineering to enhance bone formation during GBA. In an animal experiment, a stiff occlusive titanium barrier was placed over supracrestally placed high-profile dental implants with demineralized freeze-dried bone allograft (DFDBA)+saline, DFDBA+PRF, or DFDBA+rifamycin to evaluate whether bone formation was enhanced.

MATERIALS AND METHODS

The study protocol was approved by the ethics committee of Cumhuriyet University. All experiments were performed according to the Cumhuriyet University Guidelines for the Care and Use of Laboratory Animals.

Study Design

This study used 23 adult male New Zealand white rabbits, weighing 3.0-3.5 kg. In the experimental model, 23 3-mm-diameter, 10-mm-long implants were placed in the rabbit tibia, leaving the threads exposed by 2 mm in coronal section. The exposed threads were covered with a custom-made dome-shaped stiff occlusive titanium barrier with a diameter of 8 mm, height of 4 mm, and thickness of 0.3 mm. The 3-mm-diameter holes at the top of each barrier were closed with a Teflon cover. All of the barriers were cleaned in an ultrasonic bath and sterilized in an autoclave before use. The titanium barriers were filled with DFDBA (Maxxeus Community Tissue Services, Ohio, USA) combined with saline (seven rabbits), DFDBA combined with PRF (1:1 ratio) (eight rabbits), or DFDBA combined with rifamycin (250 mg; Koçak Farma, Istanbul, Turkey) (eight rabbits). The rabbits were assigned randomly to the three groups.

PRF Preparation

For each of the experimental animals, PRF was prepared before surgery. Five milliliters of blood were withdrawn under sedation from the central auricular artery of each animal into a blood-collection tube without anticoagulant. The blood was centrifuged immediately after collection at 3000 rpm for 12 minutes. This produced a fibrin clot that was removed from the tube using forceps under sterile conditions. The PRF clot was cut into small pieces and combined with graft material.

Surgical procedures

All operations were completed under general anesthesia, achieved with 2% xylazine (Rompun 2%; Bayer, Istanbul, Turkey) and 1% ketamine (Ketalar; Eczacibasi Warner Lambert, Istanbul, Turkey). The experimental side was shaved and cleaned with povidone–iodine. After the incision was made, the tibia bone was exposed with subperiosteal dissection.

Implant beds 3 mm in diameter and 8 mm deep were prepared, and an implant (Adin Dental Implant Systems, Toureg–NP 3.0×10 mm; Afula, Israel) was inserted with primary stability. Healing caps were screwed on the implants. This left the implants exposed by 2 mm (Figure 1). Six small holes were drilled with a 1-mm-diameter round burr to induce bleeding from the marrow space. All implants were covered with a titanium barrier, and the barriers were filled with DFDBA+saline, DFDBA+PRF, or DFDBA+rifamycin. The tissues were sutured tightly in two layers using absorbable sutures (Pegelak, poly (glycolide-co-lactide) (PGLA); Doğsan, Trabzon, Turkey). Postoperatively, the rabbits were given ceftriaxone 50 mg/kg (Rocephin; Deva, Istanbul, Turkey) and carprofen 4 mg/kg (Rimadyl; Pfizer, New York, IL, USA) intramuscularly once daily for 3 days.

The animals were sacrificed 4 weeks after implantation. The implants were dissected with the bone, and any signs of unusual healing were documented (Figure 2).

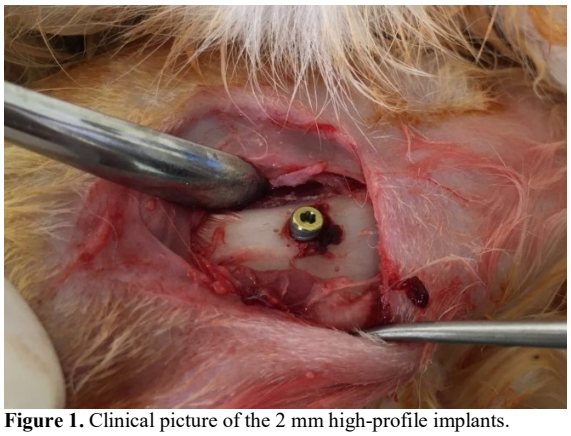

Figure 1. Clinical picture of the 2 mm high-profile implants.

