Microscopic Analysis of Reconstructed Maxillary Alveolar Ridges Using Autogenous Bone Grafts from the Chin and Iliac Crest

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Purpose: *The aim of this study was to histologically analyze the bone repair of maxillary areas reconstructed with autogenous bone grafts using 2 different donor sites, the ilium and the chin.* ***Materials and Methods:*** *Specimens were retrieved with trephine burs positioned transversely in augmented ridges 4 months after placement of the grafts. To analyze bone conditions, a histomorphometric study of ground sections was performed using a special template to identify 3 specific regions of the specimens: cortical bone, cancellous bone, and the region of transition between the alveolar ridge and the graft. Ten patients, 5 men and 5 women, with a mean age of 47 years (range 28 to 67) were evaluated.* ***Results:*** *Results indicated good incorporation of the grafts in this period, demonstrated by intense osteogenesis indicating an active remodeling process.* ***Discussion:*** *In both groups, the improvement in bone quality of the receptor site was evident, independent of the size of the reconstruction, although chin grafts presented better bone quality.* ***Conclusions:*** *From this study it was possible to conclude that a period of 4 months is sufficient for the placement of osseointegrated implants in reconstructed areas, where chin or iliac autogenous grafts have been used.* (INT J ORAL MAXILLOFAC IMPLANTS 2002;17:507–516)

Key words: *bone marrow transplantation, endosseous dental implants, osteogenesis*

The success of alveolar ridge rehabilitation using endosseous implants is directly related to bone quality and quantity.[1–3] There are various alternative surgical techniques and materials available for improving ridge conditions that meet these requirements. Iliac crest and chin are frequently used as donor sites for the reconstruction of atrophic ridges.[4–15] The embryologic origins of donor and receptor sites have been also discussed, since these may be the main cause for the higher levels of bone resorption in areas reconstructed with iliac crest when compared to intramembranous bone.[7,16] However, when autogenous bone grafts are used, regardless of the donor site used, immediate or late placement of implants may be chosen.[4,5,17–19] When the implants are placed in 2-stage surgery, different waiting periods have been cited in the literature, varying from a few to several months. A 2-stage procedure is often chosen because of the high rates of success and more predictable results.[19] However, there is no agreement as to the period of time necessary between graft surgery and implant placement in the literature reviewed for this study; this period varied from 3 to 18 months[7,11–13,15,18,20–25] depending on the type of reconstruction and the donor site used.

Considering the different results reported, the present work aimed to analyze the events of bone repair in maxillary regions reconstructed with iliac crest and chin autogenous bone grafts 4 months

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Table 1 Patient Data

Patient no./ initials	Sex	Age (y)	Reconstructed region	Region	Donor site	Anesthesia	Size of blocks
1/AMRS	M	41		12–21	Iliac	General	5 × 2 × 0.7 cm (PT)
2/ARP	M	67		13–16	Iliac	General	7 × 2 cm (FT)
3/NOS	F	51		16–26	Iliac	General	7 × 2 cm (FT)
4/NMN	F	36		16–26	Iliac	General	6 × 2 cm (FT)
5/ARP	M	67		24–26	Iliac	General	7 × 2 cm (FT)
6/RAP	M	47		14–15	Chin	Local	1.2 × 0.6 cm
7/SFC	F	28		21–23	Chin	Local	1.8 × 0.7 cm
8/JBR	M	31		11–22	Chin	Local + sedation	2.5 × 0.8 cm
9/NATO	F	55		24–26	Chin	Local	1.8 × 0.8 cm
10/VAAZ	F	40		14–15	Chin	Local	1.4 × 0.8 cm

FT = full thickness; PT = partial thickness.
Tooth numbers are FDI system.

after this procedure. In addition, differences were sought in integration of the grafts and the bone quality provided by these alternative donor sites.

MATERIALS AND METHODS

Ten patients, 5 men and 5 women with a mean age of 47 years (range 28 to 67), received autogenous bone grafts from the iliac crest and chin for reconstruction of atrophic areas of the maxilla for future rehabilitation with endosseous implants. Five of them received iliac grafts, and the other 5 received chin grafts. The defects found in these patients corresponded to Class IV, V, and VI according to Cawood and Howell[26] and demonstrated inadequate bone width and height (Table 1).

