Bone Augmentation of the Inferior Floor of the Maxillary Sinus With Autogenous Bone or Composite Bone Grafts: A Histologic-Histomorphometric Preliminary Report

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Thirteen patients were treated for elevation of the maxillary sinus floor with autogenous or composite (autogenous bone + hydroxyapatite granules) bone grafts and endosseous implants. Bone biopsies were taken both at graft positioning and at implant placement. Histologic and histomorphometric results suggest that graft architecture, conformation, and composition may influence the mean bone content at the end of the healing. Iliac bone grafts contained 53% bone, while chin bone grafts contained 66% bone. Composite bone grafts had the smallest percentage of bone: 44.3%. Chin block bone grafts had the largest percentage of bone content (69.3%), while chin particulate bone graft conformation had 62.6%. Although particulate grafts healed in only 4 months, bone quality and quantity allowed earlier implant placement.
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Key words: atrophic maxillae, autogenous bone graft, bone graft, composite graft, sinus lift

In completely and partially edentulous patients with advanced maxillary atrophy, treatment with fixed prostheses supported by endosseous implants is often problematic. In these patients, grafting may be necessary to increase bone quantity. Grafting material must integrate with the host site, so as to reconstruct the primary supporting function of the bone and provide biologic response to mechanical loading. The ideal characteristics of grafting material are:[1] *(1)* an osteoconductive matrix, which is a nonviable scaffolding conducive to bone ingrowth; *(2)* osteoinductive factors, which are the chemical agents inducing the various stages of bone repair; *(3)* osteogenic cells, which have the potential to differentiate and facilitate the various stages of new bone formation; *(4)* structural integrity.

Autogenous bone grafts satisfy most of these requirements[2] and are considered the ideal standard for any other grafting.[3] The literature contains a number of clinical studies investigating autogenous bone grafts to reconstruct the atrophied maxillae.[4–10] There are three main grafting techniques: *(a)* placement of bone grafts as inlays on the sinus floor; *(b)* placement of bone grafts as onlays to augment the alveolar ridges; and *(c)* placement of bone grafts as inlays on the floor of the nose and the maxillary sinuses, following a Le Fort I osteotomy.

Sinus floor elevation with particulate cancellous bone grafts harvested from the iliac crest and immediate implant positioning was reported by Kent and Block[4] in 1989. After a 4-year follow-up period, survival rate of implants was 100%. Keller et al[5] used corticocancellous block bone grafts harvested from the ilium and placed as inlays on the floor of the sinus and on the nasal cavities. Of a total of 83 implants placed simultaneously with the bone grafts,

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6% were lost during a follow-up period of 1 to 6 years. Jensen and Pedersen[6] and Jensen et al[7] obtained similar results in two studies of block bone grafts harvested from intraoral donor sites and used as inlays on the sinus cavity floor or as a combination of inlay and onlay bone grafts positioned over the alveolar crest. After a follow-up period ranging from 12 to 58 months, 6.5% of 291 implants positioned immediately in the grafted areas had to be removed.

Nystrom et al[8] harvested autogenous bone grafts from the iliac crest as horseshoe-shaped cortico-cancellous blocks. These were positioned as onlays over the atrophied maxillary alveolar crest in conjunction with implant placement. After a 2-year follow-up, the implant survival rate was 77.4%. A subsequent study[9] on the same group of patients found marginal resorption of the graft volume. Loss of volume at the apical implant site of those patients followed was nonlinear, with a maximum during the first 3 to 6 months of follow-up (–2 mm in height and –3.6 mm in width).

In 1989, Sailer[10] described a new method of placing endosseous implants in extremely atrophic maxillae. Iliac bone blocks were positioned as inlays on the floor of nasal and sinus cavities previously exposed with a Le Fort I osteotomy. Endosseous implants were placed simultaneously. Later, Isaksson et al[11] applied this method and reported an implant loss of 21% after a follow-up of 11 to 24 months.

