Microcomputed and Histologic Evaluation of Calvarial Bone Grafts: A Pilot Study In Humans

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Two evaluation techniques (histology and microcomputed tomography [micro-CT]) were synergistically applied to calvarial bone graft to verify whether additional bone information can be obtained for the assessment of bone grafts. Ten extensive bone defects in the anterior and posterior maxilla or mandible involving crestal bone were treated by grafted blocks and chips of autogenous calvarial bone. The grafts were fixed with lag screws and left to heal for 4 months. No complications were observed. At surgical reentry for implant placement, a cylindric bone biopsy of both graft and native bone was retrieved and analyzed with both micro-CT and standard histology. Two- and three-dimensional (2D, 3D) micro-CT analyses allowed bone connectivity indices to be evaluated. This is useful for estimating bone strength and observing bone structure. The integration of the grafted calvarial bone with the residual bone of the recipient site was considered satisfactory. Histologic analysis allowed observations to be made at a higher resolution. Calvarial bone grafts seem to have positive effects when used as grafting materials. The application of both histologic and micro-CT techniques allows a better evaluation of grafted bone by concurrently allowing 2D and 3D visual and morphometric analysis of bone vitality, structure, turnover, and strength. (Int J Periodontics Restorative Dent 2011;31:e29–e36.)

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Implant-supported prosthetic rehabilitation is frequently compromised by atrophy of the maxilla or mandible after tooth loss, trauma, or resection. Distraction osteogenesis has been proposed, but in severe atrophies, the osteotomy and anchorage sometimes cannot be properly executed.[1] Clinical success has been obtained in the posterior maxilla by grafting the sinus,[2–4] obtaining histologic evidence of new bone formation,[5] but sinus graft procedures do not coronally rebuild the vertical dimension of the bone crest.[1] Also, guided bone regeneration methods using reinforced membranes have been proposed,[6] but in extensive reconstruction, autogenous bone block grafting is considered the gold standard technique.[7] Several studies have reported that grafted bone blocks resorb rapidly[8]; however, membranous bone has been shown to be more effective than endochondral bone in maintaining volume.[9] Intraoral donor sites should be considered to retrieve membranous bone to rebuild small defects, whereas calvarium is a useful donor site for other purposes.[10] In 1929, Dandy[11] grafted the orbital wall with autologous calvarial bone, while Tessier[12] and Tulasne et al[13] described various methods for harvesting and grafting parietal bone in maxillofacial surgery. Boyne and James[4] described sinus and alveolar ridge augmentation via bone blocks and chips harvested from the calvaria.

Dental research employs qualitative and quantitative morphometry for evaluating the bone integration of grafts, dental implants, biomaterials, and regenerative procedures.[14] Bone volume, bone structure, and several dynamic parameters can be assessed to evaluate the development of bone tissue during the healing period or when subjected to a functional load.[15–17] In addition to standard bone morphometric analysis, which is generally based on two-dimensional histologic sections, microcomputed tomography (micro-CT) outlines and quantifies bone, implants, and biomaterials in three dimensions.[18–20] The synergy between histologic and micro-CT analysis allows the examination of bone structure, tissue, and cells.[19,20] The development of grafted bone tissue has not been fully clarified from a biomechanical viewpoint, and questions remain regarding the reasons for bone graft resorption and differences in healing and maintaining volume, in particular between membranous and endochondral bone.[21] A three-dimensional (3D) analysis of the bone graft and recipient bed could better clarify the biomechanical qualities, healing mechanisms, and development of grafted bone. The aim of the present study was to use micro-CT in addition to histology for evaluating the bone biopsy of a grafted site.

Method and materials

Ten patients in good general health requiring extensive bone reconstruction for the placement of dental implants (Fig 1a) received calvarial autogenous bone grafts in block form for complete reconstruction of the bone crest. Prior to treatment, all patients signed an informed consent form. In some patients, simultaneous maxillary sinus grafting with autogenous bone particles was performed. Bone grafts were harvested from the parietal bone using the split calvarial graft technique in situ.[10,12] Grafts were prepared so that the bone ridge could be rebuilt with lag screw fixation (Fig 1b) and left to heal for 4 months, completely covered by soft tissue. Temporary splints were not used in the edentulous region to prevent wound dehiscence. During healing, a surgical guide was prepared for correct positioning of the implants. Reentry was performed after 4 months (Fig 1c), and implants were placed in the grafted bone (Fig 1d). The grafted area was checked clinically and evaluated radiographically using endooral radiographs (panoramic and CT; Figs 2a and 2b). In one patient, at surgical reentry, a cylindric bone biopsy of grafted and native bone was retrieved using a trephine bur along the implant insertion axis to perform micro-CT and histologic analysis. Thirty-two rough endosseous titanium dental implants (3.7 to 5 mm in diameter, 10 to 13 mm in length; WINSIX, BioSAF)[6] were placed in the augmented areas. The implants healed submerged for 3 months before being progressively loaded with a provisional fixed partial denture.

