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Maxillary alveolar ridge reconstruction with monocortical fresh-frozen bone blocks: A clinical, histological and histomorphometric study

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ABSTRACT

Background: This investigation is a clinical and histological assessment of fresh-frozen bone use in the reconstruction of maxillary alveolar ridges. The study evaluates the effectiveness of this material as a bone filler prior the placement of dental implants.
Patients and methods: Sixteen patients with atrophic maxillary ridges underwent maxillary reconstruction with fresh-frozen tibial human block grafts prior to implant placement. Sampling procedures were carried out 4, 6 and 9 months later when a bone core was removed from the grafts for histological and histomorphometric analysis.
Results: Eighteen blocks were placed, and each patient received either 1 or 2 blocks. During the sampling procedures, all of the grafts were found to be firm in consistency, well-incorporated, and vascularized. A total of 34 implants were placed into the grafts with a minimum of 40-Newton-cm torque in all cases. The follow-up period ranged from 18 to 30 months. No implants were lost. The histological analysis revealed vital bone with mature and compact osseous tissue surrounded by marrow spaces.
Conclusion: Bone allografts can be used successfully as graft material for the treatment of maxillary ridge defects. This type of bone graft can be used safely in the areas of implant placement as a suitable alternative to autogenous grafts.

1. Introduction

The treatment of the edentulous atrophic posterior maxilla with a fixed partial denture or a fixed prosthesis is a major challenge because implant-supported rehabilitation requires adequate volume and quality of the alveolar bone, The pattern of ridge resorption also contributes to severe jaw atrophy and eventually to an unfavorable maxillomandibular relationship (Cawood and Howell, 1991).

Reconstruction of severely resorbed jaws requires different surgical procedures depending on the severity of the bone atrophy. These procedures often involve the use of bone substitutes or the harvesting of autogenous bone from a donor site. Autogenous bone is believed to be the most effective bone graft material, and is still regarded as the “gold standard” for augmentation procedures because of its osteogenic potential, but this graft has limited availability and the surgical harvesting procedures can cause additional morbidity to the patient (Nkenke et al., 2002; Cricchio and Lundgren, 2003; Acocella et al., 2010).

These shortcomings have therefore focused attention on the use of allogeneic bone graft materials. Allogeneic bone grafts, including fresh-frozen, freeze-dried or demineralized freeze-dried, and cryopreserved grafts, are all harvested from cadaveric sources, and are successively processed and stored in different ways (Gazdag et al., 1995). Allografts have proven to be clinically useful when autologous bone is limited in supply because any size and shape of graft needed may be provided by tissue banks (Perrott et al., 1992; Dallari et al., 2006). The use of bone allograft offers several benefits when compared to autogenous bone grafts by reducing operative and anaesthesia time, with less discomfort and morbidity to patients (Kao and Scott, 2007). Despite these benefits, the documented use of block allografts in the treatment of alveolar ridge atrophy is limited in the literature. It is estimated that since 1972 in the United States, 40,000 persons have received allogeneic grafts in the

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maxillomandibular region annually. In addition, the use of human bone allografts has increased more than 400-fold, with more than 800,000 transplantations performed annually in the United States (Burchardt et al., 2005). Because of the development of specialized centres in the manipulation and processing of skeletal tissues, the use of allogeneic grafts has become an alternative for the treatment of the atrophic maxilla and mandible (Buck et al., 1990).

Osteogenesis is the most important basic process for total integration of bone grafts. The new bone from the patient is produced by the osteoinduction process, whereby mesenchymal cells differentiate into osteoblast cells that produce new bone. This differentiation process is coordinated by glycoproteins, such as bone morphogenetic protein (BMP). BMPs are present in both autogenous and allogeneic bone. The process of osteoinduction determines the 3-dimensional growth of capillary vessels, perivascular tissues, and osteoprogenitor cells from the patient to the graft (Mulliken et al., 1981). The incorporation process of the graft happens through the gradual replacement of bone until the formation of new bone occurs, beginning with the acute inflammatory process and finished with a gradual substitution (Mulliken et al., 1981; Köndell et al., 1996).

Fresh-frozen bone is harvested aseptically from live or cadaveric donors, and then frozen. Immediately after removal from the human body, the bone tissue is stored at −80 °C and, if there are no contraindications emerging from the results of the screening procedures, then the fresh-frozen bone (FFB) can be used for implantation (www.aatb.org, 1996; www.eamst.org, 1997). There is no additional preparation, and the osteoinductive proteins are preserved. Guidelines on donor selection, tissue procurement, tissue preservation, tissue storage, and adequate record-keeping procedures have been designed by bone banks to ensure the supply of safe allogeneic bone (Tomford et al., 1983; Palmer et al., 1999; Hofman et al., 2005).

A concern with bone allograft is its antigenicity. However, long-term evaluation of the use of fresh-frozen allograft has been reported by some authors. They did not observe any significant allergic reactions, rejection, or any unexpected antibodies after allograft transplantation in a 30-year period (Virolainen et al., 2003). Histological and immune-response assessments showed no signs of antigenic reaction to the use of fresh-frozen allograft in the treatment of large bone defects (Aho et al., 1998).

This study presents a clinical, histological and histomorphometric analysis on the use of fresh-frozen allogeneic onlay bone grafts for ridge augmentation left to heal for period varying from 3 to 9 months. This study's objective was to gain insight into the vitality of bone grafts in the human jaw with emphasis on the survival of osteocytes used as sign of bone vitality.

2. Materials and methods

Sixteen patients (11 males, 5 females, mean age 41.06 ± 9.51) requiring unilateral or bilateral alveolar ridge reconstruction participated in this study (Table 1). They were all offered the choice between the use of conventional grafting materials (hydroxyapatite, β-tricalcium phosphate, an organic deproteinized bovine bone, etc) and the allograft material (FFB allograft). Patients were fully informed about the full range of risks including the possibility of HIV, HCV, and HBV contamination. Patients were enrolled between October 2006 and November 2007.