Analysis of the size of the defects and the quantity of required bone graft was mandatory for choosing the donor site, noted by the graft size difference. In the majority of cases the iliac crest graft was harvested with both cortices, with the exception of 1 patient in whom the external cortex was preserved because of the anatomy of the maxillary defect. Patients receiving iliac crest bone grafts were treated under general anesthesia. Local anesthesia was used for mandibular grafts; in 1 patient intravenous sedation was associated with local anesthesia.

The grafts were harvested using an oscillating saw and chisels, adapted to the receptor site, and fixed with 2.0-mm titanium screws (Synthes; Stratec Medical, Oberdorf, Switzerland). Despite the variety of defects, the type of reconstruction was very similar; the onlay technique was used to improve the thickness of the ridges by positioning bone blocks on the buccal aspect. No specific preparation of the receptor site was performed and no topical antibiotic was used.

At the time of implant placement, 4 months after bone-grafting surgery, an incisional biopsy was performed using a 2.6-mm trephine bur (Ace Surgical, Brockton, MA), positioned transversely to the ridge. This permitted the acquisition of representative material from the alveolar ridge and bone graft (Fig 1) in a previously selected area and did not interfere with the positioning of the implants. From this procedure, the specimens were retrieved within the total width of the new ridge, presenting bone graft and bone ridge from the maxilla, since the bur reached the palate. Five specimens corresponding to sites of the maxilla reconstructed with iliac crest and 5 specimens corresponding to sites reconstructed with chin grafts were obtained. The reconstructive surgeries and the biopsies were performed by a single operator.

The specimens were fixed in buffered formalin, demineralized in 10% ethylenediaminetetraacetic acid (EDTA) and stained with Harris' hematoxylin, eosin, and the Mallory trichromic technique. The slices were analyzed by light microscopy and repair of the grafted area was observed.

Histomorphometric analysis was performed and bone quality evaluated from measurement of the mineralized bone matrix present in 3 distinct regions of the specimens: A, the superficial portion of the specimen, representing the external cortex region of the graft; B, the intermediate portion of the specimen, representing bone graft marrow; and C, alveolar portion of the specimen, representing the site of transition between ridge and graft, or the original ridge (receptor area). To make measurement possible, the slices related to the largest diameter of the specimen were projected using a Macronucleo projector (Biotec, Rio de Janeiro, Brazil) over a white sheet of paper, using this as a template to register the regions to be analyzed (Fig 2).

In the present work, the slices presenting the largest diameter of the specimen were selected and divided into 3 proportional sections. During retrieval of the specimens, care was taken to identify the portion of the bone cylinder that corresponded to the outer surface and to the deepest regions, representing the cortical layer of the graft and alveolar ridge or its interface with the bone block, respectively. This division was made in an attempt to find differences in bone quality among these regions, and also to determine whether the graft procedure offered improvement under local conditions of implantation. The middle third was chosen for its clinical importance, since a representative part of the implant surface rests in this region.

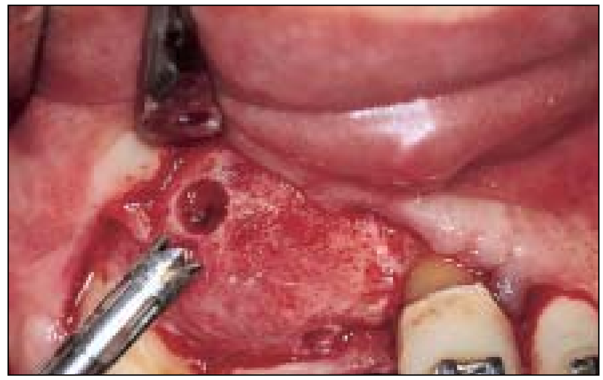

Fig 1 Specimens were obtained 4 months after the graft surgery using a trephine bur.