A recent comparative clinical study[12] evaluating these three techniques reported an 86% survival of endosseous implants (after follow-up of 33 to 95 months) for inlay grafting of the maxillary sinus, 83% for onlay augmentation of the alveolar crest, and 68% for inlay grafting of the floor of the nose and the maxillary sinuses following Le Fort I osteotomy.

All of these studies evaluated results based on the survival rate of endosseous implants positioned in the grafted areas. Only a limited number of clinical studies have investigated grafting materials and procedures from a histologic or histomorphometric perspective. In 1993, Moy et al[13] carried out a histomorphometric study of five patients who underwent maxillary sinus augmentation; at the time of implant placement, eight bone biopsies were taken from seven graft sites. Bone composition of four different grafting materials was quantitated: particulate autogenous chin bone grafts contained 59.4% bone; composite grafts of hydroxyapatite granules and chin bone contained 44.4% bone; grafts of hydroxyapatite granules alone contained 20.3% bone; grafts of demineralized bone powder alone contained 4.6% bone. A comparative study by Nishibori et al[14] reported the histologic results of two patients treated with sinus floor elevation using autogenous iliac bone grafts in one, and with allogenetic demineralized freeze-dried bone (DFDB) grafts in the other. After an 8-month healing period, the autogenous bone grafts contained new bone tissue adequate in quantity and quality, while the allogenic graft, 16 months after surgery, still contained remnants of graft material and a small quantity of qualitatively poor bone tissue. Similarly, Wallace et al,[15] in serial histologic examinations of a single patient, observed slow bone formation in xenografts composed of a mixture of anorganic bovine matrix (80%) and autogenous bone (20%) placed into the maxillary sinus cavities. Twelve to 20 months of healing were required for graft material resorption and remodeling to vital bone.

The aim of this study was to investigate, using histologic and histomorphometric analysis, formation of new bone tissue in different graft materials for augmenting the bone quantity of the maxilla.

Materials and Methods

This study comprised 13 white patients, 8 men and 5 women, ranging in age from 41 to 62 years. All patients suffered from maxillary atrophy classified as Cawood and Howell[16] group V or VI, in conjunction with partial edentulism in the distal regions of the maxillary alveolar ridge. All were treated by a two-stage surgical procedure involving grafting and the placing of endosseous implants.

Preoperative planning consisted of clinical examination of the maxilla and radiographic assessment with panoramic radiographs and computerized tomography (CT) scanning. All patients were in good health, and no localized or general pathology was present; no patients were undergoing chronic drug therapy. Graft volume, material, and conformation were determined on the basis of the clinical and radiographic examinations; the quantity of bone required determined the anatomic selection of the donor site. For bilateral maxillary sinus floor elevation, bone was taken from the iliac crest, while for unilateral maxillary sinus floor elevation, bone was taken from the chin region.

Patients were divided by operative plan into four groups (Table 1). Four patients underwent bilateral sinus floor augmentation with autogenous bone grafts conformed as blocks and harvested from iliac crest (Group 1); healing before implant placement was 6 months, to limit the graft volume resorption that has been reported with iliac bone grafts.[9,17,18] A second group of three patients underwent unilateral sinus floor elevation with autogenous bone grafts harvested from the chin region and conformed as blocks; healing ranged from 8 to 12 months before implant placement to achieve sufficient revascularization and

Table 1 Operative Plan for Grafting Material

	Case no.	Sex	Age	Classification*	Surgery	Bone graft	Healing (mo)
Group 1	1	M	60	VI	Bilateral	IBB	6
	2	M	55	V	Bilateral	IBB	6
	3	F	43	VI	Bilateral	IBB	6
	4	F	49	VI	Bilateral	IBB	6
Group 2	5	M	51	V	Unilateral	CBB	8
	6	M	44	V	Unilateral	CBB	12
	7	M	62	VI	Unilateral	CBB	12
Group 3	8	M	56	V	Unilateral	PCB	4
	9	M	54	VI	Unilateral	PCB	4
	10	F	44	V	Unilateral	PCB	4
Group 4	11	F	57	V	Unilateral	Composite	12
	12	F	55	V	Unilateral	Composite	12
	13	M	41	V	Unilateral	Composite	12

