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Original research

A STUDY ON THE USE OF DIFFERENT MATERIALS COMBINED WITH ALLOGRAFT ON OSSEOINTEGRATION AND BONE REGENERATION OF DENTAL IMPLANTS WITH CORONAL DEFECTS IN A RABBIT MODEL

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ABSTRACT

Objectives: To assess differential effects of different materials combined with allograft on bone-to-implant contact and newly formed bone formation in dental implants with coronal defects histomorphometrically.

Materials and Methods: The study was conducted on 24 male New Zealand white rabbits. Dental implants (3.0 × 10 mm) were placed at the center of defects (9 mm diameter, 4 mm depth) created in the tibial bones of the rabbits. Graft (GF, n=8), graft + rifamycin (GR, n=8) and graft + black cumin oil via orogastric route (GB, n=8) were applied on the coronal aspects of the implants for 28 days. Undecalcified histomorphometric analyses were conducted on slides stained with toluidine blue.

Results: Bone-to-implant contact was 46.57% ± 3.59% in the graft (GF), 67.12% ± 3.64% in the graft + rifamycin (GR) and 55.62% ± 4.37% in the graft + black cumin oil (GB) groups. The percentage of new bone formation at the defect area was 34.71% ± 4.11% in the graft, 55.37% ± 4.89% in the graft + rifamycin, and 45.75% ± 3.69% in the graft + black cumin oil groups. In terms of new bone formation and bone-to-implant contact, graft + rifamycin and graft + black cumin oil groups were significantly different from the graft group. The differences between the graft + rifamycin and graft + black cumin oil groups were also statistically significant.

Conclusions: Allograft + rifamycin and orogastric black cumin oil were found to have positive effects on bone healing at sites with coronal defects. Rifamycin showed significantly greater favorable effects on bone-to-implant contact and new bone formation compared to black cumin oil.

Key Words: Allograft, rifamycin, black cumin oil, histomorphometry, dental implant.

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INTRODUCTION

Long-term success has been demonstrated clinically for dental implants used in dentistry and dental implants have become a widely preferred, alternative therapeutic option. Compared to traditional dental prosthesis, patients have reported greater satisfaction and improved quality of life with dental prosthesis supported by dental implants in terms of comfort, stability and esthetics.[1]

While dental implants are the most preferred and effective treatment modality, their effectiveness depends largely on successful osseointegration during the healing process. Branemark *et al.*[3] described osseointegration as the direct structural and functional connection of living bone to load-bearing endosseous implants and several factors are involved in the establishment of such connection. Thus, this is an area that still requires a great deal of further research and development.[2]

Implants can be placed in fresh sockets after tooth extraction using the immediate implant loading method, allowing for shortening of the long healing period. However, this may lead to formation of bone defects resulting from size difference between the implant and the extraction socket.[4]

Several grafting materials are used for peri-implant bone defects. Autogenous bone grafts have osteoinductive, osteoconductive and bone regenerative properties and the use of autogenous bone grafts is the gold standard for repairing bone defects owing to their biocompatibility and rapid revascularization. However, challenges associated with harvesting the autograft, requirement for a second surgical operation, inability to harvest sufficient volumes of autograft and complications such as postoperative pain limit routine use of autogenous bone grafts. Consequently, allografts with osteoinductive and osteoconductive capabilities have been introduced as an alternative to autogenous bone grafts.[5] In osteoconduction, graft material does not activate bone formation but rather serves as a scaffold or physical matrix for new bone growth that is perpetuated by the native bone. At this stage, some bone grafts are reabsorbed and finally disappear completely as new bone formation, but others are not reabsorbed and help healing of the defect by filling the spaces between graft fragments and newly formed bone.[6] Osteoinduction involves the stimulation of osteogenesis mechanism and owing to growth factors and signalling proteins they contain some graft materials exhibit osteoinductive property by stimulating mesenchymal stem cells in the recipient tissue to differentiate into osteoblasts.[7]