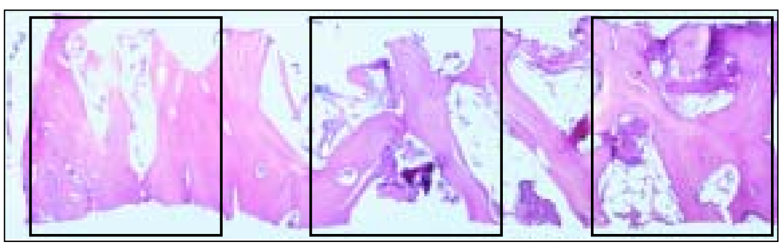

Fig 2a Specimen obtained after the biopsy, representing the whole extent of the reconstructed ridge. Note the selection of the region corresponding to the external cortex.

Fig 2b Projection of this region to outline of the mineralized bone surface.

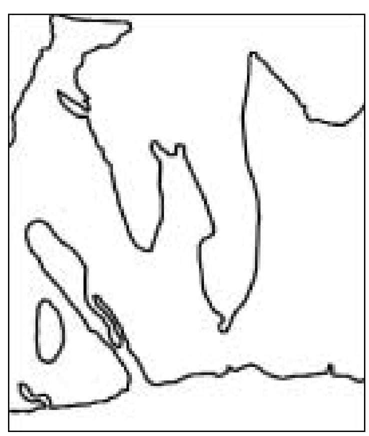

Fig 2c Example of the outline ready to be digitized.

Figs 3a to 3d Specimens retrieved from areas reconstructed using iliac grafts.

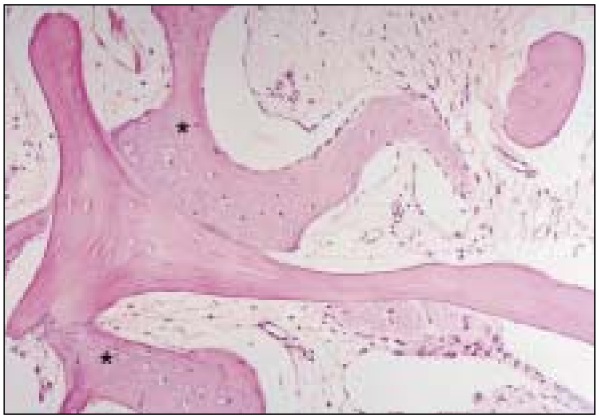

Fig 3a Osteogenesis was identified by the presence of woven bone *(asterisks).*

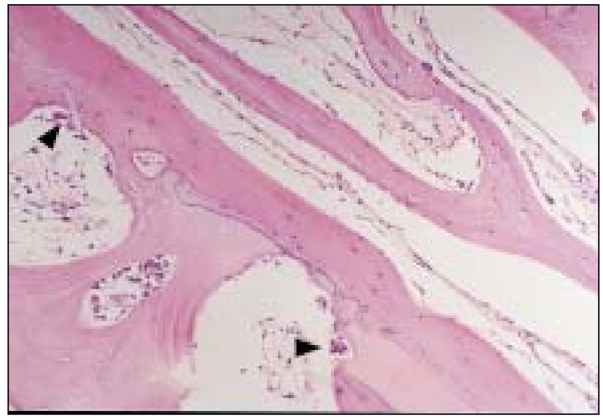

Fig 3b Eventual clasts were seen in the specimens demonstrating the remodeling process *(arrowheads).*

