Evaluation of the Ideal Implant Insertion Time in Human Bone Biopsies After Sinus Elevation Using a Combination of Autologous Bone and Graft Substitute

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Purpose: *To evaluate the ideal implant insertion time in human bone biopsies after sinus elevation with a composite graft consisting of an equal amount of biomaterial and autologous bone, by comparing the bone regeneration obtained 4 to 5 months after surgery with that obtained after 6 to 8 months, and using the adjacent native bone as reference.* ***Materials and Methods:*** *Twenty-six biopsy specimens of 11 patients were analyzed. Two groups were created depending on the time of implant insertion: group t1 at 4 to 5 months (n = 13) and group t2 at 6 to 8 months (n = 13). The same volume of grafted bone and native bone were analyzed for each biopsy with microcomputed tomography (microCT) and gene expression analysis.* ***Results:*** *Statistically significant differences were found in bone mineral density (BMD), bone volume fraction, and trabecular separation (TbSp) between native and grafted bone in both groups, with higher grafted bone values, except for the variable TbSp, which was lower in the grafted bone. This decrease in TbSp in the grafted bone in both groups can be explained by the significant increase in trabecular thickness in group t2 and the trabecular number in group t1, compared with native bone. No significant differences were found between the two groups in the morphometric parameters and BMD of the grafted bone. Also, no significant changes in the messenger RNA (mRNA) levels of bone formation, bone resorption, and inflammatory markers were found between both groups, with the exception that alkaline phosphatase mRNA levels were significantly lower in group t1 relative to native bone.* ***Conclusion:*** *This composite graft showed no differences in three-dimensional microstructure, BMD, or at the molecular level between 4 to 5 months and 6 to 8 months of healing time. Thus, this time can be shortened to 4 months with the security of a grafted area of mature bone.*
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Inadequate bone height on the lateral side of the maxilla is a contraindication for implant surgery. This condition can be treated with an internal increase of the maxillary sinus floor, referred to as sinus elevation or sinus lift (SL).[1] SL is a surgical procedure that prepares a door hinge on the lateral side of the maxillary sinus and internally rotates to a horizontal position. The new elevated sinus floor, together with the schneiderian membrane, creates a space that can be filled with graft material. The SL was proposed by Tatum[2] in 1976, and a description was first published by Boyne and James[3] in 1980. It is considered adequate to treat those cases with more than 4 mm bone height (height from alveolar crest to sinus floor) in a single surgical procedure with simultaneous SL and implant placement. This procedure is called simultaneous implant placement, and primary stability is obtained on the patient's native bone. In cases with lower bone heights (≤ 4 mm), it is recommended to place the implants in a delayed mode. In these cases, SL is first done, and then implants are placed after graft consolidation.[4–7] This

Table 1 Oligonucleotide Sequences Used in the Real-Time PCR of Reference and Target Genes

Gene	Forward	Reverse
18S rRNA	5'-GTAACCCGTTGAACCCCATT-3'	5'- CCATCCAATCGGTAGTAGCG-3'
ACTBL2	5'-AAGGGACTTCCTGTAACAATGCA-3'	5'-CTGGAACGGTGAAGGTGACA-3'
GAPDH	5'-TGCACCACC-AACTGCTTAGC-3'	5'-GGCATGGACTGTGGTCATGAG-3'
COL1A1	5-CCTGACGCACGGCCAAGAGG-3'	5'- GGCAGGGCTCGGGTTTCCAC-3'
ALP	5'-CCGCTATCCTGGCTCCGTGC-3'	5'-GGTGGGCTGGCAGTGGTCAG-3';;
RUNX2	5'- CTGTGCTCGGTGCTGCCCTC-3'	5'- CGTTACCCGCCATGACAGTA-3'
OC	5'-GAAGCCCAGCGGTGCA-3'	5'- CACTACCTCGCTGCCCTCC-3'
H+-ATPase	5'- GGTGATGTCACAGCAGAAGTTATG -3'	5'-TGCTCAATTCAGTGCCAAAGGAGT-3'
TRAP	5'- CATGACCACCTTGGCAATGTCTC-3'	5'- CTGTGGGATCTTGAAGTGCAGG-3'
IL6	5'-AGGAGACTTGCCTGGTGAAA-3'	5'- GCATTTGTGGTTGGGTCAG-3'
IL10	5'-TTATCTTGTCTCTGGGCTTGG-3'	5'-ATGAAGTGGTTGGGGAATGA-3'
TNFA	5'- CTATCTGGGAGGGGTCTTCC-3'	5'-GGGGGTAATAAAGGGATTGG-3'

procedure is known as delayed implant placement. The most important variables in the SL are the type of approach (lateral or crestal), the timing of implant insertion in relation to the graft, the type of graft placed, and the type of implant.[8]

