Volumetric Changes in Anterior Maxillary Reconstruction using DBBM Associated with PRF Membrane and Titanium Mesh – case series

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Abstract

Aim: This study aimed to assess the horizontal and volumetric bone augmentation obtained by using deproteinized bovine bone mineral (DBBM) associated with platelet-rich fibrin (PRF) membrane and titanium mesh at anterior atrophic region of the maxilla.

Materials and methods: Eight healthy patients requiring horizontal and vertical bone reconstructions were included. The amount of horizontal bone gain was measured on computed tomographic images and the thickness at three levels (7,11,13 mm from the crest) before and six months after reconstructive surgery. Each graft was plotted on sagittal image at the mid-face by using the OnDemand 3D Dental TM software and volumetric analysis was performed by using the InVesalius 3.0 software.

Results: The initial height was 15.61 ± 4.3 mm and the final one was 17.94 ± 5.2 mm ($p = 0.001$), resulting in a gain of 2.33 mm and the initial thickness 5.962; 6.762; 7.918 (7,11,13 mm from the crest) change to 8.627; 10.05; 10.63 respectively. The volume increased 42.939± 16.856 mm^3, which corresponds to a variation of 45 - 160%. depending on the defect. A total of 20 implants were successfully installed.

Conclusion: The use of DBBM, PRF and titanium mesh is a safe choice with promising results.

Keywords: Deproteinized bovine bone mineral; platelet-rich fibrin; implantology; bone augmentation; titanium mesh.

Introduction

Oral rehabilitation with dental implants is an effective treatment for replacing lost teeth with high success and survival rates. However, long-term success and stability of the implants are directly related to the quality and quantity of bone available at the implant site (Urban, 2019 *et al*; Roca-Millan *et al*., 2020).

The great challenge of reconstructing the atrophic anterior maxilla prior to rehabilitation with implants lies in the long period of treatment and multiple surgical procedures (Roca-Millan *et al*., 2020). Besides the wide variety of clinical outcomes, they are reported when several vertical bone augmentation techniques and different biomaterials are used to restore the aesthetic zone in the maxilla (Demetriades *et al*., 2011).

Among the available options, autogenous bone graft is still considered as gold standard for these reconstructions (Barone and Covani, 2007; Myamoto *et al*., 2012). However, the use of autogenous bone has disadvantages, such as need for another donor surgical site, higher morbidity, increased risk of infection and psychological factor of the patient.

Earlier studies have demonstrated that deproteinized bovine bone mineral (DBBM) is a well-accepted alternative biomaterial to autogenous bone graft not only due to its increased clinical and histological improvement in the long term, but also due to the high

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implant survival rates and well-established biological properties (Artzi *et al.*, 2001). Although the majority of studies reported the use of DBBM in association with autogenous bone graft, regardless of the ratio some studies found high volumetric stability and newly bone formation when DBBM was used alone as autogenous bone substitute (Starch-Jensen *et al.*, 2020; Kim *et al.*, 2020).

The role of the platelet-rich fibrin (PRF) in bone augmentation is not well understood as there are contradictory findings in the literature (Tatullo *et al.*, 2012; Yoon *et al.*, 2014). However, the association with PRF requires less quantity of graft material and one can also speculate that biological properties are improved at the site. In addition, PRF membranes can prevent graft exposure, which is the most common complication in bone reconstruction procedures. For consolidation of large three-dimensional bone defects, titanium meshes are well known for functioning as a mechanical scaffold and providing stability of the graft used (Hartmann *et al.*, 2019).

To our knowledge, there is no study in the literature investigating the association between deproteinized bovine bone mineral graft without autogenous bone and PRF with titanium mesh for reconstruction of atrophic anterior maxillary.

Therefore, the aim of this study was to assess the horizontal and volumetric bone augmentation obtained by using DBBM associated with PRF membrane and titanium mesh at anterior atrophic region of the maxilla, previous to the placement of osseointegrated implants and subsequent prosthetic rehabilitation.

Materials and Methods

This study was submitted to and approved by the Research Ethics Committee of the University of Santo Amaro according to protocol number CAAE 09095619.0.0000.0081.