Sample preparation

After retrieval, the biopsy was sent to Dr Paola Trisi, director of the laboratory of the Biomaterials Clinical-Histological Research Association, Genova, Italy, and processed for micro-CT and histologic examination. The sample was initially fixed in 10% neutral buffered formalin and analyzed using 3D micro-CT (Scanco Medical). The specimen was then dehydrated in an ascending series of alcohol dilutions and embedded in Remacryl resin (Cesare Scala). After polymerization, micro-CT analysis was performed, and later, the specimen was processed into thin ground sections for histologic examination.

Micro-CT processing

A high-resolution microtomography system (microCT-40, Scanco Medical) was used in multislice mode. Each 3D image data set consisted of approximately 400 micro-CT slice images (1,024 × 1,024 pixels with 16-bit gray levels).[19,20] The specimen was scanned in high resolution mode with an x, y, and z resolution of approximately 20 μm.

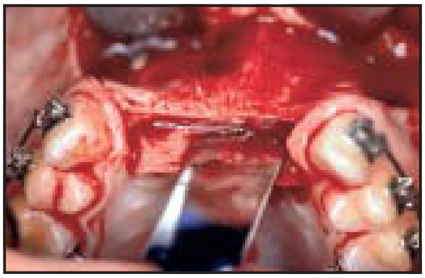
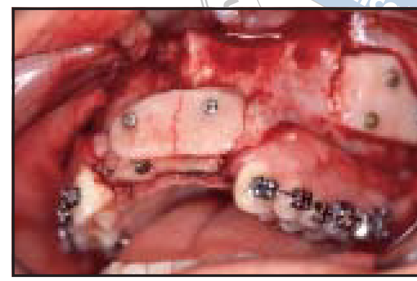


Fig 1a (left) *Clinical view of the surgical area. In this patient, bone atrophy following trauma caused extreme resorption of the crestal maxillary bone, requiring extensive vertical and lateral bone reconstruction.*

Fig 1b (right) *Surgical phase of bone reconstruction with calvarial bone grafts prepared to rebuild the bone ridge through lag screw fixation. Grafts were harvested from the parietal bone using the split calvarial graft technique in situ, which allowed sufficient bone to be harvested for an extensive maxillary reconstruction.*



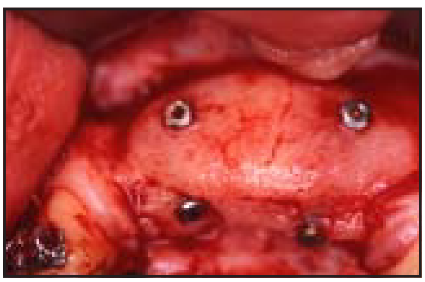


Fig 1c (left) *Reentry procedure. The calvarial graft appeared well integrated and firm, with no visible signs of resorption.*

Fig 1d (right) *During reentry, two implants were placed, and primary stability was obtained.*

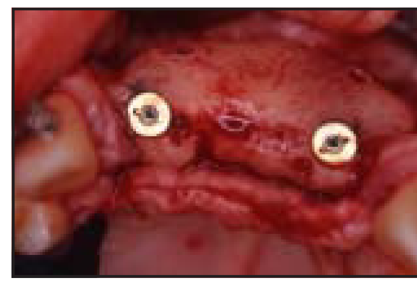
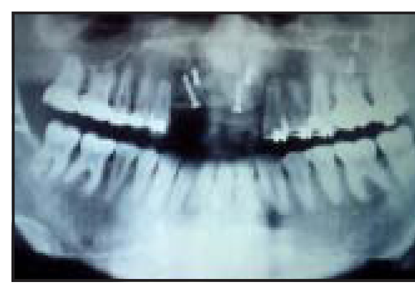


Fig 2a (left) *CT scan performed 4 months after implant placement showed good integration of the grafts and implants. It also showed that the rebuilt alveolar bone crest of the treated area had the typical anatomy of normal maxillary bone.*

Fig 2b (right) *Panoramic radiograph 4 months after implant placement.*



The voxel size was 15 × 15 × 15 μm^3. Scanning time was approximately 4 hours.

