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ARAŞTIRMA / RESEARCH

Effects of dentin graft on bone mineral density of newly formed bone: an experimental animal study

Dentin greftinin yeni oluşan kemikteki kemik mineral yoğunluğu üzerindeki etkileri: deneysel bir hayvan çalışması

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Abstract

Purpose: The aim of this study was to evaluate the bone mineral density of dentin used as bone graft material in bone defects.

Materials and Methods: Sixteen male New Zealand white rabbits were used. Two critical-size calvarial bone defects were created in each rabbit and filled with different types of grafts. The bone defects were divided into four groups. The defects in group A1 were filled with processed dentin from human teeth; group A2 was filled with processed dentin from rabbit teeth; group B1 was filled with bovine bone (Bio-Oss), and the defects in group B2 were left empty. Bone mineral density was evaluated using dual-energy X-ray absorptiometry at the 4th and 12th weeks. **Results:** The bone mineral density values at the 4th week were not statistically different among groups A1, A2 and B1. However, the bone mineral density values at the 12th weeks were significantly higher for group B1 than the other groups. Histopathologic evaluations showed better bone-healing for group B1 than group A1 and A2.

Conclusion: Dentin grafts and bovine bone graft have similar outcomes according to the bone mineral density values at the early term of bone-healing (4 weeks). However, in the late term of bone-healing (12 weeks), bone mineral density values of bovine bone were better than the other groups.

Key words: Dentin, bone mineral density, DEXA, bone graft.

Öz

Amaç: Bu çalışmanın amacı, kemik defektlerinde kemik grefti materyali olarak kullanılan dentinin kemik mineral yoğunluğunu değerlendirmektir.

Gereç ve Yöntem: Çalışmada onaltı erkek Yeni Zelanda beyaz tavşanı kullanıldı. Her bir tavşanın kalvaryum kemiğinde ikişer kritik büyüklükte defekt oluşturuldu ve farklı greft materyalleri ile dolduruldu. Kemik defektleri dört gruba ayrıldı. A1 grubundaki defektler; insan dişlerinden elde edilmiş dentin ile; A2 grubundaki defektler; tavşan dişlerinden elde edilen dentin ile dolduruldu. B1 grubu sığır kemiğiyle (Bio-Oss) dolduruldu ve B2 grubundaki (kontrol) defektler boş bırakıldı. Kemik mineral yoğunluğu DEXA ile 4. ve 12. haftalarda değerlendirildi

Bulgular: 4. haftada kemik mineral yoğunluğu değerleri A1, A2 ve B1 grupları arasında istatistiksel olarak anlamlı bulunmadı. Bununla birlikte, 12. haftadaki kemik mineral yoğunluğu değerleri grup B1 için diğer gruplara göre anlamlı derecede yüksek bulundu. Histopatolojik değerlendirmeye göre; B1 grubu, A1 ve A2 gruplarına kıyasla daha iyi bir kemik iyileşmesi gösterdi.

Sonuç: Dentin greftleri ve sığır kemik grefti kemik iyileşmesinin erken dönemindeki (4. hafta) kemik mineral yoğunluğu değerlerine göre benzer sonuç sergilemiştir.. Bununla birlikte, kemik iyileşmesinin geç döneminde (12. hafta) sığır kemiğinin kemik mineral yoğunluğu değerleri diğer gruplardan daha iyi bulundu.

Anahtar kelimeler: Dentin, kemik mineral yoğunluğu, DEXA, kemik grefti.

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INTRODUCTION

Maxillofacial defects can develop as a result of surgery, congenital malformation, infection, trauma, malignancy or atrophy. Different kinds of bone grafts are used for the reconstruction of bone defects in maxillofacial surgery[1,2]. Autogenous bone grafts have been widely used for reconstruction of such defects. They are the gold standard to which all other grafting materials are compared because autogenous bone grafts are the host itself and there is also an absence of antigenicity, early revascularization, and resistance to infection[3]. Despite all these benefits, autogenous bone grafts have some disadvantages such as limited donor sites, donor site damage as a result of surgery, extended surgery time, and resorption of the graft material[4]. Due to these disadvantages, researchers are seeking alternative materials that can be used as substitutes for autogenous bone grafts such as allogeneic bone, xenogeneic bone, and synthetic materials[5]; scientists have tried to develop better and more ideal bone grafting materials.

