
Grafting heterologous bone blocks in the atrophic anterior maxilla as an alternative option to autogenous bone. Preliminary short-time results from a split-mouth prospective study

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KEYWORDS

Cone beam computed tomography, Guided Bone Regeneration, Heterologous bone, Volume change.

ABSTRACT

Aim *This clinical study was designed to investigate the clinical behavior and assess the volumetric dimensional changes of a cancellous heterologous bone graft, when used to treat anterior maxilla bone defects, in comparison to cortical-cancellous autogenous bone blocks.*

Material and Methods *Seven patients presenting with bone atrophy of the anterior maxilla area received two block grafts in a split mouth design: an autogenous bone block collected from the ascending ramus and an equine-derived bone block. Cone beam computed tomography (CBCT) scans were recorded before the graft surgery (T0), 15 days (T1) and 6 months after the grafting surgery (T2). Bone and graft volume were analyzed using the SimPlant Crystal Pro® software (Materialise, Lueven - Belgium).*

Results *No complications were observed and all implants could be placed into the planned positions. Equine-derived bone blocks showed a significant smaller volume reduction than autogenous ones (9.2±1.6% vs.14.6±1.2%, respectively, p<0.05).*

Conclusion *Preliminary results of this clinical study suggest that heterologous bone blocks might be used as a valid alternative to autogenous, intra-orally collected, bone block grafts to obtain sufficient bone volume for the reconstruction and rehabilitation of the atrophic anterior maxilla.*

Introduction

After tooth loss, progressive and irreversible alveolar bone resorption occurs in all regions of the maxilla and mandible, resulting in the reduction of the height and width of the alveolar ridge. Such atrophic process is more enhanced in the first year following the onset of edentulism, and continues over the following years at a slower rate (1, 2). The resorption rate of the buccal cortical plate is greater than that of the palatal one, resulting in the displacement of the center of the alveolar ridge to a more palatine position (3). The extent of bone loss may be such that restoring, at least partially, the volume of the alveolar ridge becomes necessary to achieve rehabilitation through osseointegrated implants (4). Different grafts (allogeneic, heterologous and alloplastic materials) have been used to recover the bone volume required for implant placement (5), even though autogenous bone is still considered the gold standard for bone grafting (2, 6-8). Autogenous grafts may be collected either from intraoral or extra-

oral sites, according to the extent of augmentation. Disadvantages in using autogenous bone involve the need for a second surgical site, implying additional risks of vascular and neurological injuries, infection, and increased postoperative morbidity. Additionally, the limited amount of donor tissue that may be collected at the donor site may pose a limit to the augmentation surgery, or forcing the surgeon to collect autogenous bone from extra-oral sites, with additional risks (2, 6-8). The use of alternative grafts in the treatment of atrophy of the jaws aims to prevent most of the disadvantages related to autogenous grafts. Heterologous bone grafts represent a promising alternative as they share their architecture and mineral composition with those of human bone (9,10) providing them with similar osteoconductive properties. Yet, they usually do not exert any osteoinductive effect (5). Their biological and mechanical properties depend strictly on the different methods of processing, and on the origin of, the xenogeneic bone (11). Their use may



Figure 1 Patient presenting atrophic anterior maxilla, calling for horizontal ridge augmentation

also allow overcoming possible allergic and antigenic reactions to allogeneic bone (12, 13) whose availability is anyway strictly dependent on a sufficient number of donors and on properly functioning tissue banking. Among heterologous bone grafts, a form of enzyme-deantigenic equine bone has been used successfully for bone reconstruction both in fields different from oral surgery (14) and in a wide range of oral applications (15-20) including augmentation surgeries using bone blocks (21-24). Yet, to the Authors' knowledge, still no prospective clinical studies have been published comparing directly in the same patients the effectiveness of such heterologous bone blocks to that of autogenous bone blocks in the augmentation of the atrophic maxillae. Therefore, this work aims to report the preliminary short-term results of a split-mouth clinical study that was specifically designed to assess the clinical effectiveness, and the post-grafting volumetric dimensional changes of such cancellous equine-derived, enzyme-deantigenic heterologous bone blocks grafts when used to treat horizontal defects of the anterior maxilla, in comparison to intra-orally collected autogenous bone blocks.

