

Calvarial Versus Iliac Crest for Autologous Bone Graft Material for a Sinus Lift Procedure: A Histomorphometric Study

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Purpose: The aim of this study was to compare, through histomorphometric analysis, the use of donor autogenous bone graft from calvarial or iliac sources for maxillary sinus lift procedures. **Materials and Methods:** Sixteen patients requiring maxillary sinus augmentation were included in this study. One group of 10 patients was alternatively selected to receive autologous calvarial bone particles, and another group of 6 patients received autologous iliac bone particles. Five months after surgery, bone biopsy specimens were obtained at the time of implant procedure and analyzed through histomorphometry. To compare mean values between the calvarial and iliac crest groups, the Student t test was performed. The level for statistical significance was set at $P < .05$. **Results:** All patients completed the healing period following sinus augmentation procedure without complications. In the calvarial group, an average total bone volume (BV) of $73.4\% \pm 13.1\%$ was found. Nonvital bone constituted an average of $5.5\% \pm 6.3\%$ of the total tissue volume. The percentage of vital bone (VB) showed an average of $67.9\% \pm 16.1\%$. In the iliac group, the average total bone volume was $46.6\% \pm 17.4\%$, with an average of $12.6\% \pm 7.7\%$ of NVB and an average of $34.0\% \pm 21.5\%$ of VB. A significant difference was observed between calvarial and iliac bone grafts with respect to BV, VB, and NVB ($P < .05$). **Conclusion:** From this present histomorphologic study, it might be concluded that grafted bone obtained from calvarial sources for sinus lift procedure presented a significantly higher degree of bone volume and vital bone volume in contrast to bone harvested from the iliac crest. INT J ORAL MAXILLOFAC IMPLANTS 2007;22:527-532

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Implant placement in the posterior maxilla is complicated by residual ridge resorption or sinus proximity.¹ The sinus augmentation technique was proposed² to restore vertical bone height, allowing the placement of implants. For this procedure, autologous bone is still considered the gold standard in sinus augmentation surgery,³ since it improves the healing process by stimulating undifferentiated mesenchymal cells, osteoblasts, cytokines, and growth

factors⁴ and promotes neoangiogenesis, a fundamental process for the revascularization^{5,6} and remodeling^{7,8} of the bone graft.

The iliac crest, which is endochondral, may be used as a donor site⁹⁻¹⁴ when a large amount of autogenous cancellous bone is needed. Indeed, previous histomorphometric studies⁹ of grafting with iliac crest bone have shown an increase in the median percentage of cancellous bone with a healing period of 4.5 months and little resorption of the graft. Other authors¹⁰ evaluated the outcome of grafting with iliac bone clinically and radiologically; they found that iliac grafts could be used predictably in bone augmentation procedures, with a small amount of resorption. Barone et al¹¹ analyzed autologous iliac bone grafted in human maxillae with a histomorphometric study. After 5 months of healing, a total bone volume percentage of $70\% \pm 19.9\%$ was found. Triplett and Schow¹² defined the iliac bone as the best autologous graft material for fast revascularization and integration.

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Moreover, Iturriaga and Ruiz¹⁵ reported encouraging results with calvarial (membranous) bone grafts; they observed a high rate of bone remodeling, with no vital bone resorption and many large proliferative osteoblasts.¹⁶ Lizuka et al¹⁷ found that after a mean follow-up time of 19.6 months, bone resorption measured radiologically was minimal; endosseous dental implants were successfully placed and maintained, and satisfactory prosthetic rehabilitation was achieved in all patients. However, differences between iliac and calvarial bone when used as graft material were observed in animal studies. Kusiak et al¹⁸ found faster revascularization for membranous bone, and Sullivan and Sz wajkun¹⁹ observed enhanced revascularization by endochondral bone. Regarding volume maintenance, Ozaki and Buchman²⁰ demonstrated a good outcome with membranous bone grafts, probably because of their intrinsic microarchitecture rather than their embryologic origin.²¹ The aim of this study was to compare histomorphometrically the use of autogenous bone graft from calvarial versus iliac bone for maxillary sinus augmentation procedures.

MATERIALS AND METHODS

Patient Selection

Between January 2004 and June 2005, patients with severe maxillary atrophy were treated with autogenous bone grafts harvested from extraoral sources at the Department of Dentistry, San Raffaele Scientific Institute, Milan, Italy.

Inclusion criteria were absence of systemic disease, aged between 35 and 70 years old, monolateral residual maxillary sinus floor of less than 2 mm (class V according to Cawood and Howell classification²²), and partial or total edentulism. Exclusion criteria were presence of diabetes, presence of blood disorders, and smoking more than 10 cigarettes per day. A total of 16 patients met these criteria and thus were retrospectively studied.

