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Doi: 10.7126/cumudj. 475498 *RESEARCH ARTICLES*

HISTOMORPHOMETRIC ASSESSMENT OF THE IMPACT OF BOVINE DEMINERALIZED BONE GRAFT ON BONE HEALING VERSUS AUTOGENOUS, ALLOGENEIC AND SYNTHETIC GRAFTS IN EXPERIMENTALLY- INDUCED CRITICAL SIZE BONE DEFECTS IN RATS

Ratlarda Deneysel Olarak Oluşturulan Kritik Boyutlu Kemik Defektlerine Uygulanan Sığır Kaynaklı Deminarelize Kemik Greftininin Kemik Iyiyleşmesine Olan Etkisinin Otojen, Allojenik ve Sentetik Greftlerle Karşılaştırılmasının Histomorfometrik Olarak İncelenmesi

T. Peyami HOCAOĞLU[1], Sadık GENÇOĞLAN[2], Murat ARSLAN[1],

M.Emre BENLİDAYI[3], Mehmet KÜRKÇÜ[3]

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ABSTRACT

Objectives: Bone tissue has the ability to heal itself (regeneration) and may restore its morphology and function when injured. However, healing may be limited in the case of large wounds. 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. Autogenous bone grafts have been regarded as "gold standard" for treatment of critical-size bone defects. Known drawbacks of autogenous bone graft have led to research efforts focusing on different graft materials and resulted in several alternative substitutes including xenografts, allografts and synthetic graft materials.

The aim of the present study was to perform a histomorphometric study to investigate the effect of bovine demineralized bone graft on bone healing in comparison to autogenous, allogeneic and synthetic graft materials when applied into critical size bone defects with a diameter of 5 mm.

Materials and Methods: Experimental animals were divided into 4 groups, each having 8 rats. In the control group, a mandibular defect was created and then filled with a bovine graft (Integros Bone Plus XS Adana/Turkey). In the experimental groups, autogenous bone was reinserted into the critical-size defect which was created using a trephine bur in Group I (autogenous group) and Group II received a human graft (Korea Bone Bank (KBB) Gasandong Keumcheongu Seoul/South Korea) to fill the critical-size defect. For Group III, a synthetic bone graft β-tricalcium phosphate (Cerasorb North Carolina/USA) was applied on the critical-size bone defect. Specimens were obtained for histomorphometric examination and rats were sacrificed on day 28.

Results: Histomorphometric examination performed on day 28 to evaluate the relative effects of different graft materials on new bone formation showed no significant difference in the volume of newly formed bone between groups receiving autogenous bone graft, allograft and bovine xenograft but a significant difference was observed versus synthetic bone graft group.

Conclusion: While autogenous bone graft is currently regarded as the gold standard for bone regeneration, the difficulties in harvesting and application of autografts limit their use. Our results demonstrate that bovine bone graft may be used as a safe and effective alternative to autogenous bone graft.

Keywords: dental graft, autogenous graft, allogeneic graft, xenograft, bone regeneration

ÖZ

Amaç: Kemik dokusu iyileşme özelliğine (rejenerasyon) sahiptir ve yaralanan kemik dokusu şekil ve fonksiyonunu yeniden kazanabilmektedir. Fakat yaralanmanın boyutu büyük olduğu zaman iyileşme sınırlı kalabilmektedir. Kritik boyutlu kemik defekti; kemik dokusunda, canlının yaşamı boyunca, şekil ve fonksiyon olarak, kendiliğinden tamamen iyileşmesinin mümkün olmayacağı boyuttaki defekt anlamına gelir. Kritik kemik defektlerinde tedavi için otojen greft uygulaması altın standart olarak kabul edilir. Otojen kemik greftinin bazı dezavantajları nedeniyle araştırmacılar çalışmalarını farklı greft materyalleri üzerinde yoğunlaştırmışlardır. Bu çalışmalar neticesinde Ksenogreft, Allogreft ve sentetik greft materyalleri gibi seçenekler ortaya çıkmıştır.

Çalışmamızın amacı 5mm çapında kritik boyutlu kemik defektlerinde sığır kaynaklı demineralize kemik grefti uygulamasının kemik iyileşmesine etkisi ile aynı çaptaki defektlere otojenik, allojenik ve sentetik greft materyali uygulandığı zaman elde edilen iyileşmelerin histomorfometrik olarak incelenmesidir.