Materials and methods

Study design

The present prospective split-mouth clinical study involved recruiting patients among those presenting at the Oral Implantology Clinic, University of Guarulhos, Sao Paulo, Brazil, with horizontal atrophy of the anterior maxilla and requiring implant-supported rehabilitation. Patients were included in the present study only if presenting horizontal bone atrophy, having a residual bone thickness <3 mm as measured in the cross-sections of cone beam computed tomography (CBCT) scans at the first visit before surgery (baseline, T0), (Fig. 1). Additionally, patients had to be non smokers, at least 21 years old,

and presenting general good health conditions while showing no general contraindications to regenerative bone surgery. The protocol design involved grafting one side of the anterior maxilla with an autogenous cortical-cancellous bone block collected from the ascending ramus of the maxilla (control graft), and the other side with a heterologous bone block (test graft). Patients provided their informed consent to the treatment. The clinical investigation was approved by the local Ethical Committee.

Heterologous bone blocks

The heterologous bone blocks (Cancellous Blocks, Bioteck, Arcugnano, Vicenza, Italy) were collagen-preserving, enzyme-deantigenic, equine-derived bone blocks. In short, to achieve such bone blocks equine femurs are subjected to sectioning to blocks of the desired size and shape. These subsequently undergo a chemical-physical antigens elimination process. The process (Zymo-Teck, Bioteck, Arcugnano, Vicenza, Italy) involves using hydrolytic enzymes to get the selective degradation of antigens, while preserving useful molecules such as bone collagen that, in order to be made non-antigenic, undergo only C- and N-terminus cleavage. Additionally, as the process occurs at relatively mild temperatures (<50°C) the chemical-physical properties of bone apatite are not altered. Finally, bone blocks are sterilized by beta-irradiation and may be stored in their hermetic packaging at room temperature for five years.

Surgical protocol

The patients received the following medication: amoxicillin 875 mg, for 7 days; nimesulide 100 mg/12h for 4 days, starting one hour before surgery; and paracetamol 750 mg/6h.

A local anaesthetic (2% mepivacaine with 1:100,000 epinephrine, DFL®, Rio de Janeiro, Brazil) was administered, and a muco-periosteal flap was elevated. The side to be grafted with the heterologous bone block was subjected to random choice by tossing a coin. The heterologous bone block was first shaped using rotating burs under irrigation, and the receiving site prepared by drilling holes in it to help bleeding. The block was then further adapted to the receiving site to achieve the maximum possible intimate contact and fixed with two self-drilling osteosynthesis screws, 1.5 mm in diameter (screw head - square key) and with variable lengths of 8, 10, 12 and 14 mm, which promoted initial stability (Fig. 2a, 2b). Bone blocks managed to withstand a certain degree of compression that allowed to reduce the gaps between the block and the receiving site. In addition, granular heterologous material (Cancellous-Cortical Mix Granules, Bioteck, Arcugnano, Vicenza, Italy) was used for additional bone augmentation and

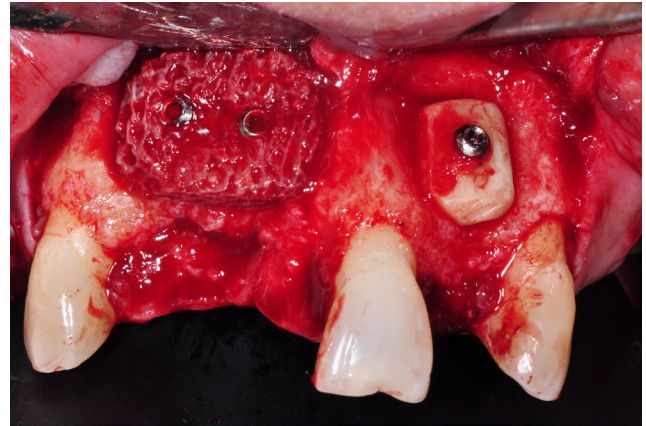


Figure 2 The heterologous cancellous block (left) and the autogenous cortical-cancellous block (right) are adapted to the receiving sites (a) and fixed with self-drilling screws (b)

further fill any residual gap between the block and the patient's bone (Fig. 3). Finally, a collagen membrane (Biocollagen, Bioteck, Arcugnano, Vicenza, Italy) was used to cover the grafted site and protect it from soft tissue invasion while regenerating (Fig. 4).

