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ORIGINAL RESEARCH

WILEY CLINICAL ORAL IMPLANTS RESEARCH

Comparison of autogenous and allograft bone rings in surgically created vertical bone defects around implants in a sheep model

Mehmet Emre Benlidayi[1] | **Ufuk Tatli**[1] | **Fariz Salimov**[1] | **Hüseyin Can Tükel**[1] | **Orcan Yüksel**[2,3]

[1]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Cukurova University, Adana, Turkey

[2]Private Practice, Zahnarztpraxis Dr.Yüksel & Kollegen, Frankfurt, Germany

[3]Academic Teaching and Research Unit, Medical Faculty of Goethe-University Frankfurt, Frankfurt, Germany

Correspondence
Mehmet Emre Benlidayi, Cukurova Universitesi Dis Hekimligi Fakultesi, Cene Cerrahisi Klinigi, Adana, Turkey.
Email: emrebenlidayi@yahoo.com

Abstract

Objectives: The aim of this study was to compare autogenous and allograft bone rings in surgically created vertical bone defects.

Material and methods: Four male, 1-year-old sheep were used in this study. In each sheep, eight vertical bone defects 7 mm in diameter were created using trephine drill in the iliac wing. Autogenous and allograft bone rings 5 mm in height and 7 mm in diameter were used for vertical augmentation around implants. The study consisted of four groups according to the bone ring type and amount of vertical augmentation, autogenous 2 mm, allograft 2 mm, autogenous 4 mm, and allograft 4 mm. Two of the animals were sacrificed after 4 months, and the remaining two animals were sacrificed after 8 months. Undecalcified sections were prepared from harvested samples. Histological assessment and histomorphometric analysis were performed.

Results: Autogenous 2 mm group showed higher values than allograft 2 mm group, and autogenous 4 mm group showed higher values than allograft 4 mm group in terms of bone area and bone-to-implant contact (BIC) after 4 months. However, allograft 2 mm group showed higher bone area and BIC values than autogenous 2 mm group after 8 months. Also, autogenous 4 mm and allograft 4 mm groups showed comparable results after 8 months. Allograft 2 mm and allograft 4 mm groups showed higher bone area and BIC values at 8 months compared with 4 months.

Conclusions: Allograft bone ring looks promising in augmentation of surgically created vertical bone defects around implants after 8 months of healing.

KEYWORDS
animal experiments, bone regeneration, bone substitutes, bone–implant interactions, guided tissue regeneration, morphometric analysis

1 | INTRODUCTION

Unfavorable local conditions due to atrophy, trauma, and periodontal disease may cause insufficient bone volume or an unfavorable interarch relationship, which does not allow placing the implants in a correct position. Alveolar bone augmentation procedures are used to allow implant placement in an optimal position to achieve long-term function and acceptable esthetic outcome. Vertical bone augmentation is a challenging procedure especially in the anterior esthetic zone. Many techniques have been described for vertical bone augmentation, such as the use of particulate bone substitutes and guided bone regeneration (GBR); autogenous, allogenic, and xenogeneic block grafts; and distraction osteogenesis (Felice et al., 2008; McAllister & Haghighat, 2007; Petrungaro & Amar, 2005; Rachmiel,

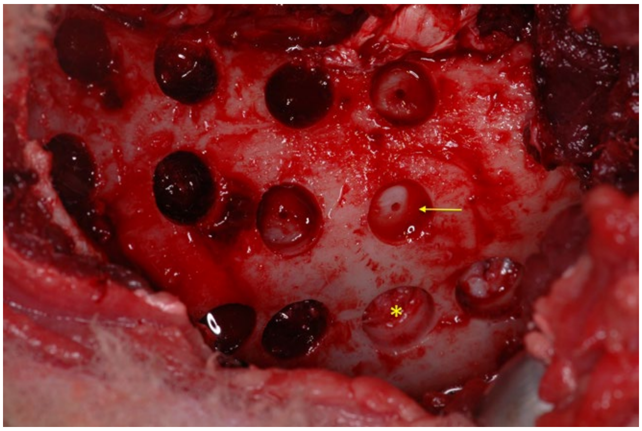

FIGURE 1 Intraoperative view of surgically created standard vertical bone defects in the iliac wing (yellow arrow). Donor sites of autogenous bone rings in the same iliac wing (yellow asterisk)

Shilo, Aizenbud, & Emodi, 2017; Rocchietta et al., 2016; Simion, Trisi, & Piattelli, 1994; Todisco, 2010). Autogenous block bone grafts are still considered the gold standard in the majority of cases with vertical bone deficiency due to osteoconductive, osteoinductive, and osteogenic potential (Chiapasco, Gatti, & Gatti, 2007; Rocchietta et al., 2016). The implants can be placed either simultaneously or in a second procedure.