*Cawood and Howell[3] classification.
IBB = iliac bone block; CBB = chin bone block; PCB = particulate chin bone.

viability of this type of graft, essentially consisting of compact bone[2,19] (Group 2). A third group of three patients underwent unilateral sinus floor elevation with autogenous bone grafts harvested from the chin region and conformed as particulate bone (Group 3); in this case, the healing period was reduced to 4 months, since the original compact bone structure had been removed by the particulation process, and graft volume resorption could be presumed to occur during the early phases of healing. A fourth group of three patients underwent unilateral sinus floor elevation with composite bone grafts (Group 4) consisting of autogenous bone harvested from the chin region and combined (50% of graft volume) with porous hydroxyapatite granules (Interpore 200, Interpore International, Irvine, CA). The healing was prolonged to 12 months to allow adequate new bone formation, as indicated for this type of graft.[13]

After graft healing, all patients received threaded titanium implants (Nobel Biocare, Göteborg, Sweden), ranging in length from 10 mm to 18 mm and in diameter from 3.75 mm to 4.0 mm, in the grafted areas. Both at graft positioning and at implant placement, bone biopsies were taken in the grafted regions using a trephine bur (Nobel Biocare) measuring 4.8 mm in diameter; biopsy specimens were evaluated histologically and histomorphometrically.

Surgery. Patients requiring bilateral maxillary sinus floor elevation underwent hospitalization, and bone grafts were harvested from the inner aspect of the anterior iliac crest under general anesthesia. After exposure of the region, corticocancellous blocks were removed with a micro-oscillating saw (Stryker, Kalamazoo, MI) under abundant irrigation. Iliac bone blocks were then tested in place and adapted to the contours of the sinus cavity that had previously been opened by a fenestration in the denuded anterolateral wall of the maxillary sinus with a round bur. Blocks were firmly secured to the sinus floor with two titanium screws measuring 15 mm in length and 2 mm in diameter (Aesculap, Tuttlingen, Germany). In patients requiring unilateral maxillary sinus floor augmentation, the autogenic bone was harvested from the chin region under local anesthesia (mepivacaine 20 mg and adrenalin 0.010 mg, vials 1.8 mL, Astra Farmaceutica, Milan, Italy) without hospitalization. Once the region was exposed, a round tungsten carbide bur (H23 008, Komet CMS, Milan, Italy) or a micro-oscillating saw (Stryker) was used under abundant irrigation to harvest block bone grafts. For particulate grafts, bone cores were harvested from the chin region by means of a 4.8-mm-diameter trephine bur (Nobel Biocare) until the desired quantity of bone was obtained. Bone cores were placed into a fragmenting device (Mini Mill Bone Mill, Bio Comp, Ventura, CA) for particulation. Chin block bone grafts were adapted in shape, placed on the sinus floor, and fixed with a single titanium screw measuring 15 mm in length and 2 mm in diameter (Aesculap). Particulate chin bone grafts were directly packed to fill the floor of the sinus cavity without any fixation.

Histologic Analysis. Bone biopsies were fixed in 10% neutral formalin for 48 hours, decalcified in Osteodec (Bio-Optica, Milan, Italy), and then dehydrated by multiple passages in progressively diluted alcoholic solutions and in xylene. Thereafter, specimens were embedded in paraffin wax and sectioned to about 7 µm using a microtome (Leitz 1208, Wetzlar, Germany). Sections were stained with hematoxylin-eosin, embedded in resin, and observed under an optical microscope (Laborlux D, Leica, Wetzlar, Germany). The various components of the