Dentin and bone have similar biochemical organic and inorganic components. The dentin matrix consists of 70-75% inorganic components, 20% organic components, and 10% water[6]. Alveolar bone has a similar structure to dentin, consisting of 65% inorganic components, 25% organic components, and 10% water. Dentin and bone contain similar proportions of body fluid (10%), collagen (18%), non-collagenous proteins (2%), and hydroxyapatite (70%)[7].

Human tooth has been used as a bone substitute in previous experimental studies[8]. It has been reported that demineralized dentin stimulates new bone formation, as an osteoinductive and osteoconductive implant material[9,10]. The advantages of particulate dentin include: lack of foreign body reactions, osteoconductive effect, lower cost, and it is easy to obtain[11-13].

Recent studies evaluated clinical applications of dentin grafts for implant surgery, such as repairing bone defects or sinus floor augmentation procedures. Clinical studies with long-term follow-up demonstrated successful outcomes of dentin grafts for implant surgery[7,14]. Despite the relatively high number of studies, the exact bone-healing capacity of dentin as a graft is unknown. Bone mineral density (BMD) is an important component for bone mass and quality. BMD is an effective issue for implant surgery regarding primary implant stability and insertion torque[15,16]. There is a paucity information about the effect of dentin as a graft on the BMD of new bone formation in the literature.

In this animal study, we aimed to evaluate the early- and late-term bone-healing capacity of dentin grafts used as allografts and xenograft material in bone defects and compared them with bovine bone according to histopathologic and dual-energy X-ray absorptiometry (DEXA) results.

MATERIALS AND METHODS

The surgical procedures for this research were performed according to protocols approved by the animal care and ethics committee of Ondokuz Mayis University (protocol number: P.Y.O DİS.1904.10.009). Sixteen mature adult male New Zealand white rabbits weighing an average 3.5 kg were used. The rabbits were divided into 2 main groups as A and B. Two identical 15-mm-wide, full-thickness, critical-size calvarial bone defects were created in each rabbit, one on each side of the midline of the cranium. Eventually, 32 defects were created in 16 rabbits and 8 rabbits were included in each group. The defects were filled with processed dentin from human teeth in group A1; processed dentin from rabbit teeth in group A2; processed bovine bone (Bio-Oss) in group B1, and left empty in group B2 (control group).

Preparation of the dentin grafts

Extracted human and rabbit teeth were washed with water, cleaned, and all soft tissue including the periodontal ligament was removed from the root. After the enamel had been removed with a high-speed diamond bur, the teeth were separated vertically to remove pulp. The teeth were immersed in isopropanol for 2 h to remove any remaining soft tissue or fat. After isopropanol, the teeth were washed with distilled water several times to eliminate organic solvent and then boiled in water at 100°C[17]. The teeth were fragmented into smaller pieces using a QUADRO COMIL (QUADRO, Ontario, CA) to pass through from special 1200-1500–μm sieve. After this procedure, another device called a Malvern Mastersizer 2000 (Malvern, Worcestershire, UK) was used to standardize the size of the dentin particles between 1200-1500 μm. The dentin particles were packed and sterilized using

25 kGy γ-irradiation.

Surgical procedure

The surgical areas of the experimental rabbits were shaved and washed with a 0.2% chlorhexidine solution. Anesthesia in the experimental rabbits was administered using an intramuscular injection of ketamine HCL (22-50 mg/kg) and xylazine (3-10 mg/kg). At the calvarial region, a longitudinal vertical skin incision was made and periosteum was removed to expose the calvarial bone. A special guide was used to make a standard critical-sized calvarial defect, which was 15 mm in diameter, bilaterally in the parietal bone[18,19]. The rabbits were divided into two groups, group A and B. The defects made on the same rabbit were named A1 and A2 or B1 and B2. With the exception of the control group whose defects remained unfilled, each rabbit had two defects on their parietal bone that were filled with different graft materials (Figure1). After the grafting procedure, the periosteum was closed with Vicryl 5-0 sutures (Ethicon, Edinburgh, UK), and the skin flaps were closed using Silk 3-0 sutures (Doğsan, Trabzon, TR). The rabbits were given antibiotics once daily for 5 days (amoxicillin + clavulanic acid) (Sylunox, Pfizer, IT), and analgesics for 2 days at 2 mg/kg (tramadol HCl IM) (Contramal, Abdi İbrahim, Türkiye). The rabbits were euthanized 12 weeks after the surgery with 30 mg/kg suxamethonium chloride (2% Lysthenon, FAKO, İstanbul, TR) for histologic examination.

