Clinical Similarities and Histological Diversity Comparing Fresh Frozen Onlay Bone Blocks Allografts and Autografts in Human Maxillary Reconstruction

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ABSTRACT

Background: In the absence of autologous bone for harvesting, fresh-frozen bone allografts turned into an alternative for bone reconstruction procedures.

Purpose: The purpose of this study was to make a histological analysis of fresh-frozen onlay bone allografts (ALs), compared with autografts, in patients who needed maxillary reconstruction prior to dental implants placement.

Materials and Methods: Twelve patients with bone deficiencies (width inferior to 4 mm) in the sites where the implants were planned were enrolled in the study. From these, six were elected to be treated with autogenous (AT) bone grafts and six with fresh-frozen bone AL. This last group included the patients who had absence of a convenient amount of bone in donor sites. Each patient received from one to six graft blocks, totalling to 12 ATs and 17 ALs. Seven months after grafting procedures, biopsies of the grafts were made using 2-mm internal diameter trephine burs, and processed for histological analysis. One biopsy was retrieved from each patient.

Results: Clinically, all grafts were found to be firm in consistency and well-incorporated to the receptor bed. Histological analysis showed a large amount of necrotic bone surrounded by few spots of new-formed bone in the AL group, suggesting low rate of graft remodeling. In the AT group, an advanced stage of bone remodeling was seen.

Conclusions: Human fresh-frozen bone block AL showed clinical compatibility for grafting procedures, although associated to slow remodeling process. Further studies are needed to define, at long term, the remodeling process chronology the clinical longitudinal results for fresh-frozen bone AL.

KEY WORDS: atrophic maxillae, bone allograft, histological analysis

INTRODUCTION

The achievement of high success rates in implantology is directly connected to the presence of an adequate bone volume, to permit and maintain the osseointegration of dental implants through the time.[1–3] Bone volume is normally diminished by tooth loss, trauma, periodontal disease, and other pathologies,[4,5] and the most predictable process to rehabilitate patient's bone architecture is bone grafting, before implants placement.[6–8]

The gold standard for bone grafting is autogenous (AT) bone,[9,10] harvested from extra-[11–14] or intra-oral[15–17] donor sites. AT bone graft is accepted as a highly predictable and effective method, without immunologic impediments.[9,18–20] However, this technique is associated to disadvantages such as postsurgical pain, increased

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blood loss, risk of paresthesia and infection, and in several cases, with deficiencies in the quality and quantity of available bone donor sites, providing an insufficient bone volume to be grafted prior to implants placement.[21]

In the absence of AT bone for harvesting, fresh-frozen bone allografts (ALs) turned into an alternative, in view of the fact that it provides a reasonable source for grafting material.[6,7,22–24] During the last two decades, the use of fresh-frozen bone AL has significantly increased.[25,26] This fact is directly related to the establishment of severe guidelines for bone processing, which defined the donor selection, how the bone must be harvested, processed, and stored, together with the record-keeping procedures that must be respected. The increased the safety of this bone graft, and the recent absence of reports on cross-contamination in its clinical use, considering diseases such as hepatitis or HIV, makes the fresh-frozen bone AL an alternative to autografts.[27–40] Another concern about bone ALs is their possible antigenicity, but there is still limited information regarding this issue.[41]

The purpose of this study was to make a histological analysis of fresh-frozen bone onlay ALs, compared with autografts, in patients who needed maxillary reconstruction prior to implants placement.

MATERIALS AND METHODS

This research protocol was approved by the Araraquara School of Dentistry Ethics Committee (CEP-FO/Car) and by the National Research Ethics Committee (CONEP-MS), under protocol number 36/08. All treatments were performed in the Department of Periodontology, Araraquara Dental School (UNESP – Univ. Estadual Paulista), Araraquara, São Paulo, Brazil.

A total of 12 partially or totally edentulous patients (five males and seven females, age ranging from 25 to 60 years), who desired oral rehabilitation with titanium implants and had at least one site with severe bone deficiency (i.e., <4 mm alveolar ridge width) not allowing the placement of a regular size implant, were treated. Alveolar ridge width was determined on the cross-sectional image section view of cone beam computed tomography (CBCT) (i-CAT Classic, Imaging Sciences International, Hatfield, PA, USA), generated images (DICOM-based data sets) 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. None of the patients presented with systemic diseases affecting bone turnover, or were pregnant or lactating, or had habits that could interfere with treatment (for example, smoking, alcoholism, and drug use).Treatment allocation in the present study was not randomly assigned. Patients judged as not having adequate amounts of donor (autologous) bone were treated with fresh-frozen bone ALs. This decision was taken based on the clinical screening and CBCT examinations and depended on the amount of bone resorption and/or number of sites requiring reconstruction in each patient. Following these criteria, six patients were elected to be treated with ATs and six with fresh-frozen bone ALs.