The high regenerative capacity of the maxillary sinus allows most graft materials to regenerate bone correctly.[9] Autologous bone, allografts, xenografts, and synthetic biomaterials have been used either alone or in combination with autologous bone. Studies in the literature have shown similar results for these different types of grafts.[9–14] One of the major advantages of biomaterials over autologous bone grafts is the absence of donor site morbidity. One option to avoid this is by using a scraper that allows the obtention of an autologous bone specimen from the maxillary malar area through the same incision made during the SL procedure.[15]

The combination of different graft materials enhances the advantages of each graft. Thus, autologous grafting accelerates bone regeneration because of its osteoprogenitor cell content (exclusive to this type of graft) and its richness in growth factors.[16] Furthermore, bovine hydroxyapatite and biphasic calcium phosphate are excellent osteoconductive materials[16–18] that are longer acting than the autologous graft because it is resorbed very slowly.[19] Between these two types of biomaterials (Bio-Oss and BoneCeramic), no important differences have been found in the resulting three-dimensional (3D) microstructure and bone mineral density (BMD) when combined with autologous bone.[20]

The use of biomaterials alone for bone regeneration needs a prudent healing time of 9 to 12 months before the placement of dental implants.[21] However, the combination of autologous bone with biomaterials has been proposed to shorten the healing period.[16] Nowadays, many surgeons re-enter the site 4 to 5 months after SL with composite graft or only autologous bone without any strong evidence of the maturity of this graft.[22–28] Thus, based on this fact and on the findings of Hallman et al,[16] the aim of the present investigation was to evaluate the ideal implant insertion time in human bone biopsies after maxillary sinus floor elevation (SL) with a composite graft consisting of an equal amount of biomaterial and autologous bone obtained by using a bone scraper.[15,29] The authors compared the bone regeneration obtained 4 to 5 months after surgery with that obtained after 6 to 8 months (which is recognized as mature bone in the literature), and used the adjacent native bone as reference.[8–10,16,17,21,30,31]

MATERIALS AND METHODS

Study Design

Patients older than 18 years with a bone height requiring an SL procedure to place one or more dental implants were randomly eligible for inclusion in this prospective study. The residual bone height of the lateral-posterior segments of the edentulous maxilla below the floor of the maxillary sinus had to be 4 mm or less, as measured with computed tomography (CT). Furthermore, residual bone width had to be at least 6 mm, measured with CT. In total, 11 patients (four men and seven women) were included in the study. They underwent 26 grafting procedures and were divided into groups based on two time points, 4 to 5 months (group t1) and 6 to 8 months (group t2) after SL (Table 1). The study was conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki and was approved by the Ethics Committee of Balearic Islands (CEI-IB). All participants provided written informed consent.

Systemic and local exclusion criteria were defined as any factors interfering with implant surgery: smokers of more than 15 cigarettes per day, severe liver or

kidney disease, history of radiotherapy in the head and neck region, concomitant chemotherapy to treat malignant tumors, decompensated diabetes, active periodontal disease, diseases of the oral mucosa (planus lichen in the treatment area), previous destructive surgery or ear, nose, and throat (ENT) specialist contraindication for SL, poor oral hygiene, period less than 2 months after dental extraction, uncooperative patients, and bisphosphonates in the previous 3 months.

Surgical Procedure

Antibiotics were administered to the patients 24 hours before surgery (875/125 mg amoxicillin/clavulanate potassium and 300 mg clindamycin to those who were allergic to penicillin). Smokers were advised to cease smoking.

Local anesthesia solution of articaine with 0.5% mg epinephrine was injected into the buccal and palatal maxillary area. The incision was made on the top of the alveolar ridge, or slightly on the palatal side, through the keratinized, attached mucosa. This way the wound closure could be solid and with sufficient overlap to deal with a possible dehiscence. Then, a mucoperiosteal flap was raised, exposing the anterior and lateral maxillary sinus wall and the maxillomalar buttress.