This is study with nine healthy male and female patients over 18 years old at the time of the surgery, with most being partially edentulous patients needing bone reconstruction in the maxilla. Only patients with three-dimensional bone defects were included.

The following inclusion criteria were used: being older than 18 years old, presenting edentulous anterior maxilla with bone defects and insufficient bone to place osseointegrated implants, having no disease or systemic condition and having proper oral hygiene. The exclusion criteria were the following: being pregnant or lactating, presence of any systemic impairment, use of any medication that could interfere with bone metabolism, medical and general contraindications for the surgical procedure, and being heavy smokers (> 10 cigarettes/day).

All patients included in the study underwent tomographic examination before the surgical procedure and after six months (post-surgery) to remove the titanium mesh and place osseointegrated implants.

Surgical Procedure

All PRF membranes were prepared according to described elsewhere (Choukroun *et al.*, 2017). Four to six 10-ml blood collection tubes were used for collecting samples from each patient according to the defect size. Peripheral blood was collected into a white Vacutainer tube (VACUETTE* Intralock) and centrifuged (IntraSpin™, Intralock Iberia) at 2700 rpm for three minutes. After centrifugation, 3 ml of the supernatant was pipetted with a sterile syringe and stored. Next, 2 to 4 red Vacutainer tubes (BD Vacuteiner ®) were used as needed for sample collection and then centrifuged at 2700 rpm for 12 minutes. Once the PRF membranes were obtained, two ones were cut with scissors and mixed with DBBM, resulting in a biomaterial mixture to which was added the stored supernatant.

Midline incision was made at the recipient site with intramuscular and vertical releasing incisions, and after achieving a full-thickness resection, the flap was reflected and the graft site was prepared. Perforations were made in the primary bone with a drill so that it could have blood supply. Bio-Oss® (Geistleich Pharmaceutical, Wollhausen, Switzerland) was used for all grafts, in which it was combined with the PRF membrane before being placed onto the prepared site. The titanium mesh was fitted over the screw to ensure that the grafted material is stable and supplied. Each mesh was attached to the residual bone with titanium osteosynthesis screws. After adaptation of the titanium mesh, PRF membranes were placed over it so that sutures could be made in the region and tissue adaptation occurs with no tension in the surgical area. All patients were treated with Amoxycycline 500 mg (Medley, Campinas, Brazil) (3 times daily) for seven days, Decadron 4 mg (Achê, São Paulo, Brazil) (2 times daily) for three days and dipyrone in case of pain. All surgical procedures were performed by the same experienced implant doctor.

Linear Analysis

The images of the patients' maxillary arch were acquired by using a PreXion scanner (X Trillion Inc, Tokyo, Japan), which was operated with a cubic field of view of 8 cm^3, 90kVp 4mA and exposure time of 37 seconds for the best image quality. Later, the images were exported in DICOM format (Diagnostic Imaging and Communication in Medicine) with voxel size of 0.160-mm. The amount of bone augmentation was determined by measuring the bone graft before surgery and postoperatively at six months (Figure 1).

All measurements were made by a previously trained and calibrated examiner and whose calibration protocol followed similar methods described by Araujo *et al*., 2003. Data analysis was performed to determine intra-examiner reliability in which the standard error of measurement was used for continuous variables. The examiner was considered calibrated when the standard error of measurement was ≥ 0.8.

All files were exported in DICOM format to allow visualization with OnDemand 3D DentalTM software (Cybermed Co., Tustin, USA) and measurements. Firstly, the images were captured and saved with DICOM system. Next, the patient's head angular position (Axis & Reslice) was determined by a tracing an arch/curve over the region to select the area of interest for measurements.

The presence of teeth adjacent or opposite to the toothless area can help in determining the position measured in both situations. Each graft was plotted on sagittal image at the mid-face by using the OnDemand 3D DentalTM software. Figure 2 exemplifies how this measurement was performed.

Next, a perpendicular line was drawn along the residual bone and three horizontal lines were recorded in width at distances of 7, 11 and 13 mm from the crest, as shown in Figure 2 (adapted by Monje *et al*., 2015).

Figure 1. Axial section showing the region traced with the arch/curve and selection of the area of interest.