Densitometric and histologic evaluation

The BMD of the defects was evaluated using a DEXA device at the 4th and 12th postoperative weeks (Bone Densitometer, HOLOGİC, Bedford, MA, USA) in Ondokuz Mayıs University Medical Faculty, Department of Nuclear Medicine. The numeric values of the BMD (g/cm^2) were evaluated using the QDR 44 for Windows XP Operating System (Standard Software Configuration, HOLOGİC, Bedford, MA, USA) of the Hologic QDR Discovery (Bone Densitometer, HOLOGIC, Bedford, MA, USA) (Figure 2).

The animals were euthanized at the 12th postoperative week. Samples were washed and kept in 10% formic acid solution prepared with 2% paraformaldehyde for about 3 weeks for decalcification; the solution was changed every 3 days. The samples were embedded in paraffin blocks and then 5-µm sections were prepared using microtomes (RM-2125 RT, Leica Microsystems Gmbh, Germany)..

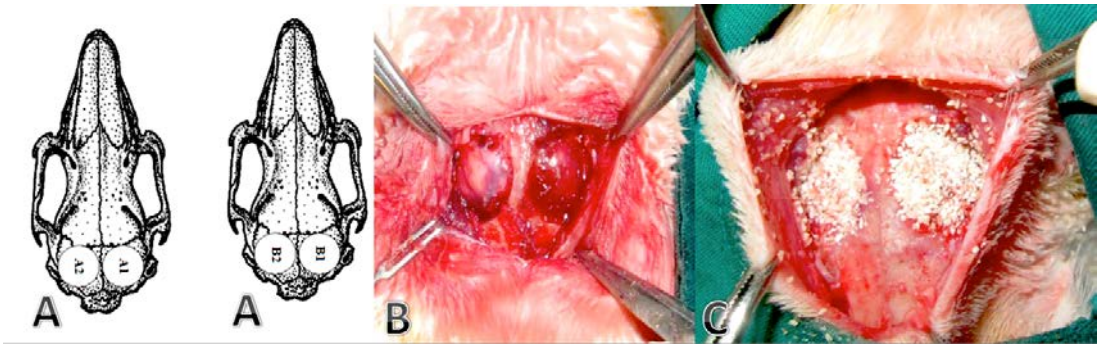

Figure 1: A) Figure showing the 2 main groups and 4 subgroups of study. Rabbit's cranium were named A1 and A2 or B1 and B2 Groups: A1; processed dentin from human teeth, A2; processed dentin from rabbit teeth; B1; processed bovine bone (Bio-Oss), B2; control group. B) Rabbit had two defects on their parietal bone. C) Except the control group defects were filled with different graft materials.

After the samples were deparaffinized at 60°C degrees using xylol in an incubator, the blocks were rehydrated through a series of decreasing concentrations of alcohol. The slices were stained with hematoxylin-eosin within 5 minutes and then washed with tap water. The slices were decolorized with 2% acid alcohol and stained with 2% lithium carbonate. The slices were washed with distilled water and stained with eosin for 3 minutes. The dehydrated tissues, which were passed through the graded alcohol series, were left for 45 minutes in xylol and mounted using Entallan. The sections

were examined under a light microscope (Nikon Eclipse E600W, Nikon L1-DS5M, Japan).

Inflammation, formation of fibrous tissue around bone graft material, formation of bone tissue around the bone graft material, resorption of the bone graft, and formation of bone marrow were evaluated semi-quantitatively. Each parameter was assessed using a scoring technique as: 0 (none), 1 (poor), 2 (average), and 3 (rich) for each defect[19]. The histologic results of the four groups were compared for the 12 weeks

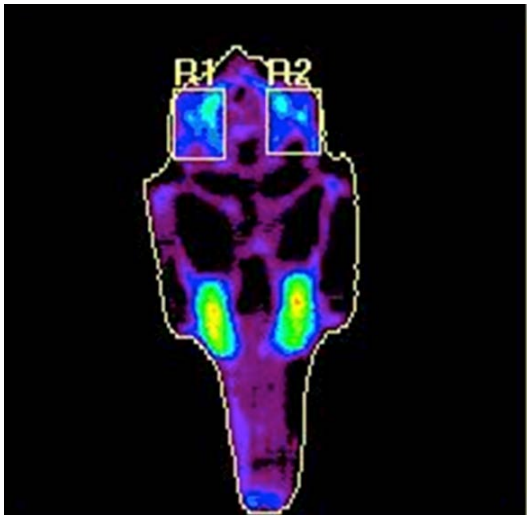

Figure 2. At 12th weeks DEXA image, R1; group A1 DEXA image and R2; is group A2 DEXA image.