The start of the graft harvesting was normally with the Micross bone scraper (Meta Advanced Medical Technology). Bone specimens from the maxillomalar buttress and the lateral wall of the sinus were collected using the bone scraper as a part of the antrostomy by pushing the end of the device toward the bone surface and simultaneously pulling the device itself backward. Collection of 2 mL of bone specimen was feasible with a mean surgical time of 10 minutes for this harvesting purpose. The collected bone specimen was preserved in a sterile environment until grafting. The graft was mixed 1:1 with Bio-Oss (Geistlich Pharma) or BoneCeramic (Straumann). All patients were treated using the same surgical technique consisting of sinus floor augmentation via a lateral approach.[2]

A large, round, diamond bur was used, which could not easily damage the membrane or perforate the bony wall. A round bony window was created on the lateral side of the sinus and internally rotated to a horizontal position (trap-door technique). Then, it was lifted along with the schneiderian membrane using special sinus floor elevation instruments (designed by Tatum[2]) that worked with different angled active tips. If a membrane perforation was performed, its size was written down according to the classification described by Hernández-Alfaro et al.[32]

To homogenize the combination of autologous bone and biomaterial, 1 mL of sterile physiologic serum was used. Once the graft was properly positioned in the new space created, the reconstructed site was covered with a resorbable collagen membrane (Bio-Gide, Geistliche Pharma). The use of dentures was not permitted until the dentures had been adjusted and refitted at least 2 weeks after surgery.

After 4 to 5 months or 6 to 8 months of healing, implant sites were prepared in a second surgical stage with a trephine bur of 2.5-mm diameter to obtain bone biopsy specimens from the same sites where implants had to be placed. Every attempt was made to keep the bone biopsy inside the trephine bur. Clinical and radiographic evaluation was performed. Panoramic radiographs were used to assess new bone formation. At this time, a total of 26 implants were placed under local anesthesia. Straumann and Nobel Biocare implants were used. In all patients, biopsy specimens of the grafted area and native bone were obtained (n = 26) through this surgical approach before implant insertion. The final peak of the insertion torque of each implant was measured when the implant was fully seated. Implant stability quotient (ISQ) readings were also obtained for each implant at placement time using the Osstell ISQ system.

Radiologic evaluation included preoperative pantomography and maxillary CT. All the panoramic radiographs were taken with the same pantomograph (Ortopantomograph OP 200 D, Instrumentarium Dental) and corrected for a constant magnification of 25%. The radiographic records consisted of pantomography taken before bone graft surgery and at 4 to 5 months and 6 to 8 months (at the time of implant placement).

MicroCT Analysis

The purpose of microCT analysis was to evaluate the 3D architecture parameters and the volumetric bone mineral density of the grafted bone structure and native bone at different healing times, 4 to 5 months and 6 to 8 months later (Fig 1). The specimens were examined using a microCT machine (Skyscan 1172, Skyscan). Specimens were placed in 200-µL sterile microtubes containing RNAlater and in Parafilm (American National Can) to avoid degradation of samples and movement, respectively, on a sample holder in a vertical position to ensure parallel scanning conditions. The resolution was set at 7.8-µm voxels with radiographic tube current of 100 µA and voltage of 100 kV with a 0.5-mm aluminium filter. Specimens were rotated 360 degrees around the long axis (z-axis) of the sample. Three absorption images were recorded every 0.4 degrees of rotation. The beam hardening was set to 20%, smoothing to 1, and ring artefact reduction to 6, during the reconstruction of the axial images (Nrecon v.1.4.4, Skyscan). Post alignment was optimized automatically.

After reconstruction, the same volume of interest (VOI) was applied as inside the reconstructed images of the two phantom rods. The same VOI was chosen for

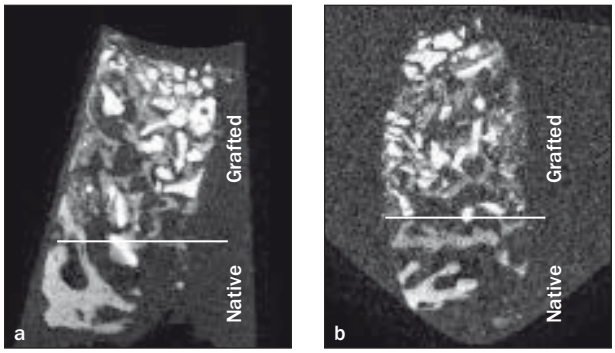
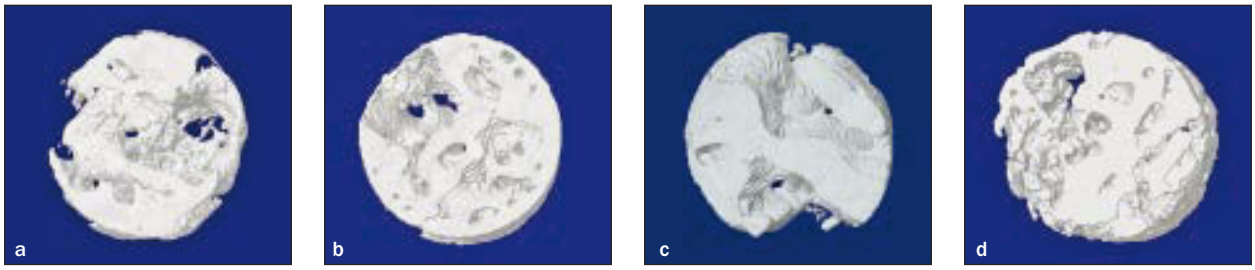


