Introduction

An adequate alveolar bone volume is required for successful functional–aesthetic prosthetic implant rehabilitation. Unfortunately, the placement of guided dental implants may be impossible in the case of alveolar bone resorption. During the last ten years there has been an increased request for prosthetic implant rehabilitation in patients with alveolar ridge atrophy. Implant insertion can be performed by surgical techniques such as osteotome sinus floor elevation (OSFE) and splitting crest in minor cases of atrophy, but otherwise it is first necessary to increase bone volume. This may be achieved by sinus lifting, bone-added osteotome sinus floor elevation technique (BAOSFE), future site technique, guided bone regeneration (GBR) or osteodistraction, but the most common technique used for three-dimensional ridge construction is the use of bone grafts.

Abstract

Statement of the problem: Although autologous bone is considered to be the gold standard grafting material, it needs to be harvested from patients, a process that can be off-putting and can lead to donor site morbidity. For this reason, homologous fresh-frozen bone (FFB) was used in the current study as an alternative graft material.

Purpose of the study: The aim of this study was to evaluate the effectiveness of FFB as a grafting material in complex maxillary sinus lift with immediate implant insertion.

Methods: FFB was obtained from the Veneto Tissue Bank and preserved at -80°C. Twenty-one patients were surgically treated with FFB block grafts in 26 maxillary sinus rehabilitations, with 47 immediate implant insertions, with a reopening phase after six months. All patients underwent orthopanoramic X-rays and CT scans before, immediately after and four months (X-ray only) post surgery. Bone biopsies were performed in order to evaluate the volume and density of the bone grafts, which all showed optimal adherence without complications.

Results: Four months post surgery, 64% of grafts showed no evidence of bone resorption or resizing. In all other cases resorption was slight. All implants were clinically osseointegrated, with only one implant failure during the provisional prosthetic loading stage (97.8% success rate). Histological studies confirmed these results, showing the presence of new bone and sparse osteoclastic activity four months post implantation, with 80% mature bone material observed after 12 months.

Conclusions: Use of FFB permits effective bone-adding surgery and immediate implant insertion under local anaesthesia, decreasing both chair time and patient discomfort.

Key words: Fresh-frozen bone; sinus lift; bone block grafts; jaw atrophy; implants.
Although autologous bone is considered to be the best grafting material due to its particular physiological properties (e.g., osteoinduction, osteoconduction, osteogenesis, biocompatibility), this type of bone harvesting involves both pain and possible complications. For example, bone harvesting in the hip may cause oedema as well as pain for four to five days at the donor site. Additional adverse effects include the necessity to use crutches for two weeks and reduced mobility for up to a month. Surgical complications such as lateral thigh anaesthesia, lesion of the inguinal ligament and abnormal iliac fractures have also been shown to occur in some cases.

To prevent the possibility of donor site morbidity due to the use of autologous bone, other grafting materials available on the market may be chosen. These include homologous bone (e.g., demineralised freeze-dried bone allograft [DFDBA], freeze-dried bone allograft [FDBA]), heterologous bone (e.g., bovine, equine or porcine derivatives), and alloplastic materials (e.g., ceramic, bio-glass, hydroxy-apatite and tricalcic sulphate). While none of these materials are osteoinductive, they are all osteoconductive and may therefore be considered as alternatives to autologous bone (Table 1).

In the present study, homologous fresh-frozen bone (FFB) obtained from the Veneto Tissue Bank in Treviso, Italy. The FFB was obtained from the Veneto Tissue Bank in Treviso, Italy. The requirements for homologous bone donors are more stringent with respect to those of organ donors. The presence of risk factors such as contagious disease, neoplasm, rheumatic and/or degenerative disease and sepsis necessarily disqualifies the donor. In order to detect infectious agents, the following tests are performed on donor blood samples taken within eight hours of death: anti-HIV-I/II antibody (Ab); anti-HCV Ab; hepatitis B virus (HBV) surface antigen (HbsAg); anti-HBV core (Hbc) Ab; anti-HBV surface (Hbs) Ab; anti-human T-lymphotropic virus (HTLV)-I/II Ab; anti-Ag Treponemal Ab; anti-cytomegalovirus (CMV) immunoglobulin (Ig)G Ab; anti-CMV IgM Ab; anti-Toxoplasma IgG Ab; and, anti-Toxoplasma IgM Ab. A haemoculture is also performed to detect aerobic and anaerobic bacteria, mycobacteria and mycotic agents. As a further safety precaution, a serological follow-up is conducted using PCR techniques to detect any viral RNA or DNA of HIV, HCV and HBV. This method reduces the “diagnostic window period” of seven days for HIV, HCV and HBV. At the base of these time margins, the possibility of viraemia at time of donation may be extrapolated. These values are theoretical, since they are calculated