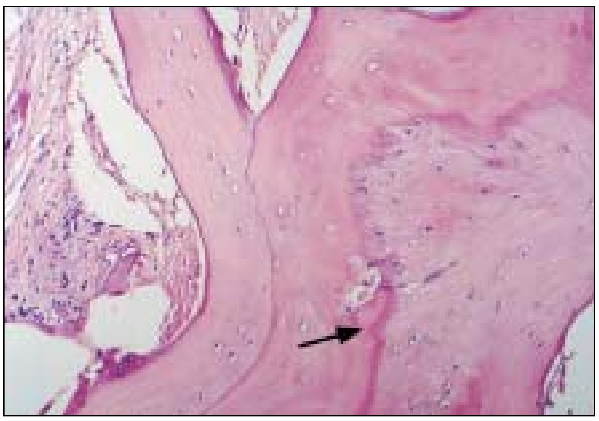

Fig 3c Reversal lines denoting new bone formation *(arrow).*

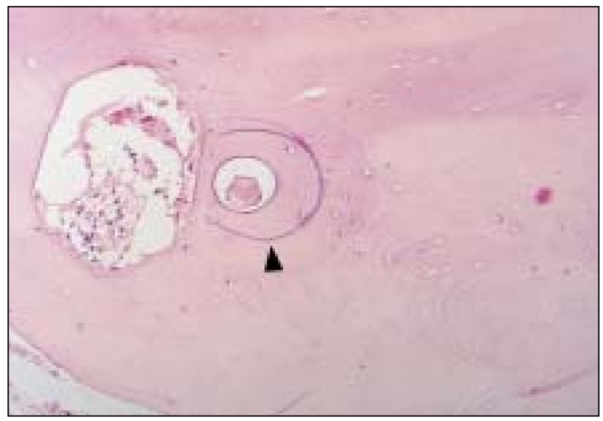

Fig 3d New bone formation was also seen in cortical bone *(arrowhead)* (hematoxylin-eosin; original magnification ×40).

Choice of the segment of interest in each portion of the specimen was made using a rectangular template over which the images of the chosen regions were projected. The size of this template followed the maximum possible proportion of the smaller specimen. Also, considering the different thickness of the grafts, the shortest specimen was used as a parameter for the other specimens and to determine the size of the template. Outlines of the mineralized bone matrix were then measured using a digital table for acquisition of the total area of the previously determined regions.

Only the grafted areas were analyzed. No previous biopsies of the donor sites or of the receptor sites alone were made, since the aim of the study was to observe and compare the behavior of these different bones as graft sources in maxillary regions after the reconstruction.

The data obtained from the different grafts were compared using analysis of variance (ANOVA), the *F* test, and the Tukey test ($P \leq .05$).

RESULTS

Specimens Corresponding to Iliac Crest Grafts

When sites were observed microscopically, bone tissue characterized by thin trabeculae (most of them non-viable) was noted in association with regions of new bone formation characterized by disorganized tissue, a large number of osteocytes, and reversal lines (Fig 3a). On the surface of the trabeculae, a thin layer of osteoblasts was present. Eventual areas of resorption could be seen, as well as a small number of clasts (Fig 3b). Evident reversal lines were noted in the areas of new bone formation. Predominantly fatty medulla was found in the analyzed specimens, but vascularized fibrous connective tissue was also noted, weakly infiltrated with mononuclear leukocytes (Fig 3c). In cortical bone, large Haversian systems were observed, in some areas filled by newly formed bone, demonstrating the remodeling process (Fig 3d).

Figs 4a to 4d Specimens retrieved from areas reconstructed using chin grafts.

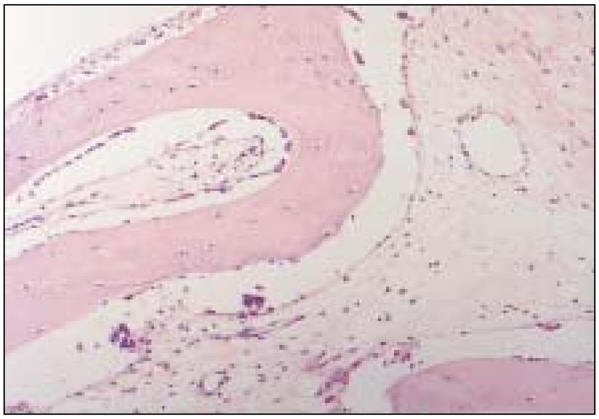

Fig 4a Viable trabeculae were noted containing osteocytes in the lacuna.