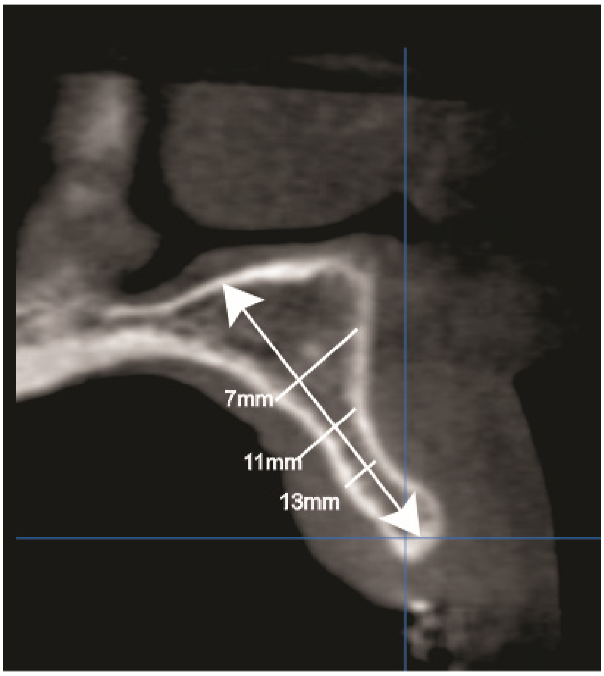

Figure 2. Representation of linear measurements of the surgical area on CT image.

Volumetric Analysis

The DICOM files of each patient were exported to the InVesalius 3.1.1 software (2017) (Center for Information Technology Renato Archer, Campinas, SP, Brazil). With this software, it was possible to assess the anatomical structures three-dimensionally based on a set of two-dimensional images, which were acquired with computed tomography, thus allowing the generation of three-dimensional models of the regions of interest. After reconstructing the DICOM images three-dimensionally, the software allows the generation of 3D files in STL format.

The images were exported as a DICOM file by using the software's option for importing DICOM file. The file was selected so that it opened in a multiplanar view, in which four slices were selected (i.e. axial, coronal, sagittal and volume slices). Next, the threshold volume was adjusted for selection of the region of interest and volumetric measurement after the surface was created. These data were obtained from DICOM files before and after the bone grafting procedure in each patient, with the difference being calculated to obtain the volumetric gain.

All measurements were made by the same operator in triplicate and the average was recorded. The resulting data were analysed by using the GraphPad Prism 5.0 statistical software. The differences in the height and width between baseline and reopening surgery were compared by using paired t-test. The significance level was P= 0.05 for all analyses.

Results

A total of eight patients, five patients male and three female, age ranging from 34 to 69 years old (48.00 ± 11.48) were included in the study for implant placement, Eight areas were selected totalizing 16 tomographic images. The demographic data are shown in Table 1 and the initial measurements of height and thickness are shown in Table 2.

In the first surgical procedure patients had mild edema and reported moderate pain; one patient had gingival dehiscence without exposure of the titanium mesh at 2 weeks post-operatively, but there was no compromise of the graft, with healing after 4 weeks. No sequelae at the time of mesh removal was observed and there was no problem with implant placement either.

Table 1. Demographic data of the patients and areas evaluated in the study.

Patient	Age	Gender	Area	Smoking	# implant	Cause of absence
1	43	M	12, 11, 21, 22	No	2	Trauma
2	69	M	13, 12, 11	Yes	2	Fratura
3	49	M	12, 11, 21, 22	No	3	Caries
4	56	M	21, 22, 23	No	2	No information
5	42	M	11	No	1	Trauma
6	36	F	12, 11, 21, 22	No	4	No information
7	34	F	11, 12	No	2	No information
8	55	F	13,12,11, 21, 22, 23	No	4	Caries

Table 2. Data on bone height and thickness (in millimeters) before and after the grafting procedure (mean and standard deviation).