The AL was processed according to American Association of Tissue Banks (AATB) guidelines[42] in an bone tissue bank (UniOss, Marília, Brazil) authorized by the Brazilian Ministry of Health. During the first surgical phase and under local anesthesia, a total flap was attained to provide a full visualization of the bone defect. Any reminiscent of soft tissues were removed from the bone surface, and delicate burs were used, always under intense saline solution irrigation, to etch the host cortical bone allowing the vascularization to occur in an easier way toward the grafts.

In the AL group, the cortical bone blocks were removed from the freezer and were put into sterile saline solution for 10 minutes before surgical procedure, to hydrate and gradually get to room temperature. The cortical ATs were harvested from the mandibular retromolar region, following the classic technique.[43] For both groups, all graft blocks were prepared with careful trimming using #700 cylindrical and maxi-cut burs, always under abundant sterile saline solution irrigation, and adjusted to the host site. The graft blocks were fixated to the host bone site using 1.5×10-mm screws (Neodent, Curitiba, Brazil) and covered by a collagen membrane (Genius Baumer, São Paulo, Brazil) prior to the interrupted nylon 4-0 suture (Figure 1). Each patient received from one to six graft blocks, totalling to 12 ATs and 17 ALs.

All patients received antibiotics (Amoxicillin 500 mg, three times daily for 7 days), nonsteroidal anti-inflammatory treatment (Nimesulide 100 mg, two times daily for 5 days), and analgesics (Acetaminophen 750 mg, according to individual needs). Patients continued to rinse with Chlorhexidine digluconate for the following 7 days. Sutures were removed 14 days after surgery.

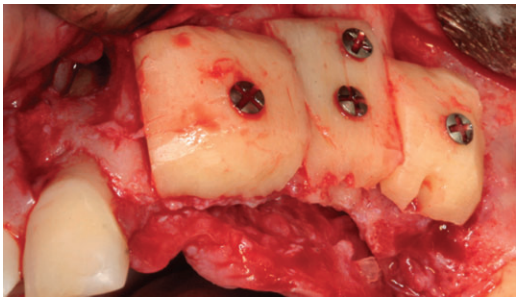

Figure 1 One of the treated cases in the bone allografts group. The grafts trimmed and installed with fixation screws.

At the second surgical phase, 7 months after the grafting procedure, a 2-mm internal diameter trephine bur was used to retrieve a biopsy containing the grafted and native bone areas. The biopsy was carefully obtained perpendicularly to the graft (Figure 2). One biopsy was retrieved from each patient.

The retrieved biopsies were routinely processed for light microscopy, and 6-mm thin sections stained with hematoxilin-eosin were used for descriptive histological analysis using a DIASTAR light microscope (Leica Reichert & Jung products, Wetzlar, Germany) connected to a Leica Microsystems DFC-300-FX digital camera (Leica Microsystems, Wetzlar, Germany). Observations were made in the whole biopsy looking for a comparison between grafted and the native bone that allowed the distinction of three areas: (1) area comprising the pre-existing residual bone (e.g., residual maxillary bone); (2) area comprising the interface between the grafted bone and the host bone; and (3) area comprising the still unremodeled area of the graft bone. The delineation of the unremodeled bone was made based in histological criteria, e.g., absence of nucleus in osteocytes lacunae and local absence of capillaries.

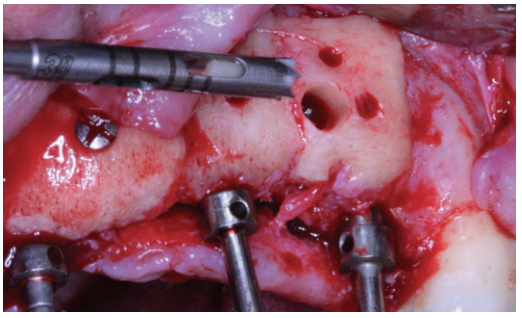

Figure 2 Biopsy retrieval, in a place that does not interfere in implants placement. The biopsy contain sample of the grafted and native bone areas.