Fig 4b Evident osteoblast layer could be seen around bone trabeculae *(arrowhead).*

Fig 4c The presence of apposition lines denotes remodeling process *(arrows).*

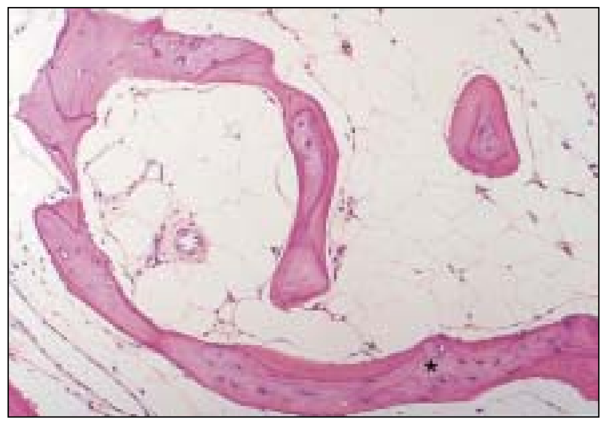

Fig 4d Woven bone was seen associated with non-viable trabeculae *(asterisk)* (hematoxylin-eosin; original magnification ×40).

Specimens Corresponding to Chin Grafts

Bone tissue constituted by thick trabeculae was observed, presenting osteocytes containing lacunae, and were thus considered morphologically viable (Fig 4a). Evident reversal lines were observed in areas of woven bone, indicating new bone formation. On the surface of the trabeculae a regular layer of osteoblasts could be seen; in the specimens where bone formation was exuberant, the osteoblastic layer was more evident (Fig 4b). New bone formation was also noted next to lamellar bone. In marrow spaces, fat tissue was predominantly found, but the presence of associated hematopoietic tissue was noted. Although connective tissue filled the medulla, the vascularization was intense (Fig 4c). Non-viable trabeculae were seen, isolated or juxtaposed to a highly cellular woven bone (Fig 4d). In the cortical bone, evident Haversian systems were seen showing irregular surfaces, with few areas of new bone formation.

Histomorphometric Analysis of Total Mineralized Bone Area

This study evaluated the area of 3 previously established regions of the specimens taken from iliac crest and chin grafts, making possible a comparison between the different regions and the donor sites used. The resulting numbers corresponding to the areas are presented in Table 2. From these numbers, 2 factors were correlated in this analysis: grafts (chin and iliac crest) and regions (A, B, and C).

Statistical Analysis

The mathematic model for variance analysis was of completely randomized design in the split plot scheme.

Significant differences were detected between the mean of the areas (at 0.43% level) and a tendency to significant difference was seen for the grafts (at 7.42% level), while for the interaction of regions (combination of grafts with the regions), the F test

Table 2 Bone Surface Measurements of Regions A, B, and C

Graft	Region	Patient	Total bone area (mm²)
Iliac	A	1	2,938.040
Iliac	A	2	2,797.398
Iliac	A	3	5,270.600
Iliac	A	4	3,484.033
Iliac	A	5	3,271.106
Iliac	B	1	616.930
Iliac	B	2	2,853.964
Iliac	B	3	2,975.465
Iliac	B	4	3,274.930
Iliac	B	5	3,963.867
Iliac	C	1	1,367.940
Iliac	C	2	1,899.633
Iliac	C	3	2,992.365
Iliac	C	4	1,831.428
Iliac	C	5	3,559.700
Chin	A	6	5,102.134
Chin	A	7	2,552.866
Chin	A	8	6,719.266
Chin	A	9	5,072.500
Chin	A	10	5,777.600
Chin	B	6	5,622.367
Chin	B	7	2,877.297
Chin	B	8	4,902.999
Chin	B	9	2,484.762
Chin	B	10	4,348.383
Chin	C	6	2,543.099
Chin	C	7	3,627.966
Chin	C	8	3,468.030
Chin	C	9	1,803.830
Chin	C	10	3,210.137

Biopsies obtained from chin and iliac grafts using a 2.6-mm trephine bur.