Gereç ve Yöntemler: Deney hayvanları her grup 8 deney hayvanından oluşan 4 gruba ayrıldı. Kontrol grubunda mandibulada defekt oluşturulduktan sonra defekt sığır kaynaklı kemik grefti (Integros Bone Plus XS Adana/Türkiye) ile dolduruldu. Daha sonraki deney gruplarında; I. grupta oluşturulan kritik boyutlu defekte trefin frezle çıkartılan otojen kemik tekrar konuldu. II. grupta oluşturulan kritik boyutlu defekte insan kaynaklı kemik grefti (Korea Bone Bank (KBB) Gasandong Keumcheongu Seoul/Korea) uygulandı. III. grupta oluşturulan kritik boyutlu kemik defektine ise sentetik kemik grefti grubunda yer alan β-trikalsiyum fosfat (Cerasorb North Caroline/USA) uygulandı. 28 gün sonra ratlar öldürüldü.Her grup sakrifiye edilerek histomorfometrik incelemeye alındı.

Bulgular: Farklı greft materyallerinin 28. günde yeni kemik oluşumuna olan etkisinin histomorfometrik olarak incelendiğinde otojen kemik grefti, allogreft ve sığır kaynaklı kemik grefti uygulanan gruplar arasında yeni oluşan kemik hacmi bakımından anlamlı bir fark bulunmazken, sentetik kemik grefti uygulanan grupla aralarındaki fark anlamlı bulunmuştur.

Sonuç: Otojen kemik grefti günümüzde hala altın standart olarak kabul edilmesine rağmen, elde edilmesi ve uygulanmasındaki zorluklar nedeniyle çalışmamızda kullandığımız sığır kaynaklı kemik greftinin otojen kemik greftine alternatif olarak güvenilir ve etkili biçimde kullanılabileceği belirlenmiştir.

Anahtar kelimeler: dental greft,otojen greft,allojenik greft,ksenogreft,kemik rejenerasyonu,

[1] Cumhuriyet University, Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, Sivas, Turkey
[2] Private Practice, İstanbul, Turkey
[3] Çukurova University, Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, Adana, Turkey

INTRODUCTION

Bone tissue has the ability to regenerate and may restore its morphology and function when injured.[1] However, healing may be limited in the case of large wounds. Bone healing after a bone defect varies in relation to the size of the defect and the animal species.

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.[2,3] During healing, critical-size bone defects are filled with fibrous connective tissue rather than bone tissue.[4-6] While oral and maxillofacial surgeries depend primarily on bone regeneration for healing, critical-size bone defects do not heal spontaneously and several alternative materials are used to trigger healing process. Ideally, the best method would be filling of the defect with another bone tissue having similar size, shape and antigenic properties with the original bone.[7,8]

Repair and restoration of bone defects date far back in history. In the 30th century B.C., surgeons have used gold or silver plates as graft material for reconstruction purposes. Later on, especially in the 20th century, studies have extensively focused on graft materials and allografts, xenografts and alloplastic materials have been largely used as an alternative to autogenous grafts.[9]

Autogenous bone grafts derive from the individuals themselves and in terms of osteogenesis, autogenous bone grafts are the only most effective graft material among all bone grafts. Autogenous bone graft combines osteoconductive, osteoinductive, and osteogenic characteristics. Autogenous bone grafts have been regarded as "gold standard" for repairing bone defects.[10-14] The advantages conferred by autogenous bone grafts include direct osteogenesis via living osteoblasts and osteoprogenitor cells in the bone marrow, osteoconduction through collagen matrix and osteoinduction by BMPs (bone morphogenetic proteins).[15] On the other hand, autogenous graft use has certain important limitations including requirement for a repeat surgical procedure for bone harvesting which adds to discomfort of the patient in the postoperative period and creation of a new defect in the donor site poses risk of infection or morbidity.