Recently, research on filling bone defects has focused on local or systemic substitutes (e.g., antibiotics or platelet-rich plasma) that could possibly promote effectiveness of graft materials in new bone formation.[4,8] Antibiotics have been used for many years in various fields of medicine. Numerous antimicrobials were used to achieve aforementioned purpose including tetracycline, tobramycin, cefalotin, vancomycin, nitrofurazone, rifamycin, povidone iodine and chlorhexidine.[9] More recent studies have increasingly examined the effects of rifamycin on promoting bone formation with overall successful results.[10,11] In a study on experimental animals, Carvalho *et al.*[10] reported that irrigation with rifamycin led to better bone formation in extraction sockets where fibrinolytic alveolitis was produced compared to control group. In one study by Tasdemir *et al.*[11] contaminated autologous onlay bone grafts harvested from experimental animals were decontaminated with rifamycin and reintroduced and histological examination showed earlier revascularization and osteogenesis in the decontamination group compared to control group.

Nigella sativa is an herbaceous annual plant that belongs to the *Ranunculaceae* family which grows from its seeds. The seeds of *N. sativa* are 2-3 mm triangular grains with an intense black color. *N. sativa* seeds contain water, ash, crude protein, crude fiber, and carbohydrates (68%) and a substantial amount of volatile oil (32%). The major active chemical component of the volatile oil is thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone).[12] Thymoquinone (TQ) was reported to have antihypertensive, hypoglycemic, antifungal, antibacterial, antiallergic as well as immunopotentiating activities.[13,14] TQ also has

anti-inflammatory and antioxidative properties and accelerates new bone formation.[15] Ozdemir *et al*.[16] evaluated the effectiveness of black cumin oil administered by orogastric route on alveolar bone resorption in rats with experimental periodontitis and reported much less alveolar bone loss in the TQ groups versus control group.

Considering these data, we believe that black cumin oil, when given by orogastric route, may accelerate bone formation and promote faster healing and thereby shorten the osseointegration time in the treatment of peri-implant defects using allografts. To our best knowledge, there are no similar studies in literature on treatment of peri-implant bone defects with both rifamycin and black cumin oil. Thus, the aim of the current study was to compare the effects of rifamycin locally administered at the time of grafting and systemic black cumin oil on osseointegration in implants with coronal defects versus control group histomorphometrically.

MATERIAL AND METHODS

The experimental animal group consisted of 24 male New Zealand white rabbits *(Oryctolagus cuniculus L.)* of 6 months of age and weighing 2.5 to 3.5 kg. Rabbits were divided into 3 equal groups to be applied graft (GF, n=8), graft + rifamycin (GR, n=8) and graft + black cumin oil via orogastric route (GB, n=8). Food and water were supplied to the animals without any restriction. Standard rabbit feed was used as forage. Standard conditions (22-24ºC, 55-70% humidity, 1 ATM) were applied for the rabbits in the animal room. During the study, experimental animals were placed into stainless steel cages (50 × 80 × 50 cm) such that there was one animal in each cage. After their transfer to the laboratory, all animals were subjected to one month of treatment and monitoring to ensure optimal health conditions and protection from infections and to allow for adaptation of animals to their new habitat prior to surgical procedures.

Approval for the conduct of the study was obtained from the Animal Experimentation Ethics Committee of Sivas Cumhuriyet University before initiation of the study (date 25.12.2014 and no. 63) and principles of ethical treatment of experimental animals set forth in the Article 13 of the Code of Ethics of Sivas Cumhuriyet University were followed.

Supported by Sivas Cumhuriyet University Scientific Research Projects Unit (CUBAP), the present research study (code no. DIS-158) was conducted at the Experimental Animal Laboratory of Sivas Cumhuriyet University and the Research Laboratory of Erciyes University, Faculty of Dentistry, Department of Oral and Maxillofacial Surgery.

