Bone allograft has become an alternative to autogenous bone due to its decreased operative trauma and the almost unlimited supply of reconstructive material. The aim of the present study was to histologically evaluate the suitability of fresh-frozen bone graft (test group) used in maxillary ridge augmentation, comparing it to autogenous bone (native maxilla: control group). During the re-entry procedures, 9 months after the fresh-frozen allogeneic bone blocks were placed in the atrophic maxillary ridges, bone cores were removed with a trephine bur from test and control treatments in the same patient. Routine histologic processing using hematoxylin and eosin and Picrosirius staining was performed. Mature and immature collagen area and density analysis were carried out for both groups under polarization. The results of Student’s t test for paired samples ($P > .05$) showed no statistically significant difference in mature and immature collagen area or density percentage between test and control groups. Histologically similar bone formation patterns were observed in both groups. We concluded that fresh-frozen bone allograft is a biologically acceptable alternative for augmentation of the deficient alveolar ridge, showing a similar collagen pattern to that of autogenous bone.

**Key Words:** bone allograft, histologic analysis, picrosirius, collagen

**Introduction**

The increasing popularity of dental implant surgery has created a heavy demand for dentoalveolar reconstruction. Insufficient alveolar contours may require bone grafting procedures to restore adequate bone volume before implant placement to counteract potentially harmful results, such as higher failure rates and unsatisfactory esthetic results.$^{1,2}$

Bone may come from the host (autograft), a donor (allograft), or other sources such as xenograft, ceramics, and demineralized bone matrix.$^{3}$ Autogenous bone graft remains the gold standard for the reconstruction of bony defects because it is the only graft material that exhibits the 4 desired properties of bone graft materials: osteogenesis, osteoinduction, osteoconduction,
and osseointegration. Nevertheless, considerable drawbacks to its use have been noted: high morbidity at the donor site, limited quantity of bone, unpredictable quality of bone, increased blood loss, increased operative time, and donor site infection.

Allogeneic bone is the most commonly used alternative to the autogenous harvest, and the advantages of its use include convenience for the surgeon, decreased operative trauma and blood loss, absence of donor site morbidity, and greater availability of bone.

The properties of the allograft are directly related to the steps taken in processing the material. Frozen bone is harvested under aseptic conditions and is processed by storing the sterile specimen at $-80^\circ$C until the transplant moment. No additional preparation is needed, and currently, frozen bone is considered a safe material from a viral point of view because of rigorous donor screening and aseptic proprietary processing programs. Freezing also decreases the immunogenicity of a bone allograft. Although it is frequently used by orthopedic surgeons with positive long-term results, some studies suggest that although meaningful clinical healing does occur with cortical allografts, the graft is never entirely replaced.

Most of the research on fresh-frozen bone allografts has been conducted in the field of orthopedic reconstructive surgery; the published oral and maxillofacial literature is limited in scope. In addition, no quantitative analysis has studied the pattern of collagen fiber organization in fresh-frozen bone grafts used in maxillary ridge augmentation as compared with autogenous bone. The aim of the present study was to histologically evaluate the suitability of fresh-frozen bone graft (test group) used in maxillary ridge augmentation, comparing it with autogenous (native) bone (control group) from the same patient, using the Picrosirius-polarization method.

**Patients and Methods**

**Patient selection and reconstructive surgical procedure**

Approval for this study was obtained from the Ethics Committee in Research at Pontificia Universidade Católica do Paraná, Brazil, number 1480. All subjects signed a consent form to participate in the study.

From April 2005 to June 2006, 18 patients from the postgraduate course in Implantology at the Universidade Federal do Paraná who had atrophic maxillary ridge necessitating bone block grafts before implant placement were consecutively admitted to this study. Patients without sufficient compliance with therapy and those with systemic medical conditions were excluded. The group consisted of 6 males and 12 females, and the average age was 41.5 years, with a range from 27–61 years.