This study was approved by the ethical committee of University of Florence in accordance with the revised Helsinki Declaration. All patients signed a specific written informed consent form.

The inclusion criteria were the following: the need for alveolar ridge reconstruction and implant placement in a 2-stage procedure, the presence of severe maxillary bone atrophy, and the presence of good health without any disease that would contraindicate reconstructive bone surgery. General exclusion criteria were acute myocardial infarction within the past 6 months, uncontrolled coagulation disorders, uncontrolled metabolic diseases (diabetes mellitus, bone pathologies), patients treated with radiotherapy to the head/neck district within the past 24 months, patients treated with intravenous biphosphonates, patients with psychological or psychiatric problems, and heavy smokers (>10 cigarettes/day).

Local exclusion criteria were oral infections and uncontrolled periodontal disease.

All patients underwent clinical and panoramic radiographs and study models. CT scans with a template were performed to study the programmed implant sites as well as to evaluate the morphology of the bony defect.

2.1. Graft material

The FFB, obtained from the Musolo-skeletal Tissue Bank of Tuscany (Florence, Italy), is mineralized, non-irradiated, disinfected, and frozen homologous bone. Bone harvesting is performed from the tibia in the first 12 h after the donor's death. The bone is then disinfected, for at least 72 h at −4 °C in a polychemotherapeutic solution of vancomycin, polymyxin, glazidine, and lincomycin. Afterwards, the sample is irrigated with sterile saline solution. The sample is then subdivided into cortical blocks, packed in double sterile casing, and frozen at −80 °C.

The requirements for homologous bone donors are more stringent than those for organ donors. The presence of risk factors such as contagious disease, neoplasms, rheumatic disease, degenerative disease, and sepsis disqualifies the donor. To detect infectious agents, the following tests are performed on donor blood samples taken within 8 h of death: anti- HIV-I/II Ab, anti-HCV Ab, HbsAg, anti-HBc Ab, anti-HBs Ab, anti-HTLV-I/II Ab, anti-Ag treponemal Ab, anti-CMV IgG Ab, anti-CMV IgM Ab, anti-toxoplasma IgG Ab, and anti-toxoplasma IgM Ab. A culture is also performed to detect aerobic and anaerobic bacteria, mycobacteria, and mycotic agents. As a further safety measure, a serologic follow-up uses polymerase chain reaction techniques to detect any viral RNA or DNA of HIV, HCV, and HBV. This method reduces the "diagnostic window period" to 7 days for HIV, HCV, and HBV.

2.2. Surgical procedure

In 7 out 16 patients, surgery was performed under general anaesthesia. Each patient was draped to guarantee maximum asepsis. The skin was disinfected using iodopovidone 10% (Betadine®, Purdue Purdue Pharma L.P.; Stamford (CT), U.S.A.) and the patients were asked to rinse with chlorhexidine mouthwash 0.2% (Curasept; Curaden Health Care Srl, Milano, Italy) for 30 S. The maxillary surgical sites were infiltrated with a local anaesthetic containing a vasoconstrictor (Ultracain D-Suprarenin® 40 mg/ml+5 μg/ml, Hoechst, Frankfurt am Main, Germany).

To create the recipient site, a crestal incision (at the top of the edentulous alveolar crest) and 2 vertical releasing incisions were performed. A full-thickness flap was raised and the palatal flap was held with a 3–0 (Vicryl Rapid, Ethicon, Johnson & Johnson Roma, Italy) resorbable suture. The bone defect was evaluated to determine the size and shape of the block needed (Fig. 2a). The recipient site was prepared in order to make it suitable for receiving the graft (perforated to promote the bleeding). The allogeneic monocortical bone block that had been deep-frozen (Tissues Bank, Careggi Hospital, Florence, Italy) was restored in a rifamycin solution and adapted to the atrophic maxilla (Fig. 1). The block allografts were positioned over the recipient site with the endosteal side facing the cortical bone. The blocks were stabilized on the residual ridge with

Table 1
Patient data and results of histomorphometry means.

Patient	Sex	Age	No. of i mplants	Healing time (months)	Non-vital bone (%)
1	M	39	3	4	72.5
2	F	21	1	4	68.5
3	M	38	2	4	79.8
4	F	53	2	6	59.2
5	M	37	2	9	38.4
6	M	41	2	6	62.3
7	M	32	1	4	65.4
8	F	43	2	4	70.2
9	M	56	2	6	62.4
10	M	57	2	4	69.3
11	M	42	6	6	62.8
12	F	35	2	6	63.5
13	M	38	2	9	41.5
14	M	46	1	6	58.3
15	M	31	2	4	73.8
16	F	48	2	9	43.6

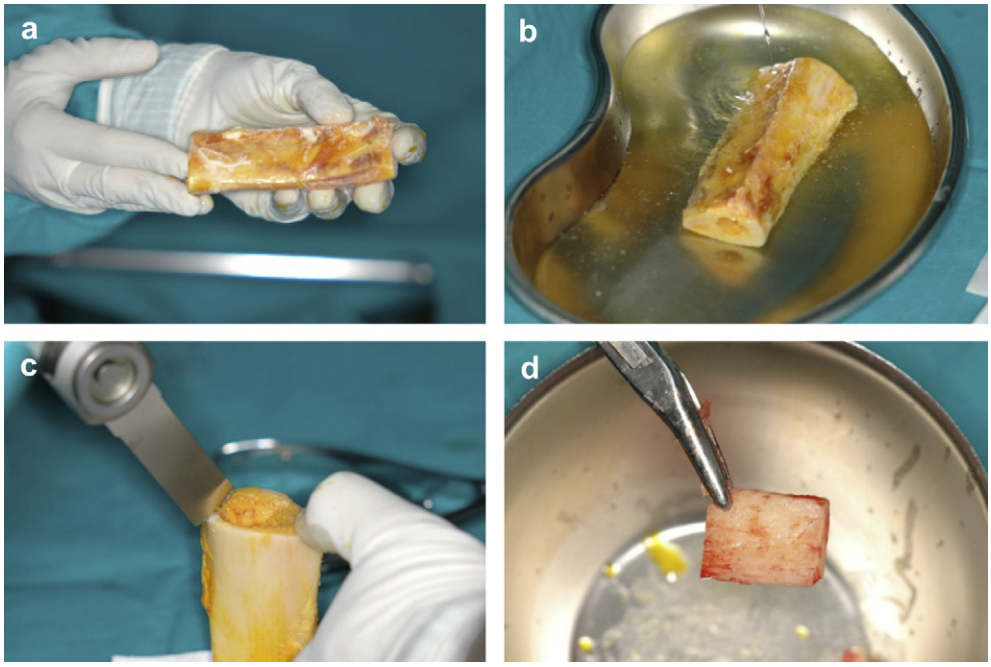

Fig. 1. Fresh-frozen bone FFB obtained by tibia.