Before				
	Heigh 1	**Thickness 1**		
		7	11	13
N	27	27	26	18
Minimum	9.18	2.22	3.16	3.2
Maximum	21.61	11.55	10.52	12.79
Mean	14.52	5.962	6.762	7.918
Std.Deviation	3.136	2.487	1.979	2.536
Std. Error	0.6034	0.4786	0.3882	0.5977
After				
	Heigh 2	**Thickness 2**		
		7	11	13
N	27	27	27	24
Minimum	12.24	5.27	6.17	6.54
Maximum	25.05	13.48	15.93	15.57
Mean	16.76	8.627	10.05	10.63
Std.Deviation	3.497	1.681	2.169	2.279
Std. Error	0.6731	0.3234	0.4175	0.4652
P value	0.0001	0.0002	<0.0001	<0.0001

Std. D- standard deviation, mm- milimeters.

After 6 months a new tomography was evaluated before implant placement. Table 2 shows the height and thickness after the grafting. Reopening surgery for removal of the titanium mesh was performed after six months. A total of 20 implants were placed after removal of the surgical mesh and the success rate was 100%.

Volumetric measurements are shown in Table 3. Data were obtained before and after the grafting procedure. We can observe in the reconstructed areas a volumetric gain found on average of 42.939.362 ± 16.856.559 mm3, which corresponds to a variation of 45 to 160%.

In Figures 3A, B one can observe the region of the bone defect linearly, which guaranteed us to make the measurements to assess the bone gain. Figures 4 and 5 show the volume of the regions before and after the grafting procedure, whereas Figure 6 shows the region of the volumetric gain after it.

Table 3. Graft volume changes before and after the surgery (data in mm^3).

Patient	Volume Before	Volume After	Difference	% Of Gain
1	71.688	104.533	32.844	45.81
2	38.733	102.496	63.763	164.62
3	29.684	77.153	47.469	159.91
4	32.922	68.447	35.525	108.52
5	34.367	52.412	18.044	52.50
6	90.563	126.932	36.368	40.16
7	52.138	121.654	69.516	133.33
8	58.377	98.360	39.982	68.49
Media	51.059	93.998	42.939	97.000
Std. Deviation	21.571	25.960	16.856	51.583

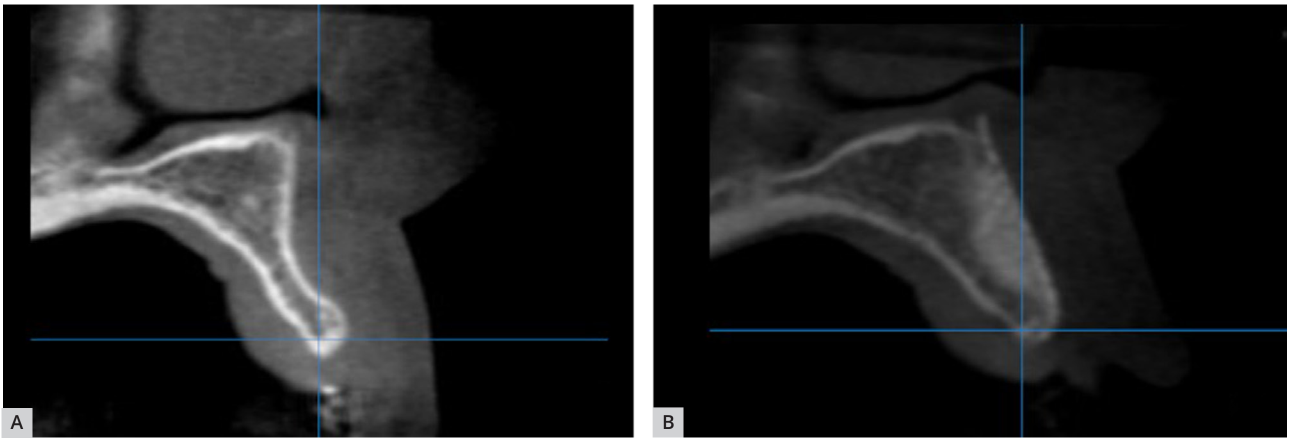

Figure 3. A) Axial section showing the region traced with the arch/curve, election of the area of interest and linear measurement. B) Representation of the final tomographic image and linear measurement.

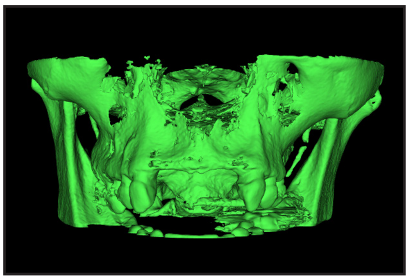

Figure 4. 3D Representation of the tomographic image showing volumetric result prior to surgery.