### Table 1: Qualities of reconstructive grafting materials.

<table>
<thead>
<tr>
<th>Material</th>
<th>Osteogenesis</th>
<th>Osteoinduction</th>
<th>Osteoconduction</th>
<th>Structural strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autologous bone</td>
<td>++ (medullary)</td>
<td>+ (cortical)</td>
<td>++++ (medullary)</td>
<td>- (medullary)</td>
</tr>
<tr>
<td>DFDBA</td>
<td>-</td>
<td>+ / - ?</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>FDBA</td>
<td>-</td>
<td>+ / - ?</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Xenograft material</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Alloplastic material</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>BMP rh</td>
<td>-</td>
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DFDBA: demineralised freeze-dried bone allograft; FDBA: freeze-dried bone allograft; BMP rh: bone morphogenetic protein.
based on the statistical incidence of the same infectious agents in the reference population; in reality the processing and preservation techniques notably diminish the infective power of viral agents to such an extent that several studies confirm the possibility of infection from a homologous graft to be 1 in 1,000,000.16 The use of this material is well known in Italy, particularly in orthopaedic surgery, so no approval for its use was required by the local ethics committee. Written informed consent was obtained from patients prior to the procedure. Ethical approval has been obtained for studies of similar design from our Department (i.e., Dept of Maxillofacial Surgery) at the Civil Hospital Castelfranco using FFB.18,19 The tissue bank has a quality management system for the harvesting, processing, preservation, validation and distribution of human tissues for clinical use. In May 2003, the tissue bank obtained the following certification: ISO 9001:2000 UNI EN ISO 9001:2000 IQ NET IT/25398. The tissue bank conforms to the national tissue guidelines and has been accredited (by inspection) by the national transplant centre.

Upon completion of all safety tests, eventual tendinous or periostic residuals are removed and the bone is then disinfected for at least 72 hours at -4°C in an antibiotic solution of vancomycine, polymyxine, glazidine and lincomycine. With FFB there is no lipid oxidation since homologous bone is not radiated. The sample is then subdivided into cortico-medullary blocks, packed in double sterile casing and frozen at -80°C. Bone, unlike organs, may be preserved at such temperatures for up to five years, with a shelf life of six days after unfreezing (Figure 1).

**Surgical protocol: reconstruction phase**

Between November 2003 and September 2005, FFB bone block grafts were used to perform 26 maxillary sinus rehabilitations (with 47 immediate implant insertions) on 21 patients (13 females and eight males between 39 and 71 years of age) who had not accepted autologous bone harvesting. Cortico-medullary inlay block grafts were performed in 13 of the cases for isolated vertical defects with irregular prosthetic space.

All surgical procedures were performed under local anaesthesia and under aseptic conditions. A full-thickness incision was made within the keratinised gingiva from the distal aspect of the first tooth contiguous to edentulous space to the distal end of the edentulous ridge. Vertical releasing incisions were made at the mesio-buccal line angle of the second to last existing tooth and at the distal aspect of the crestal incision. The buccal and lingual flaps were reflected with a periostal elevator, avoiding damage to the anatomic structures and the periosteum. The sinus membrane was released carefully from the inferior and lateral sinus walls and lifted superiorly. The type of procedure performed depended on the residual thickness of the alveolar ridge: bicortical grafts for bone thickness <4mm and monocortical grafts in all other cases (Figures 2 and 3). In three cases where the maxillary sinus had an inadequate height and prosthetic
space, inlay and onlay block grafts were reconstructed (Figure 4). In the remaining ten situations there was a combination of vertical defects and narrow alveolar ridges, and inlay and veneer grafts were therefore performed. Sutures were removed 10 to 14 days post surgery following the application of 0.2% chlorhexidine gel for two minutes to minimise bacterial contamination of the wound. At this stage the provisional prosthesis was removed. Patients were instructed to rinse twice a day with chlorhexidine until completion of the bone maturation period. Patients were subsequently assessed once a week for the first month and then once a month until second-stage surgery.