Fig 1 *(left)* Micro-CT slice of bone biopsies showing native and grafted bone for the two groups studied. *(a)* 4 to 5 months (t1) and *(b)* 6 to 8 months (t2).

Fig 2 *(below)* Three-dimensional model of the volume of interest analyzed in the *(a, c)* native and *(b, d)* grafted area in two biopsy specimens after *(a, b)* 4 months (t1) and *(c, d)* 6 months (t2) of SL.



all the samples: a cylinder with a diameter of 2.6 mm and height of 1 mm for the grafted and native bone for each biopsy specimen (Fig 2). A 3D morphometric analysis was conducted with CTan software (Skyscan) to determine the architecture of the newly formed bone in the grafted area. The greyscale threshold was set between 100 and 255. The 3D images (Fig 2) were prepared in a volume rendering program (CTvol, Skyscan). Calcium hydroxyapatite phantom cylinders of a density of 0.25 and 0.75 g/cm^3 were used for the BMD calibration and analyzed in the same manner as the bone samples. The standard unit of x-ray CT density (Hounsfield unit) was calibrated, followed by the conversion from Hounsfield unit to BMD. Volumetric BMD measurements were obtained for the grafted and native bone contained for each biopsy specimen.

Total RNA Isolation

Frozen bone biopsies were cut with a bone saw to take 1 mm of grafted bone and 1 mm of native bone from each sample. Then, samples were pulverized and used immediately for total RNA isolation using a monophasic solution of phenol and guanidine isothiocyanate (Tripure, Roche Diagnostics) according to the manufacturer's protocol. Total RNA was quantified at 260 nm using a Nanodrop spectrophotometer (NanoDrop Technologies).

Real-Time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

The same amount of total RNA (150 ng) from each sample was reverse transcribed to complement DNA (cDNA) using a High Capacity RNA-to-cDNA kit (Applied Biosystems) according to the protocol of the supplier. Each cDNA sample was diluted, and aliquots were stored at –20°C until the PCR reactions were carried out.

Real-time PCR was performed using the LightCycler FastStart DNA Master PLUS SYBR Green I (Roche Diagnostics) following the manufacturer's instructions. Real-time PCR was performed for three reference genes: 18S ribosomal RNA (18S rRNA), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and beta-actin (ACTBL2), and nine target genes related to bone formation (collagen type I [COL1A1], alkaline phosphatase [ALP], runt-related transcription factor 2 [RUNX2], and osteocalcin [OC]), bone resorption (vacuolar type proton ATPase [H+-ATPase], tartrate-resistant acid phosphatase [TRAP]), and inflammation (interleukin-6 [IL6], interleukin-10 [IL10], and tumor necrosis factor α [TNFA]). A negative control without cDNA template was run in each assay. Each reaction contained 500 nmol/L of the corresponding oligonucleotide primers (Table 1), 5 µL of LightCycler FastStart DNA Master PLUS SYBR Green I (Roche Diagnostics), and 3 µL of cDNA in a final volume of 10 µL.

The amplification program consisted of a preincubation step for denaturation of the template cDNA (5 minutes at 95°C), followed by 45 cycles consisting of a denaturation step (10 seconds at 95°C), an annealing step (10 seconds at 60°C), and an extension step (10 seconds at 72°C) for all, except h-ALP and h-OC, for which 45 cycles were used consisting of a denaturation step (10 seconds at 95°C), an annealing step (5 seconds at 68°C), and an extension step (12 seconds

at 72°C). After each cycle, fluorescence was measured at 72°C. A negative control without a cDNA template was run in each assay.