Surgical Procedure

All operations were performed on rabbits under general anesthesia induced by Xylazine (10-20 mg/kg) (Rompun 2%, Bayer, Istanbul, Turkey) and Ketamine HCl (50 mg/kg) (Ketalar, Eczacıbaşı-Warner Lambert, Istanbul, Turkey). Experimental side of the tibia was shaved and cleaned with povidone-iodine. Surgical area was prepared by applying a sterile film on the tibia sections covered with a sterile surgical drape. A skin incision of 2-3 cm was made from the medial proximal metaphysis of the tibia towards the distal. Subcutaneous and muscle layers were crossed by blunt dissection and the surface of the tibia bone was reached. By use of a trephine drill, bony defects (9 mm diameter, 4 mm depth) were created under saline solution irrigation. (Figure 1)

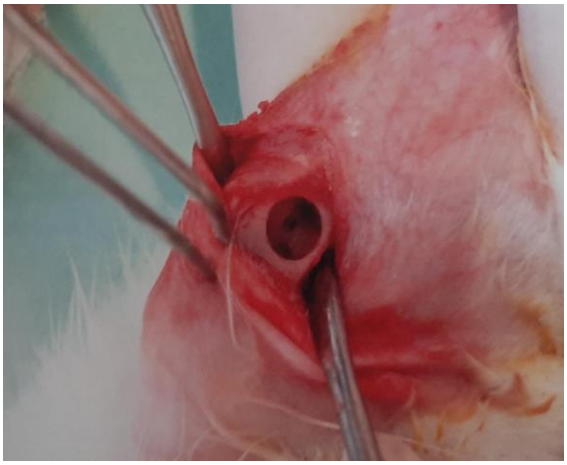


Figure 1. Bone defect created on tibia with trepine drill.

Implant beds (3 mm diameter, 6 mm depth) were prepared in the center of each defect according to the manual of the implant system. (Figure 2)

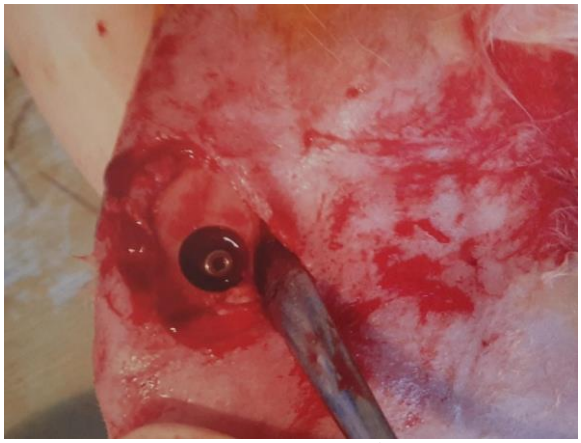


Figure 2. Dental implant placed in bone defect

After drilling, the implant beds were rinsed with saline solution, and implants (Touareg-NP, 3.0x10 mm; Adin Dental Implant System, Afula, Israel) were inserted with primary stability (6-mm depth). The upper part of the implant was free (4 mm depth) at the center of the created defects. Healing caps were screwed on the exposed portion of the implants. In all groups, an allograft (Maxxeus Community Tissue Services, Ohio, USA) was used for the coronal part. GF group received graft alone, while GR group received graft + rifamycin (RİF, Koçak Farma, Istanbul, Turkey). Black cumin oil (2 mg/kg daily) was administered by gastric feeding until the animals were killed on day 28.

After the surgery, muscle and subcutaneous fascia were sutured in the elevated epidermal flaps. The tissues were sutured in 2 layers using degradable sutures (Pegelak, poly [glycolide-co-lactide]; Dogsan, Trabzon, Turkey). 50 mg/kg Ceftriaxone (Cephaxon; Toprak, Istanbul, Turkey) IM and Carprofen, 4 mg/kg SC (Rimadyl; Pfizer, New York, NY) were given once daily for three days to experimental animals postoperatively. Animals were sacrificed at 4 weeks after implantation. The implants with surrounding bones were dissected, and any signs of unusual healing were documented.

