Incorporation and Remodeling of Bone Block Allografts in the Maxillary Reconstruction: A Randomized Clinical Trial

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ABSTRACT

Background: Severe alveolar atrophy often presents a challenge for the implant surgery. The significant lack of bone in the alveolar ridges may compromise the final restorations both from the aesthetic and functional standpoints.

Objectives: To evaluate the behavior of bone block allografts for the maxillary augmentation and to investigate its incorporation, remodeling, and implant survival rates in two different healing time points.

Material and Methods: Sixty-six consecutive patients (52 female/14 male, mean age: 57.95 ± 9.06 years old), presenting 113 atrophic alveolar ridges underwent maxillary augmentation with fresh-frozen allogeneic bone blocks from tibia. Patients were randomly assigned in two groups: Group 1—patients who would wait 4 months for implant placement after grafting, and Group 2—patients who would wait 6 months. Events of infection, suture dehiscence or mucosal perforation were recorded. Cone-beam computed tomography scans were compared volumetrically between the time of the grafting surgery and reentry procedure after incorporation. Biopsies were collected and subjected to histological, histomorphometric and immunehistochemical analysis.

Results: A total of 305 implants were placed in the reconstructed sites. The mean resorption rate in Group 1 (13.98% ± 5.59) was significantly lower than Group 2 (31.52% ± 6.31). The amount of calcified tissue, newly formed bone and remaining graft particles demonstrated no difference between groups. The samples showed evident immunolabeling for the podoplanin protein in both groups. The implants cumulative survival rate was 94.76%.

Conclusions: The findings of the present study indicate that there is a significant difference regarding the resorption of the grafts when waiting 4 or 6 months before placing the implants, even though no difference was found in the histological, histomorphometric, and immunohistochemical features. Both 4-month and 6-months healing times are suitable for the implant placement.

KEY WORDS: alveolar ridge reconstruction, bone allograft, bone augmentation, bone grafting, edentulous atrophic maxilla, implant survival, randomized controlled trial, histological analysis

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INTRODUCTION

The rehabilitation of fully and partially edentulous patients with the use of oral implants today is considered as a routine treatment modality and it is very well-documented in literature.[1] High success rates achieved with the techniques currently available allows the planning of complex cases, extending the indications of these strategies in dentistry.[2]

Severe alveolar atrophy often presents a challenge for the implant surgery. Extensively reabsorbed ridges cannot only lead to an insufficient bone volume for the placement of dental implants but may also lead to unfavorable

maxillo-mandibular prosthetic relationships. Therefore, the significant lack of bone in the alveolar ridges may compromise the final restorations both from the aesthetic and functional standpoints.[3,4]

The reconstruction of the alveolar ridges aiming for the implant placement should provide sufficient structure for fixation of the screws in a favorable tri-dimensional position, as well as a healthy and physio-logically active environment for the osseointegration. Thus, it is imperative that the grafting material used in the reconstructions is able to regenerate the lost tissue with a new organized calcified scaffold, proper-ly vascularized, which would allow the appropriate healing around the implants similarly to the native bone.[4,5]

Ideally, bone grafts should have sufficient struc-tural integrity to maintain space for the growth, mat-uration, and bone consolidation. Furthermore, they should be capable of promoting cell recruitment with the potential to form bone within the graft. Although controversial, another feature commonly cited in lit-erature as preferable in the graft materials is that they must be fully absorbed and replaced by viable native bone. As with any desirable technique or material, the grafts must present predictable and reproducible results.[6,7]

The incorporation of grafted material to the new site is given through intramembranous ossification by migration and vessel growth within the graft and a subsequent slow remodeling.[8,9] During this process, as the material undergoes engraftment, it also decreases in volume as a result of the remodeling activity. It is known that different materials have dif-ferent behaviors and different optimal waiting periods prior to implant placement, resulting in distinctive degrees of incorporation and resorption.[3]

The gold standard block graft material in litera-ture is the autologous bone.[6,10–12] The use of this material in alveolar reconstruction with high rates of success and good predictability is widely reported in scientific publications. The bone collected from the same patient has osteogenic, osteoinductive, and osteoconductive properties given by the viable cells, morphogenetic proteins and its scaffold, respective-ly.[10] However, the use of autologous bone results invariably in increased morbidity inherent to the har-vesting surgery.[6,13–15] Likewise, limited availability, the possibility of injury to vital structures, excessive

swelling, loss of blood, increased morbidity and com-plications associated with the second surgical access, encourage the search for other bone substitutes.[11,16]

In the past decade, several studies have demon-strated the application of fresh-frozen bone allografts (FFBA) as a viable option to replace the autogenous tissue.[17–20] The use of bone allografts has been described extensively in orthopedic procedures, and today can be considered as a safe material from the immune and contamination standpoints.[21] Even though it is well documented in case reports and case series, the FFBA still requires more well-designed studies to guide the clinical practice.[16]

The waiting time after the grafting procedure for the second surgery (implant placement) is not a con-sensus yet in literature.[20,22–28] It is assumed that the greater degree of incorporation of the grafts, the more beneficial for the implant placement. However, the extension of the waiting period for the second surgical stage also seems to imply a higher resorption of the grafted bone.[29] The incorporation times and resorption rates of allografts are not yet well defined and many studies are based on the data from other biomaterials.[21,27,29,30]

The aim of this study is to evaluate the clinical behavior of allogenic bone block grafts for ridge aug-mentation and to investigate its incorporation, remodeling, and implant survival rates in two differ-ent healing time points.

MATERIAL AND METHODS

This research protocol was approved by the Ethics Committee of The University Hospital Pedro Ernesto under the protocol number: CEP-HUPE, 2762/2010. All of the eligible patients signed informed consent forms. The treatments were performed by trained sur-geons with comprehensive experience in oral reconstruc-tive surgery at the Department of Oral Implantology, Pontifical Catholic University of Rio de Janeiro [Rio de Janeiro, Brazil]

Patient Selection

A total of 66 consecutive partially or totally edentu-lous patients (52 female/14 male, mean age: 57.95 ± 9.06 years old, ranging from 37 to 75 years), presenting 113 atrophic alveolar ridges and looking for oral rehabilitation with dental implants were included in this study during a 4-year period (2010–

2014). The inclusion criteria was: present at least one edentulous site with severe bone deficiency [Cawood and Howell class IV atrophy][31] that required horizontal alveolar augmentation prior to implant placement. The diagnosis for all the eligible patients was confirmed by cone-beam computerized tomography scanning (CBCT) [i-CAT Classic, Imaging Sciences International, Hatfield, PA, USA]. The images were acquired with a resolution of 96 dpi, 14-bits gray scale and 0.25 mm voxel size, and set to 120 kVp, 5 mA, with a 20-second exposure time. The exclusion criteria were: smoking, systemic diseases, current, or previous therapy with oral or intravenous bisphosphonates and irradiation at the head and neck in the past 5 years and noncompliance with the study protocol. The interventions were classified as anterior, posterior, or full-arch.