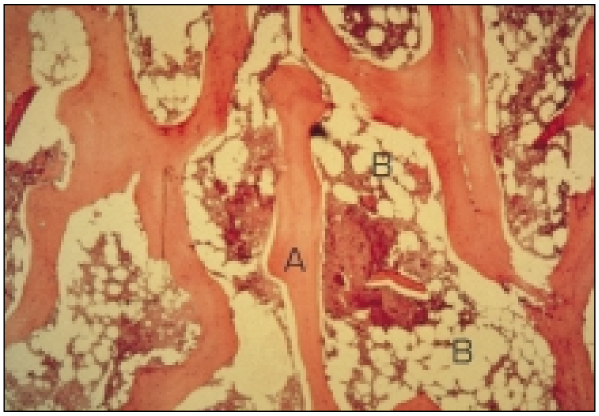

Fig 1 Histologic appearance of block bone graft from the iliac crest at the first surgical stage: typical cancellous structure with thin trabeculae (A) and large marrow spaces (B) (hematoxylin-eosin stain; magnification ×20).

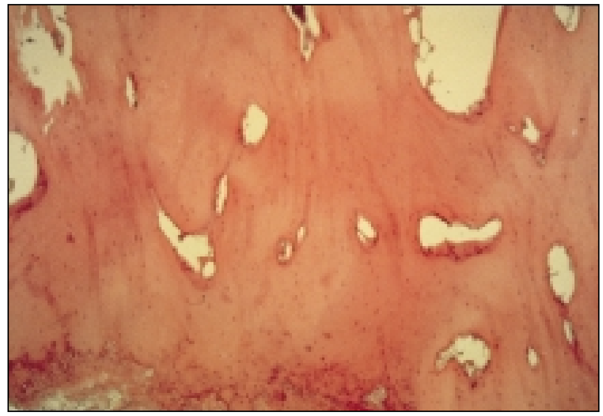

Fig 2 Block bone graft harvested from the symphyseal region at first surgical stage. The graft is almost entirely constituted of cortical bone and small cavities filled with fibrous tissue (hematoxylin-eosin stain; magnification ×25).

specimens were evaluated semi-quantitatively and assigned a score ranging from – to ++++ for:

- Amount of mineralized tissue and soft tissue
- Number of osteoblasts
- Bone vitality, expressed as number of osteocytes with clearly recognizable nuclei
- Number of blood vessels in the stroma

Subsequently, these values were reported as percentages according to the following: – = 0%; + = 10% to 20%; ++ = 30% to 50%; +++ = 60% to 70%; ++++ = 80% to 100%.

Image Analysis (Histomorphometry). After conventional microscopic examination, computer-assisted morphometry was performed. Sections were examined under a light microscope (Leitz Ortholux) equipped with a charge-coupled device camera connected by means of a PC Vision Plus 512 frame grabber (Imaging Technologies, Woburn, MA) to an MS-DOS 486 computer using automated image-analysis software (Java, Jandel Scientific, Corte Madera, CA). All measurements were made using a 10× microscope lens; for each specimen, four counts were made after random choice of section, measuring the fractional area (video counts/total number of pixels) covered by trabecular structures in the area of interest, by tracing the perimeter of each structure manually with a mouse. The hard tissues were selected by the manual threshold method on the grey-level scale of the image. Because of differences in background and intensity of staining between different slides, the upper and lower grey-level limits were chosen differently for each section, according to the window that appeared to be most consistent with the microscope image.

Results

None of the patients undergoing surgery at the iliac site complained of significant pain or difficulty in walking. Similarly, in the chin region, no signs or symptoms of pain were reported. Second-stage biopsies were performed at different times according to the operative plan, and direct comparison of the results was therefore not possible. Results were grouped by common embryonic origin, conformation, and composition of the bone grafts.