Preparation of Histological Sections and Histomorphometric Examination

Bones surrounding the implants were removed along with 1 cm-wide intact bone and then stored in 4% buffered formalin solution for 24 hours. Subsequently, specimens were dehydrated with 60%, 80%, 96%, and 100% ethanol in alcohol tanks for one day. Dehydration of specimens in an ascending series of ethanol rinses was followed by embedding in a methyl methacrylate-based resin (Technovit 7200 VLC; Kulzer and Co, Wehrheim, Germany). These blocks were subjected to light polymerization over 8 hours at 40°C under a wavelenGBh of 450 nm and then adhered on plexiglass slides under vacuum.

300-350 μm thick sections were obtained from the specimens using a high-precision diamond disk saw (Exakt 300 CL, Exakt Apparatebau, Germany). Then, sections were ground down to 40 μm using abrasive papers from a microgrinding system (Exakt 400 CS, Exakt Apparatebau, Norderstedt, Germany), and four sections were prepared from each specimen and stained with toluidine blue.

For histomorphometric evaluation, all sections were visualized by a light microscope (Olympus® CX41, Tokyo, Japan) and a digital camera mounted on the microscope (Olympus® DP 25, Tokyo, Japan). Images were downloaded to a personal computer and analyzed histomorphometrically using a Bioquant Osteo II image analysis software (Bioquant Image Analysis Corp., Nashville, TN).

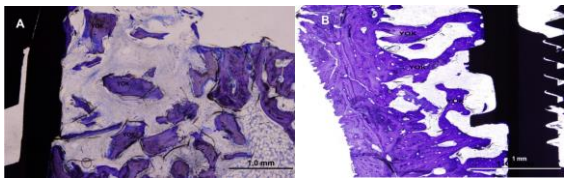
The percentage of bone-to-implant contact (BIC) was calculated from the implant shoulder to the end of the gap (4 mm) and new bone formation on each section was expressed as a percentage of bone volume.

Statistical Analysis

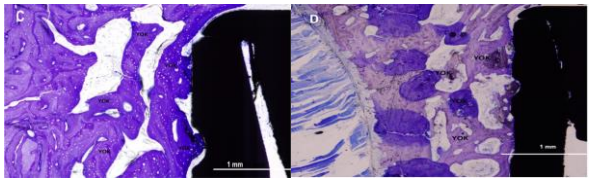
The data of the study were loaded into SPSS program (version 14.0 for Windows) and normal distribution was determined by Kolmogorov-Smirnov test in the evaluation of the data. The differences between the groups were evaluated by parametric test, ANOVA variance analysis test. Significant differences were determined according to test results. Tukey test, one of the post-hoc tests, was used to determine the cause of this difference. The data were expressed as arithmetic mean ± standard deviation in the tables and p value was taken as 0.05.

RESULTS

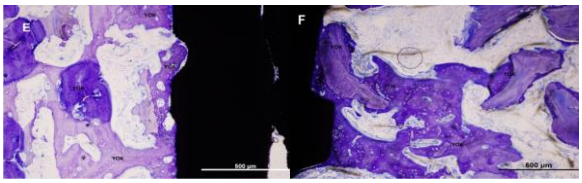
Rabbits had no weight loss after surgery. Following a 28-day period of non-problematic healing, successful osseointegration was achieved in all implants. New bone formation was present in peri-implants in all groups. (Figure 3)



A-B= Group receiving only allograft after 28 days of surgery.



C-D= Group receiving allograft mixed with rifamycin after 28 days of surgery.



E-F= Group receiving allograft and orogastric feeding with black cumin oil for 28 days. Toluidine blue staining shows new bone formation around dental implant.

Figure 3. Histologic evaluation of experimental defect area (toluidine blue stain, with scale bar equal to 1 mm and 500 μm)

Bone-to-implant contact was 46.57%±3.59% in the graft (GF), 67.12% ± 3.64% in the graft + rifamycin (GR) and 55.62%±4.37% in the graft + black cumin oil (GB) groups. The percentage of new bone formation at the defect area was 34.71%±4.11% in the graft, 55.37% ± 4.89% in the graft + rifamycin, and 45.75% ± 3.69% in the graft + black cumin oil groups (Table 1).