The maxillary reconstructions were made with human block grafts of tibial fresh-frozen chips obtained from the Clinics Hospital Bone Bank of Universidade Federal do Paraná, which follows all strict regulations adopted by the American Association of Tissue Banks (AATB), meeting uniformly high safety and quality control measures. The bone bank comprehensively screens its donors through a thorough assessment of medical and lifestyle stories. In addition to historical screening, serologic tests for syphilis, Chagas disease, human immunodeficiency virus (HIV), hepatitis A, B, and C, and human T-cell lymphotropic virus-1 (HTLV-1) and culture for fungi and aerobic and anaerobic bacteria are performed.

Maxillary ridge resorption was evaluated through clinical and radiologic examination. A panoramic radiograph and routine blood examination, including hemogram, coagulogram, creatinine, and glycemia, were performed for all patients. Those who presented altered blood tests or any other systemic disease that would lead to any risk during
the surgical procedure or during the healing period were excluded from the study.

Patients were premedicated 1 hour before surgery with amoxicillin (Amoxil, Glaxo SmithKline, Rio de Janeiro, Brazil) and dexamethasone 8 mg (Decadron, Ache´r´ios Farmace´uticos, Guarulhos, Sao Paulo, Brazil).

All surgeries followed the same routine: The bone was thawed for 1 hour, and then the blocks were sculpted with chisels and rotary instruments to adequately fit the osseous defect with no gap. An appropriate local anesthetic (Articaine 4% epinephrine 1:100.000, DFL, Rio de Janeiro, Brazil) was administered, and a full-thickness mucoperiosteal flap was elevated. After careful site preparation, the blocks were perfectly adapted to the maxillary wall and were fixed with miniscrews (Neodent, Curitiba, Parana, Brazil). Miniscrews were placed through the central portion of the blocks and rested in the palatal portion of the defect to prevent micromovement of the graft; when larger blocks were used, 2 miniscrews were placed in each lateral portion of the graft. After the blocks were fixed, some perforations were made to enhance marrow space bleeding (Figure 1).

The number of blocks that each patient received ranged from 1 to 4. The flaps were repositioned without tension, and silk sutures (4.0, Ethicon Inc, Somerville, NJ) were used for closure. One sample of the allogeneic bone that was not used in the grafting procedure was sent for histologic analysis. Postoperatively, systemic antibiotic (amoxicillin 1 g, twice a day) was prescribed for 1 week, and paracetamol with codeine (one 30 mg tablet every 4 to 6 hours for 48 hours) was prescribed for pain control. Dexamethasone (4 mg 3 times a day) was administered for 2 days to minimize edema. Antiseptic mouthwash (0.2% chlorhexidine gluconate) was used twice daily for 2 weeks. Sutures were removed after 7 days.

The reopening surgery was carried out an average of 9 months after bone graft (range, 8–11 months). After the implants (Systhex Sistema de Implantes Osseointegrados, Curitiba) were placed, a bone core from the graft bone (test group) was removed with a 2.5 mm diameter trephine bur for histologic analysis (Figure 2). The bone cores were all removed with the bur in a buccopalatal direction, thus guaranteeing that the biopsies contained only allograft bone without

Figures 1–3. Figure 1. Fresh-frozen bone block allograft fixed to the maxillary ridge with titanium miniscrews. Figure 2. Bone core removal from the allograft for histologic analysis. Figure 3. Transoperative view during the reopening surgery for implant installation. Note the excellent incorporation of the graft.
any portion of native bone. For the control group, a bone core from the autogenous bone (native maxilla) of the same patient was removed with a trephine bur of the same size, from a neighboring nongrafted area. Panoramic radiographs were routinely performed in the first and sixth postoperative months to evaluate the osseointegration of the implant.

**Histologic analysis**

The bone specimens were routinely processed for serial decalcified sections. Specimens were fixed in 10% neutral buffered formalin solution for 48 hours and decalcified in 5% trichloroacetic acid for 15 days, then were embedded in paraffin. Blocks were cut to 6-μm-thin slides and were stained with hematoxylin-eosin (HE) and Picrosirius. The sections stained with HE were used to evaluate bone morphology. Those stained with Picrosirius were viewed with a light microscope (10× at objective 6.3× Optovar 1.25) (Olympus BX50, Olympus, Japan) under polarized light coupled to a computer containing Image ProPlus 4.5 software (Media Cybernetics Inc, Bethesda, Md) for image analysis of the collagen area and density. The maximally bright position of collagen fiber bundles was determined before evaluation. Birefringent shades of yellow to red bands are indicative of mature collagen, and a greenish birefringence indicates immature collagen.13,14 The percentage of mature and immature collagen area and density were measured in 2 fields of each section of test and control groups. The mean percentage was obtained for each of the examined sections.