titanium micro-screws, 1.5 mm in diameter (Fig. 2b, Fig. 3a). Any sharp angles in the block grafts were smoothed to avoid perforation of the overlaying flap (Fig. 2b). Periosteal incision was performed at the base of the buccal flap in order to obtain a tension-free adaptation of the wound margins. The flap was then sutured with a resorbable suture that was removed after 10 days (Fig. 2c). Postoperative management was prescribed, and it included systemic antibiotics 1000 mg amoxicillin 2 times a day for 7 days (Zimox, Pfizer, Latina, Italy), chlorhexidine 0.2% (Curasept; Curaden Health Care Srl, Milano, Italy), mouthwash (twice a day for 10 days), and analgesics (Brufen; Abbott SpA, Campoverde (Lt), Italy). During the healing period, all patients were seen once a month until the time of implant placement.

A bone graft was considered to be successful if the following criteria were met: 1) absence of graft exposure and postoperative infection, 2) incorporation of the graft within the recipient site, 3) absence of radiolucency, 4) bleeding from the bone graft after removal of the stabilization screws, and 5) the possibility for implant placement.

At the time of implant surgery, 18 bone cores were harvested from the alveolar crest using a 3.0×10 mm diameter trephine under cold sterile saline solution irrigation; the bone cores were marked for orientation and processed for light microscopy.

After the biopsies, 34 implants (PrimaConnex® Tapered Calcium phosphate ceramic blasted Keystone Dental, Inc., Dallas, TX) were placed with torque not less than 40 Ncm (Fig. 2d; Fig. 3b,c).

Implants were buried, and the second stage of the surgery was carried out after an additional healing period of 4 months (Fig. 3d).

2.3. Biopsy procedure for histology and histomorphometric analysis

All specimens were immediately fixed in 4% formaldehyde solution in 0.1 M phosphate buffer (pH 7.3) at 4 °C for 24 h. They were then rinsed 3 times with 0.1 M phosphate buffer and, finally, stored in 70% ethanol at 4 °C, until ready to be embedded. All the biopsies were cold embedded in methylmethacrylate with 20% plastoid. Undecalcified, 5 μ thick sections were made along the axis of the biopsy using a Jung K microtome. All specimens were then, stained with haematoxylin and eosin. A Leica DM RA microscope connected to a computer using an electronic stage table and a Leica DC 200 digital camera were used for histomorphometric measurements. Leica QWin© software (Leica Microsystems Image Solutions, Rijswijk, the Netherlands) was used to process and measure the digitised images. This software allows selection of parts of an image by setting a threshold to the colour of the pixels, which can then, be measured.

All measurements were carried out at ×200 magnification in order to allow clear distinction between empty and full osteocyte lacunae. The sections stained with haematoxylin and eosin were used for the measurements of total bone volume and non-vital bone grafted (NVBG) volume. The mineralized bone tissue containing areas of empty osteocyte lacunae was defined as NVBG. NVBG was expressed as a percentage of the total bone volume and its measurement was made semi-automatically by outlining the area of empty osteocytes lacunae (Fig. 4).

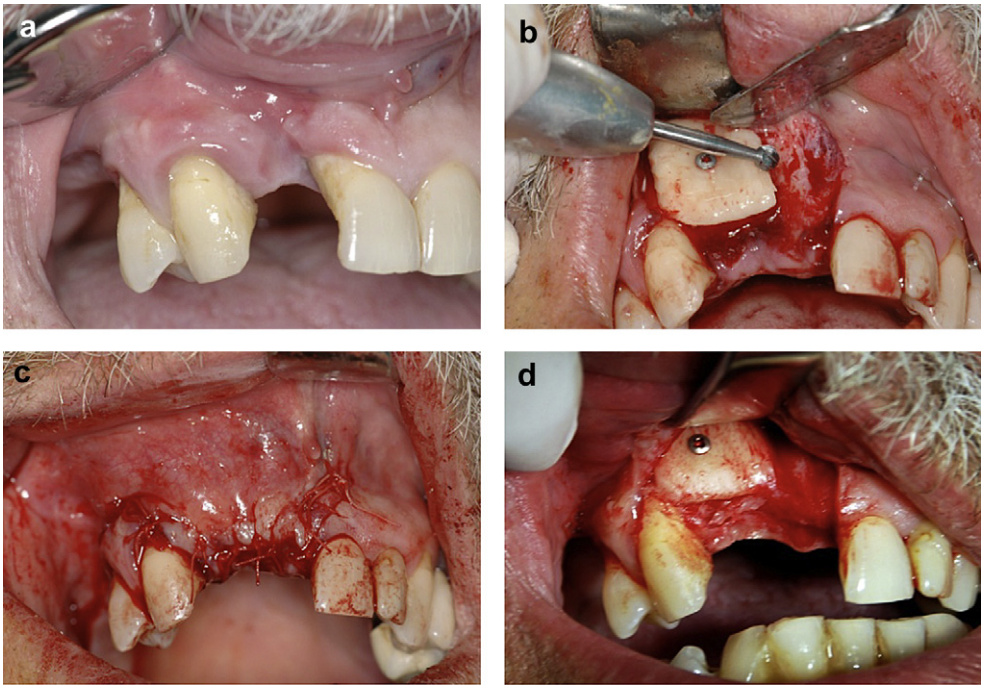

Fig. 2. View of a maxillary a narrow alveolar ridge (a); adaptation and fixation of the graft to the maxillary recipient site (b); primary flap closure without tension (c); re-entry surgery after the healing period (d).