Preoperative diagnosis was assessed through orthopantomography and CT scan.

All patients signed an informed consent form. Before positioning implants, after 5 months of healing, radiographic examinations (orthopantomography and computed tomography [CT]) were performed by a radiologist.

Surgical Procedures

A single surgeon performed both graft harvesting and maxillary sinus augmentation with the patient under general anesthesia.^{23,24} Vertical incisions were made in a palatal area of the alveolar crest anteriorly

and posteriorly. Mucoperiosteal flaps were elevated, and a bony window was created on the lateral wall of the maxillary sinus using a round diamond bur under irrigation with sterile saline solution. The Schneiderian membrane was carefully elevated, and the bony wall was gently pushed inside the sinus cavity, creating a cavity for bone augmentation. Donor bone was obtained from the anterior iliac crest and the parietal bone according to Tessier technique.²⁵

One group of 10 patients alternately selected received autologous calvarial bone particles, and another group of 6 patients received autologous iliac bone particles. Larger particles of bone were reduced to fine chips by a bone grinder. A resorbable Bio-Gide membrane (Geistlich Biomaterials, Wolhusen, Switzerland) was used to cover the bony sinus windows; no fixation was used. The mucoperiosteal flap was sutured using nonresorbable 5.0 sutures (GTAM; W.L. Gore & Associates, Flagstaff, AZ). In the postsurgical procedures, patients received antibiotics (Augmentin, 500 mg 4 times daily; Beechman-Wulfing, Neuss, Germany). Sutures were removed after 1 week.

Five months after surgery, all patients received at least 2 implants on the grafted side of the maxilla. A full-thickness flap was raised, the bone was inspected, and samples for biopsy were obtained from the occlusal aspect of the alveolar ridge using a trephine drill under copious irrigation with sterile saline solution. One cylindrical biopsy specimen was taken from each patient, for a total of 16 biopsies. All the biopsy specimens were approximately 2 mm in diameter and 10 mm in length, and were marked on the occlusal sides for the orientation during the histologic process.

Titanium implants of 3.7 to 5 mm in diameter and 10 to 13 mm long (WINSIX: BioSAF, Milan, Italy) were placed at the same sites from which the biopsy specimens were obtained. A total of 45 implants were placed in posterior maxillary sites.

Histomorphometry

The samples were fixed in 4% buffered formaldehyde, then dehydrated in graded series of alcohols from 50% to 100% and embedded in Epon (Hexion, Columbus, OH) according to a previously described procedure.²⁶

Undecalcified, 30 ± 10 mm thick sections along the axis of the biopsy were obtained with Isomet Buehler microtome (Buehler, Lake Bluff, IL); the sections were stained with toluidine blue and observed with a microscope by Normasky differential interference contrast (DIC) (Fomi III; Carl Zeiss, Oberkochen, Germany).

A FOMI III microscope (Zeiss) connected to a computer and a Leica DC 280 digital camera (Leica

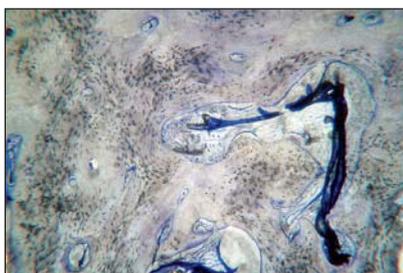


Fig 1 Lines of osteoblasts and osteocytes around nonvital empty osteocyte lacunae in iliac autologous bone (toluidine blue; original magnification $\times 32$).



Fig 2 Nonvital empty osteocyte lacunae delimited by osteoblasts and osteocytes in iliac crest autologous bone (DIC; original magnification $\times 250$).

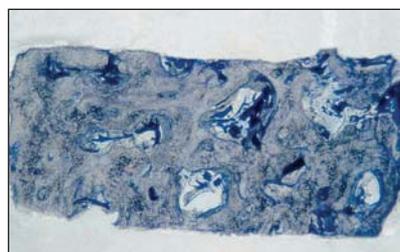
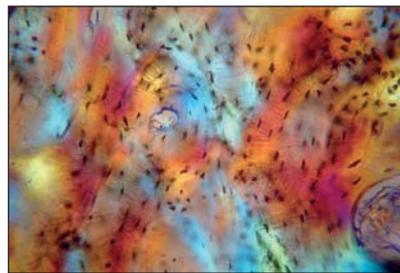
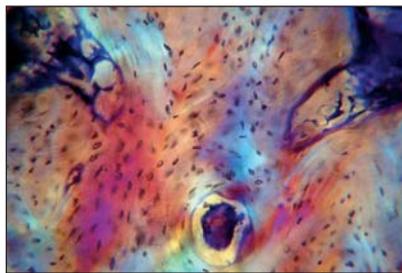


Fig 3 Histologic section of grafted calvarial bone. A high density of vital lamellar bone and reduced medullary space can be observed (original magnification $\times 10$).