RESULTS

The surgical approach used in the patients presented in this study allowed implant placement without significant inflammatory reaction, for both treated groups. During the healing period, the patients used tooth-supported, fixed or removable, prostheses. Healing proceeded within normal limits with no complications and the bone grafts showed adequate bone adaptation when the sites were reopened, permitting that implants were placed and bone biopsies were retrieved without any loss for the patient. All retrieved biopsies (a total of 12) were suitable for macro and microscopic analysis.

Implants (a total of 40) were placed with a minimum of 35 Ncm of torque in all cases, in the augmented sites. All patients were able to proceed to the prosthetic phase of the treatment.

Clinically, the AL group showed signs of a delayed remodeling compared with the AT group, because there was the perception of large amounts of unremodeled bone in the former group (Figure 3, A and B). The macroscopic analysis of the biopsies, using a

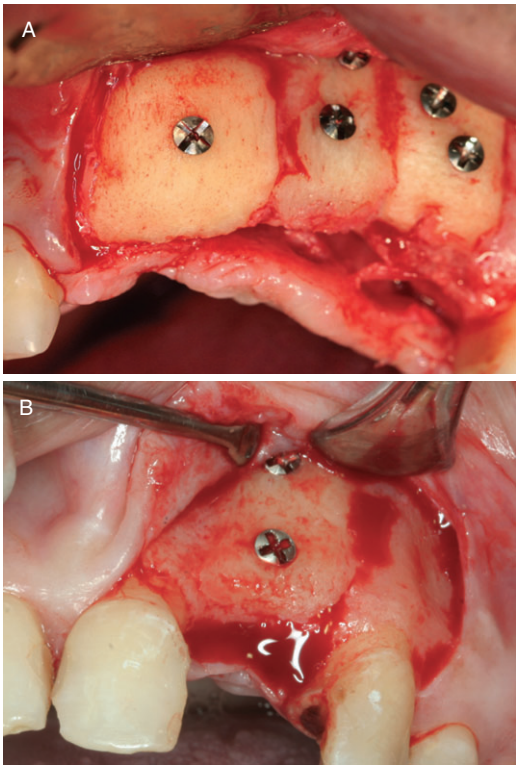

Figure 3 Examples of the grafted sites seven months after first surgery. In the bone allografts-treated group (A), the macroscopic difference between the host bone and the grafted material is clear, suggesting not complete bone remodeling. In the autogenous bone grafts group (B), the macroscopic analysis of the grafted material shows signs of advanced remodeling.

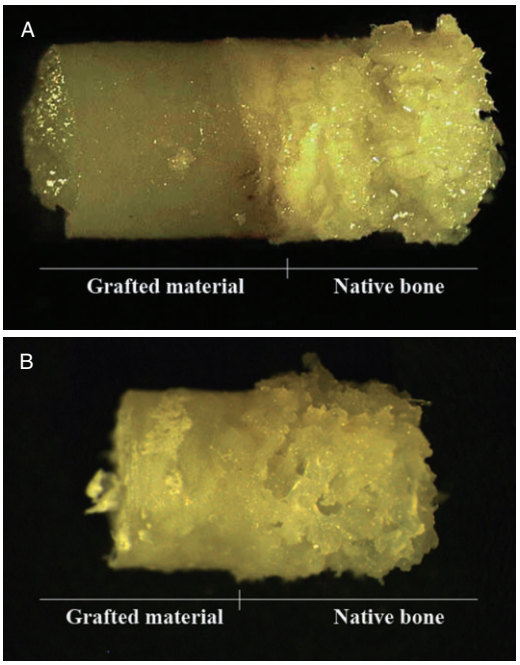

Figure 4 Stereomicroscopic view of one of the retrieved biopsies. In the bone allografts group (A), the difference between the native bone and the grafted material is clear, although there is evidence of continuity and biocompatibility between these two segments. In the autohenous bone grafts group (B) is difficult to delimitate the boundaries between the non-remodeled and remodeled grafted material.

stereomicroscope, confirms the clinical perceptions, showing in all six cases of AL group reminiscent and apparently quiescent large areas of the original cortical of the AL. In the AT group, those boundaries were unclear and it was not visible as in the AL group (Figure 4, A and B).