Histologic processing

After micro-CT scanning, the specimen was sectioned into 200- to 250-μm sections with a Micromet high-speed rotating blade microtome and subsequently ground to approximately 40 to 50 μm using an LS2 grinding machine (Pace Technologies). A routine stain with toluidine blue and acid fucsin was applied to the slides. Three sections of the biopsy were produced, and histomorphometric analysis was conducted by digitizing the microscope images via a JVC TK-C1380 Colour Video Camera (JVC Victor) and a frame grabber. Routine analysis of the digitized images followed, using the image analysis software IAS 2000 (Delta Sistemi). The images were acquired using a 5× lens.

Fig 3a (left) *Photograph of a treated patient 1 year after an accident that caused facial trauma and bone atrophy (photograph taken prior to surgical treatment).*

Fig 3b (right) *Photograph of the treated patient 9 months after surgical and prosthetic treatment.*

Morphometric measurements

The morphometric parameters calculated with micro-CT and histology were: total volume (TV), bone volume (BV), relative bone volume (BV/TV), connectivity density, trabecular number, trabecular thickness, and trabecular separation. The morphometric values obtained with micro-CT were compared with those obtained by standard histomorphometric analysis. Micro-CT measurements were calculated using the mean of the values obtained from 400 slices (sections), while the histomorphometric measurements were calculated using the mean values of the 3 sections of the sample.

Results

Clinical findings

The 4-month healing period yielded no complications for any grafting procedure. Upon clinical and radiographic examination, the healed bone grafts appeared to be well integrated and in tight contact with the recipient buccal plate. Implant surgery in the grafted sites was successful, and after 4 months, bone density evaluated by hand drilling appeared mostly medium/normal (D2 to D3) or hard (D1), and all implants placed in the grafted sites presented good primary stability. The autogenous bone chips used as filling material for the minor defects between bone blocks and the recipient bone plate appeared to be incorporated within the newly formed alveolar ridge. At periodic recalls after implant loading, radiographic bone loss was not observed to be excessive, and clinical signs of failure were absent. The 3D reconstruction of the sequence on CT images from the micro-CT analysis allowed the correlation of the 2D section with the 3D structures. After 6 months of submerged healing, a definitive ceramic prosthesis was placed. All patients showed good functional and esthetic results (Figs 3a and 3b).

Micro-CT histologic and histomorphometric results

Morphometric results of the bone micro-CT evaluation were as follows: TV = 31.80 mm^3, BV = 12.25 mm^3, BV/TV = 38.50%, and connectivity density = 485.42 mm^3.

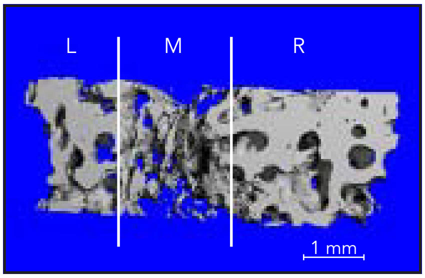


Fig 4 *3D micro-CT reconstruction of the entire biopsy retrieved from the grafted area. Morphologic 3D analysis allowed observation of the dense bone of the graft on the left* (L)*, the thin trabeculae of the junction between native bone and graft in the middle* (M)*, and the thick trabeculae of the native bone on the right* (R)*. In the junction, thin trabeculae of woven bone connected the graft to the recipient site. In this portion of the sample, several bone fragments are also evident (original magnification ×10).*