**Statistical analysis**

All data were tabulated and statistical tests were performed with the Statistical Package for the Social Sciences (SPSS) for Windows, version 13.0 (SPSS Inc, Chicago, Ill). The normality of the data was tested using the Kolmogorov-Smirnov test at a 5% significance level. Student’s t test (P < .05) for paired samples was used to detect significant differences in mature and immature collagen areas and density percentages in the test and control groups.

**Results**

A total of 39 fresh-frozen bone blocks were grafted, and clinical success was observed in all patients. No case of infection was reported. During the reopening surgeries, all blocks were firm in consistency, well incorporated and vascularized (Figure 3). None of the blocks was dislodged at the time of implant placement, and additional grafting was not necessary in any case. A total of 58 implants were placed into the grafts; all are currently accompanied by implant-supported restorations.

Histologic analysis of the sections stained with HE revealed a typical lamellar arrangement around Haversian canals interspersed with osteocytes in lacunae, characteristic of secondary bone, in all specimens of the test group. No evidence of acute or chronic inflammatory infiltrate was found in any of the samples. Similar histologic aspects were observed in the control group (Figure 4).

Margins of the grafted bone revealed osteoblast-like cells and the presence of a large quantity of osteocytes in lacunae. The central portions of the grafted bone revealed fewer osteocytes with a higher number of empty lacunae; blood vessels were always present (Figure 5).

Margins of the grafted bone revealed osteoblast-like cells and the presence of a large quantity of osteocytes in lacunae. The central portions of the grafted bone revealed fewer osteocytes with a higher number of empty lacunae; blood vessels were always present (Figure 5).

Picrosirius-polarization analysis was used to obtain the area and density mean percentage of mature and immature collagen in test and control groups. The Kolmogorov-Smirnov test revealed that data showed a normal distribution for mature and immature collagen area and density percentage, when the average values of test and control groups were compared (P > .05).
Student's t test revealed no significant difference ($P > .05$) in collagen area or density percentage in test and control groups (Table). In both groups evaluated by polarization, the arrangement of collagen fibers in test and control groups did not differ (Figure 6).

The nongrafted allogeneic bone under polarization revealed exclusively the presence of yellow to red birefringent shades, characteristic of mature collagen. Nine months after the graft, the same tissue presents greenish birefringent shades characteristic of immature collagen (Figure 7).

**TABLE**

Mean and standard deviation of area and density percentage of immature and mature collagen of test and control groups*†

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*Student's t test (paired samples): $P < .05$.
†N indicates number of patients; ica, immature collagen area; mca, mature collagen area; icd, immature collagen density; mcd, mature collagen density; T, test group; C, control group.
Reports of the use of bone allograft appeared as early as the 1800s; however, it was the establishment of large-scale tissue banks that facilitated the routine use of these allografts.\(^\text{15}\) Most allograft studies were performed in the orthopedic field, where they have been used for a long time for many applications such as trauma, spine fusion, revision arthroplasty, tumor surgery, and nonunion.\(^\text{11,16–18}\) Very few published studies have investigated the oral and maxillofacial areas, and most of these were executed in animals\(^\text{1,5,19}\) or were limited to case report series.\(^\text{2,8,20,21}\) To our knowledge, this is the first study comparing the presence of mature and immature collagen in fresh-frozen block allografts in maxillary ridge augmentation and autogenous bone in humans, using polarized microscopy.