Bone ring technique was described as a one-stage procedure for vertical augmentation, in which an autogenous corticocancellous block bone graft is stabilized with a simultaneously inserted dental implant. This technique has been documented in several case reports and series but there is a lack of information to demonstrate its efficacy (Fukuda, Takahashi, & Yamaguchi, 2000; Giesenhagen, 2006; Giesenhagen & Yuksel, 2010; Omara, Abdelwahed, Ahmed, & Hindy, 2016; Stevens, Emam, Alaily, & Sharawy, 2010; Tekin, Kocyigit, & Sahin, 2011).

The advantages of this one-stage method are reduction of the number of surgical interventions and overall treatment period. The disadvantages of autogenous bone grafting are additional surgery and possible donor-site morbidity. It was reported that almost 50% of patients have postoperative temporary paresthesia in the first month after bone harvesting from symphysis (Nóia et al., 2011). Moreover, autogenous bone used in bone ring technique has a fracture risk during graft harvesting or implant placement (Omara et al., 2016; Stevens et al., 2010).

Alternative treatment modalities have been investigated to avoid these disadvantages. The allograft bone ring is a pre-fabricated allogenic cancellous graft, which allows bone augmentation, and implant placement in a one-stage procedure. The benefit of this technique is the avoidance of harvesting of autogenous bone. However, there are not enough data in the literature about the success of this graft material.

There is not enough data in the clinical studies in which allograft bone ring was used (Flanagan, 2016; Yuksel, Giesenhagen, & Chmielewski, 2014). Flanagan (2016) used allograft bone ring for vertical augmentation around implants in eight patients. In this pilot study, implant failure was not observed for over 1-year function. Yuksel et al. (2014) demonstrated a new method for reducing treatment time in sinus augmentation. In this technical report, one-stage sinus augmentation and implant placement using allograft bone ring were presented. In this context, histological evaluation and histomorphometric analysis of allograft bone ring are required.

The aim of this study was to compare the autogenous and allograft bone rings in surgically created vertical bone defects in an iliac sheep model after 4 and 8 months of healing.

2 | MATERIAL AND METHODS

2.1 | Animals

Four male, 1-year-old sheep were used in this study. The animals received standard feed and water ad libitum. The study was approved by Animal Experiment Ethics Committee of Cukurova University (No. 2016-7).

2.2 | Surgical procedures

All surgical interventions were performed under systemic anesthesia using ketamine (10 mg/kg i.m.; Ketalar®, Pfizer Ilac Ltd. Sti., Istanbul, Turkey) and xylazine (3 mg/kg i.m.; Rompun®, Bayer Veteriner Ilaclari, Istanbul, Turkey). General anesthesia was induced with intravenous administration of 15 mg/kg thiopental sodium (Pental Sodyum®, Ibrahim Etem Ulagay Ilac San., Istanbul, Turkey). After endotracheal intubation, a mixture of anesthesia gas (1.8%–2.0% isoflurane, 20%–30% nitrous oxide, pure oxygen) was delivered for maintenance of anesthesia. Local anesthesia (Maxicaine®, Vem Ilac San., Istanbul, Turkey) was used for intraoperative analgesia. All animals received 1,000 mg cefazolin sodium (Cefamezin®, Eczacıbasi Ilac San., Istanbul, Turkey) as antibiotics and 75 mg diclofenac (Dikloron®, Deva Holding, Istanbul, Turkey) as analgesics intraoperatively and for 7 days after surgery. The right iliac wing was exposed surgically, and eight vertical bone defects 7 mm in diameter were created using a trephine drill in each sheep. Four of the defects were 3 mm in depth, and the other four defects were 1 mm in depth. Also four autogenous bone rings were harvested using trephine drill from the iliac bone (Figure 1). All the autogenous bone rings and allograft bone rings (Maxgraft®; Botiss Dental, Berlin, Germany) were 5 mm in height and 7 mm in diameter, and the center of the rings was prepared for the 3.5 mm diameter implant (Ankylos®, Dentsply Sirona Implants, Sweden). All the bone rings were fixed with 1-mm subcrestally placed implants, and cover screws were inserted into the implants. Each sheep received eight implants (Ankylos® Ø3.5 mm, length 11 mm), and thus, two implants were placed for each group with either autogenous bone ring or allograft bone ring (Figure 2). Thus, a total of 32 dental implants were placed in four sheep. The study consisted of four groups (Figure 3).