Statistical analysis

Densitometry results were examined for normality using the Kolmogorov-Smirnov test before the statistical analysis. According to the test results, the paired-samples t-test and independent-sample t-test were used for comparison between two groups. The Wilcoxon and Mann-Whitney U tests were used to compare pathology results between two groups. All statistical results were evaluated using the MINITAB statistical program.

RESULTS

In group A1 and group A2, BMD was performed at the 1st (early term) and 3rd (late term) month. Group A1e: at the end of the 1st month (early term) for group A1 (processed dentin from human teeth); group A2e: at the end of 1st month (early term) for group A2 (processed dentin from rabbit teeth); group A1l: at the end of the 3rd month (late term) for group A1 (processed dentin from human teeth); group A2l: at the end of the 3rd month (late term) for group A2 (processed dentin from rabbit teeth). According to the statistical results for DEXA in the evaluation of BMD; the difference between group A1e and A1l was statistically significant (P=0.001). The difference between group A2e and A2l was statistically significant (P=0.007). There was no significant differences between group A1e and group A2e (P=0.632) and also between group A1l and group A2l (P=0.842) (Table 1).

Table 1. Statistical results for early and late term BMD evaluation between subgroups of dentin grafts (A)

Groups	**A1e (early)**	**A1l (late)**	**A2e (early)**	**A2l (late)**
Mean ± SD	0.945±0.060	0.991±0.057	0.934±0.066	0.994±0.067
	A1e-A2e	**A1e-A1l**	**A2e-A2l**	**A1l-A2l**
P values between groups	0.632	0.001	0.007	0.842

Paired-Samples "t" test, *p*: 0.05; A1e: At the end of 1st month (early term) for group A1 (processed dentin from human teeth), A2e: At the end of 1st month (early term) for group A2 (processed dentin from rabbit teeth); A1l: At the end of 3rd month (late term) for group A1; A2l: At the end of 3rd month (late term) for group A2.

Table 2. Statistical results for early and late term BMD evaluation between subgroups of groups B.

Groups	**B1e (early)**	**B1l (late)**	**B2e (early)**	**B2l (late)**
Mean ± SD	0.907±0.092	1.073±0.047	0.964±0.106	1.022±0.075
	B1e-B2e	**B1e-B1l**	**B2e-B2l**	**B1l-B2l**
P values between groups	0.052	0.000	0.049	0.002

Paired-Samples "t" test, p: 0.05

B1e: At the end of 1st month (early term) for group B1 (Bio-Oss); B1l: At the end of 3rd month (late term) for group B1. B2e: At the end of 1st month (early term) for group B2 (control); B2l: At the end of 3rd month (late term) for group B2.

In group B1 and group B2, BMD was performed at the 1st (early term) and 3rd (late term) month. Group B1e: at the end of 1st month (early term) for group B1 (processed bovine bone) (Bio-Oss); group B2e: at the end of 1st month (early term) for group B2 (control group); group B1l: at the end of 3rd month (late term) for group B1 (processed bovine bone) (Bio-Oss); group B2l: at the end of 3rd month (late term) for group B2 (control group). According to the statistical results for DEXA in the evaluation of BMD; the differences between group B1e and group B1l (P<0.001), group B2e and group B2l (P=0.049), group B1e and group B2e (P=0.05), and also between group B1l and group B2l (P=0.002) were satistically significant. (Table 2)In the comparison of densitometry results for independent groups, there was no significant difference between group B1e and group A1e (P=0.335) and group B1e and group A2e (P= 0.505); however, the difference between group B1l and group A1l (P=0.007) and group B1l and group A2l were statistically significant (Table 3).