Allografts are bone grafts that are harvested from another individual of the same species with a different genotype and most commonly used as an alternative to autogenous grafts. Allografts do not have the disadvantages of the autogenous grafts such as donor site injury and limited quantity of bone available for harvesting. Allografts are obtained from living humans or cadavers and stored in bone banks. Allograft use has been long reported in the literature for defects involving long bones and posttraumatic bone defects.[16-18] Allografts are available in different shapes.[19-22]

Donor and recipient species are different in heterogeneous bone grafts. Bovine, porcine, coral and equine bones are mostly used as a source of graft. Natural hydroxyapatite is synthesized from the calcium carbonate skeleton of the coral and the resulting material is highly biocompatible. In recent years, bovine xenografts have been the major focus of studies.[23] Bovine bone graft is a biocompatible and osteoconductive material.[24,25] Concerns have been raised recently over the risk of prion infection through bovine graft materials causing Bovine spongiform encephalopathy (BSE) in the cattle and Creutzfeldt-Jacob disease in humans. Heterogeneous bone grafts are associated with these risks.

Recently, biocompatible synthetic materials have been manufactured to overcome the limitations of allografts and xenografts.[26] Synthetic materials are commercially available with a wide range of products.

Combinations of different grafts have been used in research studies for treatment of defects that maxillofacial surgeons deal with. Thus, synthetic materials can be used in combination

with autogenous and/or allogeneic bone grafts with osteoinductive properties.[27-29]

The advantages of alloplastic materials include no risk of cross-infection, ease of access, sterilizability, biocompatibility and easy storage. However, they do not have osteogenic and osteoinductive features, which constitute a major disadvantage.[30]

The aim of the present study was to examine the potential of aforementioned diffirent graft materials in inducing bone formation in critical-size bone defects by histomorphometric means.

MATERIALS AND METHODS

Approval for the conduct of the study was obtained from Cumhuriyet University Ethics Committee for Animal Experimentation before initiation of the study (Approval No. 170, 04/06/2009). Throughout the study, the 13th item of Adherence to Ethical Principles of Cumhuriyet University Ethics Committee Directive was followed.

The study was conducted on 32 adult Wistar albino rats with a mean age of 12 weeks and approximate weight of 250-300 grams. All rats were examined and confirmed to be in good health by a veterinarian. Study rats were supplied by Cumhuriyet University Experimental Animals Laboratory.

Stratification of Experimental Animals

Experimental animals were divided into 4 groups, each having 8 rats. In the control group, a mandibular defect was created and then filled with a bovine graft (Integros Bone Plus XS Adana/Turkey). In the experimental groups, a critical-size defect was created using a trephine bur and autogenous bone was reinserted in the area in Group I (autogenous group) and Group II received a human graft (Korea Bone Bank (KBB) Gasandong Keumcheongu Seoul/South Korea) to fill the critical-size defect. KBB was chosen as the supplier of the human graft because it has been certified by both KFDA (Korean Food and Drug Administration) and FDA (US Food and Drug Administration) and their grafts were used in many studies in Korea and globally due to its established safety and effectiveness. For Group III, a synthetic bone graft β-tricalcium phosphate (Cerasorb North Carolina/USA) was used to fill the critical-size bone defect. We used Cerasorb because of its proven safety and efficacy demonstrated through several scientific studies.[31]

Surgical Technique

Anesthesia of the experimental animals was induced by intramuscular injections of 3 mg/kg xylazine (Rompun 2%, Bayer, İstanbul, Turkey) and 90 mg/kg Ketamine HCl (Ketalar, Eczacıbaşı-Warner Lambert, Istanbul, Turkey). Rats were sacrificed on day 28. Adequate depth of anesthesia was confirmed by observation of the loss of pupillary reflex and the skin overlying the angulus mandibularis area was shaved bilaterally (Figure1).

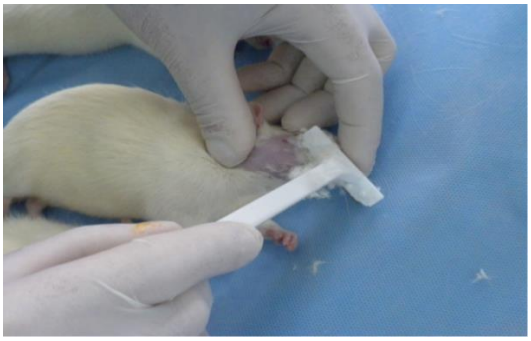

Figure 1. Angulus mandibularis area was shaved bilaterally)

Betadine® was used to stain and disinfect the perimandibular area (Figure 2).