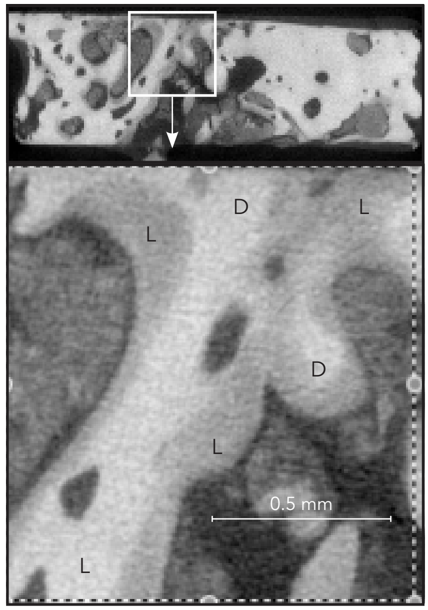


Fig 5a (left) *Native bone was denser when compared to grafted bone. New bone apposition during the healing period at both native and grafted bone was evident, especially in the cancellous bone of the junction between native bone and graft (original magnification ×10). In fact, at higher magnification (white outline, magnification ×20), it is possible to clearly see less mineralized younger bone* (L) *apposing older bone* (D).

Fig 5b (above) *Micro-CT 2D evaluation showing thin trabeculae of a low density, a possible expression of thin woven bone trabeculae similar to that found in bone fractures healing through a callus formation. Fragments of bone are also visible both in the central and in the grafted portions (right side of biopsy), probably autogenous cancellous chips of the graft (original magnification ×10).*

Morphologic description of 2D and 3D micro-CT images

Visual assessment of the bony structure obtained by micro-CT produced several 2D slices and a 3D computed reconstruction of the specimens. The 3D micro-CT reconstruction gave a precise representation of the bony trabeculae and allowed visual exploration of the bone microarchitecture in every part of the biopsy. A 3D reconstruction of the maxillary bone and calvarial grafts was obtained; morphostructural micro-CT investigation showed satisfactory integration of the grafts, with similar structure to native bone. Biopsies showed a similar dense structure in both basal bone and grafts connected in the middle by cancellous bone composed of thin trabeculae and large medullary spaces (Fig 4).

The 2D slices showed that the structure of the grafted bone was very similar to that of native bone. In nature, the bony structure of calvarial bone is often similar to the structure of maxillary bone. Native bone was slightly denser on radiographs if compared to the grafted bone. Many of the trabecular surfaces on both sides of the biopsy were covered with newly formed bone, confirmed by the cementing lines visible in the micro-CT 2D sections. New bone apposition both at the native and grafted bone areas was also evident, especially in the cancellous bone at the junction between bone and graft (Fig 5a). The 2D micro-CT analysis showed a dense structure composed of thick trabeculae well connected to each other by bone bridges both in the grafted area and in the native bone. Radiopaque particles that were probably autogenous calvarial chips were evident in contact with mineralized bone or in the marrow in some slices (Fig 5b). The mineralized bone, as measured by micro-CT, occupied 61.50% of the total space of the biopsy. At the interface between native bone and graft, very thin trabeculae of a low radiographic density were present, a possible expression of the thin woven bone trabeculae that usually characterize bone healing fractures with a scar. Histologic analysis confirmed the micro-CT observations: Remodeling took place both in recipient sites and grafted sites of the sample (Fig 6). Layers of osteoid matrix covered at least 30% of the bone surface.

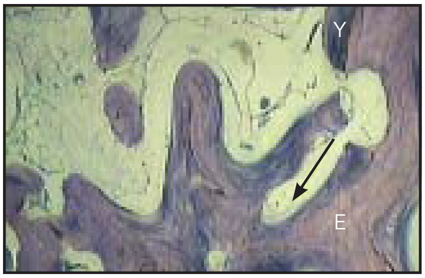


Fig 6a (left) *Histology confirmed the presence of younger bone (Y) and osteoid* (arrow), *revealing active bone remodeling and new bone apposition to pre-existing trabeculae in the grafted bone. E = native bone. (original magnification ×25).*

Fig 6b (right) *Histology also showed younger bone and osteoid in recipient sites. Both native bone and graft revealed similar bone remodeling patterns (original magnification ×25).*

Discussion

Loads applied in the posterior maxilla are heavy, but the bone is often soft in quality and lacking in quantity.[22] Bone rebuilding of the atrophic posterior maxilla, according to functional and esthetic requirements, needs good bone quality and quantity for safe implant placement. Sinus grafting, bone regeneration, and osteodistraction procedures have been proposed but are limited to select favorable cases; the mechanical properties of the newly formed bone are often low, even after several months of healing.[1] The quality of newly formed bone depends on an undisturbed healing period[6,23] and on the density of the pre-existing bone.[24] In the case of severe skeletal alterations, autologous bone grafts may be a suitable alternative.[8] Good intrinsic mechanical properties of bone grafts are able to avoid the risk of micromovements of fixation screws, grafts, and implants throughout the healing period during either bone reconstruction or implant osseointegration.