Histologic Results. *First-Stage Biopsies.* Specimens of iliac crest bone harvested at the first surgical stage were essentially composed of cancellous bone, characterized by a thin trabecular structure containing a large number of osteocytes and surrounded by an abundant hematopoietic marrow (Fig 1). Specimens harvested from the chin region consisted of a prevalent cortical structure and a limited number of thick trabeculae with a lower degree of cellularity (Fig 2). In all cases, the bone showed typical histology.

Embryonic Origin. Specimens taken from iliac block bone grafts (endochondral, Group 1) appeared, after 6 months of healing, to maintain their cancellous structure, characterized by thin trabeculae with high vitality as indicated by the large number of osteocyte nuclei detectable inside the osseous lacunae. Soft and mineralized tissues appeared to be present in equal amounts (Fig 3). In chin block bone grafts (membranous, Group 2), bone tissue still prevailed over soft tissue 8 to 12 months after placement (Fig 4). Compactness remained, but vitality within the cortical component varied to a great extent. Some limited areas of the graft were not populated with

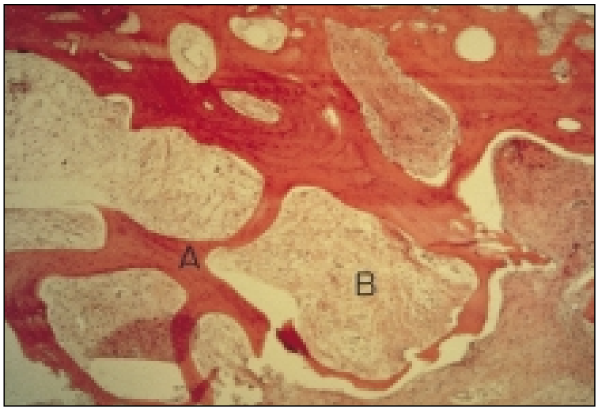

Fig 3 The same case as shown in Fig 1 after a healing period of 6 months. The cancellous architecture is almost entirely conserved: a thin trabecular network (A) surrounds large marrow cavities occupied by fibrous connective tissue (B). The graft appears uniformly viable, as evidenced by the large number of osteocyte nuclei detectable inside the osseous lacunae (hematoxylin-eosin stain; magnification ×20).

Fig 4 Block bone graft from the chin after 12 months of healing. As in Fig 2, the initial graft architecture is maintained and a prevalence of mineralized tissues is visible (hematoxylin-eosin stain; magnification ×20).

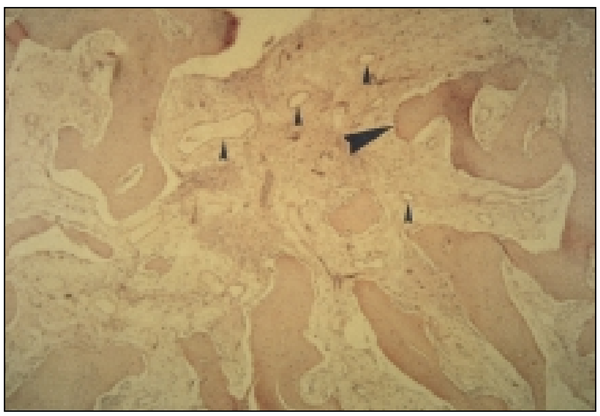

Fig 5 Particulate chin bone graft after 4 months of healing. Mineralized tissues are less prevalent than in block grafts. The bone is organized in a newly formed trabecular network with large connective spaces. On the borders of the trabeculae, numerous osteoblasts are visible *(large arrowhead);* within the connective tissue, several blood vessels are visible *(small arrowheads)* (hematoxylin-eosin stain; magnification ×12.5).