To allow relative quantification after PCR, standard curves were constructed from the standard reactions for each target and housekeeping genes by crossing point values, ie, the cycle number at which the fluorescence signal exceeds background, compared with log cDNA dilution. The crossing point readings for each of the unknown samples were used to calculate the amount of either the target or reference gene relative to a standard curve. Normalized mRNA levels were calculated as the ratio of relative concentration for the target genes relative to that for the geometric mean between the three reference genes (18S rRNA, GAPDH, and ACTBL2).

Statistics

All data are presented as mean values ± standard errors of the mean. The Kolmogorov-Smirnov test was used to assume parametric or nonparametric distributions for the normality tests. Differences between groups t1 and t2 were assessed with the Mann-Whitney or Student *t* tests. Differences between native and grafted bone were assessed using the Wilcoxon test for ratio or the paired *t* test. To measure correlation among variables, Pearson correlation analysis was used. SPSS program for Windows, version 17.0 was used. Results were considered statistically significant at $P \leq .05$.

RESULTS

Clinical Parameters

A total of 26 biopsies from 11 patients (seven women and four men) were studied (Table 2), of whom six patients had re-entry surgery after 4 to 5 months (group t1) and five had re-entry after 6 to 8 months (group t2). Patients in groups t1 and t2 had a mean age of 52 and 60 years, respectively. Mean ISQ values were 64.3 for group t1 and 68.5 for group t2. Five group I (sinus perforation < 5 mm) perforations were done, four in group t1 and one in group t2. To date, an implant survival of 100% was achieved with 12 implants placed in 2010 (between July and October), 10 in 2011 (between October and December), and 4 in 2012 (between June and December).

Bone Morphometric Parameters and BMD

Grafted and native bone samples were differentiated in each bone biopsy specimen, and the same VOI was applied before 3D morphometric and BMD analysis (Figs 1 and 2). Statistically significant differences were found in the variables BMD, bone volume fraction (BV/TV), and trabecular separation (TbSp) between native and grafted bone in the groups t1 and t2. This showed grafted bone values were higher in all except the variable TbSp, for which the values were lower in the grafted bone than in native bone (Table 3, Fig 3). The decrease in TbSp in the grafted bone for both groups can be explained by the significant increase in trabecular thickness (TbTh) in group t2 and the trabecular number (TbN) in group t1, compared with native bone, respectively. No differences were found between the groups in the trabecular pattern factor (TbPf) and the structural model index (SMI).

No differences were found in the morphometric parameters of the grafted bone at t1 and t2. No correlation was found between the primary ISQ values and morphometric parameters of the grafted bone or between primary ISQ values and the primary height from alveolar crest to sinus floor.

Table 2 Clinical Parameters Organized by Re-entry Group

Variable	t1 (4–5 mo)		t2 (6–8 mo)	
No. of biopsies	13	RS: 8 LS: 5	13	RS: 8 LS: 5
No. of patients	6 (5F, 1M)		5 (2F, 3M)	
Mean age (y)	52.0 ± 6.8		60.2 ± 4.4	
Mean ISQ	64.3 ± 13.9		68.5 ± 3.6	
Location	First premolar: 1 Second premolar: 6 First molar: 3 Second molar: 3		Second premolar: 5 First molar: 4 Second molar: 4	
Residual bone width (mm)	6–7			
Membrane perforations	Group I	2 RS 2 LS	Group I	1 LS

RS = right sinus; LS = left sinus; F = female; M = male; ISQ = Osstell implant stability quotient value; group t1: re-entry at 4–5 months; group t2: re-entry at 6–8 months; Group I: sinus perforation < 5 mm.

Gene Expression of Bone Formation, Resorption, and Inflammation Markers from Native and Grafted Bone

Figure 4 shows relative mRNA levels of bone formation–related genes (COL1A1, RUNX2, ALP, OC) and bone resorption genes (TRAP and H+-ATPase). No significant changes in mRNA levels of these markers were found between groups t1 and t2, either in grafted bone or in native bone. Only ALP mRNA levels were lower in grafted bone than in native bone in the t1 group. Gene expression profiles of proinflammatory (TNFA, IL6) and anti-inflammatory (IL10) cytokines were also investigated (Fig 5). In group t1, a significant decrease in mRNA levels of IL10 and TNFA was observed for grafted bone compared with native bone. No differences were found in inflammatory bone markers between groups t1 and t2, either in grafted bone or in native bone.

