Evaluation of Demineralized Freeze- Dried Bone in Augmentation of Buccal Defects during Implant Placement

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Introduction: Bone thickness in the anterior of the maxilla is one of the major concerns for implant placement. The aim of the present study is to evaluate stability of demineralized freeze- dried bone (DFDB) graft for augmentation of buccal defects during implant placement at the anterior of the maxilla using cone-beam computed tomography (CBCT). **Materials and Methods:** The DFDB graft was used for augmentation of buccal defects during implant placement at the anterior of the maxilla. The amount of remnant DFDB was measured in three points: Coronal, middle and apical portion of the buccal sides of implants after one year. **Results:** Twenty-two samples were included in this study. All of the exposed implants were osseo-integrated. A significant difference was detected for remnant grafting bone in the coronal and apical portion of the implants between the central site and the lateral site ($P<0.05$) without any difference in the middle portion. Analysis of data did not show any differences of remnant grafting bone thickness among one-third coronal ,one-third middle and one-third apical portion of the buccal sides of implants after one year *($P>0.05$)*. **Conclusion:** DFDB could be used successfully for augmentation of buccal defects during implant placement. It is assumed that approximately 50% of DFDB is resorbed one year after grafting. The recipient site may influence the amount of resorption rate.

Keywords : Augmentation; Demineralized freeze- dried bone; Graft; Implant; Maxilla

Introduction

The limitation of bone thickness in the anterior of the maxilla and anatomical variations such as concavity of the buccal aspects of the bone may challenge dental implant placement in the proper position to achieve aesthetic results in maxilla region. Defects at the buccal site of implants must be augmented with bone substitute materials (1). Placement of bone grafts or other biomaterials in bony defects adjacent to dental implants promote osseointegration and improve adjacent soft tissue esthetics (2). Autologous bone grafts have been considered the gold standard for reconstruction of the defects adjacent to dental implants (2). However, donor site morbidity, unpredictable resorption patterns and duration of operation are limitations of using autologous bone grafts that lead to application of other bone substitute materials such as alloplasts, xenografts and allografts. Freeze-dried bone is a well-documented bone-grafting material, utilized for oral bone grafting in periodontal bony defects, extraction sockets, maxillary sinus grafts and around dental implants (3). Freeze-dried bone can be mineralized or demineralized. The demineralization process, in removing the mineral phase, exposes the collagen and growth factors, including bone morphogenetic proteins (3). Freeze-dried bone, especially the demineralized type, may stimulate bone formation through osteoinduction or osteoconduction (4). Some early studies showed fibrous connective tissue surrounding demineralized freeze-dried bone (DFDB) particles and no new bone formation (5) and other studies demonstrated incorporation of DFDB particles with new bone and healthy osteocytes (6).

The aim of the present study is to evaluate stability of DFDB grafts for augmentation of the buccal defects during implant placement at the anterior of the maxilla using cone-beam computed tomography (CBCT).

Materials and Methods

This prospective study aimed to evaluate the stability of allograft blocks in reconstruction of the buccal bone defects during placement of dental implants in the anterior of the maxilla using CBCT. The present study was performed from September 2014 to October 2015 in the Department of Oral and Maxillofacial Surgery of Shiraz University of Medical Sciences. Also, current study was approved by the ethics committee of Shiraz University of Medical Sciences. Subjects eligible for the study had a missing tooth at the anterior of the maxilla and by CBCT results demonstrated bone defects in the buccal bone. The minimal bone thickness in all subjects was 4 mm or more. Subjects were excluded from the study if they had a previous bone augmentation by bone substitutes or bone metabolic disease. None of the subjects

Table 1. Correlation of age and remnant grafting bone thickness (RGBT) in various portions of the implants. * indicates significant difference

RGBT (mm)	Age	*P*-value
RGBT in Coronal	1.46±0.31	>0.05
RGBT in Middle	1.58±0.32	>0.05
RGBT in Apical	1.67±0.32	>0.05

Table 2. Evaluation of remnant grafting bone thickness (RGBT) between two sites (Central and lateral). * indicates significant difference

RGBT (mm)	Sites		*P*-value
	Central	Lateral	
RGBT in Coronal	1.36±0.36	1.61±0.14	<0.05*
RGBT in Middle	1.5±0.36	1.7± 0.20	>0.05
RGBT in Apical	1.59±0.38	1.78±0.16	<0.05*

Table 3. Evaluation of remnant grafting bone thickness (RGBT) between two genders

RGBT (mm)	Sex		*P*-value
	Male	Female	
RGBT in Coronal	1.55±0.27	1.20±0.29	>0.05
RGBT in Middle	1.62±0.29	1.58± 0.20	>0.05
RGBT in Apical	1.67±0.29	1.61±0.24	>0.05

Table 4. Comparison of remnant grafting bone thickness (RGBT) in various portions of the implants

Outcome	Coronal	Middle	Apical	*P*-value
RGBT (mm)	1.46±0.31	1.58±0.32	1.67±0.32	*P*>0.05

underwent a fresh socket implant surgery. Informed consent was obtained from the patients. CBCT were taken before implant placement and one year after augmentation and implant placement. A standardized protocol of the NewTom for the extended (15 ×15 cm) field of view (FOV) with 0.3 mm slice thickness and 26.9s acquisition time was used for imaging. Image processing and the measurements were performed by Mimics innovation suite version 15 (Materialise, Leuven, Belgium) bone thickness was measured in one-third coronal, middle or apical of the implant buccal side on the images.