Table 3. Statistical results for early and late term BMD evaluation between groups of bone grafts

Groups	A1e (early)	A1l (late)	A2e (early)	A2l (late)	B1e (early)	B1l (late)
Mean±SD	0.945±0.060	0.991±0.057	0.934±0.066	0.994±0.067	0,907±0,092	1,073±0,047
	A1e-B1e		A2e-B1e	A1l-B1l		A2l-B1l
P values between groups	0.335		0.505	0.007		0.017

Unpaired "t" test, p: 0.05; A1e: At the end of 1st month (early term) for group A1 (processed dentin from human teeth); A2e: At the end of 1st month (early term) for group A2 (processed dentin from rabbit teeth); A1l: At the end of 3rd month (late term) for group A1; A2l: At the end of 3rd month (late term) for group A2; B1e: At the end of 1st month (early term) for group B1 (Bio-Oss); B1l: At the end of 3rd month (late term) for group B1.

There was significant difference between group B2e and group A1e (P<0.001), group B2e and group A2e (P<0.001), group B2l and group A1l (P=0.001), and group B2l and group A2l (P<0.001).

A1 and A2 showed similar histopathologic findings. Mild inflammation was observed in the defects. Fibrotic connective tissue around the bone graft particles was at a good level. The beginning of different quantities of resorption was seen in all samples of the groups (Figure 3).

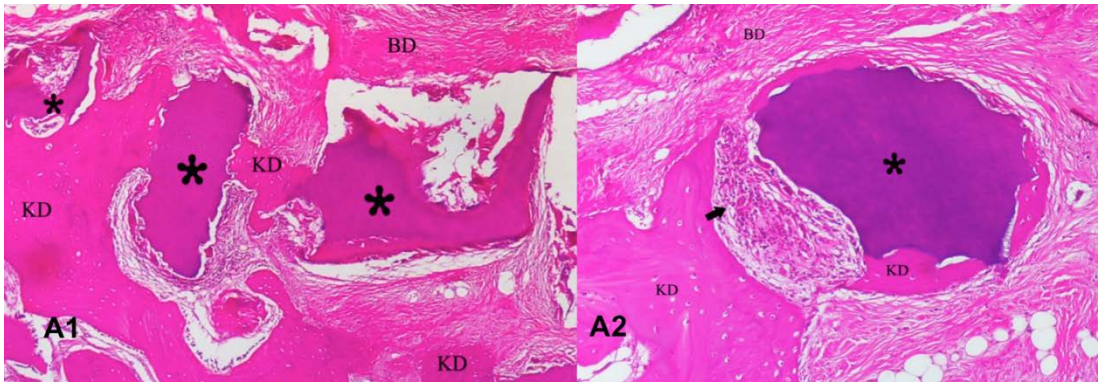

Figure 3. In group A1 *; human dentin graft, KD; Bone tissue, BD; Connective tissue. In Group A2 *; Rabbit dentin graft, arrow; inflammation area, KD; Bone tissue, BD; Connective tissue.

In particular, the bone graft particles located near the edges of the defect were surrounded by trabecular bone formed by intramembranous ossification. In group B1, no inflammation was seen in the defect area. At the edge of the defect, bone graft particles were surrounded by bone trabeculae. In the newly formed bone trabeculae, bone graft particles were not fully resorbed. The section

showed that almost all of the defect site was closed by bone trabeculae and bone graft particles surrounded by fibrous tissue remained in a very limited area. In group B2 (control group), the defect area was closed with thin fibrotic connective tissue (Figure 4).