Table 3 Bone Morphometric Parameters and BMD Analyzed in Native and Grafted Bone with Microcomputed Tomography

Variable	Native bone	Grafted bone t1	Grafted bone t2
BMD (g/cm^3)	0.646 ± 0.035	0.807 ± 0.043*	0.816 ± 0.031*
BV/TV (%)	25.9 ± 2.33	44.8 ± 4.39*	41.9 ± 6.43*
TbTh (mm)	0.162 ± 0.020	0.147 ± 0.012	0.192 ± 0.034*
TbN (mm^{-1})	1.81 ± 0.130	3.10 ± 0.315*	2.43 ± 0.362
TbPf (mm^{-1})	–2.10 ± 5.11	–3.78 ± 7.09	2.57 ± 3.927
SMI	1.58 ± 0.655	1.19 ± 0.744	1.53 ± 0.670
TbSp (mm)	0.312 ± 0.022	0.146 ± 0.019*	0.182 ± 0.029*

*$P \leq .05$. Paired t test was used to assess differences between native and grafted bone. No statistical differences were found in grafted bone between t1 and t2.
BMD = bone mineral density; BV/TV = bone volume fraction; TbTh = trabecular thickness; TbN = trabecular number; TbPf = trabecular pattern factor; SMI = structural model index; TbSp = trabecular separation.

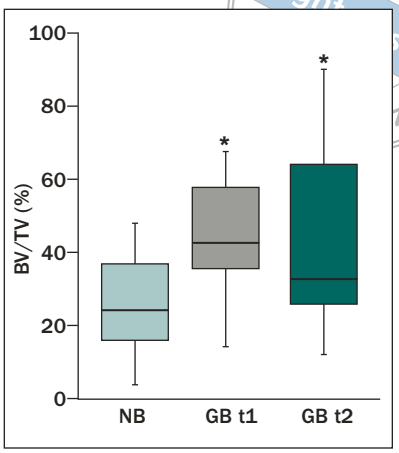
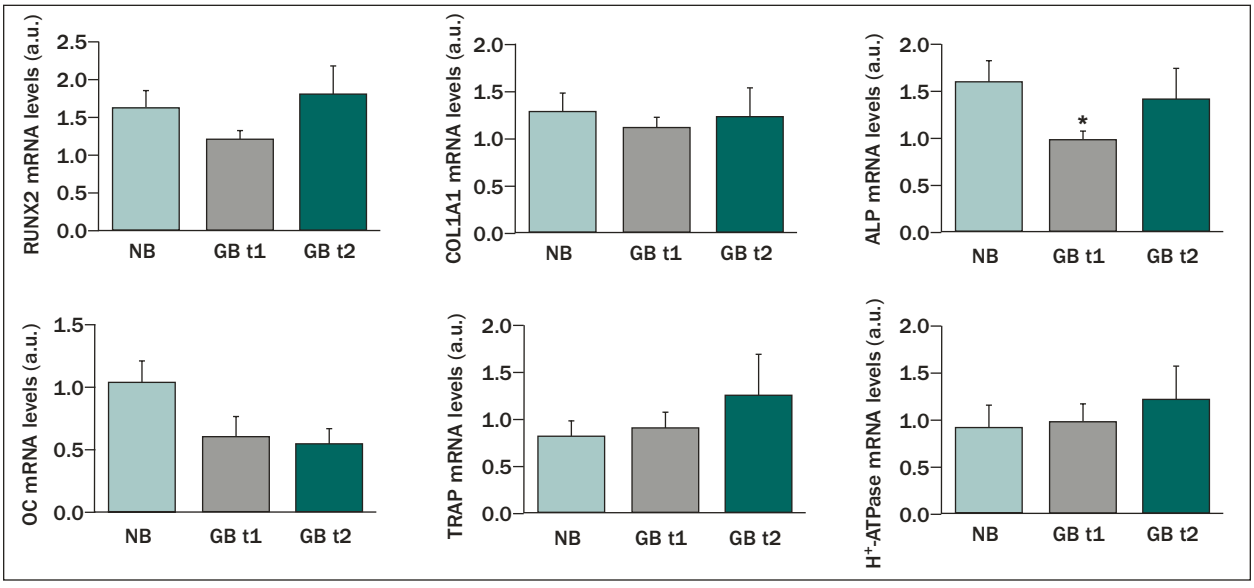


Fig 3 Bone volume fraction (BV/TV) in native (NB) and grafted (GB) bone after 4–5 months (t1) and 6–8 months (t2) of SL. Boxes represent the 25th to 75th percentiles, and horizontal lines within the boxes represent the median values. The error bars extend down to the smallest value and up to the largest. Paired *t* test was used to assess differences between native and grafted bone: *$P \leq .05$.