The FFABs were acquired for each patient according to the Brazilian National Transplant System policy from the Musculoskeletal Tissue Bank of the National Institute of Traumatology and Orthopedics [Rio de Janeiro, Brazil]. The grafts used were fragments of cortico-cancellous proximal tibia.

Before the surgeries, the patients were randomly assigned by an electronic random number generator to one of the two groups as follows: Group 1—patients who would wait 4 months for implant placement and Group 2—patients who would wait 6 months for implant placement.

Surgical Procedures

The surgical procedures have been described in details in our first study.[32] Briefly, before the surgical procedures, the patients were asked to perform a mouth rinse with chlorhexidine 0.12% for 1 minute. Antisepsis was made on the peri-oral skin with povidine-iodine solution and then the head, neck and chest were draped with a perforated sterile drape. The bone grafts were left in sterile saline for approximately 30 minutes before the procedure to thaw following the Tissue Bank recommendations. Under local anesthesia, consisting of lidocaine 2% with epinephrine 1:100,000 [DFL®, Rio de Janeiro, RJ, Brazil], a full-thickness muco-periosteal flap was elevated providing proper access to the resorbed ridge. The receptor site was prepared using a surgical drill at a low speed to create a "box" of approximately 0.5 mm in depth to increase the contact area and stabilize the bone blocks

and also to remove any remains of soft tissue adhered to the buccal plate. The defects were measured and then bone grafts were sculpted into block shapes to fit the sites appropriately. The blocks were then adapted to the receptor bed and were fixed with titanium screws of 1.5 mm in diameter and 10, 12, or 14 mm in length, depending on the needs of each region. Each block was fixed with at least two screws to ensure the mechanical stability of the grafts. The flaps were repositioned, ensuring primary closure of the wounds. To close, 4-0 silk sutures were used [Ethicon Inc.® Somerville, NJ, USA].

The patients were medicated with 500 mg oral azithromycin once daily for 3 days, NSAIDs for postoperative pain control and chlorhexidine 0.12% mouth rinse twice per day for 15 days. Sutures were removed 7 to14 days after surgery.

During the healing period, the patients were recalled for a follow-up consultation at the 30, 60, 90, and 120 days after surgery. Events of infection, suture dehiscence or mucosal perforation exposing the grafts were recorded and treated when needed.

CBCT Evaluation

After grafting surgery, all the patients underwent a CBCT scan at a maximum interval of 7 days (T_0). After the waiting period for each group, all the patients underwent a new CBCT scan at a maximum interval of 7 days before the day planned for implant placement surgery (T_F). The scans followed the same acquisition protocol, resolution, voxel size and exposure time as the scans used for the diagnosis at the first moment.

The images were generated in DICOM files and were analyzed and compared using the VoXim®/Osteo software [IVS Technology GmbH, Chemnitz, Germany]. The measurements were performed by a single trained operator. The intraexaminer calibration was performed using three CT scans of patients not included in the study, acquiring two measurements for each test with an interval of one week between them. The Kappa index was 0.95.[33]

The volume of the grafts was calculated by separating the blocks virtually from the host maxilla in the CBCT images using the Voxim software segmentation tool. This tool calculates the volume from a designated area in a mililiter scale. Using the computer mouse, the block grafts were carefully selected on
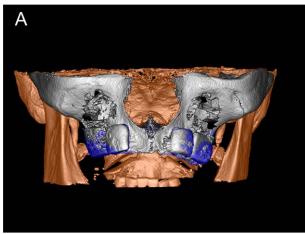
Figure 1 (A) Image fusion of the initial volume data set and the final volume data set (front view); (B) Occlusal view. Initial graft volume in blue and final graft volume in gray.

the 3D view and the final selection was then confirmed on the coronal, sagittal, and transversal plane views. The measurements were done on both scans (T_0 and T_F) and were then compared. The software fusion tool was used to visually illustrate the blocks volume alterations. It allows the superposition of two different data sets facilitating the three-dimensional (3D) understanding of the resorption pattern (Figure 1, A and B).

The volumetric changes were determined for each graft using the following formula:

$$\text{Volume reduction (\%)} = \frac{T_F\,\text{Volume} - T_0\,\text{Volume}}{T_0\,\text{Volume}} \times 100.$$

Thus, the final volume is given as a percentage of the total reduction compared with the initial volume.

Statistical analysis was performed to test if the two samples came from the same population. The Mann-Whitney test was the tool used. Significance

was determined at the 5% [$p < .05$] level. Discrepancies within each group were evaluated using the Friedman test.

Implant Placement Surgeries

The patients were scheduled for implant placement procedures according to the times determined for each group. All of the procedures followed the same routine: local anesthesia was administered using lidocaine 2% with epinephrine 1:100,000, and a full-thickness muco-periosteal flap was elevated. The screws used to fix the blocks were removed, and a graft sample was collected with a trephine drill 3.0 mm in diameter applied to a bucco-palatal depth of 10 mm (Figure 2A). The fragments were preserved in 10% buffered formalin (Figure 2B). The implants were placed using implant system drills [International Intralock ®, Boca Raton, FL, USA] according to the manufacturer's specifications (Figure 2, C and D). The flaps were repositioned and closed with 4-0 silk sutures.

The patients were medicated with 500 mg azithromycin once daily for 3 days, NSAIDs for postoperative pain control and chlorhexidine 0.12% mouth rinse twice per day for 15 days. The sutures were removed 7 days after surgery.

Histological and Histomorphometric Analysis

Samples of the grafts retrieved by a cylindrical biopsy with a trephine bur during implant placement surgery were immersed in 10% buffered formaldehyde and submitted to histological and histomorphometric analysis.

The samples were decalcified in 5% nitric acid, routinely processed, and embedded in paraffin. Three 5-µm sections from each sample were stained with hematoxylin and eosin following the standard protocol of the Oral Pathology Laboratory, School of Dentistry, and State University of Rio de Janeiro. Histological analysis was performed using a Leica DM500 light microscope [Leica Microsystems, Wetzlar, Germany] and histological images at ×100, ×200, and ×400 magnifications were obtained using a connected Leica ICC50 HD digital camera [Leica Microsystems, Wetzlar, Germany].