The microscopic analysis of the biopsies from the AL group showed large and predominant segments of necrotic bone (as seen on Figures 5 and 6, in the small frame showing the full biopsy view), with empty osteocytes lacunae and little or devoid osteoclastic activity. Frequently, blood vessels were seen invading the Haversian canals of the grafted material. Although revascularization, not completed, was a feature present at 7 months after AL, only in very few areas active osteoblasts within the Haversian canals were present indicating that a centrifugal bone remodeling process is plausible, but very limited (see Figure 6). These remodeling areas were recognized by the presence of newly formed primary bone. In addition, no direct contact between the remodeled bone and the grafted bone could be seen (see Figure 5), and there was still a clear separation between the residual bone and the grafted material,

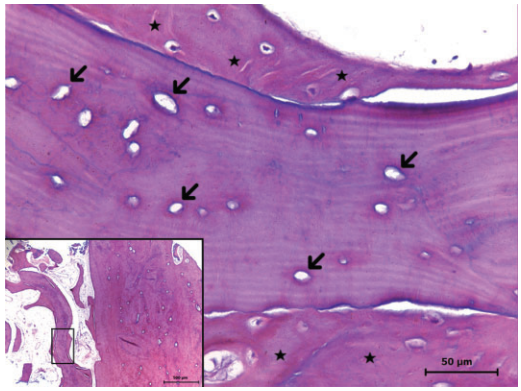

Figure 5 Photomicrograph of an overview of the biopsy area corresponding to the bone allografts, with all bone on the right side of the biopsy still necrotic (in the frame). Higher magnification (of the area corresponding to the rectangle) showing the great volume of necrotic bone, with empty osteocytes lacunae (→) reminiscent from the graft material. In very few areas, the necrotic bone was surrounded, by newly formed bone (*), showing exuberant osteocytes, although it is not directly attached to the host site.

as observed in stereomicroscopy analysis. In the interfaces between the grafted AL and the host site, the remodeling process was usually more advanced than in the boundaries of the graft.In the AL group, there were some osteoclastic activity, surrounded by a connective fibrous tissue with a lack of inflammatory cells, but newly formed bone failed to invade the graft itself.

In theAT group, small or no areas of necrotic bone could be seen in the full biopsy (as seen on Figure 7, in the small frame showing the full biopsy view), with exuberant osteocytes all over the chosen fields of view (see Figure 7). The presence of several reversion lines

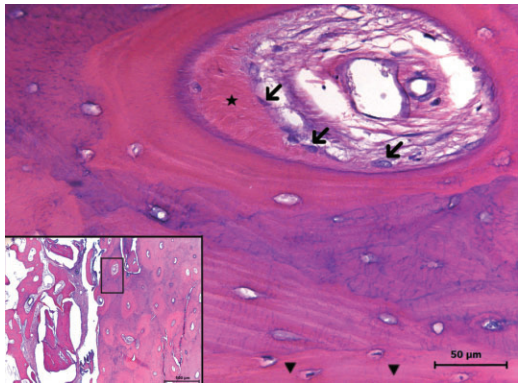

Figure 6 Photomicrograph of an overview of the biopsy area corresponding to the bone allografts (in the frame). In the higher magnification, blood vessels penetrating in the original Haversian system of the grafted bone, with active osteoblasts (→) and newly formed primary bone (*) can be seen. In the boundaries, remodeled and viable bone can be seen (▲), integrated to the grafted material (still necrotic).

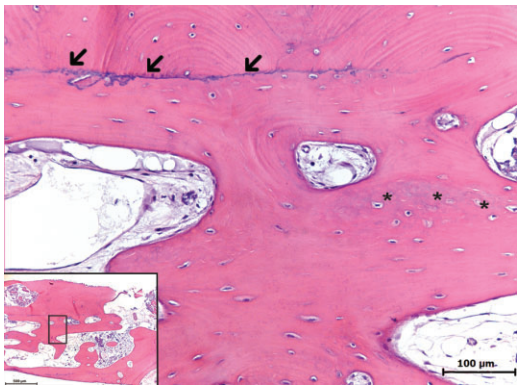

Figure 7 Photomicrograph of an overview of the biopsy area corresponding to the autogenous bone grafts (in the frame). The higher magnification shows an advanced remodeling stage with several reversion lines and lamellar bone, without the presence of necrotic bone. A large number of exuberant osteocytes in lacunae is associated to non lamellar arrangement typical of newly remodeled bone (*).

indicate advanced stage of remodeling of these grafts, with no perception of difference between the grafted and the host bone, as previously shown in the stereomicroscopy analysis (see Figure 7). Considering all blocks from the AT group, it is observed that the largest area of the grafts were completely remodeled after 7 months, and were already integrated to the host bone site because the difference between the residual bone and grafted material was almost inexistent.