An autogenous bone block was then collected from the ascending ramus of the mandible using round burs under copious irrigation with saline solution and grafted at the contralateral side, using the same fixation system (Fig. 2). Particulate autogenous bone, collected at the receiving site during its preparation with a bone scraper, was used to perform additional bone augmentation and further fill any residual gap between the block and the patient's bone (Fig. 3). Again, a collagen membrane (Biocollagen, Bioteck, Arcugnano, Vicenza, Italy) was finally used to cover the grafted site at the control side (Fig. 4). The flaps were repositioned without tension and sutured.

The patients were instructed to rinse with a solution of chlorhexidine digluconate (Periogard, Colgate-Palmolive, São Paulo, Brazil) twice a day for 10 days, starting two days after surgery.

A cone beam computed tomography (CBCT) scan was collected two weeks after the surgery (T1) in order to evaluate the adaptation and volumetric dimensions of the bone grafts. All patients were monitored monthly up to the dental implants placement. At six months after the grafting surgery (T2) a CBCT scan was collected in order to plan the implant surgery and to assess the volumetric changes of the onlay bone grafts. Implants were placed thereafter.

At implant surgery, antibiotic prophylaxis, pain management, anesthesia were performed as previously described. After elevating a mucoperiosteal flap, the grafted zone was assessed. Implants were then placed according to the manufacturer instructions and left submerged. Patients are currently controlled on a monthly basis. The protocol study involves following them up to five years after implant placement.

Graft success criteria

The graft was regarded as successful only if all the following were observed:

- 1 no change of the overlying soft tissues during the healing period,
- 2 no reported pain or discomfort,
- 3 stability of the graft after the removal of fixation screws,
- 4 absence of soft tissue between the graft and recipient bed,
- 5 no separation of the graft at the time of implant installation,
- 6 no sign of infection during the healing period.

Three-dimensional volume analysis and statistics

Three-dimensional bone volumetric analysis was carried out exporting the CBCT scans dicom files and analysing them with the SimPlant Crystal Pro® 3D analysis software (Materialise, Leuven - Belgium). The volume of each block graft (either heterologous or autogenous) of each patient at 15 days (V1) and at 6 months after grafting (V2) was calculated in cubic millimeters (mm³) as previously described (25-27). For each block, the volume change (V2-V1) between T1 and T2 was calculated. In order to investigate any differences in the dimensional change between the two kind of blocks, i.e. heterologous and autogenous, volume changes for each block type were averaged and compared by means of a Wilcoxon t-test for paired data. Difference was regarded as significant if $p < 0.05$. Statistical calculations were performed using a standard analysis software (Excel 2010, Microsoft, Redmond, USA). Values are provided as mean \pm standard deviation (SD).

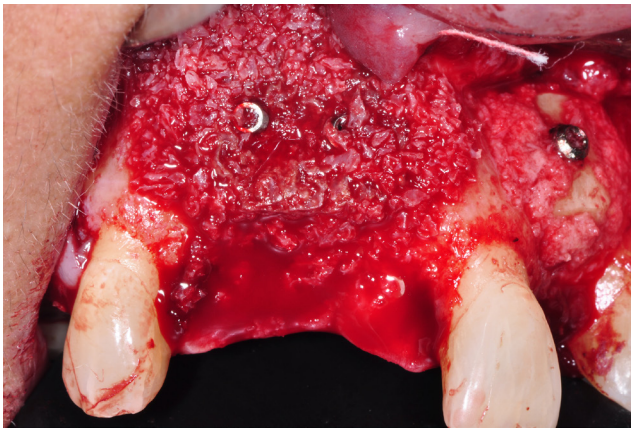


Figure 3 Cortical-cancellous heterologous and autogenous bone granules are used to fill any gap respectively between the heterologous and the autogenous blocks and the receiving site and to further augment the ridge