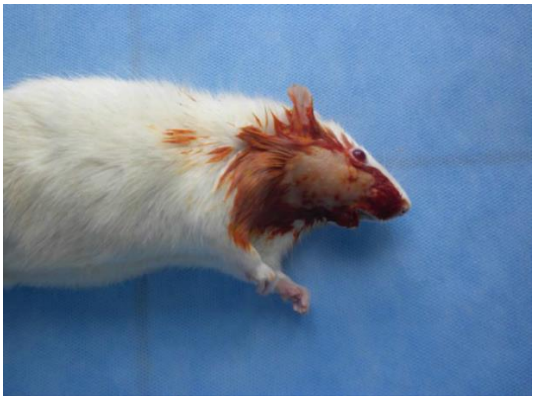

Figure 2. Disinfect the perimandibular area

An incision of 1 cm was made at the angulus mandibularis area 1 cm below the mandibular basis to remove skin, subcutaneous tissue and periosteum. Skin flap was raised to expose the bone surface (Figure3).

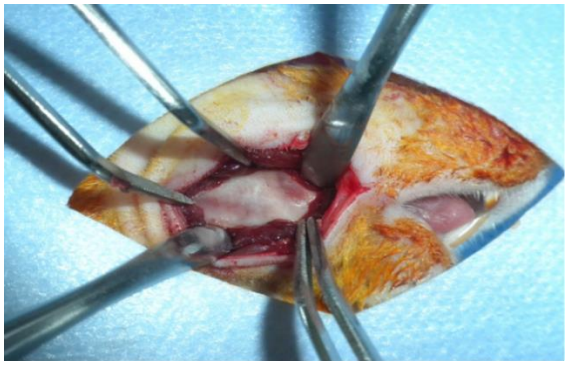

Figure 3. Skin flap was raised to expose the bone surface

A standard critical-size bicortical bone fragment with a diameter of 5 mm was removed with trephine bur under irrigation (Figure 4).

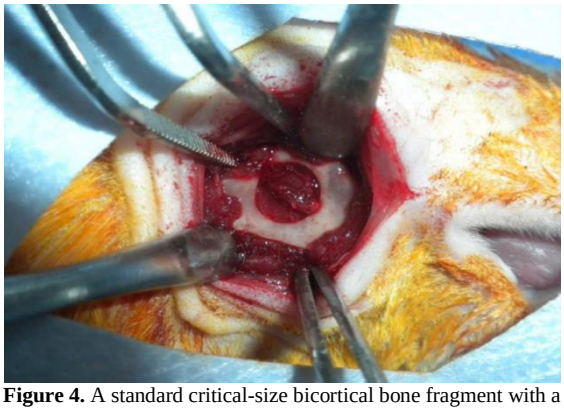

Figure 4. A standard critical-size bicortical bone fragment with a diameter of 5 mm was removed with trephine bur

Subsequently, critical-size bone defects were filled with human bone graft (Figure 5), bovine bone graft and synthetic biomaterial.

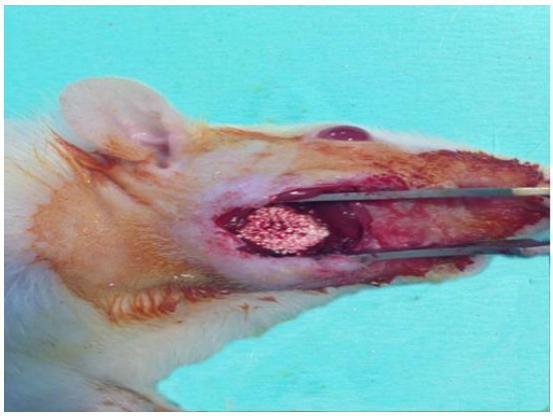

Figure 5. Critical-size bone defects were filled with human bone graft

Postoperative care of study rats and termination of experiment

All rats were given Carprofen 4mg/kg (Rimadyl, Pfizer) as analgesic and Ceftriaxone 25 mg/kg (Rocephin, Roche) as antibacterial agent postoperatively by intramuscular route for 5 days. Rats were sacrificed on the last study day (day 28) using 200 mg/kg sodium pentobarbital (Pentothal, Abbott, USA). Rat mandibles with the defect area and surrounding soft tissue were removed by dissection and placed in a 10% formalin solution.