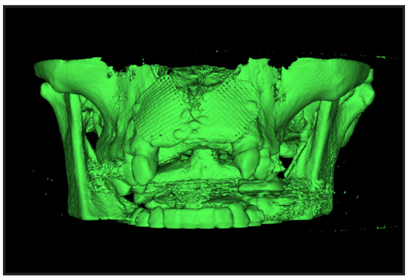

Figure 5. Representation of the final tomographic image showing volumetric result, titanium mesh and screws.

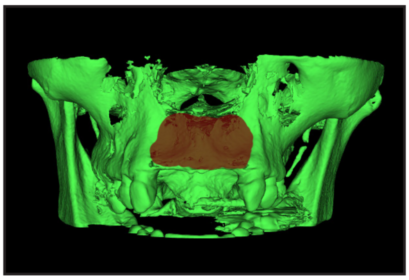

Figure 6. Final image showing grafted region superimposed on the defective area. Subtracted image.

Discussion

This study aimed at assessing reconstructions of the anterior atrophic region of the maxilla, in which DBBM was used in association with fibrin-rich plasma (PRF) membrane and titanium mesh in order to increase the bone volume, which is necessary for placement of osseointegrated implants and subsequent prosthetic rehabilitation. The success rate was 100% as all patients had prosthetic rehabilitation according to the proposed planning. Computed tomography, in turn, provides a three-dimensional image and consequently enables a more detailed diagnosis. This study assessed the bone gain linearly and volumetrically before and after the grafting procedure.

Bone is a complex connective tissue structure under constant remodeling, including cycles of bone formation and resorption. Bone tissue has considerable potential for healing, regeneration and resuming its function after injury. The healing process begins immediately after trauma to the dentoalveolar region or after a tooth extraction. After osteoblast differentiation, osteogenesis begins at the site of injury (Esfahanizadeh *et al.*, 2019).

Vertical and horizontal regeneration of resorbed alveolar ridges remains a challenge, especially in cases where bone atrophy is extensive. During the past few years, different augmentation techniques have been proposed to restore an adequate bone volume.

In a prosthetic rehabilitation, the bone quantity must be sufficient for implant placement. The anterior region of the maxilla is prone to resorption in a centripetal direction, meaning that deficiency in bone width after tooth loss is very common (Gultekin *et al.*, 2016).

Radiographic examination can be useful to assess the amount of bone remaining and the success of dental implants. Conventional two-dimensional radiographs, such as panoramic and periapical radiographs, have some limitations in assessing the success criteria for dental implants. In fact, radiographs cannot assess bone changes in the buccal-lingual direction, nor can accurately show bone resorption in the mesial-distal direction (Lim *et al.*, 2020). CT scans, in turn, provide a three-dimensional image and enable a more detailed diagnosis. Through comparative studies of these patients, we were able to verify the bone gain of the graft and also volumetric changes.

Autogenous bone is considered the gold standard graft for bone regeneration, having the advantage of using the patient's own cells, growth factors and biomolecules necessary for osteogenesis and biocompatibility, in addition to the highest degree of biological safety and mechanical properties. A limitation of its use requires a second surgical area, which increases morbidity, pain and possible complications due to longer surgery time. In the cases of bone replacement grafting, a higher rate of resorption has been reported, which is not predictable. Autogenous bone also has the limited availability of volume to be used (Fernandez de Grado *et al.*, 2018; de Azambuja *et al.*, 2019). Autogenous graft seems to have a significantly higher resorption rate compared to xenograft, that is, 24.4% versus 49% on average, respectively. This is a very relevant information when planning which type of bone grafting should be chosen for a graft surgery (de Azambuja *et al.*, 2019; Kim *et al.*, 2020).

The choice to use DBBM without association with another material was made based on advantages allowed by the type of bone, such as easy availability, osteoconductive properties and less morbidity. Other studies using DBBM in association with other materials reported good results, including lower risk of infection compared to autogenous bone grafting (Jun *et al.*, 2014).