Figure 4 A collagen membrane is placed over the grafted sites to protect them from soft tissue invasion

Results

Preliminary data reported in the present study regard seven patients, five women and two men, aged 34-65 years. Concerning the grafts, no complications were observed after the grafting surgeries, either at the test or at the control sites, and all were successful according to the above mentioned success criteria. All blocks were stable, well vascularized and incorporated (Fig. 5a-c). A certain degree of variability concerning bone block remodeling and/or incorporation could be observed between the groups and among the subjects. Resorption was lower around the head of the screws. Ridge thickness augmentation was observed in all patients, allowing for placing all implants in the pre-planned positions. All grafts remained stable during implant placement, without fracturing or separating from the pristine bone bed. A total of 16 dental implants were installed on the grafted areas. At T1, 15 days after the grafting surgery, the average bone block volume was $10.22 \pm 1.72 \text{ mm}^3$ and $11.00 \pm 1.11 \text{ mm}^3$ for the heterologous and autogenous blocks

respectively ($p > 0.05$). At T2, 6 months after the grafting surgery, a significant decrease in graft volume could be observed for both blocks type, as the average volumes were equal to $9.28 \pm 1.57 \text{ mm}^3$ and $9.39 \pm 1.16 \text{ mm}^3$ for heterologous and autogenous bone respectively ($p < 0.05$ with respect to T1). The percent dimensional changes occurred between T1 and T2 were $9.2 \pm 1.6\%$ and $14.6 \pm 1.2\%$ for heterologous and autogenous bone blocks respectively, showing a significantly higher resorption of the autogenous bone blocks ($p < 0.05$).

Discussion

The present clinical report preliminarily shows that equine-derived, enzyme-treated heterologous bone blocks may provide results comparable to those of autogenous bone, at least on a short time basis, when used to regenerate the atrophic anterior maxilla. The greater volume change showed by the autogenous bone was expected, according to the known healing process of onlay bone grafts, as the incorporation process of non-segmental bone grafts involves graft resorption until graft vascularization is complete. Sometimes, this bone volume contraction may hinder or make implant placement impossible, or make some additional bone graft material at implant placement necessary. These issues deriving from using autogenous bone have gathered increasing attention from investigators over the recent years. Even morbidity, especially when a large amount of autogenous bone is needed to regenerate large bone defects, is one of the most serious disadvantages in using autogenous bone. Using allografts, on the other hand, rise the concern of a theoretical residual risk of disease transmission and antigenicity particularly if fresh frozen allogeneic bone is concerned, even though such risk should be minimal provided that the donor's bone is properly processed (6). Being subjected to final sterilization, heterologous bone grafts do not pose problems concerning any possible residual microbial contamination. Additionally, having being subjected to antigen elimination, heterologous bone grafts do not contain cells residuals or other antigens anymore. This, beyond making them biocompatible, involves remodeling mechanism that may differ from those observed for autogenous bone as no residual, necrotic cells elimination may be enhanced by these materials. Accordingly, a lower degree of local inflammation and/or presence of lytic enzymes should be expected when such materials are being grafted. This might be a possible explanation of the present preliminary observation that such equine-derived, heterologous bone blocks undergo smaller volumetric resorption than autogenous bone blocks. In short, we suggest that the healing pattern of the two materials might not be identical, involving less inflammation and therefore

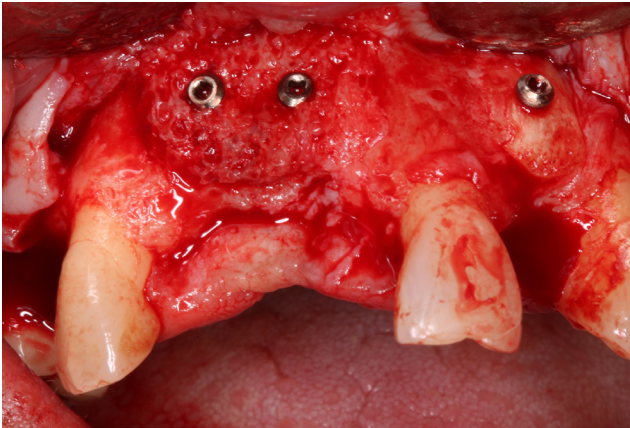


Figure 5a Six months after the grafting surgery both blocks appear stable and well-vascularized