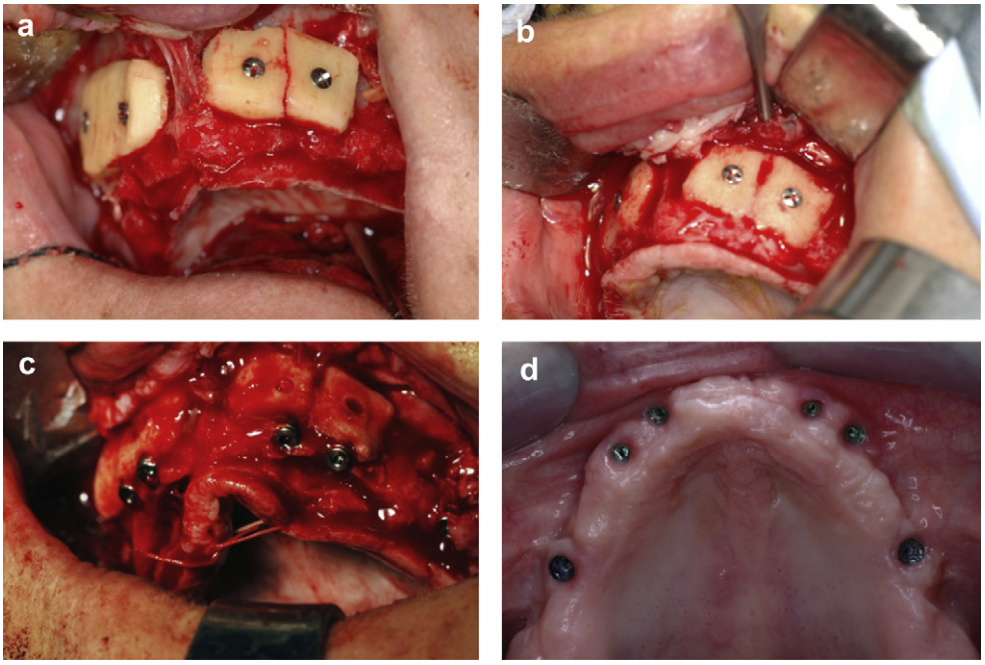

Fig. 3. Adaptation and fixation of the graft to the maxillary recipient site (a); re-entry surgery after the healing period (b); implant placement (c); after 4 months all implants were osseointegrated.

2.4. Statistical analysis

All data were analysed using SPSS for Windows 12.0. Simple regression model was applied to the data in order to examine the correlation between healing time and non-vital bone volume (NVB) (Model: $NVBi = \beta 0 + \beta 1\ moni + \varepsilon$). The data were presented as means and standard deviations. Significance was accepted when $P < 0.05$.

3. Results

3.1. Clinical evaluation

A total of 18 onlay block allografts were scheduled for lateral alveolar ridge augmentation of atrophic maxillas in 16 patients. One patient had early exposition of the block because of inadequate

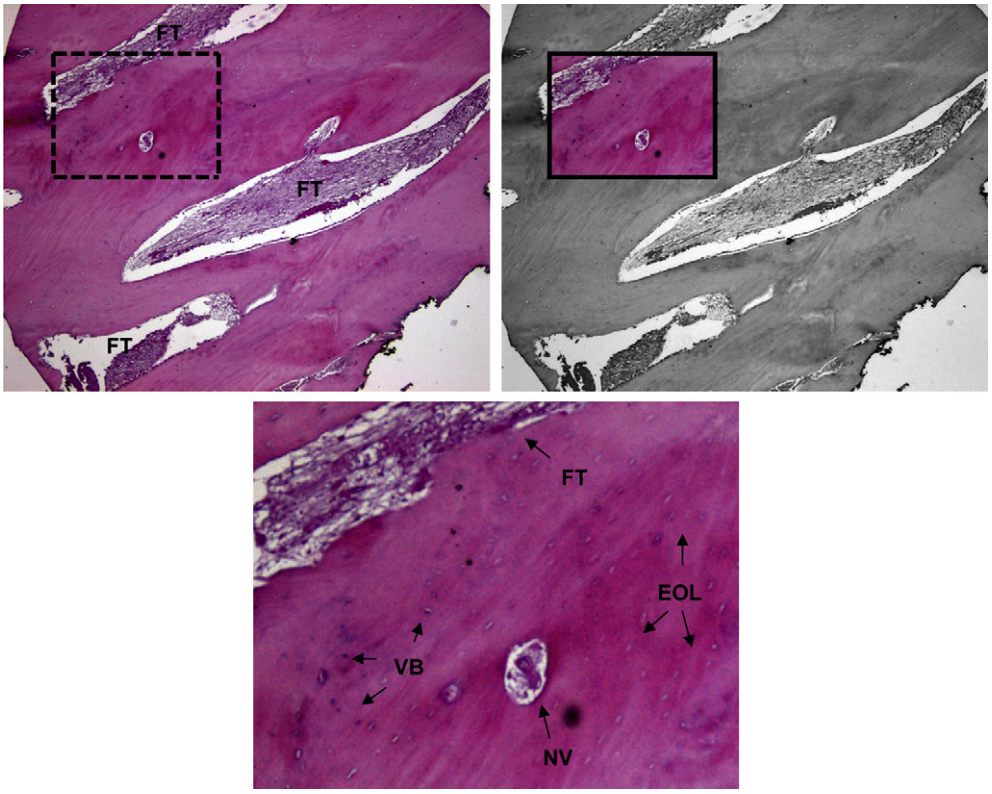

Fig. 4. Here it is illustrated how the histomorphometric analysis was carried out. Histomorphometric analysis was performed to quantify the amount of bone formation and bone ingrowth into the substitute bone blocks. The image processing program was used to identify the different tissues of interest (pre-existing bone, empty osteocytes lacune, new bone, connective tissue). The areas of these surfaces were digitally measured and expressed as surface per mm^2. Newly formed bone was defined as new bone formation noted on the surface of the fresh-frozen bone and bone formation into the bone substitute. Pictures show the compact osteonic structure with predominant empty osteocyte lacunae (EOL) and (FT). Signs of active remodelling, vital bone (VB) and new vessel ingrowth (NV), are poorly expressed (original magnification ×100).