Immunohistochemical Analysis

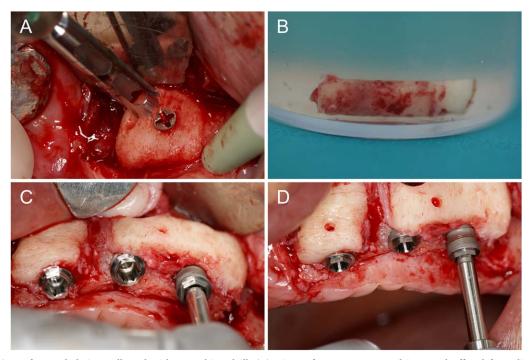Three-micrometer sections from each sample were submitted to immunohistochemistry using a mouse

Figure 2 (A) Graft sample being collected with a trephine drill; (B) Biopsy fragment preserved in 10% buffered formalin; (C and D) Implants placed in the grafted area.

monoclonal antibody anti-podoplanin [clone D2-40, dilution 1:200, DAKO, Glostrup, Denmark] through the immunoperoxidase method [LSAB, Dako, Carpinteria, US] and diaminobenzidine as the chromogen. The podoplanin is a protein expressed by osteocytes in the dendrite elongation process and, therefore, was chosen to selectively mark osteocytes viability and function.[34] The slides were counterstained with Carazzi hematoxylin and examined by a single investigator through optical microscopy at ×100, ×200, and ×400 magnifications.

For the histological and immunohistochemical analysis, the assessment was descriptive, without comparison between the groups. The parameters described were the presence of newly formed bony tissue, the presence of osteoblasts/osteocytes, the presence of vessels, the existence of inflammatory infiltrate and positivity for podoplanin marker.

The histomorphometric evaluation was performed by a single trained operator using the pictures taken from each sample in the ImageJ [NIH] software. The parameters evaluated were: percentage of calcified tissue, percentage of newly formed bone and percentage of allograft remains. To quantify each parameter, the examiner used the software to identify the areas of interest in the pictures and selecting them in an automated manner (Figures 3 and 4). When needed, the selection was corrected manually in order to maintain the precision of the measurements. The total area selected for each parameter was given by the software in a percentage of the total field.

Statistical analysis was performed to compare the mean percentage of the parameters evaluated among the two groups. The normality of the sample was tested using the Shapiro-Wilk test. The comparison among groups was performed using the Mann-Whitney test. Significance was determined at 5% [$p < .05$]. Discrepancies within each group were evaluated using the Friedman test.

Second Stage Surgery

Three months after the implant placement, the second stage surgery was scheduled in order to uncover the fixtures and connect the healing abutments. All the patients received implant-supported fixed prostheses. The follow-up period after loading the implants was set at 12 months. Events of nonintegration of the implants were recorded in order to assess the short-term cumulative survival rate (CSR). The difference in the percentage of the failed implants between the groups was assessed using the Mann-Whitey test. The possible difference in the number of
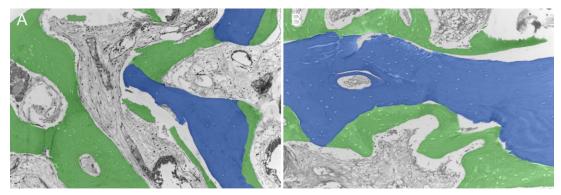
Figure 3 (A and B) Histomorphometric quantification of the remaining graft in blue and the newly formed bone in green by the ImageJ software (H&E, ×200 magnification).

lost implants between the grafted sites was assessed using the Kruskal–Wallis test.

RESULTS

Clinical Observations

Of the 66 patients included in the study, 3 developed complications that led to total graft lost and were excluded from the study. Thirteen subjects presented complications that did not jeopardize the grafting outcomes, distributed in: 2 cases of infection, 4 of suture dehiscence, 3 of mucosal perforation, 3 of infection combined with suture dehiscence and 1 of infection combined with mucosal pefuration. A summary of the demographic data and described complications are shown in Table S1. Cases of infection and dehiscence were promptly treated with debridement, antibiotic therapy, and hygiene instruction. Mucosal perforations exposing the grafted area after the initial healing were followed-up carefully to avoid secondary infection of the surgical site. The exposures were cleaned by a professional and the patients were instructed to apply a 0.2% Chlorhexidine gel twice daily during the healing period or until the perforation closing.

During the grafts reentry, all the blocks showed good mechanical stability after the fixation screws removal. A total of 305 implants were placed in the reconstructed sites. The grafts allowed a satisfactory positioning of the implants according to the prosthetic planning. All the implants were placed with the insertion torque from 20 to 45 Ncm, demonstrating good primary stability.

A summary of the variables measured is presented in Table S2.

CBCT. The mean volume reduction in Group 1 (13.98% ± 5.59) was significantly lower than Group 2 (31.52% ± 6.31), as shown in Graph 1. The pattern and direction of the changes suffered by the graft blocks were not uniform and seemed to be more evident in the cancelous portion and also from the edges to the center. Although the 6 months group have shown more volumetric loss, the resorption did not jeopardize the implant placement as planned.
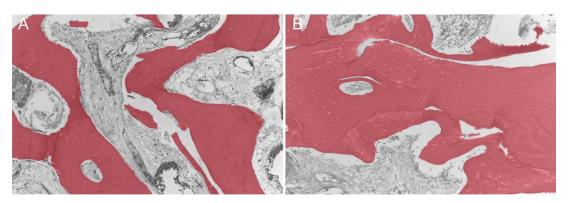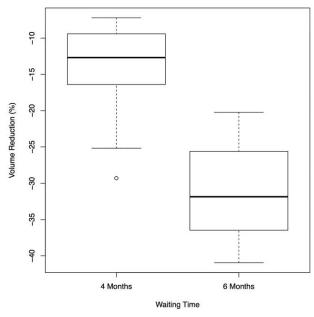

Figure 4 (A and B) Histomorphometric quantification of the calcified tissue in red by the ImageJ software (H&E, ×200 magnification).
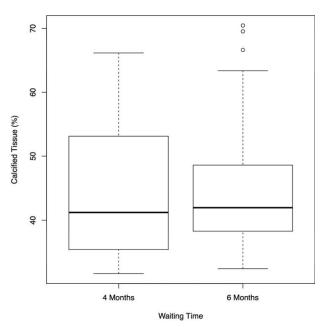
Graph 1 Boxplot of the volume reduction in both groups.

Histological. By the light microscope, the samples showed different degrees of incorporation and bone maturation. It was possible to see that the cortical plate showed less cellular activity compared to the cancelous area. Nonremodeled allograft remains were present in all the biopsies evidenced by the empty osteocytes lacunae. In both groups, the samples showed areas of new bone formation with distinctive amounts of immature calcified tissue, osteoblasts and osteocytes. Blood vessels were also found abundantly in between the graft spaces. Overall, the samples showed healthy bone contents with discrete or no inflammatory infiltrate (Figure 5).

Histomorphometric. Differences in the amount of calcified tissue found in the samples were not observed between the groups (Group 1=45.06%±11.38, Group 2=45.64%±10.97) (Graph 2). The percentage of newly formed bone also demonstrated no difference when compared to the samples from Group 2 (Group 1=20.8%±9.52, Group 2=27.2%±14.86) (Graph 3). Remaining grafted bone was found in both groups with no statistical significance between them (Group 1=24.27%±14.84, Group 2=18.44%±5.77) (Graph 4).