Autogenous 2 mm group: 2 mm vertical augmentation with autogenous bone ring.

Allograft 2 mm group: 2 mm vertical augmentation with allograft bone ring.

In autogenous 2 mm and allograft 2 mm groups, the defects were 7 mm in diameter and 3 mm in depth.

Autogenous 4 mm group: 4 mm vertical augmentation with autogenous bone ring.

Allograft 4 mm group: 4 mm vertical augmentation with allograft bone ring.

In autogenous 4 mm and allograft 4 mm groups, the defects were 7 mm in diameter and 1 mm in depth.

All augmented sites were covered with collagen membrane (Jason®, Botiss Dental, Berlin, Germany), and flap was closed primarily in layers with 3/0 absorbable suture. Two of the animals were sacrificed after 4 months of healing, and the remaining two animals were sacrificed after 8 months of healing.

2.3 | Histological analysis

The implants with surrounding bone were removed en bloc and immersed in %4 neutral buffered formaldehyde for histomorphometric analysis. The specimens were dehydrated in a graded series of ethanols and embedded in methyl methacrylate-based resin (Technovit 7200 VLC, Kulzer, Germany). Undecalcified ground sections from the implants and surrounding bone were carried out according to the method described by Donath and Breuner (1982). Serial sections were taken through the longitudinal axis of each implant with a diamond band saw (Exakt 300 CL, Exakt Apparatus, Norderstedt, Germany). The sections were reduced to a thickness of 40 μm using the micro parallel grinding system (Exakt 400 CS, Exakt Apparatus, Norderstedt, Germany). Two sections were prepared from each block and were stained with toluidine blue. The mean histomorphometric and bone-to-implant contact measurements of these sections were recorded for each implant. Images of the sections were obtained with a digital camera (Olympus DP 70; Olympus, Tokyo, Japan) attached to a microscope (Olympus BX50; Olympus) at a magnification of 4x. The images were transferred to a computer. Histological evaluation and histomorphometric analysis were done. ImageJ (National Institute of Mental Health, Washington, DC) and WinTAS image analysis software (WinTAS Trabecular Analyze System, version 1.2.9) were used for histomorphometric analysis. Region of interest (ROI) was defined as grafted site around each implant for the histomorphometric and bone-to-implant contact measurements. ROI was confined by measuring the known dimensions of autogenous or allograft bone ring around each implant on image analysis software. The following measurements were performed in the grafted site around each implant: (a) bone area (percentage of bone area to total measured area), (b) residual graft area (percentage of graft area to total measured area), (c) marrow space area (percentage of marrow space area to total measured area), and (d) mineralized bone-to-implant contact (BIC) percentage. Descriptive statistics was used for presentation of the results due to limited number of the samples (SPSS 17.0 software, SPSS Inc., Chicago, IL).

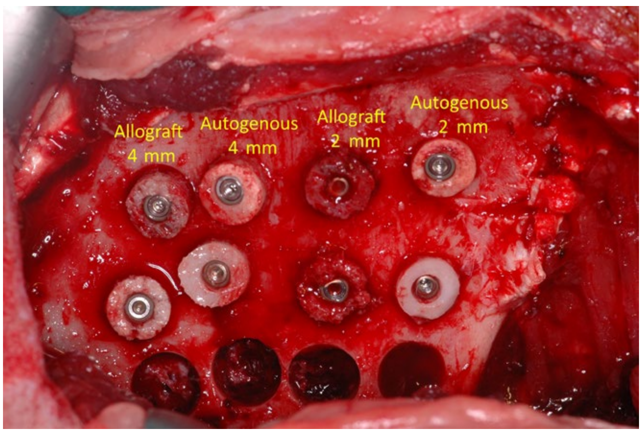

FIGURE 2 Placement of implants through the autogenous or allograft bone rings in each of the defects. Two implants were placed for each group with either autogenous bone ring or allograft bone ring

3 | RESULTS

All four animals healed uneventfully, without infection or implant failure during the healing period.