Fig 4 (left) Histologic section of grafted calvarial bone. Vital bone containing osteoblasts, osteocytes, and many blood vessels (DIC; original magnification $\times 160$).

Fig 5 (right) Histologic section from grafted calvarial bone. Vital mature lamellar bone with the presence of osteons (DIC; original magnification $\times 160$).



Microsystem, Milan, Italy) were used for histomorphometric measurements. Histomorphometry was performed with Alexasoft software (Microcontrol, Milano, Italy). The percentage of mineralized tissue was calculated for all sections of the samples, and the measurements were performed at $160\times$.

Vital bone (VB) was calculated as the percentage of the total tissue volume (TTV) that consisted of mineralized and vascularized bone tissue. Mineralized bone tissue that contained empty osteocyte lacunae was defined as nonvital bone (NVB) and was expressed as a percentage of the TTV. The total amount of vital and nonvital bone was considered the total bone volume (TBV).

Comparison between the calvarial and the iliac group was performed with an independent Student *t* test ($\alpha = .05$ or $P < .05$ was considered the threshold for statistical significance). The Bonferroni correction was used for multiple comparisons.

RESULTS

Sixteen systemically healthy patients (9 men and 7 women) were included in this study. The mean age was 51.4 ± 9.2 years (range, 38 to 65 years). Three patients were totally edentulous, and the remaining 13 patients were partially edentulous in the posterior maxilla. In all patients, preoperative diagnosis was assessed through orthopantomography and CT scan.

All patients completed the healing period following the sinus augmentation procedure without com-

plications, and no clinical symptoms of maxillary sinusitis or other complications occurred.

Two of the 6 patients in whom iliac crest bone was used reported pain around the donor site 10 days after bone harvesting; there was no donor site morbidity in the calvarial group.

Before positioning implants, after 5 months of healing, radiographic examination (orthopantomography and CT) showed good integration of the bone graft in all patients. All implants placed became osseointegrated.

Histologic evaluation of all examined sections from iliac bone grafted revealed the presence of lamellar VB and some areas of woven bone surrounding NVB tissue, which consisted of fields of empty osteocyte lacunae, observed as incremental basophilic lines mixed with interposed reversion lines (Figs 1 and 2). In the specimens where calvarial bone grafts were used, the bone grafts appeared well-included and showed continuity with the new bone tissue in comparison to the specimens grafted with crestal bone (Fig 3). Moreover, well-developed haversian and Volkmann canal systems confirmed the maturity of the bone. Osteons containing osteocytes and osteoblasts were observed (Figs 4 and 5).

Osteoid formation was particularly prominent in highly vascularized tissue. All the tissues were free of inflammatory cells. The medullary spaces were almost always filled with well-vascularized connective tissue with no signs of inflammation.

Table 1 Histomorphometric Results						
Patient	Age	Sex	Donor site	TBV (%)	VB (%)	NVB (%)
1	54	M	CAL	95.0	95.0	0.0
2	43	M	CAL	75.9	67.7	8.2
3	47	F	CAL	95.0	95.0	0.0
4	62	M	CAL	66.7	66.7	0.0
5	51	F	CAL	82.3	77.1	5.2
6	38	F	CAL	59.1	59.1	0.0
7	59	F	CAL	58.8	53.0	5.8
8	40	M	CAL	73.9	68.1	5.8
9	44	F	CAL	59.2	51.8	7.4
10	49	M	CAL	67.6	45.8	21.8
11	57	M	IL	30.4	21.5	8.9
12	39	F	IL	58.2	57.6	0.6
13	55	M	IL	32.5	6.6	25.9
14	65	F	IL	27.0	11.5	15.5
15	61	M	IL	58.2	48.1	10.1
16	58	M	IL	73.2	58.8	14.4

CAL= calvarial bone; IL= iliac bone.

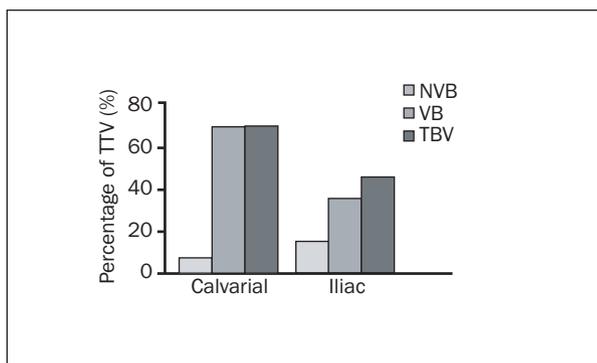


Fig 6 Histomorphometric results: calvarial versus iliac autologous bone.