Table 2
Analytical description of the amount of bone augmentation obtained and grafts resorption.

Lateral augmentation at bone grafting (mm)	Re-entry lateral augmentation (mm)	Resorption (%)
4.62 ± 0.8	4.09 ± 0.8	11.45 ± 8.37

flap design during the first surgery, and it required a second surgical procedure to cover it.

No complications occurred at the time of implant surgery.

CT scans were performed after 4, 6 and 9 months to assess the healing of the graft radiologically.

Direct bony measurements of the alveolar ridge width were taken with a millimetre graduated caliper (Acocella et al., 2010). They were taken during the first operation immediately before grafting (baseline) and immediately after grafting, and during the second operation immediately before implant placement. The amount of horizontal and vertical augmentation was calculated comparing baseline values and measurements taken immediately after grafting. Graft resorption was determined by comparing measurements taken immediately after grafting and the same measurement taken immediately before implant placement. The final bone gain obtained with the graft was calculated by comparing the amount of augmentation and the amount of graft resorption.

Table 2 tabulates the analytical data regarding the increase of the alveolar bone volume together with the increase in bone volume at the time of graft and implant placement. The mean amount of bone resorption occurring during healing time was calculated according to the record of lateral augmentation measures at bone grafting, and after 4, 6 and 9 months, at implant placement. Considering all the sites together, mean lateral augmentation at graft time, was 4.62 ± 0.8 mm, which reduced to a mean of 4.09 ± 0.8 mm at implant insertion. This is equivalent to a mean reduction in the lateral augmentation of 11.45 ± 8.37 % (min 8.3%, max 30%) during healing (Table 3). Thus, after the healing period 34 implants were placed (PrimaConnex® Tapered calcium phosphate ceramic blasted Keystone Dental, Inc., Dallas, TX) (4.1 × 11.5 mm) with a torque not less of 40 Ncm, and after 4 months of healing, all appeared osseointegrated by clinical and X-ray examinations. After the abutment connection, all patients received a cemented metal-ceramic crown. Hence, all patients were followed from 18 to 30 months after abutment connection, and according to Albrektsson criteria, no implant failed (Albrektsson et al., 1986) (Figs. 5 and 6).

3.2. Histological and histomorphometric findings

According to the histological evaluation, all the specimens showed signs of active remodelling and all the tissues were free of inflammatory cells.

The representation of vital and NVBG bone varied considerably according to each individual.

Grafted bone classed as non-vital (with fields of empty osteocyte lacunae) was predominantly lamellar type bone, while the vital bone was composed of both lamellar and woven bone.

As vital bone was in tight contact with the NVBG (osteoconductor), the first replaced the second by a creeping substitution process. Osteoclasts were rarely detected. Neo-vasculature was poorly represented in all specimens. At the interface between

Table 3
Mean and standard deviation in mm of lateral bone augmentation measured at the time of bone grafting and at the time of surgical re-entry for implant placement. Mean percentage reduction in lateral augmentation after grafts healing.

Case	Residual ridge Width (mm)	Lateral augmentation at bone grafting (mm)	Post augmentation width (mm)	Re-entry width	Re-entry lateral augmentation (mm)	Resorption (mm)	Resorption (%)
1	3	4	7.5	7	3.5	0.5	12.5
2	3.5	5	8.5	8	4.5	0.5	10
3	2.5	6	8.5	8	5.5	0.5	8.3
4	4	4	8	7	3	1	25
5	3.5	5	8.5	8	4.5	0.5	10
6	3	5	8	7.5	4.5	0.5	10
7	2.5	6	8.5	8	5.5	0.5	8.3
8	3	4	7	7	4	0	0
9	3.5	5	8.5	8	4.5	0.5	10
10	3.5	4	7.5	7	3.5	0.5	12.5
11	2.5	5	7.5	6.5	4	1	20
12	2	4	6	6	4	0	0
13	3	5	8	7.5	4.5	0.5	10
14	2	4	6	6	4	0	0
15	4	5	9	7.5	3.5	1.5	30
16	3.5	3	6.5	6	2.5	0.5	16.6

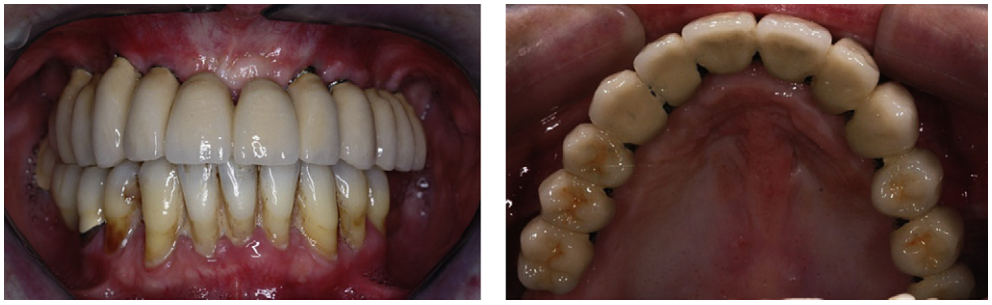

Fig. 5. Frontal and occlusal view of the partial fixed prostheses.