Several studies described graft resorption[7,25–27] as related to its microarchitecture[9]: Calvarial graft seems to be ideal because of its corticocancellous ratio. Histology and micro-CT techniques in other animal studies evaluated the healing period of calvarial grafts: Viability of bone tissue and good incorporation into the recipient bed were demonstrated.[28,29] CT scans[3] and panoramic and retroalveolar radiographs[30] are useful techniques to evaluate volume and density of calvarial bone grafts; physiologic bone adaptation during both graft healing and implant loading was found.

Preservation of calvarial graft volume was claimed to be related to functional loading through correct rebuilding of the bone crest and implant placement[3,28–31]: Bone resorption seemed to be avoided by applying physiologic strain through load application.[17,24] According to the literature, other factors involved in bone graft maintenance include periosteal preservation, distribution of mechanical forces, and interaction between microarchitectural features of bone graft and local mechanical environment.[21,31] According to the results of this study, survival was optimal when bone grafts closely duplicated the architecture of the recipient bed.[31,32] Previously published studies observed that the thick and compact cortical layer forming the membranous bone allowed lag screw fixation, gradual revascularization with more controlled resorption, and better new bone formation in comparison to endochondral bone grafts.[31–33] Lag screws seem to decrease bone graft stress and increase graft-to–recipient bone contact; graft fixation and absence of micromotion result in low-strain conditions, which minimize the early resorption phase of creeping substitution as well as stimulate direct intracortical remodeling and lamellar bone production.[33–35] With interfragmentary motion, lamellar ossification is not possible,[35] such as with large bone defects where connective tissue does not differentiate in bone tissue. Membranous bone heals with more and thicker trabeculae and lower connectivity than endochondral bone.[36] Osteogenic cells with no cartilaginous stage characterized integration of the intramembranous bone graft.[37,38]

Histomorphologic and micro-CT studies have demonstrated that integration of intramembranous bone grafts within membranous bone defects is better than endochondral grafts.[27,39] The results of this study suggest that fixed autogenous calvarial grafts are a suitable alternative to rebuild most challenging cases of large bony defects because of their characteristics and the greater quantity of bone available in the form of blocks and chips. One of the most interesting histologic observations in this study was that the 3D structure of grafted bone was very similar to that of the native bone. The 3D architecture of the bone graft after healing could explain its mechanical competence, positive clinical behavior, and the short healing time needed for dental implants.

Feldkamp et al[18] introduced a radiographic micro-CT system to create 3D images. More recent developments[40] have allowed higher-resolution 3D images and quantitative measurements.[19] Micro-CT was validated as a method for the 3D assessment and analysis of cancellous bone by Müller et al in 1998.[41] Recent studies have introduced micro-CT for dental implant research.[19,20] The mechanical properties of bone largely depend on its 3D structure,[31,41] which is measured by bone volume and connectivity indexes. In the field of bone reconstruction for dental implant rehabilitation, the ultimate goal of any clinical bone measurement is to estimate bone strength.[19,20] For this reason, it is important to quantify bone microarchitecture using a 3D technique, such as micro-CT 3D analysis.

In all patients treated in this study, the quantity and quality of bone obtained can be considered, from a clinical viewpoint, ideal for an esthetic and functional rehabilitation. Moreover, the biopsy performed confirmed the observation of a medium/normal bone quality (D2 to D3),[23] with values of BV around 38% obtained 3 to 4 months after calvarial grafting. Medium/normal bone is considered ideal for predictable implant placement,[23] and the obtained values of BV can be considered satisfying. Recent studies have confirmed that bone volume is related to expected bone-implant contact.[42] Connectivity indexes calculated using micro-CT have also indicated that grafted bone is strong enough for implant placement. Histologic analysis has demonstrated grafted bone to be bound through physiologic functional processes to bone homeostasis.

Conclusion

Micro-CT may help investigate the relative importance of bone architecture after calvarial bone grafting as a better index of bone strength. Histology allows the soft tissues and cells to be evaluated so as to verify the integration and vitality of the grafted bone. The synergy between histology and micro-CT may lend improvement to present knowledge in this field.

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