DISCUSSION

In the last years, bone has been the second most transplanted tissue – blood is the most common.[44] ALs are one of the possible alternatives for AT and, in the last decade, its use has increased 15-fold, accounting for about one-third of bone grafts performed in the United States (considering its use in dentistry and orthopedics).[7,45,46] The advantages of using bone ALs include the decreased operative time and trauma for the patient, and a theoretically unlimited supply of reconstructive material, allied to decreased blood loss and absence of donor site morbidity.[47]

However, there are some disadvantages in using AL, and the most visited are the different biological properties compared with AT,[44] and the risk of disease transmission and antigenicity.[25] Regarding the risk of disease transmission, there is a wide range of studies that state that AL represent a minimal risk to the patient.[9] If we consider the bone-tissue banks that are based on the AATB standard protocols, the risk of viral transmission by unprocessed deep-frozen, nonirradiated grafts from screened donors is currently less than 0.0005% for hepatitis C virus and 0.0001% for HIV.[48]

AL do not provoke severe marked immune responses and has acceptable compatibility with the recipient site, although there are not many long-term studies regarding this issue so far.[49] All patients treated in this study, in both AL and AT groups, presented good systemic health after the bone-grafting procedures, without any signs of contamination or immunological incompatibility problems.

Regarding the biological properties is already well established that the processing of AL tissue, even if only antibiotic washing and deep freezing are used, although lowers this risk of cross-infection, can significantly weaken the biologic and mechanical properties initially present in the bone tissue.[21,50] Through our histological evaluation, it could be seen that the AL-grafted bone showed biocompatibility, without signs of inflammatory reactions toward it. However, in disagreement to some previous results reported in the literature,[9,51] we observed that AL, in the macro and microscopic analysis, showed predominance amount of nonremodeled bone. This last (and important) characteristic was not seen in the AT group, where the majority of the grafted material was already remodeled and showed characteristics of vital bone. This agrees with Zerbo et al.'s[52] findings that the final volume of nonvital bone, evaluated from 2.5 to 7 months after AT cortical block bone grafting in the human maxilla, ranged from 1% to 34%.

Confronting our histological findings, there are some articles that state that AL can retain some osteoinductive properties, with the preservation of bone morphogenetic proteins in spite the protocols regarding this biomaterial's attainment, which allow the new-bone deposition.[53–56] Some studies of maxillary ridge augmentation using fresh-frozen bone ALs revealed living and newly formed bone incorporated with the grafted areas.[53–56] Unlike our results, the literature suggests that grafted bone would be incorporated without the reminiscence of grafted material, even at 7 months after grafting surgery.[9] In opposition to this idea, and corroborating our findings, there are also articles regarding inadequate revascularization, little creeping attachment, and decreased mineral accretion associated to a small number of cells working in the remodeling process of the fresh-frozen bone ALs.[6–8]

Recent articles even relate the impairment in these grafts remodeling process with a low level of receptor

activator of nuclear factor kappa B ligand and vascular endothelial growth factor compared with the levels related to autografts.[57] All these articles concluded that under all circumstances, bone ALs function histologically and biologically poorly compared with autografts, mainly if we consider the first 12 months following the bone-graft surgery,[6] and this is consistent with the results of the analysis of biopsies demonstrated in our study.

The delayed remodeling of the AL, evidenced in our study by little or no presence of active osteoclasts and osteoblasts, could have direct implications over its clinical use. In medical orthopedic surgery, the limited bone forming and remodeling of structural ALs is directly associated with the 25–35% failure rate within 3 years because of infection, fracture, and nonunion. The fractures at this late stage are the result of the accumulation of microcracks that cannot be repaired by the necrotic bone because there is no vascular supply. As a result of this poor clinical success, the use of structural ALs has been restricted to repair segmental defects following tumor resection in cancer patients.[58] In our study, although there was a biological delay in these grafts remodeling process, the installation of titanium implants at the correct site was possible, according to other results present in literature.[51] This makes clear that AL grafts acted only as an osteoconductive biomaterial, without any osteogenic or osteoinductive properties, such as shown by AT.[21] However, the effects of these biological events in long-term clinical results of fresh-frozen bone ALs in maxillary bones, and the success rate of implants and implant-supported prosthesis installed over them remain unclear.

CONCLUSION

Although limited, our results showed that AL is histologically inferior compared with AT, especially regarding the delayed remodeling process shown by the AL. On the other hand, AT and AL presented biocompatibility and allowed the installation of titanium implants in the planned 3-D position. Long-term studies are urgently needed to elucidate the remodeling process chronology for AL, and delimitate the clinical consequences of substituting AT by AL.

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