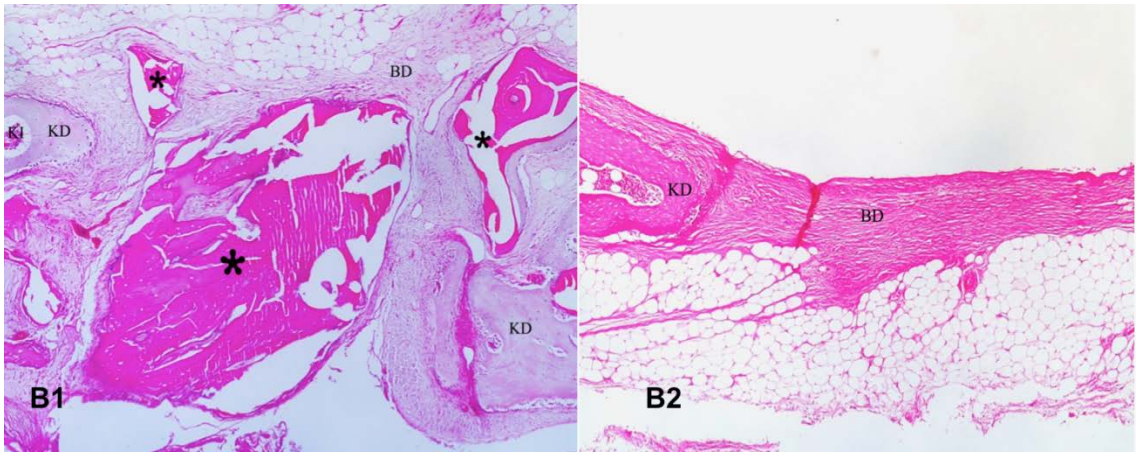

Figure 4. In group B1 *; Bio-Oss (bovine bone) graft, KD; Bone tissue, BD; Connective tissue. In group B2 (Control) edge of the defect, KD; Bone tissue, BD; Connective tissue.

There was no significant difference in inflammation (*P*=0.157), formation of fibrous tissue around the bone graft material (*P*=0.564), formation of bone tissue around the bone graft material (*P*=0.317), resorption of the bone graft (*P*=0.083), and formation of bone marrow (*P*=0.317) between groups A1 and A2 (Table 4).

Table 4. Histological evaluation of subgroups of two main groups.

Groups	A1	A2	B1	B2
INF	1.000±0.000	1.250±0.463	0.375±0.518	0.000±0.000
	P: 0.157		*P*: 0.083	
FFT	2.000±0.000	1.875±0.641	2.000±0.535	0.000±0.000
	P: 0.564		*P*: 0.008	
FBT	0.875±0.641	0.625±0.744	1.750±0.707	0.000±0.000
	P: 0.317		*P:* 0.010	
RBG	1.625±0.518	2.000±0.000	2.000±0.535	0.000±0.000
	P: 0.083		*P:* 0.008	
BM	0.375±0.518	0.250±0.463	1.250±0.463	0.000±0.000
	P: 0.317		*P:* 0.008	

Wilcoxon Signed-Rank Test, p: 0.05

Inflammation (INF), formation of fibrous tissue around bone graft material (FFT), formation of the bone tissue around the bone graft material (FBT), resorption of the bone graft (RBG) and formation of bone marrow (BM) were evaluated semi-quantitatively.

The difference in inflammation (*P*=0.009), formation of bone tissue around the bone graft material (*P*=0.026), and formation of bone marrow (*P*=0.007) were statistically significant between groups B1 and group A1; however, there was no significant difference in the formation of fibrous tissue around bone graft material (*P*=0.999) and resorption of bone graft (*P*=0.175). The difference in inflammation (*P*=0.007), formation of bone tissue around the bone graft material (*P*=0.013), and formation of bone marrow (*P*=0.003) were statistically significant between groups B1 and group A2; however, there was no significant difference in the formation of fibrous tissue around the bone graft material (*P*=0.653) and resorption of the bone graft (*P*=0.999). The statistical results between group B2 and group A1 were similar between group B2 and group A2. The difference in inflammation,

formation of bone tissue around the bone graft material, formation of fibrous tissue around the bone graft material, and resorption of the bone graft were significant between group B2 and other groups; however, there was no significant difference in the formation of bone marrow (Table 5).

Table 5. Statistical results for histological findings between groups of A and B

Groups	A1	B1	A2	B1	A1	B2	A2	B2
INF	1.00±0.00	0.375±0.518	1.250±0.463	0.375±0.518	1.000±0.000	0.000±0.000	1.250±0.463	0.000±0.000
	P: 0.009		*P*: 0.007		*P*: 0.000		*P*: 0.000	
FFT	2.000±0.000	2.000±0.535	1.875±0.641	2.000±0.535	2.000±0.000	0.000±0.000	1.875±0.641	0.000±0.000
	P: 1.000		*P*: 0.653		*P*: 0.000		*P*: 0.000	
FBT	0.875±0.641	1.750±0.707	0.625±0.744	1.750±0.707	0.875±0.641	0.000±0.000	0.625±0.744	0.000±0.000
	P: 0.026		*P*: 0.013		*P*: 0.003		*P*: 0.027	
RBG	1.625±0.518	2.000±0.535	2.000±0.000	2.000±0.535	1.625±0.518	0.000±0.000	2.000±0.000	0.000±0.000
	P: 0.175		*P*: 1.000		*P*: 0.000		*P*: 0.000	
BM	0.375±0.518	1.250±0.463	0.250±0.463	1.250±0.463	0.375±0.518	0.000±0.000	0.250±0.463	0.000±0.000
	P: 0.007		*P*: 0.003		*P*: 0.063		*P*: 0.143	