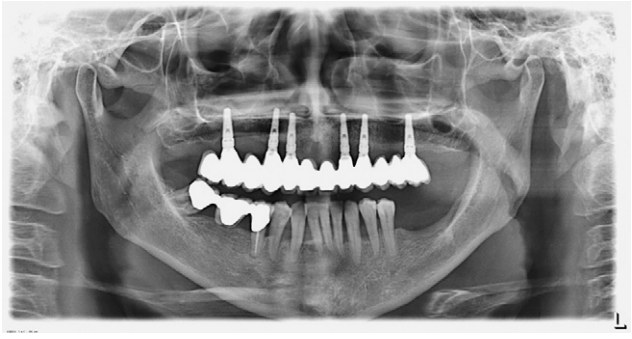

Fig. 6. Panoramic Rx after 24 months from the abutment connection.

grafted bone and recipient site, some specimens showed varying amounts of fibrous tissue mixed with new bone formation. On histological analysis alone, very little qualitative differences could be found among biopsies at different healing times. The volume of NVBG in all patients and the time of healing are presented in Table 1. The relative NVBG volume (NVBG as % of total bone volume) varied from 79.8% (in patient no.3) to 38.4% (in patient no. 5) with an overall average of 61.96% ± 11.77% of total tissue volume. After 4 months, at low magnification, all samples were free from inflammatory tissue with poor cellular activity. At high magnification, the specimens exhibited a poor amount of newly formed bone with no signs of rapid revascularization of the recipient site (Fig. 7).

After 6 months, the cellular activity was still poor and residual FFB grafts were very slowly replaced by new bone (Fig. 8). After 9 months, a high number of empty osteocyte lacunae were still present and, in addition, fibrous tissue was more present than in the samples taken at 4 and 6 months (Fig. 9). At this time new vital bone (NVB) was present surrounded by non-vital bone with empty osteocyte lacunae in way of resorption.

3.3. Statistical analysis

Two variables were investigated: NVB (non-vital bone) and healing time (months). The relationship between variables "NVB" (response variable) and "Months" (explanatory variable, length of time) looked linear ($r = -0.95$, $P < 0.001$). A straight line was fitted to the data by the least squares method. The estimated slope $\hat{\beta}_1$ was negative, indicating that the estimated decrease in NVB per month is between 4.7% and 6.9%. These data suggest that the amount of NVB decreased slowly as time of healing increased; grafted bone being completely revascularized and remodelled in 16 months at least (Fig. 10).

4. Discussion

Transplanting bone from one human to another is an idea that has been with us for hundreds of years and circumvents the problems associated with autogenous bone grafting. Over the past 20 years, the use of dental implants has become an established method to support and retain fixed posterior prostheses (Lambrecht et al., 2003; Simion et al., 2004). Originally, implant

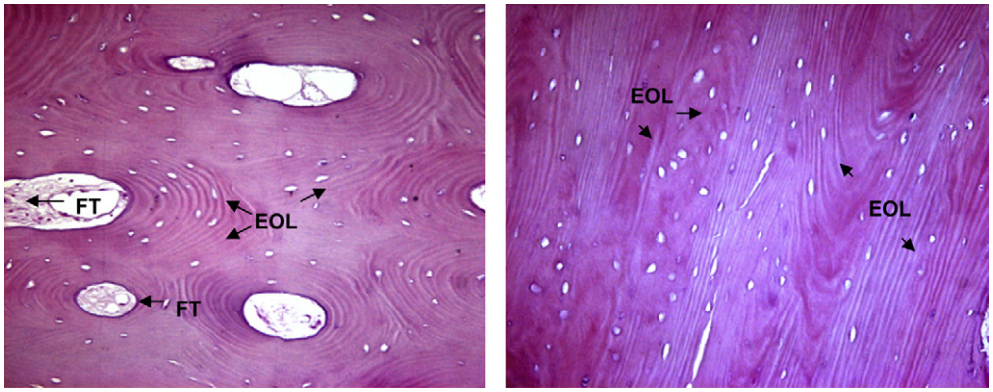

Fig. 7. Histologic view of the external cortical layer of the monocortical bone block graft after 4 months. Pictures show the compact osteonic structure with predominant empty osteocyte lacunae (EOL) and poor fibre tissue (FT). Signs of active remodelling and new vassel ingrowth are not evident (original magnification ×100 and ×200 all specimens stained with haematoxilyn and eosin).

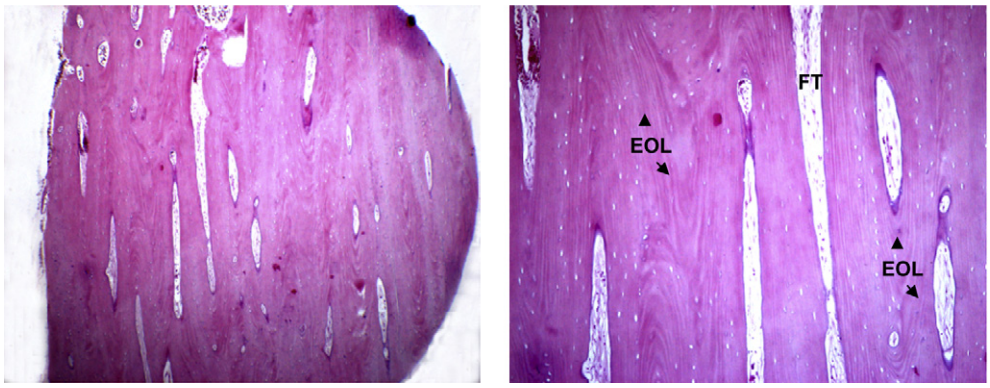

Fig. 8. Histologic view of the monocortical bone block graft after 6 months. The pictures show the typical compact osteonic structure with predominant empty osteocyte lacunae (EOL) and poor fibre tissue (FT) is still present. No signs of inflammatory tissue are evident. Signs of active remodelling and new vassel ingrowth are not evident (original magnification ×100 and ×200 all specimens stained with haematoxilyn and eosin).