The 47 cylindrical screw implants (of diameters varying between 3.75 and 4mm and lengths from 11.5 to 15mm) were positioned during this reconstruction phase and were reopened six months after surgery. All patients underwent orthopanoramic X-rays and CT scans before and immediately after surgery for control purposes. The radiological studies were repeated four months later in order to evaluate the volume and density of the bone grafts. To avoid high doses of radiation to patients, no CT images were taken four months after surgery. Radiographic images were cut with the same spiral tomography equipment, with 2mm cuts allowing comparison of each graft measurement in three dimensions. The final prosthesis was fitted after loading with provisional prosthetics for a period of six months. Further CT images will be taken three years after surgery to show the grade of bone resorption after one year of prosthetic loading.

Second-stage surgery

The study was completed by performing bone biopsies of the grafts at four, six and 12 months post surgery in order to study the histological evolution of the material. All biopsies were performed on the medullary portion of the graft vestibular to the crest. The bone biopsies were harvested with a trephine bur on the buccal side of the reconstructed alveolar ridge on medullary bone in inter-implant position. All patients gave their written informed consent. Other biopsies were not performed since it was deemed unreasonable to expect patients to undergo so many surgical procedures solely for the purposes of study. However, in two cases, bone biopsies of the graft were performed 24 months post surgery.

Bone biopsy specimen processing

The bone biopsy specimens were fixed in 4% buffered formaldehyde, dehydrated in an ascending series of alcohol rinses and embedded in a London resin. After polymerisation, the specimens were sectioned along the longitudinal axis with a high-precision diamond disk at 150µm and ground to 40µm with a specially designed grinding machine. The slides were stained with acid fuchsín and toluidine blue or with acid fuchsin and a mixture of methylene blue and Azzurro staining and observed under normal transmitted light by light microscopy. Histomorphometry was performed by connecting the light microscope to a high-resolution video camera interfaced with a personal computer. The optical system used a digitising pad and a histometry software package with image-capturing capabilities.

Results

All grafts showed optimal adherence (100%) with no evidence of complications. The resorption status of the grafts was made by comparing the post-surgery CT scans with those of four months later. At four months post surgery there was no evidence of either bone resorption or resizing in 64% of the grafts. A slight resorption was observed in 36% of the grafts, with mean values of 11.7% in height, 12.3% in width and 12.9% in thickness. These values did not interfere with the implant treatment.

At the time of reopening (six months post surgery), all implants were clinically and radiographically osseointegrated; one implant failed in the provisional prosthetic loading period, bringing the overall success rate to 97.8%. The reason for this failure may have been due to ‘fibrous integration’ of the implant, evidence of which appeared after prosthetic loading. All patients received a final prosthetic implant rehabilitation and no failure has been observed at this time.

The biopsies performed four months after surgery showed the presence of osteoids and the beginnings of bone neo-apposition (Figure 5). This is determined by the mesenchymal host cells, which differentiate into osteoblasts upon being stimulated by BMP in the bone matrix of the graft. The biopsies performed six months after surgery confirmed new bone formation and revealed the first instances of mature bone intermingled with the bone matrix of the

FIGURE 4: Upper posterior alveolar ridge (less than 4mm of residual bone and improvement of prosthetic space) treated with sinus lift (fresh-frozen bone [FFB] monocortical inlay graft) and simultaneous insertion of two implants. Radiographic images taken with the same spiral tomography equipment with 2mm cuts were utilised to compare the measures of each graft in three dimensions. Definitive dental prosthesis after 12 months.
graft. Large concentrations of osteocytes embedded in the mature bone matrix were also observed. Surprisingly, however, there was no evidence of bone resorption, which was also verified by the very limited presence of active osteoclasts. The 12-month post-surgery biopsies demonstrated a mineralised mature bone matrix percentage varying between 75 and 80%. The reactive phenomenon of bone resorption was still not present. The two bone biopsies taken 24 months post surgery in two different patients showed a percentage of mineralised mature bone matrix similar to that observed at 12 months.