3.1 | Histological assessment

3.1.1 | 2-mm augmentation groups

At month 4, in autogenous 2 mm group, bone lamellae and osteocytes were clearly distinguished. Favorable bone regeneration was seen in the coronal part of the implant. However, there were bone marrow spaces in the bone ring area. The implant was in contact with

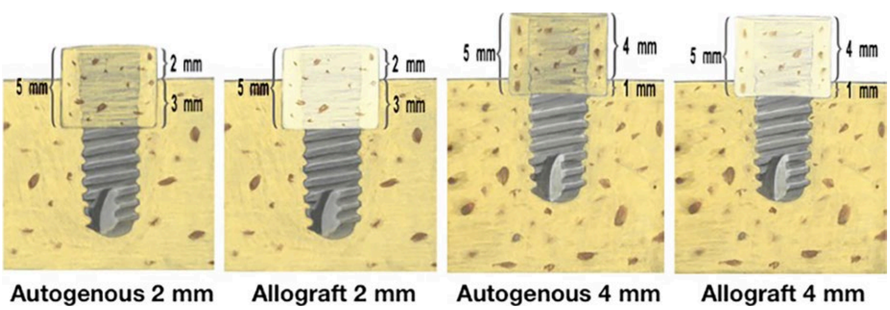

FIGURE 3 Schematic drawing of groups. The study consisted of four groups according to the bone ring type and amount of vertical augmentation

well-organized mature lamellar bone (Figure 4a). In allograft 2 mm group, it was relevant that allograft did not resorb completely. The development of lamellar bone in the allograft bone ring area was noticeable. There was a connection with the allograft and the recipient bone and also with the implant (Figure 4b).

At month 8, in autogenous 2 mm group, large bone marrow spaces due to resorption were evident in the bone ring area. The area of the bone trabeculae was obviously narrower, and the amount of lamellar bone in contact with the implant was less when compared to 4 months (Figure 5a). In allograft 2 mm group, allograft bone ring completely resorbed and replaced with mature lamellar bone. Thick bone trabeculae were observed in the bone ring area. The new bone was well consolidated to the recipient bone. Although the superior border of the bone ring showed favorable bone-implant contact, the remaining part of the bone ring submerged in the recipient bone showed minimal bone resorption (Figure 5b).

3.1.2 | 4-mm augmentation groups

At month 4, favorable bone healing and consolidation were observed in 4 mm vertical augmentation with autogenous bone ring group. The bone graft was well consolidated to the recipient bone. The margin between the bone ring and the recipient bone was unclear. The development of mature lamellar bone with haversian system was seen. The implant surface was almost completely in contact with bone in the bone ring area (Figure 4c). In allograft 4 mm group, partial resorption of the allograft was seen. Lamellar bone fusion between the allograft and the recipient bone was evident. There were some bone islands into the allograft. Also, there was a connection with allograft and the implant (Figure 4d).

At month 8, in autogenous 4 mm group, thick bone trabeculae were observed in the bone ring area. Compared to the 4 months of healing period, more bone marrow space was seen due to resorption. High amounts of lamellar bone-to-implant contact were observed (Figure 5c). In allograft 4 mm group, allograft bone ring completely resorbed and replaced with mature lamellar bone. The lacunae, osteocytes in the lacunae, and the lamellar bone, which symbolize vital bone, were observed in the bone ring area. Compared to the 4 months of healing period, higher bone consolidation to the recipient bone was seen and the amount of bone-to-implant contact was advanced (Figure 5d).

3.2 | Histomorphometric analysis

Tables 1 and 2 show the mean percentages of bone area, residual graft area, marrow space area, and BIC values for all groups after 4 and 8 months of healing.