Calvarial Group

TBV ranged from 66.7% to 95%, with an overall average of $73.4\% \pm 13.1\%$. The percentage of NVB varied from 0% to 21.8%, with an overall average of $5.5\% \pm 6.3\%$ of total tissue volume. The percentage of VB varied from 45.8% to 95%, with an average volume of $67.9\% \pm 16.1\%$ (Table 1, Fig 6).

Iliac Group

The TBV ranged from 27% to 73.2%, with an overall average of $46.6\% \pm 17.4\%$. The percentage of NVB varied from 0.6% to 25.9%, with an overall average of $12.6\% \pm 7.7\%$ of TTV. The percentage of VB varied from 11.5% to 58.8%, with an average VB volume of $34.0\% \pm 21.5\%$ (Table 1, Fig 6).

Statistically significant differences ($P < .05$) between calvarial and iliac bone grafts were found with respect to average TBV, VB, and NVB.

DISCUSSION

Ozaki and Buchman²⁰ observed the internal processes of bone graft remodeling. Cortical membranous and endochondral bone grafts were placed subperiosteally onto rabbit crania, and micro-CT scanning and histomorphometric analysis were performed to obtain detailed information regarding the microarchitecture of the cortical bone grafts. The results showed that there was no difference between the endochondral and membranous bone grafts for bone volume fraction; however, only cortical bone grafts developed a less dense, more trabecular, less organized internal superstructure. Furthermore, Peer²⁷ demonstrated clinically that membranous only bone grafts tended to resorb less over time than endochondral only bone grafts.

At histologic analysis the changes in the internal morphology of these bone grafts provided a structural framework consistent with the processes of osteoclastic resorption, bony remodeling, and new bone formation. Different authors^{20,21} have suggested that the embryologic origin of a bone graft is irrelevant (ie, that cortical bone behaves like cortical bone, regardless of the source). The clinical superiority of membranous bone grafts can be explained by the fact that membranous and endochondral bone grafts are composite grafts made up of both cortical and cancellous components. Only membranous bone grafts, therefore, resorb less over time because they have a higher proportion of cortical bone than endochondral bone grafts and not because of some innate embryologic superiority.

Le Lorc'h-Bukiet et al¹⁶ studied the structure and remodeling activities of parietal bone used for sinus grafting 10 months after grafting. The samples consisted solely of trabecular bone, which consisted of $49.4\% \pm 18.4\%$ of total sample volume. Remnants of the graft particles were embedded within new bone and showed signs of intense resorption. Bone remodeling was highly active, as shown by the presence of numerous osteoclasts resorbing new bone, together with thick osteoid seams and large osteoblasts within mineralized material. This study offered insight into cortical and trabecular bone structure and showed the low-level remodeling activity of parietal bone. The solid structure of cortical bone may be one of the main reasons that cortical only bone grafts resorb more slowly and survive better over time compared with cancellous only

bone grafts. The trabeculation of the cortical onlay grafts may be an attempt by the body to incorporate the graft with simultaneous volume reduction through osteoclastic activity,²⁰ supported by improved revascularization. Chen et al⁵ showed that cancellous bone revascularized faster than cortical bone regardless of its embryologic origin, with no difference in revascularization rate between cortical bone of different embryologic origins, supporting the concept that the trabeculation of cortical onlay bone grafts occurs concurrently and may even drive the process of revascularization. However, other studies^{28,29} have shown that embryologic origin may play a key role in intramembranous and endochondral bone healing. In fact, in a study of mandibular bone grafting, Rabie et al³⁰ demonstrated that intramembranous bone requires different cytokines and growth factors for healing than endochondral bone. Subsequently, Chung et al³¹ found that differences in molecular signaling pathways exist between intramembranous and endochondral osteogenesis. Wong and Rabie³² demonstrated that endochondral and intramembranous bone do not undergo the same healing mechanism: demineralized intramembranous bone matrix induces bone without an intermediate cartilage stage and proceeds directly through an intramembranous ossification; the mesenchymal stem cells differentiate directly to bone cells. In a study of the rat mandible, Lu and Rabie³³ attributed the difference in calvarial and endochondral bone graft healing to the different origins of the bone grafts and the recipient bed.

CONCLUSION

This histomorphologic study revealed that trabecular calvarial bone grafts used for sinus augmentation provided a significantly higher degree of bone volume and vital bone than iliac crest bone grafts. However, more studies are necessary to assess the relevance of trabecular bone arrangement to the bone healing process.

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