Table 3 Analysis of Variance and *F* Test of Variable Total Bone Area as a Function of Variation Causes (Factors) of Graft Type, Regions, and their Interactions

Factor	df	Mean squared variance	*F*	*P* > *F*
Grafts	1	9,651,290.29	4.21	.0742
Residue (A)	8	2,289,805.22		
Regions	2	6,974,206.49	7.82	.0043
Grafts × regions	2	555,465.44	0.62	.5491
Residue	16	892,306.82		
Total	29			

Table 4 Comparison (Tukey Test) of Total Bone Area Means in Relation to Each Graft Type Studied

	Graft		
Area	Chin	Iliac	Mean area (mm²)
A	5044.87	3552.24	4298.55[a]
B	4047.16	2737.03	3392.10[ab]
C	2930.61	2330.21	2630.41[b]
Mean	4007.55	2873.16	

Means followed by different letters differ at the 5% level of significance ($\alpha \leq .05$).

did not demonstrate any significant difference (Table 3). The variation coefficient obtained, 27.46%, indicated a high level of experimental precision.

Means of total bone area related to each graft, regions, and their interaction, as well as the comparison of the regions by the Tukey test, are presented in Table 4. Chin grafts presented a greater mean total bone area than iliac grafts, however, as noted in Table 3; the *F* test showed a tendency to significant difference between the graft types. The Tukey test indicated that, whereas region A presented the greatest total bone area, region C had the smallest. Region B possessed an intermediate total bone area, not differing statistically significantly from either region A or C.

The same tendency of the principal effects (grafts and regions) is followed by the interaction of the 2 factors, indicating that the effect of one factor is independent of the level of the other factor, which also can be seen by the non-significance of the interaction in the ANOVA (Figs 5a to 5c).

DISCUSSION

Autogenous bone grafts are preferred in procedures involving alveolar reconstruction because of their osteogenic capacity, low immunogenicity, and flexibility in clinical use.[27–34] The 2-stage technique apparently presents biologic advantages over simultaneous bone grafting and implant placement. Table 5 conveys the divergence among authors' opinions, where proposed healing times can vary from 3 to 18 months.[7,11–15,18,20–25] It seems that this variation is guided by clinical experience and the impressions of these authors, as well as by published results obtained from experimental animal models.

Figs 5a to 5c Means of total bone area related to each bone graft studied, to the regions, and to their interaction.

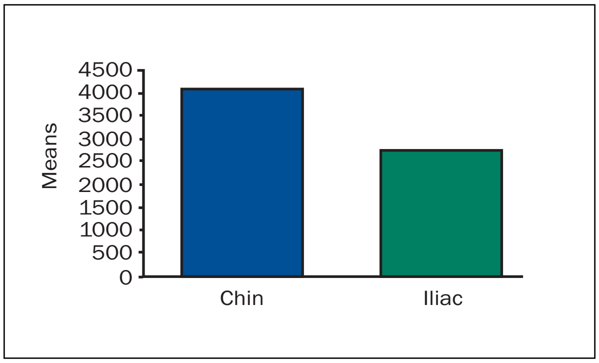

Fig 5a

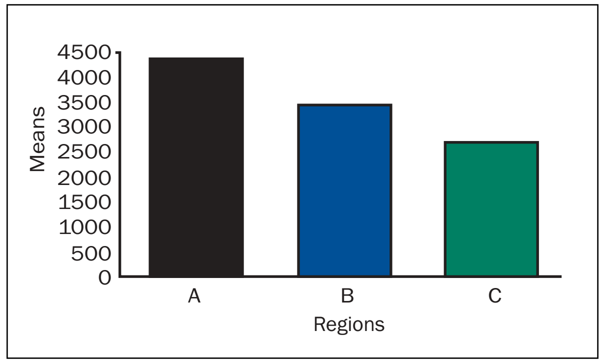

Fig 5b

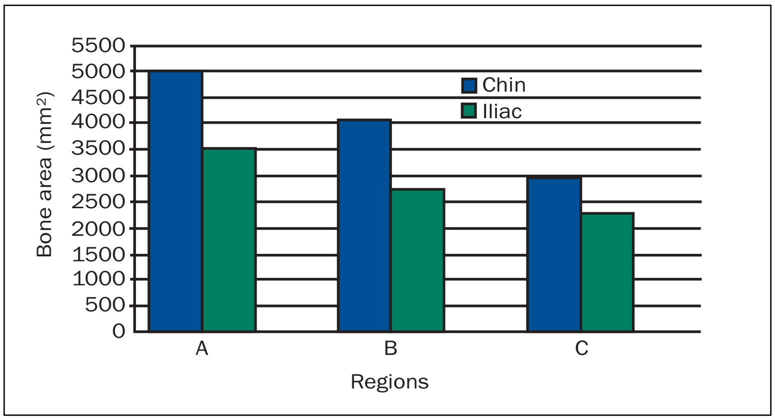

Fig 5c

Table 5 Waiting Periods for the Incorporation of Autogenous Grafts Cited by Different Authors for Placement of Osseointegrated Implants