Surgical Procedure

Access was provided by a full-thickness incision following administration of local anesthesia (2% lidocaine with 1:100,000 adrenalin, Daropakhsh, Tehran, Iran). Dental implants (Intenalhex, RBT body, Biohorizons, USA) were inserted 1mm below the buccal bone crest. DFDB blocks (Cerabone; Botiss medical, Berlin, Germany) with 3×5×10 dimensions (height×width×length) were placed on the buccal defect site and fixed with a microscrew (5 mm; Jeil, Seoul, South Korea). A resorbable membrane (Jason membrane, Botiss biomaterials, Berlin, Germany) covered the surgery site. Finally, the flap was closed by suture (5-0 Vicryl, Ethicon Inc, Sint-Stevens-Woluwe, Belgium). Patients were instructed to have soft diet on the day after surgeries and did not chew or bite on the site of augmentation for 3 weeks after the surgery and the implant were exposed six months after the surgery. Figure 1 illustrates the procedure and further evaluation.

Statistical Analysis

The statistical analyses were performed using Statistical Package for Social Sciences (SPSS 20.0.1 for windows; SPSS Inc, Chicago, IL). Continuous variables were demonstrated by mean and standard deviation and discrete variables were expressed as frequencies. Data analysis was performed by ANOVA, independent t-test and Pearson correlation.

Results

Twenty-two patients (12 males and 10 females) with mean age of 37.27±12.06 years were enrolled in this study. All of the exposed implants were osseo-integrated. The mean of remnant grafting bone thickness (RGBT) was 1.46±0.31 mm in the one-third coronal portion of the implants, 1.58±0.32 mm in the one-third middle and 1.67±0.32 mm in the one-third apical (Table 1).There was no correlation between age and the amount of remaining bone substitute materials in the coronal, middle and apical portion of the implants (P>0.05) (Table 2). The mean of RGBT was 1.36±0.36 mm in the one-third coronal portion of the implants in the central and 1.61±0.14 mm in the lateral site. A significant difference was seen for RGBT in the coronal portion between the central and the lateral site (P<0.05). The mean of remnant bone was 1.5±0.36 mm in the middle portion in the center and 1.7± 0.20 mm in the lateral site. Analysis of the data did not show any difference for RGBT between two sites in the middle portion of the implants (P>0.05).

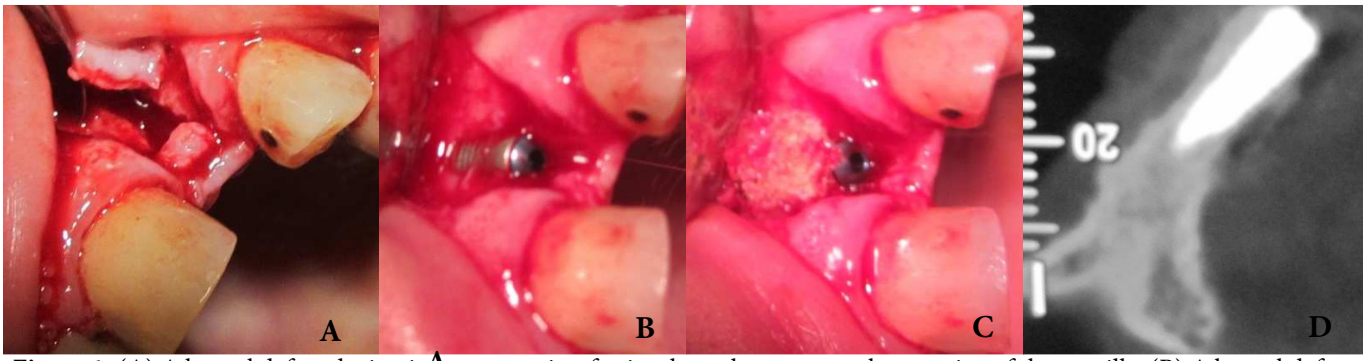

Figure 1. (A) A buccal defect during instrumentation for implant placement at the anterior of the maxilla; (B) A buccal defect during implant placement at the anterior of the maxilla; (C) Using DFDB graft for augmetaion of the defect; (D) CBCT demonstrates remnant grafting bone thickness on buccal site after one year

The mean of remnant grafting bone was 1.59±0.38 mm in the apical portion of the implants in the central site and 1.78±0.16 mm in the lateral site. Comparison of the data demonstrated a significant difference between two sites for RGBT in the apical portion of the implants (P<0.05) (Table 3). An assessment of the data using independent T test did not show any difference between two sexes for the remnant grafting bone in the coronal , middle and apical portion of the implants (P>0.05) (Table 4). Analysis of data did not show any differences of remnant grafting bone thickness among one-third coronal ,one-third middle and one-third apical portion of the implants (P>0.05).