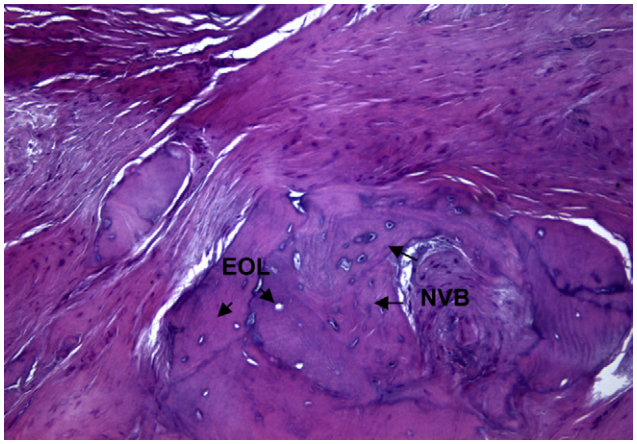

Fig. 9. Grafted bone/recipient site interface after 9 months; substantial amount of fibrous tissue, little amount of new vital bone. New vital bone (NVB) surrounded by non-vital bone with empty osteocyte lacunae (EOL), in way of resorption (creeping substitution). Original magnification ×250; specimens stained with haematoxilyn and eosin.

positioning was largely determined by the volume and location of residual bone, while the current concept for implant planning and placement is determined by satisfying prosthetic requirements (Ferrigno et al., 2006). The use of bone augmentation procedures for alveolar ridge augmentation is widely performed to allow rehabilitation with implant-supported prostheses. Allograft blocks can represent an attracting alternative treatment for alveolar ridge augmentation.

Some authors believe that the principal concern regarding, and disadvantage of, allografts is the risk of infectious disease transmission, such as acquired immunodeficiency syndrome (Buck et al., 1990; Köndell et al., 1996).

With the standard protocols applied by the bone banks, the risk of viral transmission is virtually nonexistent (Albert et al., 2006). Research to evaluate the immunologic response in patients undergoing allograft has shown no presence of antibodies in blood samples (Marx and Carlson, 1993).

With proper processing, the odds of an organism surviving allograft processing are less than one in 1 million. If a tissue bank uses the donor screening process coupled with the act of graft freezing, the risk of producing an HIV contaminated human bone allograft decreases to 1 in 8 million (Holtzclaw et al., 2008). The use of allografts is well documented in several fields of medicine; they allow a shorter operative time, reduced blood loss, and lower cost to patients when compared with autogenous bone. The use of this kind of graft has clearly increased over the last 3 years, whereas the use of autografts decreased, indicating the same trend seen in the orthopaedic field. The shortened operative time, unlimited supply, low morbidity, and painless healing period are some of the advantages that encourage us in the growing use of the allografts (Ehrler and Vaccaro, 2000; Shasha et al., 2003; Contar et al., 2009).

The findings of our study showed that deep-frozen allogeneic bone grafts represent a reliable treatment option for extensive

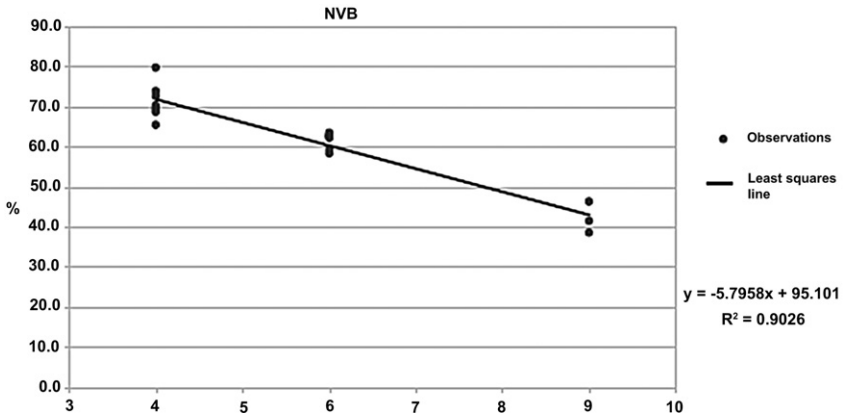

Fig. 10. Non-vital bone as percentage of total bone volume relative with time.

rehabilitation of atrophic maxillae, consistent with findings reported with the use of autologous bone. A previous clinical report indicated that fresh-frozen bone may be successfully used for the reconstruction of atrophic alveolar ridges (Perrott et al., 1992). The good results obtained with these16 patients are in accordance with the cases presented by other authors, who showed efficacy in using block allografts in areas of dental implant placement (Accetturi et al., 2002; Barone et al., 2009).

The histological evidence of new bone deposition in allografts was previously demonstrated in other cases, and confirmed in this study (Petrungaro and Amar, 2005; Stacchi et al., 2008; Acocella et al., 2011).

Although our follow-up is relatively short, a study of bone allograft with a very long follow-up period (30–35 years) proves it is a satisfactory and durable method for filling bone defects (Steinberg et al., 2004). The results support the hypothesis that fresh-frozen bone allografts can be successful as graft material for the treatment of maxillary ridge defects. If adequate surgical techniques are adopted, this type of bone graft can be safely used in regions of implant placement as a suitable alternative to autogenous grafts.

5. Conclusion

From a clinical point of view, as reported in the literature, this procedure appears to be simple, safe and effective for the treatment of localized alveolar ridge defects. Nonetheless, more studies are needed to explain the internal microarchitectural changes occurring during incorporation and remodelling processes in order to draw more definitive conclusions about the correct timing of implant insertion and loading.

The use of fresh-frozen bone allografts for the reconstruction of severely atrophic maxillae has proved to be a good treatment modality for ridge augmentation. The success rate of the block grafts was comparable to that reported for autogenous bone.