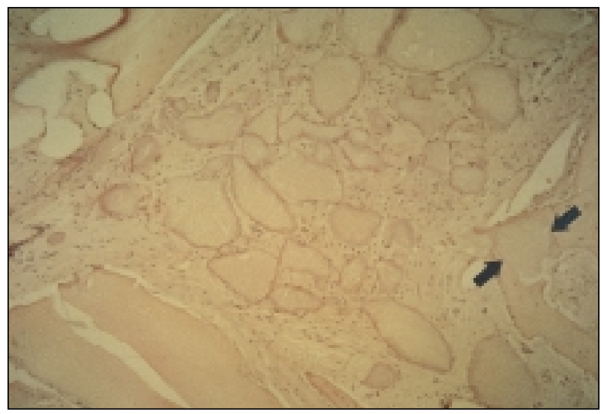

Fig 6 Composite bone graft at 12 months of healing. Fibrous connective spaces containing the hydroxyapatite granules prevail. Only in some areas *(arrows)* do the hydroxyapatite granules appear to be in direct contact with the fragments of cortical bone (hematoxylin-eosin stain; magnification ×12.5).

viable osteocytes, producing an image of empty osseous lacunae. Blood vessels were seen in larger numbers in iliac block bone grafts than in chin block bone grafts.

Geometric Conformation. In particulate chin bone grafts (Group 3), the bone tissue was slightly prevalent over soft tissue after 4 months of healing. A newly formed trabecular network could be seen in all specimens. Osteoblasts surrounding the new bone were present in a significantly higher number than in chin block bone grafts (Group 2). Osteocytes populated the trabeculae uniformly. A remarkably larger number of blood vessels was noted within the particulate bone grafts than in block bone grafts (Fig 5).

Graft Composition. In composite bone grafts (Group 4), soft tissues clearly prevailed over hard tissues. Twelve months after placement, HA granules were easily distinguishable from the other components of the graft. Most of the HA granules were surrounded by highly cellular connective tissue, and new bone tissue formed only a thin layer at the perimeter of the granules. Numerous osteoblasts were present in these areas, indicating active new bone formation. Few HA granules were integrated within the autogenous bone (Fig 6). In some regions of the connective tissue surrounding the HA granules, a slightly inflammatory reaction was noted. A moderate number of blood vessels was present.

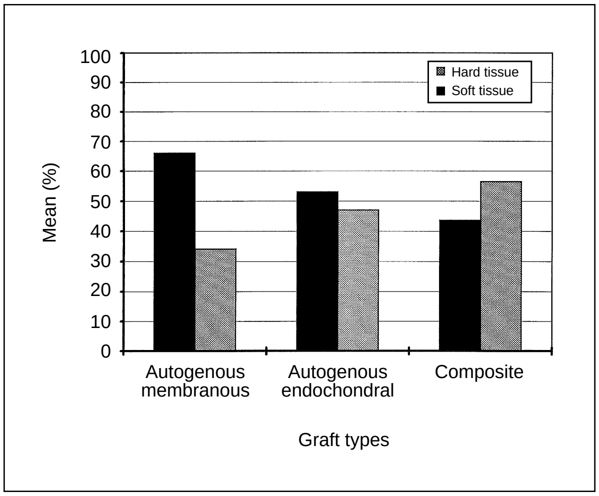

Fig 7 Histomorphometric evaluation of bone content at second-stage biopsy. The graph groups results according to embryonic origin and composition of the bone grafts; healing periods differed (see text). In all cases, autogenous bone grafts, regardless of embryonic origin and geometric conformation, had a superior quantity of hard tissues than composite bone grafts.