Histomorphometric Method

Undecalcified sections containing grafts and surrounding bone tissue were prepared by the method described by Donath and Breuner (1982).

All sections were used for histomorphometric examination. Digital images of the sections were obtained under 4 x magnification using a digital camera (Olympus® DP 70, Tokyo, Japan) mounted on the light microscope (Olympus® BX50, Tokyo, Japan). Then, images were transferred to a personal computer. Histomorphometric analyses were performed using WinTas image analysis software (WinTAS Trabecular Analyze System, version 1.2.9).

Statistical analyses

Study data were uploaded on SPSS (Version 14.0 for Windows) software. Kolmogorov-Smirnov tests,analysis of variance (ANOVA) and Tukey's test were used for data analyses. Data expressed as arithmetic mean ± standard deviation were presented in tabulated form. Type I error level was set at 0.05.

RESULTS

In each section, newly formed bone volume and unossified graft content were examined for each group histologically. Significant differences were observed when newly formed bone volumes were compared between groups ($p<0.05$). Pair-wise comparison of values

between groups revealed a significant difference between the autogenous group and Cerasorb group ($p<0.05$) but other groups showed non-significant differences ($p>0.05$) (Figure 6)

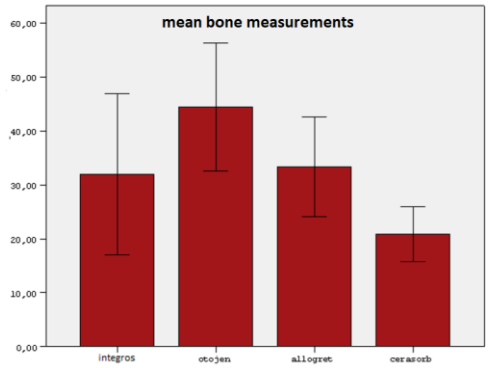

Figure 6. Results for mean bone measurements

Comparison of unossified graft content showed significant differences between groups ($p<0.05$). Pair-wise comparison of values between groups demonstrated a significant difference between Integros group and the autogenous group, between Integros group and the allograft group and between the autogenous group and Cerasorb group ($p<0.05$); there were no significant differences between other groups ($p>0.05$). (Figure 7)

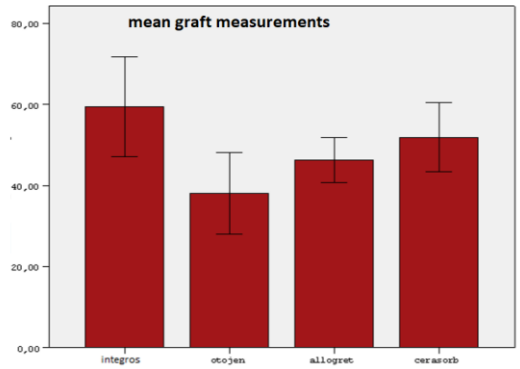

Figure 7. Results for mean graft measurements

Y axis=Mean graft volumes

X axis= Integros, Autogenous, Allograft, Cerasorb

Table 1.
Comparison of measurements for all groups

Groups	New Bone Volume $\bar{X}$±Ss	Graft Volume $\bar{X}$±Ss
İntegros	31,94 ± 14,94	59,45 ± 12,35
Otojen	44,38 ± 11,87	38,09 ± 10,12
Allogreft	33,28 ± 9,26	46,30 ± 5,51
Cerasorb	20,82 ± 5,12	51,87 ± 8,50
Results	F = 6,23 p = 0,002	F = 7,29 p = 0,001

$\bar{X}$: mean volue , Ss:Standart deviation Variance analysis : Tukeys test $p<0,05$

DISCUSSION

As with other musculoskeletal areas, bone defects in the maxillofacial area may result from infectious, degenerative, cystic, post-traumatic or neoplastic lesions and the field of maxillofacial surgery is primarily engaged in the repair of such defects. As such, investigators have put so much effort in exploring ways to contribute to the repair of these defects. Use of bone grafts has been the major focus of these studies.[32]