In the literature, DBBM is the most documented graft biomaterial for craniomaxillofacial surgery. The great advantage of DBBM is that it resembles human bone both in terms of architecture and geometry, although it depends on the origin and manufacturing process. In addition, this biomaterial has the clinical advantage of having a slow bio-absorbability, which can be very interesting to preserve the desired bone volume. As a disadvantage, DBBM lacks biological components, which limits its biological activity and poses a biological risk with potential for disease transmission (e.g. prions and retroviruses) or host immunogenic response, although these can be minimized by the manufacturing process (Fernandez de Grado *et al.*, 2018).

According to a study by Jun *et al.* 2014, Bio-Oss showed a good healing pattern and excellent maintenance of graft volume, allowing new active bone formation. Histomorphometric evaluation of a biopsy sample after four months of grafting showed osteoblasts covering the newly formed bone and accumulation of osteoids.

Several studies on the use of autogenous bone associated with bovine inorganic bone were performed, showing good results for bone augmentation (Pieri *et al.*, 2008; Monje *et al.*, 2015; Jegham *et al.*, 2017; Starch-Jensen *et al.*, 2020). ,A study conducted by our group using Bio-Oss and autogenous bone at different proportions demonstrated that dimensional stability was achieved with 25% of Bio-Oss, which was superior to the use of autogenous bone in an experimental model (Kim *et al.*, 2020).

In the present study, DBBM grafts were used without association with autogenous bone and the results obtained were satisfactory, being close to those reported elsewhere regarding autogenous bone or its association with xenogen bone. The patients evaluated showed a volumetric gain varying from 45 to 160% depending on the case.

Only one case report of bone reconstruction in a cancer patient was found in the literature, in which bone grafting was performed using DBBM associated with titanium mesh and PRF membrane. Xenogeneic bone was very effective for large areas of graft, showing to have very promising results. After eight months from the surgery, the titanium mesh was removed for placement of six implants and 6-month rehabilitation was prescribed, giving the patient a better quality of life (Lorenz *et al*., 2018).

The use of the PRF membrane covering the titanium mesh was chosen due to the risk of mesh exposure, with the PRF membrane protecting the region and minimizing the risks of contamination of the grafting material. Studies on PRF membrane have shown that its action on soft tissues can accelerate the repair process while minimizing contamination (Dohan *et al*., 2006; Miron *et al*., 2017).

The mesh used in the craniomaxillofacial region for regenerative interventions requires some properties such as biocompatibility, biological activity, occlusive properties, porosity, mechanical properties, tissue integration, exposure tolerance and biodegradability. Titanium is the most widely used material in dentistry due to its properties, such as biocompatibility, high strength, rigidity for space maintenance, low density and weight, capability to withstand high temperatures, and corrosion resistance (Artzi *et al*., 2003; Sanz *et al*.,2019; Briguglio *et al*., 2019). The results of the study by Cucchi *et al*., 2019 show that the use of titanium mesh can improve the quality of the regenerated bone and reduce the pseudo-periosteal layer.

A major complication of using titanium mesh is that exposure occurs during the healing period, which could result in infection and compromise the results. The reason why graft contamination does not occur with the titanium mesh is that it allows blood supply from the periosteum to the grafted bone (Miyamoto *et al*., 2012). In their study, exposure was found in eight meshes, of which four were removed due to infection. In addition, they reported partial bone resorption with mild infection in five cases. Louis *et al.* 2008 reported exposure of 23 meshes in 44 treated patients (52%), with only one case of graft failure with successful bone graft procedure, which resulted in 97.72%. Hartmann *et al*., 2019 evaluated 70 grafting procedures and reported 37% of exposures of titanium mesh, with implant placement not being possible in two cases only. In the present study, a small exposure to gingival tissue was observed in one case, resulting in 12.5% of exposures, which did not interfere with bone reconstruction and rehabilitation of the patient.

The present clinical study showed satisfactory gains in linear and volumetric bone by the use of titanium mesh associated with DBBM and PRF, based on the clinical and tomographic results, meaning that it can be a good alternative to autogenous bone graft. In addition, follow-up studies are needed to assess whether osseointegration in this type of bone augmentation can be maintained in the long term.

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