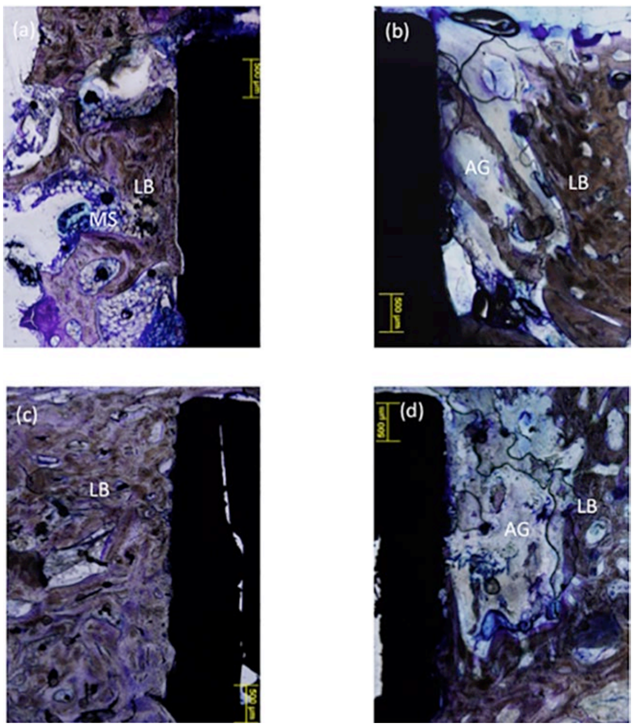

FIGURE 4 Undecalcified longitudinal section through the implants 4 months after vertical augmentation with bone ring. (a) Autogenous 2 mm group, (b) allograft 2 mm group, (c) autogenous 4 mm group, and (d) allograft 4 mm group (toluidine blue, original magnification x4). AG: allograft; LB: lamellar bone; MS: marrow space

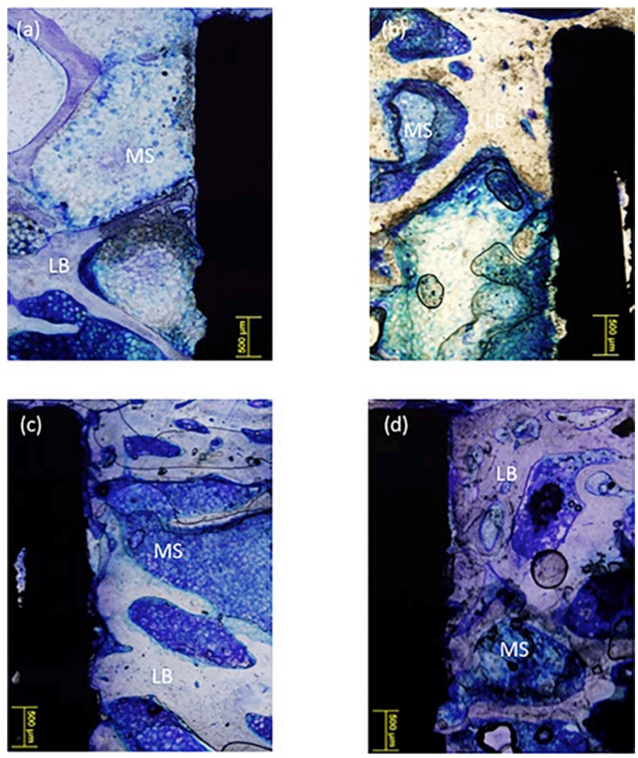

FIGURE 5 Undecalcified longitudinal section through the implants 8 months after vertical augmentation with bone ring. (a) Autogenous 2 mm group, (b) allograft 2 mm group, (c) autogenous 4 mm group, and (d) allograft 4 mm group (toluidine blue, original magnification x4). LB: lamellar bone, MS: marrow space

TABLE 1 The mean values of histomorphometric parameters in each group after 4 months of healing

Groups	Histomorphometric parameters (mean ± *SD*)			
	Bone area	Residual graft	Marrow space	BIC
Autogenous 2 mm	70.85 ± 3.51	0.00	29.14 ± 3.51	75.32 ± 7.10
Allograft 2 mm	54.53 ± 2.67	29.15 ± 3.09	16.31 ± 3.33	68.97 ± 11.25
Autogenous 4 mm	85.63 ± 3.59	0.00	14.36 ± 3.59	95.76 ± 2.97
Allograft 4 mm	66.59 ± 2.63	11.83 ± 0.96	21.57 ± 2.78	64.40 ± 4.71

Note. BIC: bone-to-implant contact.

3.2.1 | 2-mm augmentation groups

The mean bone area was higher in autogenous 2 mm group compared with the allograft 2 mm group after 4 months of healing (Table 1). The mean bone area value was lower in autogenous 2 mm group compared with the allograft 2 mm group after 8 months of healing (Table 2). Autogenous 2 mm group showed lower value, but allograft 2 mm group showed higher value at 8 months when compared to 4 months (Table 3).