Table 1. Percentage of direct bone-to-implant and new bone formation at the defect area.

Groups	BIC*	New Bone Formation
Graft (GF), %	46.57 ± 3.59	34.71 ± 4.11
Graft+Rifamycin (GR), %	67.12 ± 3.64	55.37 ± 4.89
Graft+ Thymoquinone (GT), %	55.62 ± 4.37	45.75 ± 3.69

Note: Data are presented as mean (standard deviation).
Abbreviations: BIC, bone-to-implant contact.
Statistically significant difference ($p < 0.05$) between graft (GC) and graft plus rifamycin (GR) for BIC.
Statistically significant difference ($p < 0.05$) between graft (GC) and graft plus black cumin oil (GT) for BIC.
Statistically significant difference ($p <0.05$) between graft plus rifamycin (GR) and graft plus black cumin oil (GT) for BIC.
Statistically significant difference ($p < 0.05$) between graft (GC) and graft plus rifamycin (GR) for new bone formation.
Statistically significant difference ($p <0.05$) between graft (GC) and graft plus black cumin oil (GT) for new bone formation.
Statistically significant difference ($p < 0.05$) between graft plus rifamycin (GR) and graft plus black cumin oil (GT) for new bone formation.

The percent (%) difference in new bone formation between groups was significant ($p<0.05$), with greatest new bone formation in the group treated with rifamycin (GR) and least new bone formation in the group receiving graft alone (GF). Percent BIC also significantly different between groups ($p<0.05$) with the highest percentage observed in the group receiving rifamycin, followed by black cumin oil (GB) and control (GF) groups ($p<0.05$) (Table 2).

Table 2. Statistical significance of groups

	GF-GR	GF-GB	GR-GB
BIC	p<0.05	p<0.05	p<0.05
NBF	p<0.05	p<0.05	p<0.05

BIC= Bone-to-implant contact
NBF= New bone formation
GF= Graft
GR= Graft+Rifamycin
GB= Graft+ Orogastric black cumin oil

DISCUSSION

Many conventional methods used for rehabilitation of edentulous patients have been replaced by therapeutic interventions involving dental implants which are now increasingly popular.[17] The major factor that influences the overall success of a dental implant is to achieve and maintain osseointegration.[18]

In human studies, small gaps (less than 2 mm) around the implant were reported to heal spontaneously without the need to use allograft, xenograft or membranes.[19,20] A critical-size defect is an intraosseous wound in a particular bone and species of animal that will not heal spontaneously morphologically and functionally during the lifetime of the animal. During healing, large bone defects are filled with fibrous connective tissue rather than bone tissue.[21] In a relevant study, 8-mm defect was produced, and healing was assessed histologically. With bone healing, the defect area reduced in size to 5-mm but healed by formation of fibrous connective tissue at the core of the defect.[22] However, in the present study, osseointegration was achieved in our experimental 9-mm defects that were healed with allografts without formation of fibrous tissue.

Beneficial results were reported by several studies in the reconstruction of peri-implant bone defects with grafting.[23,24] Additionally, there are some studies which used combinations of graft materials with various agents with the aim to enhance osteogenesis.[8] Differential results were reported by studies that combined rifamycin with allografts. Witso *et al*.[25] showed that allograft in combination with rifamycin or other antibiotics reduced bacterial contamination and promoted osteogenesis. In a separate study, Simsek *et al*.[8] produced 9-mm bone defects in rabbit tibias and treated control group with allograft plus saline solution and experimental groups with allograft plus platelet-rich fibrin (PRF) or allograft + rifamycin. As result, the group treated with rifamycin plus allograft showed inferior results compared to PRF group, but significantly greater BIC and new bone formation compared to control group. Contrastingly, Kaya *et al*.[26] examined osteogenesis following administration of rifamycin in combination with allogeneic, alloplastic, and heterogeneous grafts to bone defects created in rat tibias. They reported that rifamycin could reduce new bone formation when combined with allograft in bone defects. However, our current findings support prior successful results obtained with rifamycin.