Fig 4 *(below)* Gene expression of bone markers in native (NB) and grafted (GB) bone after 4 to 5 months (t1) and 6 to 8 months (t2) of SL. Data were normalized relative to reference genes (18S rRNA, GAPDH, and ACTBL2). Values represent means ± standard errors of the mean. Paired *t* test was used to assess differences between native and regenerated bone: *$P \leq .05$. a.u. = arbitrary units.



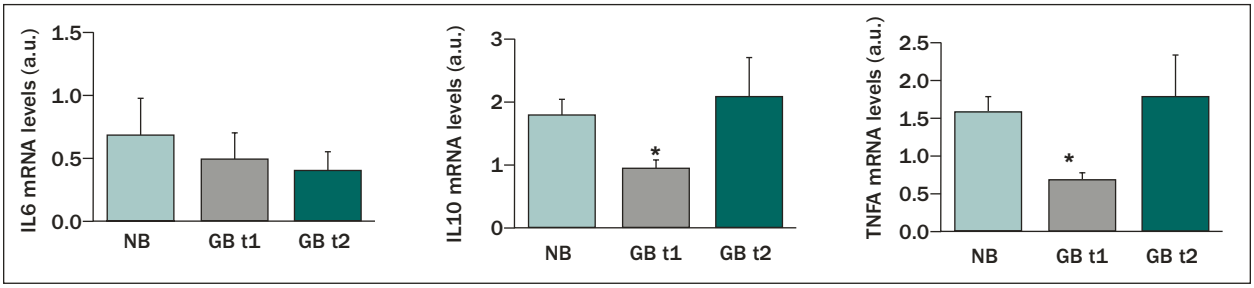


Fig 5 Gene expression of inflammation markers in native (NB) and grafted (GB) bone after 4 to 5 months (t1) and 6 to 8 months (t2) of SL. Data were normalized relative to reference genes (18S rRNA, GAPDH, and ACTBL2). Values represent means ± standard errors of the mean. Wilcoxon test was used to assess differences between native and regenerated bone: *$P \leq .05$. a.u. = arbitrary units.

DISCUSSION

The high regenerative capacity of the maxillary sinus allows most graft materials to regenerate bone correctly. However, the timing differs when using different graft materials; ie, autografts seem to reduce the healing time, whereas the use of biomaterials alone seems to have a longer healing time, with an optimum between 9 and 12 months. When using a combination of autografts and graft substitutes, many surgeons re-enter the site 4 to 5 months after SL based on their clinical experience. However, scientific evidence that the resulting grafted bone is mature at this time is lacking, because most reviews in the literature have described it after 6 to 8 months.[8–10,16,17,21,30,31] For patients, a shortened re-entry time reduces postoperative discomfort and increases their acceptance. A previous study from the present authors compared Bio-Oss and BoneCeramic (mixed as well with autologous bone) after the same healing periods following a bilateral split-mouth design model.[20] That study showed that Bio-Oss had a shorter healing time and superior osteoinductive activity (observed at molecular level), but no important differences were seen on morphometric parameters and BMD. Because of the limited amount of samples in the previous study, no evidence was found of the maturity of the grafted bone 4 to 5 months after SL. In addition, that previous study did not evaluate the native bone of each patient, so it was not possible to have the reference values of mature bone, for both microCT and gene expression analysis. The present study found no differences in the grafted bone after 4 to 5 months compared with 6 to 8 months, either at the molecular level or in the morphometric parameters and BMD. Only ALP mRNA levels were significantly lower at 4 to 5 months in the grafted bone compared with the native bone. In addition, the most important morphometric parameters and BMD were improved in the grafted bone compared with the native bone, namely, BV/TV (due to a higher TbTh or TbN, together with a lower TbSp) and BMD, because of the presence of the biomaterial in the grafted biopsy specimens at this healing time.

Resonance frequency analysis has found a good correlation during the last 13 years between its values and the stiffness grade between the bone and the implant.[33–35] But, the relationship between ISQ values and clinical perception of primary stability is not clear. It seems that the secondary ISQ values (values measured during the insertion of the gingiva former) can predict the implant's success in a significant way, not the primary values (values measured the day of implant insertion).[36] The present study found primary ISQ values similar to those found in other studies,[36,37] and with the same results in implant survival in both groups (100%). A threshold ISQ value of 60 was used to stratify implants by stability (stable/nonstable).[36] Mean ISQ values were 64.3 ± 13.9 in group t1 and 68.5 ± 3.6 in group t2. No statistically significant differences were found between both groups. No statistical correlation was found between the primary ISQ values and the morphometric parameters of the grafted bone, concordant with the study of Rozé et al.[37] There was no correlation between the primary ISQ values and the primary height of the maxillary sinus floor.