Autogenous 2 mm and allograft 2 mm groups showed comparable BIC results after 4 months of healing (Table 1). The mean BIC value was lower in autogenous 2 mm group compared with allograft 2 mm group after 8 months of healing (Table 2). When the mean BIC values were compared between 4 and 8 months of healing, autogenous 2 mm group showed lower value, but allograft 2 mm group showed higher value at 8 months compared with 4 months (Table 3).

3.2.2 | 4-mm augmentation groups

The mean bone area was higher in autogenous 4 mm group than the allograft 4 mm group after 4 months (Table 1). Autogenous 4 mm and allograft 4 mm groups showed comparable results in terms of bone area after 8 months (Table 2). When the mean bone area percentage was compared between 4 and 8 months of healing, autogenous 4 mm group showed lower value, but allograft 4 mm group showed higher value at 8 months compared to 4 months (Table 3).

The mean BIC value was higher in autogenous 4 mm group compared to allograft 4 mm group after 4 months of healing (Table 1). Autogenous 4 mm and allograft 4 mm groups showed comparable BIC results after 8 months of healing (Table 2). When the mean BIC values were compared between 4 and 8 months of healing, autogenous 4 mm group showed lower value but allograft 4 mm group showed higher value at 8 months compared with 4 months (Table 3).

4 | DISCUSSION

In this particular type of vertical augmentation, allograft bone rings seem to be more efficient than autogenous bone rings in case of 2 mm augmentation and equally efficient as autogenous bone rings in case of 4 mm augmentation after 8 months of healing.

Different animal models have been used for evaluation of bone regeneration. Ravaglioli et al. (1996) reported that mineral composition and both metabolic and bone remodeling rates are similar to humans in sheep model. In the present study, iliac wing of a sheep model was selected because this is a valid model for human bone turnover and remodeling activity. In addition, crista iliaca is a suitable region for placing dental implants in sheep (Anderson, Dhert, & deBruijnetal, 1999; Newman, Turner, & Wark, 1995; Scarano, Lorusso, Ravera, Mortellaro, & Piattelli, 2016). The sheep mandible model was not preferred because of the chewing activities of sheep, which may cause flap dehiscence, graft, and implant failure after vertical augmentation of the alveolar bone. Minimum number of animals was used in the present study in accordance with 3Rs Principle (Reduction, Refinement and Replacement). Sartoretto et al. (2016) suggested to place up to 12 implants (in the final size for marketing) per sheep from an ethical point of view. In the present study, four defects were created to harvest autogenous bone rings, additionally 8 standard defects were created, and 8 dental implants were placed in the center of these defects in each sheep. This experimental pilot study in a small number of sheep might suggest that autogenous and allograft bone rings are equivalent in non-oral sites; however, quantitative analysis in an oral model with adequate

TABLE 2 The mean values of histomorphometric parameters in each group after 8 months of healing

Groups	Histomorphometric parameters (mean ± *SD*)		
	Bone area	Marrow space	BIC
Autogenous 2 mm	53.58 ± 3.21	46.42 ± 3.21	52.95 ± 5.28
Allograft 2 mm	66.55 ± 2.64	33.45 ± 2.64	73.39 ± 4.19
Autogenous 4 mm	72.41 ± 5.09	27.59 ± 5.09	89.45 ± 9.06
Allograft 4 mm	70.98 ± 4.19	29.02 ± 4.19	83.25 ± 10.59

Note. BIC: bone-to-implant contact.

TABLE 3 The mean values of histomorphometric parameters according to different healing time points in each group

Groups	Healing period 4 months	8 months
Autogenous 2 mm		
Bone area	70.85 ± 3.51	53.58 ± 3.21
Marrow space	29.14 ± 3.51	46.42 ± 3.21
BIC	75.32 ± 7.10	52.95 ± 5.28
Allograft 2 mm		
Bone area	54.53 ± 2.67	66.55 ± 2.64
Marrow space	16.31 ± 3.33	33.45 ± 2.64
BIC	68.97 ± 11.25	73.39 ± 4.19
Autogenous 4 mm		
Bone area	85.63 ± 3.59	72.41 ± 5.09
Marrow space	14.36 ± 3.59	27.59 ± 5.09
BIC	95.76 ± 2.97	89.45 ± 9.06
Allograft 4 mm		
Bone area	66.59 ± 2.63	70.98 ± 4.19
Marrow space	21.57 ± 2.78	29.02 ± 4.19
BIC	64.40 ± 4.71	83.25 ± 10.59

numbers of animals is required to truly answer questions about the two techniques.