Mann Whitney U, *p: 0.05;* Inflammation (INF), formation of fibrous tissue around bone graft material (FFT), formation of the bone tissue around the bone graft material (FBT), resorption of the bone graft (RBG) and formation of bone marrow (BM) were evaluated semi-quantitatively.

DISCUSSION

Various bone graft materials are used for the reconstruction of bone defects that occur in the maxillofacial region. An ideal bone substitute should be biocompatible, non-antigenic, resistant to infection, gradually replaced by new bone, and have osteoinductive and osteoconductive properties[20,21]. Studies showed that demineralized dentin and bone had similar organic and inorganic structure. These studies reported that dentin could be used as a bone graft[22,23,24]. We used extracted human first premolar teeth due to orthodontic treatment and extracted rabbit teeth to obtain dentin graft materials.

In this study, rabbits were preferred because of some advantages such as the ease of obtaining dentin graft material from rabbit teeth, standardization of experimental conditions, repeatability, inexpensive material, and high bone turnover[25]. The size of the defect area is one of the important factors for the bone-healing period. A critical-size defect is the minimum bone defect that will not heal during the lifetime of the organism[26,27]. Dodde et al. created different sizes of defects in rabbit calvarial bone and showed that defects with a 15-mm diameter were critical-size defects for rabbit calvarial bone[18]. In 2009, Findikcioglu et al. used the same critical-size defect to evaluate bone-healing in calvarial bone defect areas[19]. In their study, an acrylic guide that had 15 mm diameter was used to create standard defects. For better evaluation of dentin grafts as allografts and xenografts and to eliminate local and systemic factors that affect healing, which depend on the animal, bilateral critical-size defects were created in the same animal.

Different methods have been used to obtain dentin grafts. Catanzaro et al. used extracted dogs' mandibular canine teeth to obtain demineralized dentin[23]. Teeth were embedded in 2°C 0.6N hydrochloric acid (HCl) for 5 days with the aim of removing the periodontal ligament and pulp[23]. Kim et al. used extracted human teeth and dentin particles were placed in a high-temperature furnace at 950°C (1742°F) for 30 min and were exposed to ethylene oxide gas for sterilization. In their different studies, they used a furnace at 1200°C[11,12,28]. Gomes et al. used extracted rabbit teeth and they immersed teeth in 0.6 N HCl solution at 2°C until completely demineralized and then the particles were placed ethyl alcohol 70°/gentamicin (5 mL/0.2 sol)[9,10]. Park et al. used extracted human teeth for their research and demineralized the dentin with 0.6N HCl. Park et al. compared the affect of different demineralization times of dentin on the new bone formation[24]. In another study, the researchers stored extracted bovine teeth at -80°C for 24 h and the teeth were demineralized in 0.6 N HCl for a week, then placed into chloroform methanol for 24 hours to obtain the dentin graft[29]. The authors sterilized the dentin graft with ethylene oxide gas. Some researchers used ethylene oxide for dentin graft sterilization but others used high temperatures such as 1200°C to eliminate viruses, bacteria, and fungi[4,13]. In the study by Moharamzadeh et al., dentin obtained from extracted bovine teeth was sterilized using gamma (ɣ) -irradiation[17]. Gamma

irradiation is a widely accepted procedure and a 25,000 Gray dose is recommended for bone sterilization[30,31]. Studies proved that irradiation was necessary to achieve a reduction of infectivity of the most resistant viruses such as HIV-2, hepatitis A virus (HAV), poliovirus (PV-1), and pseudorabies virus (PRV) and bacteria such as; Staphylococcus aureus (S. aureus) and Staphylococcus epidermidis (S. epidermidis)[32,33,34]. In 2016 Singh et al. published a review and showed that allograft tissues such as bone, skin and soft tissues could be sterilized with gamma radiation and made safe for clinical use[35]. Gomes et al. and Catanzaro et al. used a microtome blade to obtain 2x2-mm and 8-µm thickness dentin particulates[10,23]. Kim et al. used a high-temperature furnace at 950°C (1742°F) to get a dentin ash, the tooth was pulverized by means of mortar and pestle than dentin particles was filtered by 100-mesh screen[11,12]. In other studies, the same authors used a high-temperature furnace at 1200°C to obtain ash and grinded dentin ash into a powder using a mesh tray (sieve no. 100; 0.149 mm). They mixed this tooth ash with plaster of Paris[4,28]. In 2010, Kim et al. used autogenous teeth crushed to a powder, the size of the particles was between 200 and 1000 µm, but in another study, they used dentin particles between 0.5 to 1.0 mm[13,36].