Immunohistochemistry. The samples showed evident labeling for the podoplanin protein in both groups. The protein expression revealed long osteocyte dendrite formation within the grafted area, which is impossible to see with usual histology staining. The podoplanin activity was detected in areas of new
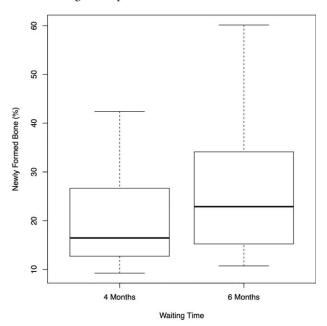

Graph 2 Boxplot of the quantification of calcified tissue in both groups.
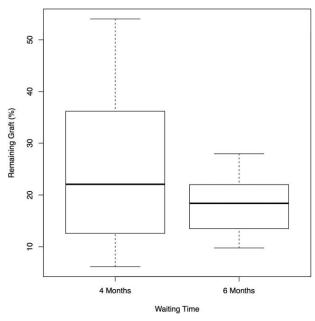
bone formation, immature bone, and also in lamellar bone areas. As expected, the allograft remains particles were negative for the immuno-labeling (Figure 6)

Implants Survival

From the 305 implants placed in the reconstructed areas, 16 implants (5.24%) were lost in the period assessed in the study, showing a CSR of 94.76%. When using the patient as the unit, 21% of the

Graph 3 Boxplot of the quantification of newly formed bone in both groups.

Graph 4 Boxplot of the quantification of remaining graft in both groups.

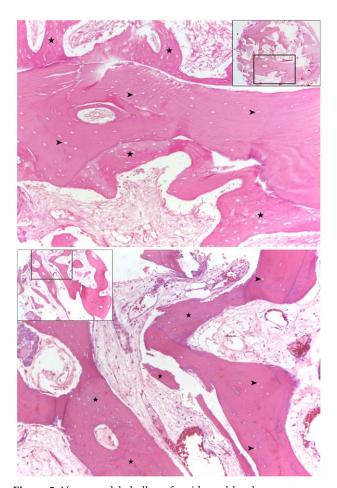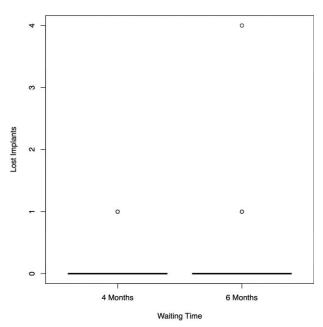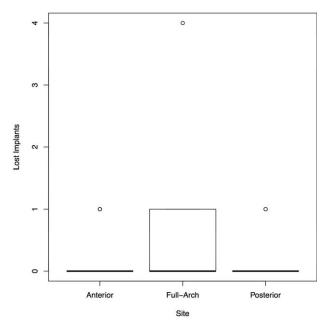**Graph 5** Correlation between of the number of lost implants and the groups.

Figure 5 Nonremodeled allograft evidenced by the empty osteocytes lacunae (arrows). Newly formed bone presenting osteoblasts and osteocytes (stars). Discrete inflammatory infiltrate (H&E, ×200 main picture, ×100 localization picture).

Graph 6 Correlation between of the number of lost implants and the grafted area.

individuals presented implant failures. There was no correlation between the number of lost implants and the groups [$p = .673$] and also no statistically significant correlation with the grafted sites [$p = .252$] (Graphs 5 and 6, respectively).

DISCUSSION

The use of onlay bone blocks grafts to reestablish maxillary width and height prior to implant
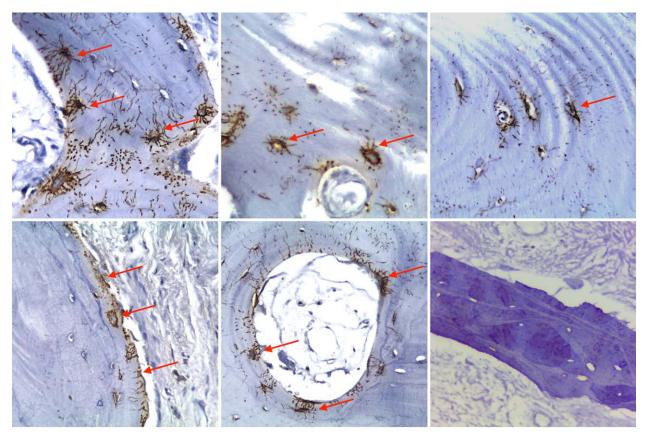
Figure 6 Podoplanin (D2-40) immunostaining showing long osteocyte dendrite formation within the areas of new bone formation, immature bone and also in lamellar bone grafted (red arrows). Allograft remains particles negative for the immuno-labeling (Peroxidase immunostaining, Hematoxilin counterstain, ×400 magnification).

placement is a well described and predictable technique.[5,16] High success rates, low complication rates and the long-term survival of dental implants in these grafts make it a good alternative for reconstructing extremely atrophic ridges. It has been well documented that all bone grafts undergo some resorption in the incorporation phase, and the resorption rates seem to vary depending on graft origin (autogenous, allogenous, xenogenous, or synthetic) as well as graft source (Ilium, tibia, calvaria).[29,35–37] These reductions in size, depending on the amount, can lead to insufficient bone volume for subsequent implant placement.[29,38]

Bone graft incorporation is a complicated process with multiple variables influencing rate, pattern, and completeness.[39] The time necessary to ensure the proper incorporation of bone block allografts prior to implant placement is still a matter of debate.[28] The physiological and biological events of incorporation of bone grafts are still poorly understood.[39,40] Frozen grafts are considered to be highly osteoconductive,[41] weakly osteoinductive and nonosteogenic.[42,43]

Therefore, bone formation rates can be expected to be inferior to that of autograft, even though studies have considered this affirmation either inconclusive or contradictory.[39,44] Apparently, more healing time leads to better incorporation but also higher resorption of the graft. The assessment of time-related remodeling of bone block grafts is of paramount importance in determining the best incorporation/ resorption relationship in order to reassess the site and place the implant. However, most of the available data on the behavior of block grafts are from animal models and cross-sectional studies.[38,45–47]

The studies available in the literature have shown reductions in the size of grafts ranging from 9 to 50% 3 to 8 months after the surgery.[35,36,48] Although these resorption rates are well described, in most of the studies, measurements are made either clinically or in a 2D perspective.[49] In a human model, the most reliable and accurate way to measure three-3D time-related changes in block grafts after surgery appears to be by means of CBCT. The low radiation dose and precise measurements allow assessment of

the grafts' behavior at different times with no harm to patients.[50–54] The time-related evaluation of changes after grafting procedures such as sinus grafting, major maxillofacial reconstructions (such as cleft palate grafts) and harvest of onlay blocks from calvaria[29,36,55,56] were well described using CBCT scans.