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References

Accetturi E, Germani KB, Cavalca D: Reconstruction of bone defects in the maxilla and mandibula through the use of frozen human bone. Transplant Proc 34: 531–533, 2002

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 placamento. J Craniomaxillofac Surg 38: 222–230, 2010

Acocella A, Bertolai R, Nissan J, Sacco R: Clinical, histological and histomorphometrical study of maxillary sinus augmentation using cortico-cancellous fresh frozen bone chips. J Craniomaxillofac Surg 39: 192–199, 2011

Aho AJ, Eskola J, Ekfors T, Manner I, Kouri T, Hollmen T: Immune responses and clinical outcome of massive human osteoarticular allografts. Clin Orthop Relat Res 346: 196–206, 1998

Albert A, Leemruse T, Druez V, Delloye C, Cornu O: Are bone autografts still necessary in 2006? A three-year retrospective study of bone grafting. Acta Orthop Belg 72: 734–740, 2006

Albrektsson T, Zarb G, Worthington P, Eriksson AR: The long-term efficacy of currently used dental implants: a review and proposed criteria of success. Int J Oral Maxillofac Implants 1: 11–25, 1986

American Association of Tissue Banks. Standards for tissue banking. American Association of Tissue Banks, www.aatb.org; 1996 [accessed 15.02.08]

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 67: 1300–1306, 2009

Buck BE, Resnick L, Shah SM, Malinin TI: Human immunodeficiency virus cultured from bone. Implications for transplantation. Clin Orthop Relat Res 251: 249–253, 1990

Burchardt H, Mindell ER, Enneking WF, Tomford W: Allograft safety and ethical considerations. In: Proceedings of the fourth symposium sponsored by the Musculoskeletal Transplant Foundation, September 2003. Clin Orthop Relat Res, vol. 435; 2005, 2–117, 2005 Edinburgh, Scotland, United Kingdom

Cawood JI, Howell RA: Reconstructive preprosthetic surgery. I. Anatomical consideration. Int J Oral Maxillofac Surg 20: 75–82, 1991

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

Cricchio G, Lundgren S: Donor site morbidity in two different approaches to anterior iliac crest bone harvesting. Clin Implant Dent Relat Res. 5: 161–169, 2003

Dallari D, Fini M, Stagni C, Torricelli P, Nicoli Aldini N, Giavaresi G: In vivo study on the healing of bone defects treated with bone marrow stromal cells, platelet rich plasma, and freeze-dried bone allografts, alone and in combination. J Orthop Res 24: 877–888, 2006

Ehrler DM, Vaccaro AR: The use of allograft bone in lumbar spine surgery. Clin Orthop Relat Res 371: 38–45, 2000

European Association of Musculo Skeletal Transplantation. Common standard for musculo-skeletal tissue banking. European Association of Musculo Skeletal Transplantation, www.eamst.org; 1997 [accessed 15.02.08]

Ferrigno N, Laureti M, Fanali S: Dental implants placement in conjunction with osteotome sinus floor elevation: a 12-year life-table analysis from a prospective study on 588 ITI implants. Clin Oral Implants Res 17: 194–205, 2006

Gazdag AR, Lane JM, Glaser D, Forster RA: Alternatives to autologous bone graft. Efficacy and indications. J Am Acad Orthop Surg 3: 1–8, 1995

Hofman A, Konrad L, Hessmann MH, Hofmann A, Konrad L, Hessmann MH: The influence of bone allograft processing on osteoblast attachment and function. J Orthop Res 23: 846–854, 2005

Holtzclaw D, Toscano N, Eisenlohr L, Callan D: The safety of bone allografts used in dentistry: a review. J Am Dent Assoc 139: 1192–1199, 2008

Kao ST, Scott DD: A review of bone substitutes. Oral Maxillofac Surg Clin North Am 19: 513–521, 2007

Köndell PA, Mattsson T, Astrand P: Immunological responses to maxillary on-lay allogeneic bone grafts. Clin Oral Implants Res 7: 373–377, 1996

Lambrecht JT, Filippi A, Kunzel AR, Schiel HJ: Long-term evaluation of submerged and nonsubmerged ITI solid-screw titanium implants: a 10-year life table analysis of 468 implants. Int J Oral Maxillafac Implants 18: 826–834, 2003

Marx RE, Carlson ER: Tissue banking safety: caveats and precautions for the oral and maxillofacial surgeon. J Oral Maxillofac Surg 51: 1372–1379, 1993

Mulliken JB, Glowacki J, Kaban LB, Folkman J, Murray JE: Use of demineralised allogeneic bone implants for the correction of maxillocraniofacial deformities. Ann Surg 194: 366–372, 1981

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 13: 514–521, 2002

Palmer SH, Gibbons CL, Athanasou NA: The pathology of bone allograft. J Bone Joint Surg Br 81: 333–335, 1999

Perrott DH, Smith RA, Kaban LB: The use of fresh frozen allogeneic bone for maxillary and mandibular reconstruction. Int J Oral Maxillofac Surg 21: 260–265, 1992

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

Shasha N, Krywulak S, Backstein D, Pressman A, Gross AE: Long-term follow-up of fresh tibial osteochondral allografts for failed tibial plateau fractures. J Bone Joint Surg Am 85: 33–39, 2003

Simion M, Fontana F, Rasperini G, Maiorana C: Long-term evaluation of osseointegrated implants placed in sites augmented with sinus floor elevation associated with vertical ridge augmentation: a retrospective study of 38 consecutive implants with 1-to 7-year follow-up. Int J Periodontics Restorative Dent 24: 208–221, 2004

Stacchi C, Orsini G, Di Iorio D, Breschi L, Di Lenarda R: Clinical, histologic and histomorphometric analyses of regenerated bone in maxillary sinus augmentation using fresh frozen human bone allografts. J Periodontol 79: 1789–1796, 2008

Steinberg EL, Luger E, Zwas T, Katznelson A: Very long-term radiographic and bone scan results of frozen autograft and allograft bone grafting in 17 patients (20 grafts) a 30- to 35-year follow-up. Cell Tissue Bank 5: 97–104, 2004

Tomford WW, Doppelt SH, Mankin HJ, Friedlaender GE: Bone bank procedures. Clin Orthop Relat Res 174: 15–21, 1983

Virolainen P, Heikkila J, Hirn M, Aro HT, Aho AJ: 30 Years of bone banking at Turku bone bank. Cell Tissue Bank 4: 43–48, 2003