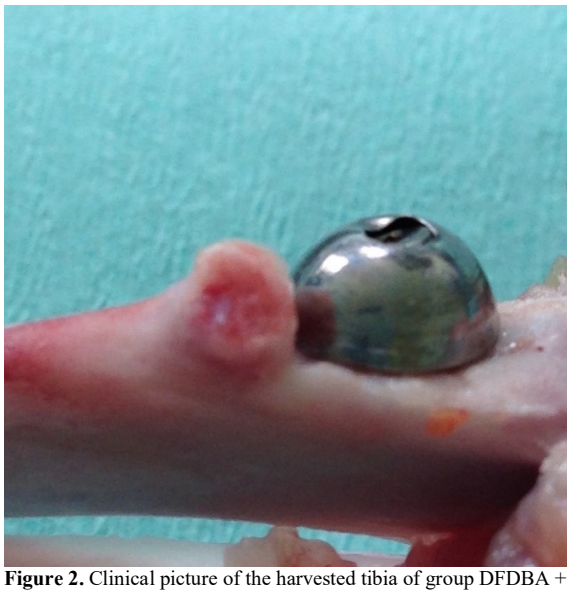

Figure 2. Clinical picture of the harvested tibia of group DFDBA + PRF with the removed titanium barrier.

Specimen preparation

The bone with the implant was removed *en bloc* and immersed in 4% neutral buffered formaldehyde for histological assessment. Dehydration with increasing percentages of ethanol was followed by embedding in a methyl methacrylate-based resin (Technovit 7200 VLC; Kulzer&Co, Wehrheim, Germany). Undecalcified

ground sections from the implants and surrounding bone were prepared according to the method described by Donath and Breuner.[13] Sections of all implants were taken through the same longitudinal plane and reduced to a thickness of 50 µm with diamond grinding. Four sections were prepared from each specimen and stained with toluidine blue.

Bone histomorphometry

All sections were evaluated histomorphometrically. Images were captured using a light microscope (Olympus BX50; Olympus Optical, Tokyo, Japan) with an attached digital camera system (Olympus DP 70; Olympus Optical, Tokyo, Japan). All images were downloaded to a personal computer and evaluated using BIOQUANT Osteo II (BIOQUANT Image Analysis Corporation, Nashville, TN, USA) image-analysis software. The percentages of bone-to-implant contact (BIC) and new bone formation were calculated.

Data analysis

Analysis of variance, Tukey's range test, and *t*-tests to examine differences between pairs were used for the statistical evaluation. SPSS 14.0 for Windows (SPSS, Chicago, IL, USA) was used for the statistical analysis. A difference was considered statistically significant if the *p*-value was less than 0.05.

RESULTS

All animals had uneventful recoveries. At sacrifice, no clinical signs of adverse tissue reactions were seen. All implants were still *in situ* at sacrifice, and all tissue specimens were available for analysis.

Figure 3 shows regenerated tissue representing healing. The area of newly formed bone and the BIC were increased in the PRF and rifamycin groups compared with the saline group. The results of the histomorphometric analysis are shown in Table 1. Morphometric measures of BIC differed significantly between the DFDBA+PRF and DFDBA+saline groups and between the DFDBA+rifamycin and DFDBA+saline groups ($p<0.05$). In terms of new bone formation, the DFDBA+PRF and DFDBA+rifamycin groups differed significantly from the DFDBA+saline group ($p < 0.05$).

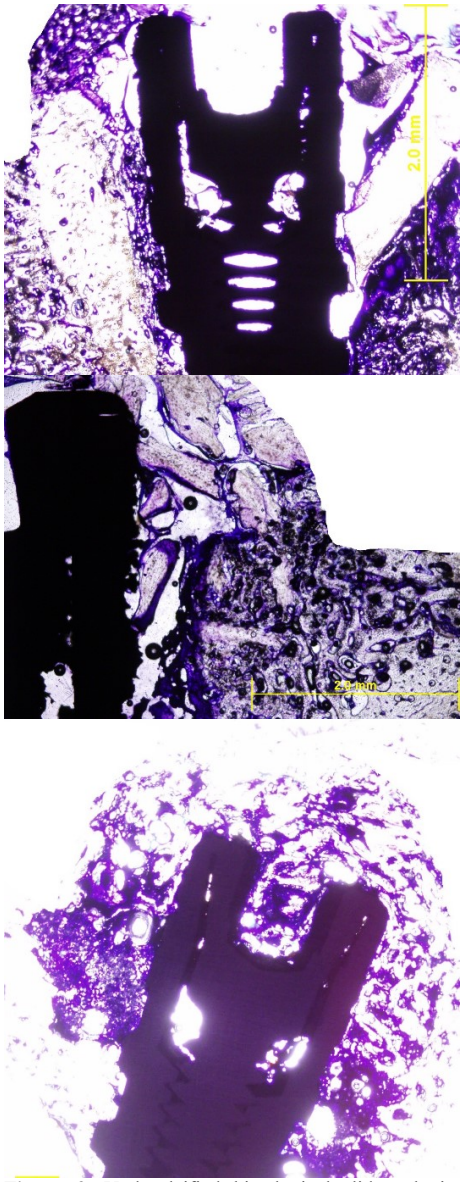

Figure 3. Undecalcified histological slides obtained from the specimens. A, Sample from DFDBA+saline group (Scale bar = 2 mm). B, Sample from DFDBA+rifamycin group (Scale bar = 2 mm). C, Sample from DFDBA+PRF only group (Scale bar = 1 mm). (Toluidine blue)

Table 1: Percentage of direct bone-to-implant contact and new bone formation.