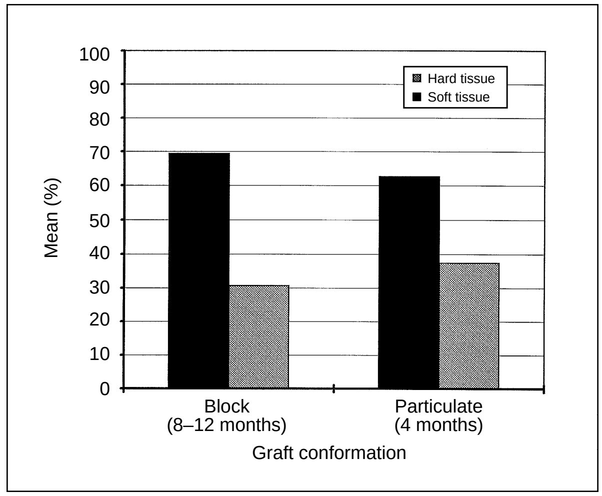

Fig 8 Histomorphometric evaluation of bone content at second-stage biopsy. The two types of bone graft shared embryonic origin (membranous) but differed for geometric conformation (block or particulate). After only 4 months of healing, particulate grafts contained a slightly lower percentage of hard tissue than block grafts after a considerably longer period (8 to 12 months).

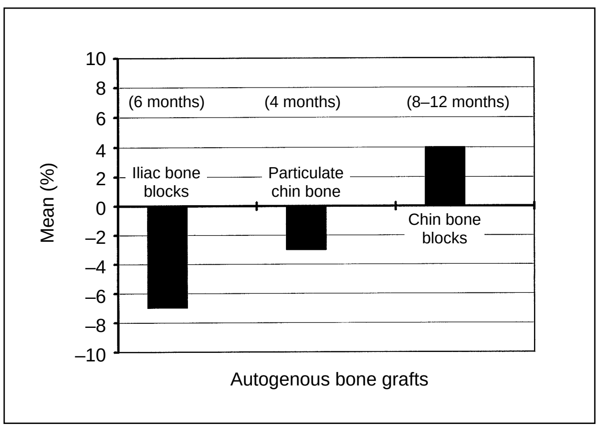

Fig 9 Variations in mineralized tissue content after varying healing periods for different types of autogenous bone grafts.

Histomorphometric Results. Relating embryonic origin to mean percentage of hard tissues, membranous bone grafts (chin, Groups 2 and 3) contained 66%, whereas endochondral bone grafts (iliac crest, Group 1) contained 53% (Fig 7).

Considering geometric conformation, chin bone grafts conformed as blocks (Group 2) contained a mean percentage of 69.3% hard tissue while particulate grafts (Group 3) contained 62.6% (Fig 8).

With regard to graft composition, the lowest percentage of hard tissues occurred in composite bone grafts (Group 4), with a value of 43.6% (Fig 7).

The initial mean percentage of bone found at the first surgical stage was 60% for the iliac bone grafts and 65.6% for the chin bone grafts. During healing, bone quantity was reduced by an average of 7% in iliac bone grafts, and by an average of 3% in particulate chin bone grafts. Chin bone grafts conformed as blocks exhibited an increased bone quantity (+ 4%) (Fig 9).

Discussion

In this study, differences in both anatomic location and embryonic origin of the skeletal regions selected for harvesting the autogenous bone grafts were investigated. The iliac donor site (endochondral) provides bone grafts consisting of a prevalent cancellous component, whereas the chin region (membranous) provides essentially cortical bone grafts. Several experimental studies[17,18,20] have shown that graft architecture, which relates to embryonic origin of the bone, can decisively influence the repair process during the healing period. Endochondral bone grafts undergo greater resorption than membranous bone grafts,[17,18,20] in which the final graft volume was increased.[18] In view of the above, varying healing periods were selected for iliac block bone grafts (6 months) and chin block bone grafts (8 to 12 months) to limit graft volume resorption and to achieve sufficient graft revascularization and vitality. It has been shown[2,19] that, from the early phases of healing, cancellous bone grafts are soon penetrated by new blood vessels and provide a large number of osteoprogenitor cells, encouraging immediate graft repair, while cortical bone grafts provide fewer osteoprogenitor cells and can require from 1 to 2 years for complete repair.