Materials used in bone grafting are classified according to their effect on bone healing. Osteogenesis is the process of new bone formation by cells that have the ability to produce new bone tissue. Autogenous bone is the only graft material with osteogenic properties.[33] Osteoinduction is a mechanism of bone formation whereby undifferentiated mesenchymal cells within the tissue are stimulated to develop into a bone-forming cell lineage called osteoblasts. Osteoconduction occurs when the bone graft material serves as a scaffold for new bone growth that is perpetuated by the native bone-forming cells.[34] An ideal bone graft material should have both osteoinductive and osteoconductive features.[35,36]

Autogenous bone grafts are considered as the gold standard and widely used in maxillofacial surgery for bone regeneration.[37-40] However, autogenous grafts have several drawbacks including the need for repeat surgery for a separate incision, its traumatic nature, and the risks involved such as high postoperative

morbidity, risk of infection and postoperative resorption.[41-45]

In a 2008 study, Mokbel *et al.* evaluated the healing patterns of critical-size bone defects in 6 groups of rats treated with deproteinized bovine xenograft, bovine xenograft covered with a resorbable membrane, decalcified freeze-dried bone allograft, composite bone substitute made of bovine xenograft and collagen, autogenous bone graft and no grafting (control group) respectively. Histomorphometric examination at 2 months showed the superiority of autogenous bone graft to other substitutes, achieving the highest mean bone formation of 2.97 mm^2.[46]

Pripatnanont *et al.* (2009) assessed new bone formation in bicortical skull defects in rabbits following application of autogenous bone, deproteinized bovine bone and different proportions of both. Histomorphometric examination at 2 months showed the greatest bone formation in the group receiving autogenous bone graft with a 30.223% of new bone. [47]

Shand *et al.* (2002) induced critical-size calvarial defects in rabbits and investigated the incorporation of allogeneic and autogenous bone grafts into these defects. Rabbits were sacrificed at 9 and 12 months postoperatively and the specimens were examined histomorphometrically. They reported that complete healing was achieved in the bone defects undergoing allograft, with no significant difference in comparison to the bone defects filled with autogenous bone.[48] Athanasiou *et al.* (2010) evaluated the differential histological properties of various bone graft substitutes when applied to critical-size bone defects and their effects on bone healing. They used 90 New Zealand rabbits which were divided into 6 groups. Critical-size (4.5 mm) bone defects were created in each group of rabbits and filled with various grafts including autogenous bone graft, human bone graft, bovine cancellous bone xenograft, calcium phosphate hydroxyapatite substitute and calcium sulfate substitute. The control group (group 6) underwent no filling. Rabbits were sacrificed at 1, 3 and 6 months after implantation and tissue samples from the grafted areas were examined histologically. The highest histological grades were obtained with the use of autogenous bone graft, the second best being bovine xenograft. Other bone graft materials achieved nearly identical healing. They concluded that apart from the autogenous bone graft, bovine xenograft was also associated with better biological response than other bone graft materials.[49]

Similarly, in the present study, the samples were examined histomorphometrically with the aim to investigate changes at the cellular level. Our histomorphometric findings showed no significant difference in newly formed bone volume between control group, autogenous bone graft and allogeneic bone graft groups but a significant difference was observed between groups undergoing autogenous bone graft and synthetic bone graft. Consistent with the previously published studies, our results suggest that bovine xenograft is a safe and effective grafting material for critical size bone defects as shown in the control group receiving bovine bone graft in this study. Absence of significant differences between control group and autogenous and allogeneic bone graft groups as observed in the current study suggests that this graft material which is manufactured in Turkey may be used as a good alternative to other graft substitutes in maxillofacial surgery.

CONCLUSION

While autogenous bone graft is currently regarded as the gold standard for bone regeneration, the difficulties in harvesting and application of autografts limit their use. Our results demonstrate that bovine bone graft may be used as a safe and effective alternative to autogenous bone graft. The only recognized disadvantage of the bovine bone graft is their long resorption time. However, they can be

safely used over a long period of time for critical-size bone defects.

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Corresponding Author

Turgay Peyami HOCAOĞLU

Cumhuriyet University

Faculty of Dentistry

Department of Oral and Maxillofacial Surgery

Sivas, Turkey

Phone : +90 346 219 10 10-2698

Fax : +90 346 219 12 37

E-mail : tphocaoglu@hotmail.com