In our study, the block grafts' volumetric changes have shown to be related to the time waited before the implant placement. Furthermore, the resorption rate has demonstrated to be uniform in each group with the block grafts used, corroborating with the rates found in our previous report[32] and with others in the literature.[57–59] It is suggested that the processing and the use of bony tissue can influence the behavior of the grafts. Long periods of freezing, the removal of the surface layers of the blocks and the age of the donors can influence the cell growth in the grafts in vitro.[60,61] Likewise, mechanical tests have demonstrated that different types of washing and sterilizing can lead to grafts that are more susceptible to fractures.[62,63] In animals, the surgical technique used for the placement of bone blocks have also shown to be a significant factor with regard to the resorption rate of the grafted blocks.[9] Similarly, studies have demonstrated that grafts undergo different resorption patterns depending on the embryonary origin of the grafted bone,[64–67] as well as the ratio between cortical and medullary contents.[67] Thus, we made a strong effort to standardize the grafts using the material from the same Tissue Bank, from the same bone (tibia) and presenting similar distribution between cortical and cancellous layers. This attempt was made in order to assure that we were using block grafts with comparable processing, bone origin properties, and microarchitecture features, respectively.

Similar histologic and histomorphometric findings are commonly reported in the literature for the allografts biopsies prior to implant placement. The presence of different degrees of new bone formation, bone cells (osteoblasts, osteocytes, and osteoclasts) and vascularization within the grafted areas are features of most of the evaluations available despite the variances between the studies.[20,24,32,57,58,68] Absent or discrete inflammatory infiltrate has been found in the samples. This corroborates with previous studies[11,20,24,32,58,69,70] and suggests that the graft incorporation site is a healthy environment.

The remodeling and repair of the grafts is given by the penetration of vessels and following substitution by the host bone. During the incorporation of the allogeneic bone, the grafted area becomes a hybrid structure comprising the calcified original graft and the new host bone, variably mineralized.[39] The remaining graft that has not yet been remodeled is histologically seen as a calcified tissue with lacunae void of osteocytes nucleai. Regarding these remnants of the graft after the initial incorporation, the findings are similar, but the interpretation of the results is varied. Some authors suggest that this feature is due to the slow process of remodeling suffered by these grafts and part of the process,[20,24,32,57] while other groups interpret this finding as necrotic bone.[11,58,68,71,72] In the present study, we could not find a significant reduction in the amounts of remaining graft in the two different time points. This data is not in accordance with our previous findings,[32] where the 4-months waiting group showed statistically significant more graft remains than the 6- and 8-months groups. The difference in the results can be due to the more precise histomorphometric measuring technique applied or to the larger number of patients enrolled in the present study. Moreover, no significant difference was found when comparing the new bone formation between the groups. These findings show that the two time points present extremely similar features, except by the resorption pattern.

Compared to the autogenous bone, the amount of graft remains in the receptor site in a short-term period is larger in the allografts, however, in larger time intervals, both have similar histological features which suggest a difference in the remodeling rate as demonstrated in both human and animal studies.[73–75] In rabbits, the amount of new bone formation with the use of allografts has shown to be statistically similar to the amount obtained with autogenous grafts.[74] Reikeras et al.[75] have shown that the slower creeping process depends on the osteoconductive properties of the graft, which is equivalent for the frozen allograft and the fresh autograft.

Data concerning the new bone formation and remodeling in the allografts using immunohistochemistry techniques is scarce in the literature. Regarding human samples, only few case reports are available.[76] In animal studies, different parameters have been

studied and, as the grafts remodeling and incorporation are a complex process, there is no consensus on which antibodies should be used to evaluate them.[47,69,77] When comparing the bone remodeling between autografts and allografts by immunohistochemistry, Hawthorne et al.[77] have found a similar pattern of labeling for receptor activator of nuclear factor-k and its ligand (RANK and RANK-L), osteoprotegerin (OPG), alkaline phosphatase (ALP), collagen 1 (COLI), osteopontin (OPN), osteocalcin (OC) and an increased activity of tartrate-resistant acid phosphatase (TRAP) in the allografts. In our study, we have not used a quantification method due to the variability of the human samples that can be more easily standardized in an animal model than in a human study. Therefore, we have chosen an antibody that is a marker for osteocyte function and that could be evaluated qualitatively instead of quantitatively. The podoplanin protein (D2-40) is selectively expressed by osteocytes compared to osteoblasts in response to mechanical stimulation and is increased by mechanical strain in vitro and in vivo.[78–80] This protein is also necessary for osteocyte dendrite elongation in response to shear stress.[34] As dendrite formation is an active process and not a passive mechanism, it is likely that the podoplanin protein is critical not only during dendritic formation, but also for osteocyte viability and function, and, therefore, essential for normal bone physiology.[34,80–85] In our samples, we found that the podoplanin expression is evident in both groups, demonstrating that even at the early time point, the osteocytes development, function, and expansion within the grafted area are clear. Furthermore, this finding suggests that this important feature of normal bone function is being developed in areas grafted with allogeneic material.

Reported success rates of allogeneic bone block grafts in the alveolar augmentation are compatible with other regenerative techniques.[71,86–89] The prevalence of complications involving bone blocks vary between 0[20,24,32,58,71,90] and 11.76%.[68] The most common failures identified in the studies are: partial or total loss of the blocks, problems in the soft tissues (dehiscence and late perforation of the mucosa) and infection. In the present study, a similar occurrence of the adverse events has been found. As highlighted in other studies[91,92] and in accordance with our experience, when properly addressed, the complications do not necessarily lead to total loss of the grafting procedure.[91]

The cumulative success rate of implants placed in allogenic bone reconstructed sites can be considered high and, similarly, comparable to other modalities of bone reconstruction. Rates reported vary between 94.7 and 100%,[20,59,93,94] extremely similar to our outcomes. Evaluating the number of implant failures according to the patients as individuals, 79% of the participants had no problems related to the fixtures, demonstrating that this reconstruction modality has a good previsibility.

CONCLUSIONS

The findings of the present study indicate that the volume reduction of the bone block allografts for the ridge augmentation is related to the time after the grafting procedure. Furthermore, there is a significant difference regarding the resorption of the blocks in waiting 4 or 6 months before placing the implants. There is no difference in the histological, histomorphometric and immunohistochemical features or the implant success rates between the groups, indicating that both 4- and 6-month healing intervals after the grafting surgery are acceptable for the implant placement. The alveolar ridge augmentation with the use of bone block allografts can be considered a reliable technique with success and complication rates comparable to other reconstruction modalities.

REFERENCES

1. Esposito M, Ardebili Y, Worthington HV. Interventions for replacing missing teeth: different types of dental implants. Cochrane Database Syst Rev 2014; 7:CD003815.