Fresh-frozen bone can be used up immediately on thawing; it has texture and strength characteristics similar to those of autogenous bone at the time of placement.\(^\text{3,9,20}\) It affords decreased immunogenicity without changes in biomechanical properties.\(^\text{6,9}\) The problems of potential disease transmission and antigenicity have been widely studied and represent minimal risk to the patient.\(^\text{8,10,17}\) Because of strict regulations adopted by the AATB, the rate of disease transmission has almost halted.\(^\text{4}\)

The long healing time with allograft bone can be considered a drawback when allograft is compared with autologous bone graft. With the latter, the reopening surgery...
occurs 4 to 5 months after the graft surgery. Because the use of allograft is a new procedure in maxillary ridge augmentation and a protocol has not yet been established, we decided to double the time used for autologous grafts to ensure a safe reopening surgery. The idea was to reopen after 8 to 9 months for all patients, but because of the absence of 3 patients on the scheduled days of the surgeries, the reopening occurred 10 (for 1 patient) and 11 months (for 2 patients) later. At this time (8–11 months later), we observe excellent vascularization and incorporation of all grafted blocks. However, we may have observed good results at 6 or 7 months as well. Future studies are necessary to determine the best timing for the reopening surgery.

Other authors have previously demonstrated efficacy in using block allografts in areas of dental implant placement. The good results obtained in the present study are in accordance with those previous reports: during the reentry procedures, all grafts were firm in consistency, well incorporated, and vascularized. Furthermore, implant placement in grafted areas demonstrated the functionality and strength of regenerated bone, as none of the blocks was dislodged at the time, and no additional grafting was necessary.

Bone is fundamentally composed of cells, inorganic matrix, and organic matrix. Collagen composes approximately 90% to 95% of the organic component of bone and is a fundamental building block in the process of new bone formation. Collagen molecules, being rich in basic amino acids, react strongly with acidic dyes. Picrosirius is an elongated dye molecule that reacts with collagen and enhances its normal birefringence.

The Picrosirius-polarization method is a specific histochemical procedure for collagen detection in tissue sections, where interstitial collagens display different interference colors and intensities, which reflect birefringence. Birefringent shades of yellow to red are indicative of mature collagen, which is typical of mature bone. Greenish birefringence is indicative of immature collagen, the earliest type to appear during bone formation and/or renewal. The color and intensity of the collagen birefringence also vary, depending on the fiber diameter, tissue section thickness, or both.

Bone grafts of any type can regenerate bone through 3 possible mechanisms: osteogenesis, osteoinduction, and osteoconduction. Although the osteoinduction properties of the allografts remain controversial in the literature, some authors indicate that the most important advantage of fresh-frozen bone is that the osteoinductive proteins are not destroyed in the preparation. A previous study showed that osteoblast-related cells can be grown in vitro from fresh-frozen allograft specimens, and these cells were morphologically indistinguishable from those grown out of freshly harvested trabecular bone. According to the results of the present study, we can suggest that allogeneic bone has an osteoconductive properties and a slower incorporation process due to the presence of many empty lacunae in the central portion of the graft; immunohistochemical studies are necessary to confirm its osteoinductive character.

Histologic evidence of new bone deposition in allografts has already been demonstrated by other authors. The histologic analysis in the present study supports good clinical results: Oriented and uniform bone deposition was observed and evidence of necrotic bone was not seen in any of the samples from the test group. Similar bone formation patterns were observed between the allograft and autogenous bone, with viable osteocytes and osteoblasts in both groups. The presence of a greenish birefringence in the grafted allogeneic bone, which did not appear before the grafting proce-
dure, may indicate that osteoblasts are being stimulated to produce matrix, demonstrating the presence of a bone renewal process, which would explain why younger fibers are present.

**CONCLUSION**

From the results of the present study, we can conclude that fresh-frozen bone allograft is a biologically acceptable alternative for augmentation of the deficient alveolar ridge, showing a similar collagen pattern to that of autogenous bone.

Further research and additional clinical studies of this material are recommended, as it certainly opens up a new perspective in the field of oral and maxillofacial surgery.

**ABBREVIATIONS**

AATB: American Association of Tissue Banks
HE: hematoxylin-eosin
HIV: human immunodeficiency virus
HTLV-1: human T-cell lymphotropic virus-1
SPSS: Statistical Package for the Social Sciences

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