Authors	Donor sites	Receptor sites	Waiting period (mo)
Keller et al (1987)[22]	Iliac crest	Maxilla	6 to 18
Widmark et al (1998)[25]	Iliac crest	Maxilla	3 to 4
Sailer (1989)[18]	Autogenous bone	Maxillary sinus	4
Misch et al (1992)[20]	Chin	Maxilla	4 to 6
Donovan et al (1994)[21]	Calvaria	Maxilla	6 to 8
Misch and Misch (1995)[13]	Chin	Maxilla	4 to 6
Triplett and Schow (1996)[15]	Iliac crest and manidible	Maxilla/mandible	6 to 9
Neyt et al (1997)[24]	Iliac crest	Maxilla	4
Misch (1997)[12]	Chin and ramus	Maxilla	4 to 6
Garg et al (1998)[7]	Chin	Maxilla	6 to 8
Keller et al (1999)[23]	Iliac crest	Maxilla	4 to 6
Lekholm et al (1999)[11]	Iliac crest and mandbile	Maxilla	5 to 6

The aim of this study was to identify and characterize bone repair, by means of optical light microscopy, over a period of 4 months following bone graft surgeries using iliac crest and chin and to verify whether there were any differences in bone quality between these 2 distinct donor sites. Microscopic analysis revealed similar patterns of new bone formation and resorption in the areas corresponding to the grafts in both groups. The irregular areas indicating bone resorption were easily identified. However, multinucleated cells were not often seen. Irregular basophilic reversal lines were located in woven bone, which was disorganized, highly populated with cells, and seen in most of the specimens, suggesting new bone formation.

In this study, bone trabeculae possessing lacunae filled with osteocytes were considered viable. Morphologic analysis demonstrated a noticeably greater amount of non-viable trabeculae in specimens retrieved from areas reconstructed using iliac crest. This may indicate slower revascularization and bone remodeling or even the influence of the size of the graft used, therefore requiring more time for complete replacement by new bone.

With the methodology used, total bone area included both viable and non-viable bone. This identification could not be done exclusively on the basis of light microscopy and so these were distinguished according to the presence or lack of osteocyte cell in the lacunae, once no immunohistochemical markers were used to prove the functional activity of the osteocytes present. Furthermore, interest was focused on the amount of mineralized bone matrix, because even when bone cells are absent, it maintains its role in bone formation, as osteoconductor and osteoinductor.

During the period studied, a biomechanically favorable clinical condition for implant placement was observed, denoting incorporation of the graft, especially at its interface with the receptor site, where the limits were microscopically indistinguishable. From these observations, the recommendation for a long waiting period before implant placement, as suggested by other authors, may be questioned, since a period of 4 months seems sufficient before performing the second surgery.

It was possible, however, to verify in this study the biomechanical conditions needed to perform the second surgery, although the appropriate time for implant placement, from a biologic point of view, remains to be determined. It should be noted that even in non-grafted areas, surgical trauma resulting from preparation of the alveolus creates non-viable bone areas that will undergo all the phenomena seen in this study during the period of osseointegration.[35]

Discussion of the nature and origin of the grafts seems irrelevant at this point. Although studies such as those of Zins and Whitaker,[36] Kusiak and associates,[37] and Alberius and coworkers[38] defend these differences, it is necessary to appreciate the phenomena of revascularization and remodeling of bone tissue, regardless of its origin, considering that the behavior presented by the intramembranous grafts differs according to their architecture. No differences were noted, however, among the structures or cell populations studied in the present work, conferring the differences of bone repair to the characteristics of the reconstruction.