2. Lambert FE, Weber H-P, Susarla SM, Belser UC, Gallucci GO. Descriptive analysis of implant and prosthodontic survival rates with fixed implant-supported rehabilitations in the edentulous maxilla. J Periodontol 2009; 80:1220–1230.

3. Chiapasco M, Casentini P, Zaniboni M. Bone augmentation procedures in implant dentistry. Int J Oral Maxillofac Implants 2009; 24 Suppl:237–259.

4. Misch CM. Maxillary autogenous bone grafting. Oral Maxillofac Surg Clin North Am 2011; 23:229–238.

5. Esposito M, Grusovin MG, Coulthard P, Worthington HV. The efficacy of various bone augmentation procedures for dental implants: a cochrane systematic review of randomized controlled clinical trials. Int J Oral Maxillofac Implants 2006; 21:696–710.

6. Zouhary KJ. Bone graft harvesting from distant sites: concepts and techniques. Oral Maxillofac Surg Clin North Am 2010; 22:301–316.

7. Pape HC, Evans A, Kobbe P. Autologous bone graft: properties and techniques. J Orthop Trauma 2010; 24 Suppl 1:S36–S40.

8. Andrade MGS, Moreira DC, Dantas DB, Sá CN, Bittencourt TCBDSC de, Sadigursky M. Pattern of osteogenesis during onlay bone graft healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010; 110:713–719.

9. Oh K-C, Cha J-K, Kim C-S, Choi S-H, Chai J-K, Jung U-W. The influence of perforating the autogenous block bone and the recipient bed in dogs. Part I: a radiographic analysis. Clin Oral Implants Res 2011; 22:1298–1302.

10. Deatherage J. Bone materials available for alveolar grafting. Oral Maxillofac Surg Clin North Am 2010; 22:347–352.

11. Spin-Neto R, Landazuri Del Barrio RA, Pereira LAVD, Marcantonio RAC, Marcantonio E, Marcantonio E. Clinical similarities and histological diversity comparing fresh frozen onlay bone blocks allografts and autografts in human maxillary reconstruction. Clin. Implant Dent Relat Res 2013; 15:490–497.

12. Springer ING, Terheyden H, Geiß S, Härle F, Hedderich J, Açil Y. Particulated bone grafts - effectiveness of bone cell supply. Clin Oral Implants Res 2004; 15:205–212.

13. Nkenke E, Radespiel-Tröger M, Wiltfang J, Schultze-Mosgau S, Winkler G, Neukam FW. Morbidity of harvesting of retromolar bone grafts: a prospective study. Clin Oral Implants Res 2002; 13:514–521.

14. Sittitavornwong S, Gutta R. Bone graft harvesting from regional sites. Oral Maxillofac Surg Clin North Am 2010; 22:317–330.

15. Arx T, von Häfliger J, Chappuis V. Neurosensory disturbances following bone harvesting in the symphysis: a prospective clinical study. Clin Oral Implants Res 2005; 16:432–439.

16. Waasdorp J, Reynolds DMDMA, Reynolds MA. Allogeneic bone onlay grafts for alveolar ridge augmentation: a systematic review. Int J Oral Maxillofac Implants 2008; 25:525–531.

17. Lupovici J. Revisiting the hopeless ridge: part I–challenging the gold standard. Compend Contin Educ Dent 2009; 30:130–132.

18. Leonetti JA, Koup R. Localized maxillary ridge augmentation with a block allograft for dental implant placement: case Reports. Implant Dent 2003; 12:217–226.

19. Lyford RH, Mills MP, Knapp CI, Scheyer ET, Mellonig JT. Clinical evaluation of freeze-dried block allografts for alveolar ridge augmentation: a case series. Int J Periodontics Restorative Dent 2003; 23:417–425.

20. Contar CMM, Sarot JR, Bordini J, Galvão GH, Nicolau GV, Machado MAN. Maxillary ridge augmentation with fresh-frozen bone allografts. J Oral Maxillofac Surg 2009; 67:1280–1285.

21. Macedo LGS, Mazzucchelli-Cosmo LA, MacEdo NL, Monteiro ASF, Sendyk WR. Fresh-frozen human bone allograft in vertical ridge augmentation: clinical and tomographic evaluation of bone formation and resorption. Cell Tissue Bank 2012; 13:577–586.

22. Petrungaro PS, Amar S. Localized ridge augmentation with allogenic block grafts prior to implant placement: case reports and histologic Evaluations. Implant Dent 2005; 14:139–148.

23. Keith JD, Petrungaro P, Leonetti JA, et al. Clinical and histologic evaluation of a mineralized block allograft: results from the developmental period (2001-2004). Int J Periodontics Restorative Dent 2006; 26:321–327.

24. Contar CMM, Sarot JR, Costa MB, et al. Fresh-frozen bone allografts in maxillary ridge augmentation: histologic analysis. J Oral Implantol 2011; 37:223–231.

25. Pendarvis WT, Sandifer JB. Localized ridge augmentation using a block allograft with subsequent implant placement: a case series. Int J Periodontics Restorative Dent 2008; 28:509–515.

26. Morelli T, Neiva R, Wang H-L. Human histology of allogeneic block grafts for alveolar ridge augmentation: case report. Int J Periodontics Restorative Dent 2009; 29:649–656.

27. Viscioni A, Franco M, Rigo L, Guidi R, Brunelli G, Carinci F. Implants inserted into homografts bearing fixed restorations. Int J Prosthodont 2008; 22:148–154.

28. Peleg M, Sawatari Y, Marx RN, et al. Use of corticocancellous allogeneic bone blocks for augmentation of alveolar bone defects. Int J Oral Maxillofac Implants 2010; 25:153–162.

29. Smolka W, Eggensperger N, Carollo V, Ozdoba C, Iizuka T. Changes in the volume and density of calvarial split bone grafts after alveolar ridge augmentation. Clin Oral Implants Res 2006; 17:149–155.

30. McAllister BS, Haghighat K. Bone augmentation techniques. J Periodontol 2007; 78:377–396.

31. Cawood JI, Howell RA. A classification of the edentulous jaws. Int J Oral Maxillofac Surg 1988; 17:232–236.

32. Deluiz D, Oliveira LS, Pires FR, Tinoco EMB. Time-dependent changes in fresh-frozen bone block arafts: tomographic, histologic, and histomorphometric findings. Clin Implant Dent Relat Res 2015; 17:296–306.

33. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977; 33:159–174.

34. Zhang K, Barragan-Adjemian C, Ye L, et al. E11/gp38 selective expression in osteocytes: regulation by mechanical strain and role in dendrite Elongation. Mol Cell Biol 2006; 26:4539–4552.

35. Johansson B, Grepe A, Wannfors K, Hirsch JM. A clinical study of changes in the volume of bone grafts in the atrophic maxilla. Dentomaxillofac Radiol 2001; 30:157–161.