Using of allograft bone ring technique has several advantages similar to autogenous bone ring. In addition, drawbacks of autogenous bone grafts are avoided. The disadvantages of the one-stage bone ring–implant procedure are that the graft failure also indicates implant failure, and there may be unsatisfactory osseointegration in the bone ring area. In the bone ring technique, there is a fracture risk during harvesting of autogenous bone ring or implant placement with either autogenous or allograft bone ring (Omara et al., 2016; Stevens et al., 2010). Care should be taken during preparation of both autogenous and allograft bone rings. In this study, bone ring fracture was not observed in any groups.

Scarano et al. (2016) used a bone defect model similar to the model used in the present study. They created 7 mm wide and 4 mm height defects around 4 dental implants placed in the iliac crest of sheep. Although there was no negative control group in the present study, Scarano et al. (2016) showed that the defect around negative control implants without any grafting was not filled by newly formed bone and no contact with the implant surface was observed. The results of the present study demonstrated the beneficial effect of autogenous and allograft bone rings for bone regeneration in surgically created vertical bone defects around implants in sheep.

Nakahara et al. (2017) evaluated the osseointegration of implants placed in a single-stage using autogenous bone ring technique compared with two-stage autogenous block bone grafting procedure in the mandible of Beagle dogs. They reported similar results for both groups in terms of osseointegration. In the autogenous bone ring test group, 73.28% and 65.27% BIC values were found after 3 and 6 months of healing, respectively. All groups in the present study showed satisfactory BIC% values after 4 and 8 months of healing (Alharbi et al., 2015; Bayounis, Alzoman, Jansen, & Babay, 2011; Nakahara et al., 2017).

Autogenous bone ring groups (autogenous 2 mm and autogenous 4 mm) showed higher values compared with allograft bone ring groups (allograft 2 mm and allograft 4 mm) in terms of bone area % and BIC % at 4 months of healing period. The results of bone area % and BIC % values in this study demonstrated that allograft bone ring groups showed higher and autogenous bone ring groups showed lower values after osseointegration period of 8 months when compared to 4 months of healing. The higher bone area and BIC values measured at 8 months of healing period in allograft bone ring groups can be explained by complete resorption of allograft and replacement by mature lamellar bone.

The mean bone area percentage was expected to decrease in autogenous bone ring groups because autogenous bone graft around non-loaded implants shows tendency to resorption (Blanco et al., 2013; Nakahara et al., 2016). Moreover, the reason why autogenous 2 mm group showed decrease in terms of bone area % and BIC % at 8 months when compared to 4 months might be high resorption in the submerged 3 mm part of the autogenous bone ring. According to the histological assessment and histomorphometric analysis of the present study, it can be suggested to wait more than 4 months for better bone regeneration when allograft bone ring is used. However, these findings should be interpreted with caution due to the small sample size in this study.

Nakahara et al. (2016) reported similar histological findings and median total bone area in autogenous bone ring and two-stage autogenous bone augmentation at 3 and 6 months of osseointegration period in mandible of Beagle dogs. They concluded that autogenous bone ring technique does not effect regenerative capacity of bone graft compared to two-stage technique.

In this study, all bone rings in four groups were covered with resorbable collagen membrane. Kohal et al. (1998) reported that using of barrier membranes for bone defects can increase the BIC values by preventing ingrowth of soft tissue. Collagen membrane used in the present study may lead to satisfactory bone regeneration and BIC % in all groups (Nakahara et al., 2016; Rothamel et al., 2005).

All autogenous and allograft bone rings fitted securely to the recipient bed, and primary stability of all implants was achieved in the present study. Graft stability during the early phases of bone healing is important for early vascularization and graft incorporation (Marx, 2007).

The findings of the present study showed that bone ring technique is a reliable and predictable alternative procedure for vertical augmentation of alveolar defects that allows the successful simultaneous placement and osseointegration of implants. This technique offers many advantages including one-stage surgery and shorter overall treatment time. Allograft bone ring looks promising in augmentation of surgically created vertical bone defects around implants after 8 months of healing but further studies are required with sufficient sample size in order to evaluate the success of allograft bone ring.

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ORCID

Mehmet Emre Benlidayi http://orcid.org/0000-0002-6102-9136
Ufuk Tatli https://orcid.org/0000-0003-1580-9980
Hüseyin Can Tükel https://orcid.org/0000-0002-6723-0842

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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