Culturing studies are available in literature that investigated the effects of rifamycin on human osteoblast-like cells. In these studies, rifamycin was shown to have toxic or inhibitory effects on osteoblast-like cells when used at concentrations exceeding a certain threshold.[27,28,29]

While black cumin oil is well known for its various beneficial effects against several diseases[14], its detailed effects on bone formation were examined in few studies. Wirries *et al*.[30] reported a significant impact of TQ on proliferation, differentiation, and mineralization of cultured osteoblast cells. Kara *et al*.[31] looked at the effects of TQ systemically administered during rapid maxillary expansion procedure on the bone formation and reported a considerable increase in new bone formation in the group treated with TQ compared with other groups.

A healing period of 28 days was used in the present study for recovery of peri-implant bone defects.[8] Although the current study demonstrated that rifamycin and black cumin oil can increase and/or accelerate bone formation, it was not possible to establish all potential effects in such a short period of time. Thus, further long-term studies are needed to examine the effects of rifamycin and black cumin oil on bone healing.

The current study supports the hypothesis that rifamycin and black cumin oil might promote and accelerate bone formation in peri-implant bone defects when used in combination with allografts.

CONCLUSION

The use of various materials in conjunction with allograft can speed up new bone formation and bone-implant contact. Especially in this study conducted to investigate the materials that increase the success of grafting with immediate loading, it was observed that the use of rifamycin and black cumin oil had a positive effect in the early period of osseointegration. Rifamycin gave better results than other materials. Long-term effects of these materials should be examined in further studies.

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CONFLICTS OF INTEREST STATEMENT

The authors report no conflicts of interest.

Koronal Defektli Dental İmplantların Osseointegrasyon ve Kemik Rejenerasyonunun Allogreft ile Birlikte Farklı Rejenerasyon Tekniklerinin Kullanımı

ÖZ

Amaç: *Allogreft ile kombine edilen farklı materyallerin kemik-implant teması ve koronal defektleri olan dental implantlarda yeni oluşan kemik oluşumu üzerindeki farklı etkilerini histomorfometrik olarak değerlendirmektir.* ***Gereç ve Yöntemler:*** *Çalışma 24 erkek Yeni Zelanda beyaz tavşanı üzerinde gerçekleştirildi. Dental implantlar (3.0×10 mm), tavşanların tibial kemiklerinde oluşturulan defektlerin merkezine (9 mm çap, 4 mm derinlik) yerleştirildi. İmplantların koronal kısımlarına 28 gün boyunca greft (GF, n=8), greft+rifamisin (GR, n=8) ve greft+orogastrik yoldan çörekotu yağı (GB, n=8) uygulandı. Toluidin mavisi ile boyanmış kesitler üzerinde dekalsifiye edimemiş histomorfometrik analizler yapılmıştır.* ***Bulgular:*** *Kemik-implant teması greftte %46.57±%3,59, greft+rifamisin %67,12±%3,64 ve greft+ çörekotu yağı gruplarında %55,62±%4,37 idi. Defekt alanındaki yeni kemik oluşumu yüzdesi greftte %34,71±%4,11, greft + rifamisinde %55,37±%4,89 ve greft+ çörekotu yağı gruplarında %45,75±%3,69 idi. Yeni kemik oluşumu ve kemik-implant teması açısından greft+rifamisin ve greft+çörekotu yağı grupları greft grubundan anlamlı olarak farklıydı. Greft+rifamisin ve greft+çörekotu yağı grupları arasındaki farklar da istatistiksel olarak anlamlıydı.* ***Sonuçlar:*** *Allogreft+ rifamisin ve orogastrik çörekotu yağının koronal defektli bölgelerde kemik iyileşmesi üzerinde olumlu etkileri olduğu bulunmuştur. Rifamisin, çörekotu yağına kıyasla kemik-implant teması ve yeni kemik oluşumu üzerinde önemli ölçüde daha olumlu etkiler gösterdi.* ***Anahtar Kelimeler:*** *Allogreft, rifamisin, çörekotu yağı, histomorfometri, dental implant.*

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