36. Wanschitz F, Figl M, Wagner A, Rolf E. Measurement of volume changes after sinus floor augmentation with a phycogenic hydroxyapatite. Int J Oral Maxillofac Implants 2005; 21:433–438.

37. Kirmeier R, Payer M, Wehrschuetz M, Jakse N, Platzer S, Lorenzoni M. Evaluation of three-dimensional changes after sinus floor augmentation with different grafting materials. Clin Oral Implants Res 2008; 19:366–372.

38. Adeyemo WL, Reuther T, Bloch W, et al. Influence of host periosteum and recipient bed perforation on the healing of onlay mandibular bone graft: an experimental pilot study in the sheep. Oral Maxillofac Surg 2008; 12:19–28.

39. Reikerås O. Impact of freezing on bone graft incorporation biomechanical evaluations in rats. Clin Biomech (Bristol, Avon) 2010; 25:177–180.

40. Aponte-Tinao LA, Ritacco LE, Albergo JI, Ayerza MA, Muscolo DL, Farfalli GL. The principles and applications of fresh frozen allografts to bone and joint reconstruction. Orthop Clin North Am 2014; 45:257–269.

41. Lind M, Bünger C. Factors stimulating bone formation. Eur Spine J 2001; 10 Suppl 2:S102–S109.

42. Marchesi DG. Spinal fusions: bone and bone substitutes. Eur Spine J 2000; 9:372–378.

43. Erbe EM, Marx JG, Clineff TD, Bellincampi LD. Potential of an ultraporous beta-tricalcium phosphate synthetic cancellous bone void filler and bone marrow aspirate composite graft. Eur Spine J 2001; 10 Suppl 2:S141–S146.

44. Floyd T, Ohnmeiss D. A meta-analysis of autograft versus allograft in anterior cervical fusion. Eur Spine J 2000; 9:398–403.

45. Merkx MA, Maltha JC, Freihofer HP, Kuijpers-Jagtman AM. Incorporation of particulated bone implants in the facial skeleton. Biomaterials 1999; 20:2029–2035.

46. Schultze-Mosgau S, Keweloh M, Wiltfang J, Kessler P, Neukam FW. Histomorphometric and densitometric changes in bone volume and structure after avascular bone grafting in the extremely atrophic maxilla. Br J Oral Maxillofac Surg 2001; 39:439–447.

47. Faria PEP, Okamoto R, Bonilha-Neto RM, Xavier SP, Santos AC, Salata LA. Immunohistochemical, tomographic and histological study on onlay iliac grafts remodeling. Clin Oral Implants Res 2008; 19:393–401.

48. Maiorana C, Beretta M, Salina S, Santoro F. Reduction of autogenous bone graft resorption by means of bio-oss coverage: a prospective study. Int J Periodontics Restorative Dent 2005; 25:19–25.

49. Verhoeven JW, Ruijter J, Cune MS, Terlou M, Zoon M. Onlay grafts in combination with endosseous implants in severe mandibular atrophy: one year results of a prospective, quantitative radiological study. Clin Oral Implants Res 2000; 11:583–594.

50. Rustemeyer P, Streubühr U, Suttmoeller J. Low-dose dental computed tomography: significant dose reduction without loss of image quality. Acta Radiol 2004; 45:847–853.

51. Loubele M, Jacobs R, Maes F, et al. Radiation dose vs. image quality for low-dose CT protocols of the head for maxillofacial surgery and oral implant planning. Radiat Prot Dosimetry 2006; 117:211–216.

52. Mischkowski RA, Pulsfort R, Ritter L, et al. Geometric accuracy of a newly developed cone-beam device for maxillofacial imaging. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 104:551–559.

53. Veyre-Goulet S, Fortin T, Thierry A. Accuracy of linear measurement provided by cone beam computed tomography to assess bone quantity in the posterior maxilla: a human cadaver study. Clin Implant Dent Relat Res 2008; 10:226–230.

54. Damstra J, Fourie Z, Huddleston Slater JJR, Ren Y. Accuracy of linear measurements from cone-beam computed tomography-derived surface models of different voxel sizes. Am J Orthod Dentofacial Orthop 2010; 137:16.e1–e6.

55. Zhao Y-F, Gui L, Liu X-J. Study of the dimension change after grafting of bone from the outer mandibular table to different maxillofacial recipient sites. Ann Plast Surg 2010; 65:475–479.

56. Sbordone C, Sbordone L, Toti P, Martuscelli R, Califano L, Guidetti F. Volume changes of grafted autogenous bone in sinus augmentation procedure. J Oral Maxillofac Surg 2011; 69:1633–1641.

57. Acocella A, Bertolai R, Ellis E, Nissan J, Sacco R. Maxillary alveolar ridge reconstruction with monocortical fresh-frozen bone blocks: a clinical, histological and histomorphometric study. J Craniomaxillofac Surg 2012; 40:525–533.

58. Lumetti S, Consolo U, Galli C, et al. Fresh-frozen bone blocks for horizontal ridge augmentation in the upper maxilla: 6-month outcomes of a randomized controlled trial. Clin Implant Dent Relat Res 2014; 16:116–123.

59. Nissan J, Mardinger O, Calderon S, Romanos GE, Chaushu G. Cancellous bone block allografts for the

augmentation of the anterior atrophic maxilla. Clin Implant Dent Relat Res 2011; 13:104–111.

60. Kluger R, Bouhon W, Freudenberger H, Kröner A, Engel A, Hoffmann O. Removal of the surface layers of human cortical bone allografts restores in vitro osteoclast function reduced by processing and frozen storage. Bone 2003; 32:291–296.

61. Schwartz Z, Somers A, Mellonig JT, et al. Ability of commercial demineralized freeze-dried bone allograft to induce new bone formation is dependent on donor age but not gender. J Periodontol 1998; 69:470–478.

62. Costain DJ, Crawford RW. Fresh-frozen vs. irradiated allograft bone in orthopaedic reconstructive surgery. Injury 2009; 40:1260–1264.

63. Boyce T, Edwards J, Scarborough N. Allograft bone. The influence of processing on safety and performance. Orthop Clin North Am 1999; 30:571–581.

64. Manson PN. Facial bone healing and bone grafts. A review of clinical physiology. Clin Plast Surg 1994; 21: 331–348.

65. Smith JD, Abramson M. Membranous vs endochondrial bone autografts. Arch Otolaryngol 1974; 99:203–205.

66. Zins JE, Whitaker LA. Membranous vs endochondral bone autografts: implications for craniofacial reconstruction. Surg Forum 1979; 30:521–523.

67. Ozaki W, Buchman SR. Volume maintenance of onlay bone grafts in the craniofacial skeleton: microarchitecture versus embryologic origin. Plast Reconstr Surg 1998; 102:291–299.