Discussion

Allograft is defined as a tissue harvested from one individual and implanted into another individual of the same species .The use of cadaver bone for grafting is known as bone allograft and it is considered by some to be the best available alternative to autografts due to its similar characteristics. Despite the superior properties of autografts, allografts are usually preferred by the patients because of the problems associated with donor site morbidity. Allografts are obtained from cadaver tissue banks for mineralized freeze-dried bone (FDBA) or DFDB. Both FDBA and DFDB are obtained from the cortical bone of long bones due to its high content of bone inductive proteins and less antigenic activity than cancellous bone. Bone allografts come in various configurations including powder, cortical chips, cancellous cubes, and cortical granules (7). The current widespread use of DFDB is based on the osteoinductive ability of this bone substitute. The demineralization process of the graft exposes the bone inductive proteins located in the bone matrix such as bone morphogenetic protein-2 (BMP2) and BMP7, which are capable of inducing mesenchymal cells to differentiate into osteoblasts *in vivo* (8). DFDB also provides an osteoconductive surface for cell attachment (9).

DFDB forms are processed by acid demineralization in 0.5 to 0.6 molar hydrochloric acid .As a result, 40% of the mineral content is removed leaving the organic matrix intact. This process helps the preservation of the BMPs present in bone and provides the inherent osteoinductive properties (10). Moreover, the collagen matrix present in DFDB acts as a scaffold that provides osteoconductive properties among the osteoinductive compartment. BMPs are associated with the organic matrix of bone and embedded within mineral content, so the demineralization process increases its bioavailability. BMPs induce migration of mesenchymal stem cells and differentiation into chondrocytes and finally lead into endochondral bone formation. Endochondral bone formation is attributed to an osteoinductive response, while intramembranous bone formation is indicative of an osteoconductive response. Nevertheless, osteoinductivity of DFDB has been recently questioned, since it seems that this property is highly dependent on manufacturing procedures (11).

The stability of DFDB in reconstruction of the alveolar ridge and periodontal defects is unknown (12). Our study demonstrated a successful use of DFDB for reconstruction of buccal defects during implant placement. Three points of measurement using CBCT demonstrated that the resorption rate was approximately 50% in one year after grafting. In augmentation of buccal defects by DFDB, the resorption rate was similar in the coronal, middle and apical portions of the implants.

Comparisons of FDBA and DFDB exist, and again, varied results have been shown. Since FMB is mineralized, it may calcify faster than DFDB. Sinus lifts where FMB was utilized resulted in harder bony substance when compared to DFDB, which resulted in cartilage formation after 6 months (13). DFDB can be used as a grafting material both alone and in combination with autogenous bone (14). Schwartz *et al.*, showed that some commercial preparations of DFDB are inactive, due to the lack of adequate quantities of BMP (15). Other studies have questioned the continued use of unsupplemented DFDB as an implant material for induction of bone adjacent to periodontal defects or dental implants (16). A study was performed to investigate the bone induction potential of human DFDB in large dogs. The histologic results

of a study on osteoinductive capability of DFDB in dogs demonstrated that bone chips were non vital, occasionally surrounded by woven bone, and appeared to break up and then remineralize without the presence of osteoclastic and osteoblastic activity (17). Another study investigated effect of allogeneic, freeze-dried, demineralized bone matrix on guided bone regeneration (GBR) in supra-alveolar peri-implant defects in dogs. The results suggested that DFDB did not enhance GBR in bone defects and had a limited potential to increase alveolar regeneration in this defect model furthermore the 16-week healing interval showed insufficient bone formation and maturation of demineralized bone with GBR (18). Several studies have suggested using platelet-rich plasma (PRP) with allografts to enhance osteoinductivity potential (3, 19). Use of a combination of two materials in two layers was introduced by Buser *et al.,* They applied autogeneous chips with a layer of xenograft for contour augmentation in concomitant with implant placement. They concluded that the risk for mucosal recession is low with early implant placement. In addition, contour augmentation with GBR was able to establish and maintain a facial bone wall in all 20 patients (20).

Conclusion

DFDB could be used successfully for augmentation of buccal defects during implant placement. It seems that approximately 50% of DFDB is resorbed one year after grafting. While the resorption rate was not changed on various parts of implants when a buccal wall was augmented totally.

Conflict of Interest: 'None declared'.

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