Histologic analysis at second-stage biopsies demonstrated that both endochondral and membranous block bone grafts maintained their architectural characteristics, consisting respectively of a cancellous bone structure in iliac grafts and a compact bone structure in chin grafts. After 6 months of healing, iliac block bone grafts (endochondral, Group 1) were highly vital and contained a greater number of blood vessels than chin block bone grafts (membranous, Group 2). Even though the healing period for this group was longer (8 to 12 months), graft vitality varied more than it did in the iliac grafts. Some isolated nonviable areas, consisting of empty osseous lacunae, were visible within the graft. This seems to confirm the tendency of this type of graft to require longer healing periods.[19]

Because of the differing healing periods, mean final bone content in Group 1 (53%) and Group 2 (69.3%), as measured by histomorphometric analysis, is not directly comparable. The study did not measure variations of graft volume. The mean reduction in hard tissues found in Group 1 (initial bone less final bone: –7% on average) and the increased bone quantity in Group 2 (+4%) suggest that the repair process in endochondral and membranous block bone grafts may differ; this healing might be similar to processes previously reported in experimental studies.[18,20]

In animals, a direct relationship has been found[21] between osteoclastic activity and the revascularization process, and it has been theorized that the differences in architectural structure, related to the embryonic origin of the grafted bone, could be the basis for the different biologic behavior of membranous and endochondral bone grafts. Experimentally, it has been found that revascularization occurs more rapidly in grafts consisting of prevalent cancellous (endochondral) rather than cortical (membranous) bone.[22] In the present study, a larger number of blood vessels was observed in iliac than in chin block bone grafts, which is in agreement with the above animal study. This may explain the decrease of bone percentage in the iliac bone grafts.

The geometric conformation of the graft may be another factor influencing the repair process. The literature contains few comparative histologic or histomorphometric studies of particulate or block bone grafts.[13,23] A recent clinical study[23] of 10 patients subjected to mandibular reconstruction with particulate or block bone grafts harvested from the ilium reports the results of histologic and microradiologic examinations of biopsies. The authors concluded that with particulate bone grafts, it is possible to reduce the healing period, enabling endosseous implants to be placed sooner.

The grafts with particulate conformation (Group 3) showed extensive revascularization to have occurred as early as 4 months, possibly because of the absence of the barrier effect of the cortical portion,[20,24] for which particulate bone graft may behave more like cancellous bone grafts. In light of the aforementioned relationship between resorption and revascularization, the healing period before implant positioning was reduced to 4 months in Group 3 to limit bone loss. Second-stage biopsies appeared to confirm this tendency. In particulate bone grafts, the initially grafted mineralized tissue (65.6%) decreased on average by 3%. Therefore, the mean final quantity of mineralized tissue in particulate chin bone grafts was 62.6%.

The graft in Group 2 used bone of the same embryonic origins for Group 3, but with a different geometric conformation. Although the quantity of mineralized tissue was evaluated after a more extensive healing period, the value obtained (69.3%) was not significantly more than that of Group 3 (62.6%) after only 4 months.

The histologic analysis of Group 3 specimens, after 4 months of healing, revealed that the vitality of the graft, evidenced by a large number of osteocytes, was comparable to that in chin block bone grafts. A newly formed trabecular system was clearly detectable, and the bone tissue was populated with a larger number of osteoblasts than in block bone grafts. In the authors' opinion, these data, in conjunction with the amount of hard tissue contained in these grafts, may allow earlier implant positioning.

Graft composition was another factor that could influence the repair. In this study, the lowest percentage of hard tissues was found in composite grafts, with a mean value of 43.6% after a healing period of 12 months. Grafts composed of autogenous bone only, regardless of their embryonic origin or geometric conformation, in all cases produced higher percentages of bone tissue, in agreement with histomorphometric results reported with this type of graft.[13] There is experimental proof that the use of hydroxyapatite in combination with an osteoinductive material,[25] rather than alone,[26] produces more satisfactory results. However, when composite bone grafts are used in reconstructive procedures, as in the present experience, prolonged healing periods may be required before endosseous implant placement is possible.

The limited number of patients treated with each type of graft did not allow any statistical analysis of the results. A larger number of patients would be needed to produce statistically significant conclusions.

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