There were five sinus perforations, and all were smaller than 5 mm in diameter. A sinus perforation of this size is assumed to be relatively common (10% to 53.85%) in SL procedures.[32,38–40] All sinus perforations were treated by patching the defect with a resorbable collagen membrane. No implant had failed at the time of this writing, but it is known that the survival rates of implants placed under reconstructed membranes may correlate inversely with the size of the perforations, with lower survival rates in sinuses with larger perforations (> 10 mm) compared with those with smaller perforations (< 5 mm): 74.14% vs 97.14%, respectively.[32] However, other authors failed to find any association between sinus membrane perforations and postoperative complications and implant survival.[38,39,41,42] The present authors will closely follow up the implant survival and observe if these perforations may influence the outcome.

The systematic review published by Aghaloo and Moy[10] lists the survival rates (minimum of 12 months' follow-up) found in 6,913 implants placed over 2,046 sinuses in patients from 39 studies. This analysis showed an overall survival rate of 91.49%. Interestingly, the analysis of implant survival was different when using: *(1)* only autologous bone (87.7%), *(2)* only bone substitutes (95.98%), and *(3)* mixed autologous bone and bone substitutes (94.88%). The survival rate of the present study at the time of writing was 100% in both groups, with 12 implants placed in 2010 (between July and October), 10 in 2011 (between October and December), and 4 in 2012 (between June and December). This review by Aghaloo and Moy shows how implant survival decreases 7% to 8% in the group when only autologous bone is used compared with the other two groups, which are very similar.[10] However, for those sinuses grafted with only bone substitutes, a healing time of 9 to 12 months is advised.[21]

The present study used a composite graft of 50% autologous bone and 50% biomaterial, using the study of Hallman et al[16] as a reference. In that study, biopsies were performed 6 to 9 months after graft placement using 20% autologous bone and 80% bovine hydroxyapatite. The present study found mature bone in the grafted areas of both groups, with no statistically significant difference in the morphometric

parameters and BMD between the two groups. Thus, very similar values were found in the amount of bone after the healing period, measured as bone area in the Hallman et al[16] study (after histologic and morphometric analysis) and BV/TV in the present study (after microCT scanning and morphometric analysis). Hallman et al found a bone area of 41.7%, whereas the present study found a BV/TV of 41.9% in group t2 and 44.7% in group t1.

Huang et al[26] used MicroCT to evaluate 10 biopsy specimens 5 months after autogenous bone graft sinus augmentation and compared them to adjacent native bone. They observed that the grafted bone had lower BV/TV, TbTh, and trabecular connectivity than the native bone. However, a direct comparison of the microCT results of this grafted bone cannot be made, because in the present study autologous bone was mixed with graft substitutes. Moreover, different sources were used for the autologous bone, ie, iliac crest vs maxilla, which have different regenerative potential and resorption rates. Thus, bone marrow stromal cells isolated from the maxilla and mandible have superior osteogenic capacities than those from the iliac crest in the same individuals.[43] In addition, iliac crest bone has an endochondral origin, whereas calvarian or maxillomalar bone of membranous origin has a lower resorption rate.[44] Moreover, it is known that the bone resorption rates in sinuses grafted only with autologous bone are higher than those grafted with composite graft or only biomaterial.[17–19,45]

The lack of differences found in the mRNA levels of bone formation–related genes between the two reentry times suggest that there are no differences in the bone-forming capacity and the functional status of the obtained regenerated bone. Moreover, although some bone formation markers such as OC were lower in grafted than in native bone, only significant differences were found on ALP mRNA levels in the t1 group. This suggests an appropriate bone-forming activity in the newly formed bone from both groups compared with the adjacent native bone. When considering bone resorption and inflammatory related genes, only the anti-inflammatory IL10 and proinflammatory TNFA showed lower mRNA levels in t1-grafted bone compared with native bone, which may suggest a low immune response and a good tolerance to bone grafting.

CONCLUSIONS

This study showed that the use of autologous bone harvested from the maxillomalar buttress associated with biomaterials in a 50% proportion decreases the bone regenerating time to just 4 months. This emphasizes an ideal graft for SL and shortens the time patients have to wait for finalizing the implant-supported rehabilitation. Patients in the present study are being followed up to evaluate if the re-entry time has any influence on implant survival.

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