Discussion and conclusions

The ideal bone graft must satisfy the following requirements: physiological properties (osteoinduction, osteoconduction, osteogenesis, biocompatibility); anatomical properties (corticomедullary bone and structural strength); easy availability; adequate volume; substitution with new mature bone; assurance of implant osseointegration; and, an appropriate benefit/cost ratio. Autologous bone, which is generally used in prosthetic surgery, remains the gold standard because of its high degree of biocompatibility. However, the pain and complications associated with bone harvesting are not acceptable to many patients. Use of FFB in dental surgery has become more common in recent years, and it is a reliable material for alveolar bone restoration with a predictable average of resorption. Indeed, utilisation of FFB for reconstruction of the atrophic jaw prior to implant placement can be considered a reliable alternative to autogenous bone. A study of 21 patients with 28 FFB onlay mandibular grafts prior to implant insertion (n=63) resulted in only two lost implants (i.e., survival rate = 96.8%). A much larger retrospective study of 287 implants inserted into resorbed maxillae augmented with FFB reported a survival rate of 98.3% over a mean follow-up time of 26 months, with a cumulative success rate based on defined criteria of 96% in the first year, which decreased to 40% at four years because of marginal bone loss. No differences were detected among implant diameters, lengths and implant site. Others have confirmed these findings with FFB, showing high implant survival rates irrespective of implant diameter, length or type. However, these previous studies have all assessed implant insertion into FFB with a time delay between bone grafting and insertion. For example, Carinci and colleagues showed that a four-month delay from grafting to implant insertion was a safe period to obtain a high survival rate and success rate for implants inserted into FFB. In that study, no implants were lost (100% survival rate) and no difference was detected between implants loaded after four months versus those loaded after six or more months. Our study goes one step further, being the first to use FFB as the sole bone material to increase atrophied alveolar ridge volume and to insert dental implants during the same surgical procedure, thus reducing chair time both for the patient and the surgeon. Immediate insertion of dental implants did not adversely affect the bone graft. Histological examination of our bone biopsies revealed newly formed bone integrated with pre-existing bone. Others have confirmed the excellent incorporation of FFB grafts, reporting non-discernible interface areas between new and old bone, features characteristic of mature and compact osseous tissue surrounded by marrow spaces, with no evidence of an acute inflammatory infiltrate.

The results of the present study showed that all FFB grafts demonstrated optimal adherence, with no signs of infection, pain, oedema or graft mobility in the implant placement phase observed. FFB has many advantages over autologous bone such as the absence of donor site morbidity, lack of quantity limitation and the certification of good quality material (provided by the tissue bank). However, it is worth noting that implants placed in sinuses augmented with particulate grafts show a higher survival rate than those placed in sinuses augmented with block grafts. Even though FFB does not possess osteogenic characteristics (since the freezing process causes cell death), it does have optimal osteoinductive properties due to BMP permanence and its medullar and cortical component guarantees exceptional osteoconductive properties. The availability of a large cortical component (tri- or bi-cortical) is useful for the primary stability of an implant inserted into an atrophic alveolar jawbone. Use of FFB halves surgical time and reduces the prosthetic rehabilitation period. Thanks to the reduced possibility of infection transmission (less than one per million), more than 400 patients so far have been treated at the Hospital of Castelfranco Veneto for alveolar dimension

FIGURE 5: A: Bone specimen 4h months post surgery: evidence of osteoblastic activity on the surface of fresh-frozen bone (FFB) graft (magnification: x50); B: Bone specimen six months post surgery: evidence of new bone pillar formation starting from FFB graft (magnification: x20); C: Bone specimen 12h months post surgery: biopsy shows 80% of mature bone and a very low degree of residual FFB graft (magnification: x10).
improvement using FFB, with no evidence of complications. In conclusion, FFB is an osteoinductive and osteoconductive material, which permits effective bone-adding surgery under local anaesthesia in the dental office with immediate implant insertion, decreasing both chair time and patient discomfort.

References