As a result of the multiple studies in the literature, there is no standardization about obtaining dentin grafts, the size of dentin particles, and the method of sterilization. In the present study, 25 kGy gamma irradiation was used to sterilize the human and rabbit dentin graft materials. Additionally, a special sieve (QUADRO COMIL, QUADRO, Ontario, Canada) and then another specific device were used to standardize the graft size between 1200-1500µm. A Malvern Mastersizer 2000 (Malvern, Worcestershire, UK) device was used to eliminate particles smaller and larger than 1200-1500 µm, respectively, thus a standard dentin graft at the same size was obtained. This preparation phase was important for better histologic evaluation and comparative analysis because Bio-Oss (1-2 mm) was used as a comparator with the dentin graft as an allograft and xenograft.

In some studies about bone grafts, inflammation, formation of fibrous tissue around the bone graft material, formation of bone tissue around the bone graft material, resorption of the bone graft, and formation of bone marrow were evaluated for the pathologic results of the defect area[19,37]. According to the histologic results, consistent with the literature, Bio-Oss was significantly more effective than human and rabbit dentin grafts at the end of the 12 weeks[12].

Many in vivo methods such as single-photon absorptiometry (SPA) dual-photon absorptiometry (DPA), single-energy X-ray absorptiometry (SXA), and DEXA are available for the measurement of BMD[38]. Studies showed that DEXA was widely accepted as the gold standard method of clinical bone mineral measurement[39,40,41]. Horner and Devlin showed that the relationships between two indices of mandibular bone quality and BMD measured using DEXA[39] . The results of the study regarding the bone quality index (BQI) and the mandibular cortical index (MCI) were significantly related with BMD[39]. In our study, BMD was evaluated at early and late healing periods of the dentin graft using DEXA. To our knowledge, few studies have evaluated the effect on BMD of dentin grafts at early and late-term healing of bone defects.

In the literature the healing period of bone defects were evaluated at different times in the rabbit. Some studies showed that 4 weeks was enough to evaluate the histologic and radiologic aspects for the early healing period of bone defects in the rabbit. Some authors asserted that 6, 8 or 12 weeks were better to evaluate the histologic or radiologic aspects of late-period bone-healing results[9,42,43]. In accordance with the literature, DEXA was used to evaluate BMD at 4th and 12th weeks in the present study, and the animals were sacrificed at the end of the 12th week for pathologic examination. Ozdemir et al., in their animal (rabbit) study, placed different types of bone graft materials into defects and evaluated BMD levels using DEXA 12 weeks later. The results of the study were significantly different between the groups[44]. In our study, new bone formation was shown radiographically and histopathologically in the groups. At the 4th week (early bone-healing period), according to the DEXA evaluation; Bio-Oss, human dentin grafts, and rabbit dentin grafts had similar results regarding BMD, there was no significant difference between these three groups, except for the control group. At the 12th week (late bone-healing period) according to the DEXA evaluation, the BMD results of the Bio-Oss group were better than those of human dentin grafts and rabbit dentin grafts. The BMD results of our study were consistent with the literature[14]. BMD is an important component for bone mass and quality;

besides, studies showed that BMD was an effective issue for implant surgery regarding primary implant stability and insertion torque. This research is a preliminary study; more in vivo and clinical studies are needed to improve the clinical application of dentin grafts such as those used in implant surgery.

Within the limitations of an animal experimental study and absence of clinical trial; Bio-Oss graft material was more effective than dentin graft materials on new bone formation. In the early bone-healing period, bovine bone graft and dentin grafts are similarly effective on the BMD of new bone nevertheless in the late bone-healing period, bovine bone graft is more effective than dentin grafts on the BMD of new bone.

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