	BIC	New Bone Formation
DFDBA+saline	58.43 ± 1.92 [a b]	36.90 ± 0.94 [d e]
DFDBA+rifamycin	68.34 ± 20.37 [a c]	45.26 ± 0.60 [d f]
DFDBA+PRF	80.70 ± 2.55 [b c]	51.82 ± 0.82 [e f]
P value	.001	.001

Same superscript letters indicate statistically significant difference ($p<0.05$). BIC = Bone-to-implant contact, DFDBA= Demineralized freeze-dried bone allografts, PRF=Platelet-rich fibrin

DISCUSSION

Previous studies[14,15] have used GBA with a dome-shaped stiff occlusive titanium barrier successfully for bone regeneration. Although bone is generated under the titanium barrier membrane with only a blood clot, this procedure has a limited potential. Sites with fewer surrounding osseous walls and more pronounced atrophy are more demanding and require materials or techniques that offer greater biological activity and regenerative capacity.[16,17] To enhance bone formation with GBA, tissue engineering procedures are used. Casap *et al.*[18] used recombinant human bone morphogenetic protein-2 (rhBMP-2) with a titanium occlusive barrier over high-profile dental implants for vertical ridge augmentation, and found more bone formation in the experimental group. Marx *et al.*[19] used a composite graft of rhBMP-2/collagen sponge, freeze-dried allogeneic bone, and platelet-rich plasma within a titanium mesh, and found that this method was successful for large vertical ridge augmentations. Misch *et al.*[17] evaluated the use of a composite graft of rhBMP-2 and bone allograft under a titanium mesh for vertical augmentation, and suggested that this technique leads to favorable vertical bone gains. The stimulation of bone regeneration is important, and topically applied growth factors may augment bone formation at GBA. However, growth factors are expensive.

In recent years, PRF has been shown to affect bone regeneration significantly. Ozdemir *et al.*[20] evaluated the effects of PRF alone when used with GBA and found that PRF under a titanium barrier enhanced new bone formation. Biologically active bone graft materials are needed to improve bone regeneration, and PRF is combined with bone grafts for this reason. Shah *et al.*[21] grafted periodontal intrabony defects with a combination of PRF with DFDBA and concluded that the combination produced better results than DFDBA alone. Agarwal *et al.*[22] grafted intrabony periodontal defects with DFDBA combined with PRF and found that combining PRF with DFDBA significantly enhanced bone regeneration compared with a bone graft alone. In the present study, the DFDBA+PRF sites showed significant increases in BIC and new bone formation compared with the DFDBA+saline group. Our results are in agreement with previous reports. Therefore, adding PRF to DFDBA may enhance bone formation during GBA. In this study, PRF upregulated the growth factors necessary for enhancing bone formation during GBA, and this technique was cost-effective.

Bone graft materials are combined with various antibiotics for different purposes, and the effects of these combinations on graft healing have been studied. The results of these studies have varied, which might have resulted in part from the potential toxic effects of various antibiotics and their localized concentrations.[23] Mabbry *et al.*[24] reported that the combination of gentamycin and tetracycline mixed with freeze-dried bone allografts increased osseous regeneration. Masters *et al.*[25] grafted intrabony periodontal defects using DFDBA with tetracycline HCl and found greater bone filling and defect resolution in the experimental group. Tabrizi *et al.*[26] evaluated the periodontal regenerative capacity of DFDBA alone and combined with local lincomycin. DFDBA with or without lincomycin did not offer predictable

benefits. The literature contains little information on the combined effects of DFDBA and rifamycin in bone healing. Kaya *et al.*[11] showed that topical rifampin can accelerate bone healing, but when they combined rifamycin with allogeneic bone grafts, they found that this combination reduced new bone formation in osseous defects. By contrast, our results showed that DFDBA + rifamycin significantly enhanced BIC and new bone formation compared with DFDBA + saline.

In this study, the samples were collected after 4 weeks, which represents the end of acute bone healing in rabbits. Rossi *et al.*[27] described the healing of marginal defects around dental implants in an experimental study and showed that these defects regenerate in 20~30 days.

CONCLUSION

Within the limitations of this study, the placement of DFDBA + PRF or DFDBA + rifamycin beneath an occlusive titanium barrier next to a high-profile implant resulted in significantly increased bone formation and BIC compared with DFDBA+saline. Additional research is required to fully explore the newly formed bone obtained using a stiff occlusive titanium barrier when high-profile implants are placed. Evidence supporting the use of antibiotic agents with bone grafts to enhance bone healing is presently insufficient. To determine the full potential of rifamycin in bone regeneration, further studies must examine the effects of rifamycin in bone healing.

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