In another analysis performed, total bone area was related to bone quantity. When the term "bone quality" is used, its direct application is meant. Lekholm and Zarb[39] classsified maxillary ridges in relation to their quality, depending on the amount of cortical bone present. More favorable prognosis was related to greater cortical portions, and failures were linked to the presence of predominantly cancellous bone that showed less dense trabeculae. Thus, the term "bone quality" is used in this work, not when referring to the bone cell population or its biochemical and structural conditions, but rather to the quantity of mineralized matrix. The higher the density, the better the conditions for the use of implants.

It is clear that in major reconstructions, particularly those that involve the use of iliac crest bone, the measured areas corresponded to a part of the sample only, although they were clearly representative, as demonstrated in the exploratory analysis of the results. The results obtained from this measurement were very homogeneous, permitting statistical conclusions to be made with confidence.

The superior suitability of the chin is noteworthy when compared to the iliac crest in relation to the quantity of mineralized matrix. In their investigations, Misch and coworkers,[20] Misch and Misch,[13] and Garg and associates[7] emphasized the quality of chin bone when used in alveolar reconstructions and reported that it offers better conditions for initial stability of the implants, because of its early incorporation into the receptor site. Such clinical reports mentioned the histologic backgrounds in their studies.

The amount of augmentation obtained, which was smaller when chin grafts were used, should be considered since it is a limiting factor in its clinical indication for more complex cases. Similarly, in partial reconstructions where a small quantity of iliac graft was used, better results were demonstrated. Thus, it can be deduced that what really influences these procedures is the original bone quality of the donor sites, specifically their cortical component. When minor reconstructions are required, small cortical portions of the ilium can be used, minimizing the differences found in this work. However, in major reconstructions the risk is proportionally higher, because of the difficulties in preparing the receptor site and the necessity for greater amounts of bone, including cancellous bone, which can present inferior quality for the purpose of osseointegration.

These differences in bone density, well defined by Misch,[12] remained proportional in all regions analyzed, showing that both cancellous and cortical bone of the ilium present lower density. Interestingly, analysis of the results found in region C, which corresponded to the alveolar bone, revealed that in cases rehabilitated with iliac grafts the density was lower, although it was used in severely atrophic maxillae with poor bone quality.

The clinical implication of these results focuses on the time of selection of the donor sites. It is important to interact with the patient when choosing the treatment, since several variables affect the prognosis. It is evident that chin grafts offer better local conditions for osseointegration; however, they present limitations related to the area to be rehabilitated. In partial cases, for instance, the chin can offer sufficient bone volume for implant placement but not sufficient volume for esthetic recovery of the defect. In this situation, bone quality must also be discussed with the patient when offering options for treatment.

The most interesting observation from this study is possibly the conclusion that, independently of the donor site, the graft procedures improved local bone conditions. Clelland and coworkers[40] showed that the greatest stress resulting from occlusal loading of prostheses supported by implants is on the alveolar crest. In light of this, Misch and Misch[13] called attention to this region, since dense bone is present, which offers a more favorable interface to strengthen distribution for the alveolar component.

The reconstruction of atrophic ridges is intended, first, to increase the bone volume for implant placement. Secondly, an improvement in bone quality is desired, since force, not always favorable, will be applied to these implants. The present study showed that both reconstructive procedures fulfill such necessities, representing reality in therapeutic options for the surgeon.

However, considering the caution that every surgical procedure and technique requires, it should be emphasized that such treatments are therapeutic options. It is important to stress that in this comparative study only 2 types of grafts were used, chin and iliac crest, in a specific type of alveolar reconstruction and in a single receptor site, the maxilla, after a period of 4 months following graft surgery. Thus, the data obtained in this work relate only to this clinical condition. It is possible that with different techniques, results may differ.

CONCLUSIONS

1. After 4 months of chin and iliac crest graft surgery, both grafts presented a dynamic remodeling process demonstrated by intense osteogenesis and close incorporation in receptor sites, indicating sufficient healing time before implant placement.
2. Both grafts offered better bone quality at the site of implant placement; despite the reconstruction volume, chin grafts offered higher bone quality and the possibility of better prognosis for the treatment.

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