68. Spin-Neto R, Stavropoulos A, Pereira LAVD, Marcantonio E, Wenzel A. Fate of autologous and fresh-frozen allogeneic block bone grafts used for ridge augmentation. A CBCT-based analysis. Clin Oral Implants Res 2013; 24:167–173.

69. Pedrosa WF, Okamoto R, Faria PEP, Arnez MFM, Xavier SP, Salata LA. Immunohistochemical, tomographic and histological study on onlay bone graft remodeling. Part II: calvarial bone. Clin Oral Implants Res 2009; 20:1254–1264.

70. Acocella A, Bertolai R, Colafranceschi M, Sacco R. Clinical, histological and histomorphometric evaluation of the healing of mandibular ramus bone block grafts for alveolar ridge augmentation before implant placement. J Craniomaxillofacial Surg off Publ Eur Assoc Cranio-MaxilloFacial Surg 2010; 38:222–230.

71. Wallace S, Gellin R. Clinical evaluation of freeze-dried cancellous block allografts for ridge augmentation and implant placement in the maxilla. Implant Dent 2010; 19: 272–279.

72. Spin-Neto R, Stavropoulos A, Coletti FL, Pereira LAVD, Marcantonio E, Wenzel A. Remodeling of cortical and corticocancellous fresh-frozen allogeneic block bone grafts - a radiographic and histomorphometric comparison to autologous bone grafts. Clin Oral Implants Res 2015; 26:747–752.

73. Gomes KU, Carlini JL, Biron C, Rapoport A, Dedivitis RA. Use of allogeneic bone graft in maxillary reconstruction for installation of dental implants. J Oral Maxillofac Surg 2008; 66:2335–2338.

74. Pelegrine AA, Sorgi da Costa CE, Sendyk WR, Gromatzky A. The comparative analysis of homologous fresh frozen bone and autogenous bone graft, associated or not with autogenous bone marrow, in rabbit calvaria: a clinical and histomorphometric study. Cell Tissue Bank 2011; 12:171–184.

75. Reikerås O, Sigurdsen UW, Shegarfi H. Impact of freezing on immunology and incorporation of bone allograft. J Orthop Res 2010; 28:1215–1219.

76. Giannini S, Vannini F, Lisignoli G, Facchini A. Histological and immunohistochemical analysis of an allogenic bone graft engineered with autologous bone marrow mononuclear cells in the treatment of a large segmental defect of the ulna. A case report. Chir Organi Mov 2008; 91:171–175.

77. Hawthorne AC, Xavier SP, Okamoto R, Salvador SL, Antunes AA, Salata LA. Immunohistochemical, tomographic, and histological study on onlay bone graft remodeling: part III: Allografts. Clin Oral Implants Res 2013; 24:1164–1172.

78. Nose K, Saito H, Kuroki T. Isolation of a gene sequence induced later by tumor-promoting 12-O-tetradecanoyl-phorbol-13-acetate in mouse osteoblastic cells (MC3T3-E1) and expressed constitutively in ras-transformed cells. Cell Growth Differ 1990; 1:511–518.

79. Wetterwald A, Hoffstetter W, Cecchini MG, et al. Characterization and cloning of the E11 antigen, a marker expressed by rat osteoblasts and osteocytes. Bone 1996; 18:125–132.

80. Rochefort GY, Pallu S, Benhamou CL. Osteocyte: the unrecognized side of bone tissue. Osteoporos Int 2010; 21:1457–1469.

81. Schacht V, Dadras SS, Johnson LA, Jackson DG, Hong Y-K, Detmar M. Up-regulation of the lymphatic marker podoplanin, a mucin-type transmembrane glycoprotein, in human squamous cell carcinomas and germ cell tumors. Am J Pathol 2005; 166:913–921.

82. Ariizumi T, Ogose A, Kawashima H, et al. Expression of podoplanin in human bone and bone tumors: new marker of osteogenic and chondrogenic bone tumors. Pathol Int 2010; 60:193–202.

83. Elabd C, Chiellini C, Massoudi A, et al. Human adipose tissue-derived multipotent stem cells differentiate in vitro and in vivo into osteocyte-like cells. Biochem Biophys Res Commun 2007; 361:342–348.

84. Prideaux M, Loveridge N, Pitsillides AA, Farquharson C. Extracellular matrix mineralization promotes E11/gp38 glycoprotein expression and drives osteocytic Differentiation. PLoS One 2012; 7:e36786.

85. Chen Z, Wu C, Yuen J, Klein T, Crawford R, Xiao Y. Influence of osteocytes in the in vitro and in vivo β-tricalcium phosphate-stimulated osteogenesis. J Biomed Mater Res Part a 2014; 102:2813–2823.

86. D'Aloja C, D'aloja E, Santi E, Franchini M. The use of fresh-frozen bone in oral surgery: a clinical study of 14 consecutive cases. Blood Transfus 2011; 9:41–45.

87. Monje A, Pikos MA, Chan H, et al. On the feasibility of utilizing allogeneic bone blocks for atrophic maxillary augmentation. Biomed Res Int 2014; 2014:1–12.

88. Kim S-G, Park J-S, Lim S-C. Placement of implant after bone graft using J block allograft. Implant Dent 2010; 19:21–28.

89. Milinkovic I, Cordaro L. Are there specific indications for the different alveolar bone augmentation procedures for implant placement? a systematic review. Int J Oral Maxillofac Surg 2014; 43:606–625.

90. Nissan J, Marilena V, Gross O, Mardinger O, Chaushu G. Histomorphometric analysis following augmentation of the anterior atrophic maxilla with cancellous bone block allograft. Int J Oral Maxillofac Implants 2012; 27:84–89 [PMC].

91. Chaushu G, Mardinger O, Peleg M, Ghelfan O, Nissan J, Allografts B. Analysis of complications following augmentation with cancellous block allografts. J Periodontol 2010; 81:1759–1764.

92. Chiapasco M, Zaniboni M. Failures in jaw reconstructive surgery with autogenous onlay bone grafts for pre-implant purposes: incidence, prevention and management of complications. Oral Maxillofac Surg Clin North Am 2011; 23:1–15.

93. Barone A, Varanini P, Orlando B, Tonelli P, Covani U. Deep-frozen allogeneic onlay bone grafts for reconstruction of atrophic maxillary alveolar ridges: a preliminary study. J Oral Maxillofac Surg 2009; 67:1300–1306.

94. Novell J, Novell-Costa F, Ivorra C, Fariñas O, Munilla A, Martinez C. Five-year results of implants inserted into freeze-dried block allografts. Implant Dent 2012; 21:129–135.

SUPPORTING INFORMATION

Additional Supporting information may be found in the online version of this article at the publisher's web-site:

Table S1. Summary of the demographic data, grafted sites and complications by group.

Table S2. Summary of the tomographic, histomorphometric and CSR measured variables by group.