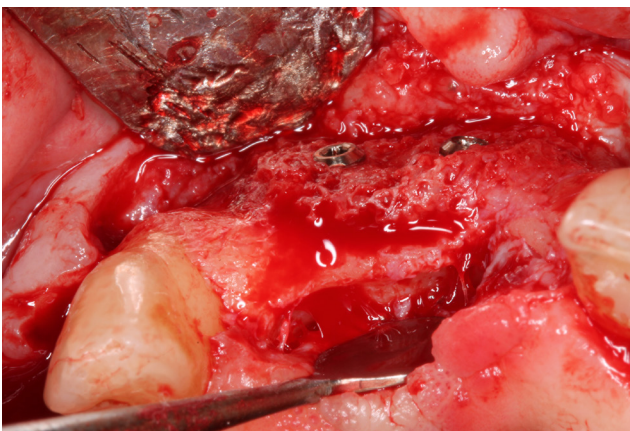


Figure 5b Occlusal view of the site grafted with heterologous block

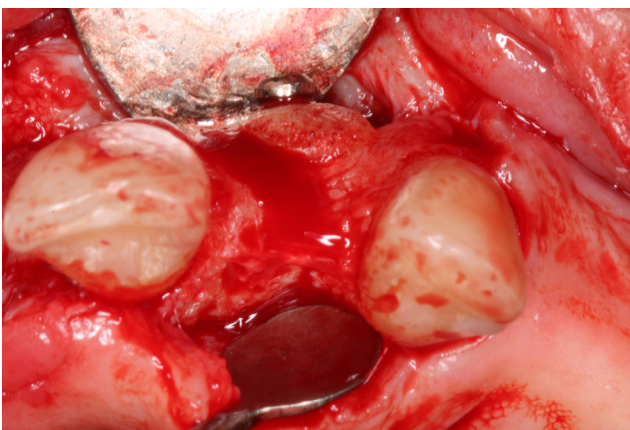


Figure 5c Occlusal view of the site grafted with autogenous block

smaller resorption when heterologous bone blocks are being grafted.

Results of the present study are consistent with those showing that processing methods do alter the resorption features of mammal bone. This makes enzyme-deantigenic equine bone substantially different

from other xenografts, such as anorganic bovine bone and others (28–31) that are obtained by eliminating all protein components (32) and other antigens through high-temperature bone processing. This difference seems to affect the cellular behavior. Indeed, when osteoclasts were cultured on enzyme-deantigenic equine bone, they adhered on this material in greater numbers and exerted a more intense degrading activity (33) than they did under the same experimental conditions on anorganic bovine bone (34), possibly because of the presence of preserved collagen in the equine xenograft (35). If such behavior would be confirmed for heterologous onlay bone grafts too at a histological and histomorphometric level, showing an osteoclastic remodeling similar to that of autogenous bone, and if long-term graft stability, associated to high implant success rate were to be observed, such heterologous bone blocks would be confirmed as a valid alternative option to the use of autogenous bone in treating the horizontal atrophy of the anterior maxilla. Long-term results of the present clinical study should provide clear data on this question, and further clinical prospective study should be carefully planned and carried out to further investigate the matter.

Conclusion

Preliminary results of this split-mouth prospective study show that heterologous, equine-derived, enzyme-deantigenic bone blocks provide appropriate clinical and tomographic results at 6 months after being grafted for the reconstruction of horizontal defects in the anterior maxilla, showing a smaller resorption rate than autogenous bone blocks. Prospective longitudinal studies using heterologous bone grafts and a higher number of patients are needed in order to understand the behavior of this graft, its stability and the survival rate of dental implants.

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