To overcome the vertical deficiency of the upper-posterior maxilla, sinus floor augmentation has been proposed as one of the treatment approaches to correct this deficiency. Regardless of the surgical technique used, the lateral window1 or crestal approach2,3 technique, materials used as the filler remain one of the interesting topics among clinicians. Hydroxyapatite and tricalcium phosphate4,5 are the most widely used synthetic materials. Anorganic bovine-derived hydroxyapatite4,5 is the preferred heterologous material. Mineralized human bone1 represents a very good alternative choice for the homologous material, although according to many authors, the gold standard continues to be autologous bone.6,7 Many studies, comparative or not, performed in animals or humans have focused on the outcomes of the grafting of those materials, but the parameters evaluated were almost always the percentage of bone, graft, and fibrous tissue content.

Limited studies on topographic disposition of bone and graft of regenerated sites are available in the literature. Most authors do not differentiate preexisting (basal or native) bone from newly formed bone,8–10 but few11,12 did, and only one paper analyzed some sectors of the regenerated tissue.13 All these authors performed analysis to study the osteoconductive capability of the graft used, but none topographically quantified the amount of mineralized materials along the whole analyzed biopsy specimen.

Therefore, the aim of the present study was to topographically analyze the material present in human biopsy specimens after sinus floor augmentation by mineralized allograft.

MATERIALS AND METHODS

The patients were evaluated in a private dental office. They were selected from a pool of subjects who required maxillary posterior implants and needed unilateral sinus augmentation. Additional inclusion criteria were < 2 mm of remaining crestal bone height below the sinus floor, as measured by CBCT. Patients enrolled in the study needed to have good general health, be...
nonsmokers, and have an absence of diseases that affect bone metabolism or wound healing, absence of any maxillary sinus pathologies, and no regular medication consumption for more than 3 months. In total, 32 patients (14 men and 18 women, aged between 38 and 77 years [mean ± SD] = 57.1 ± 8.73 years) fulfilling all the inclusion and exclusion criteria were enrolled in this study. All patients signed the informed consent form detailing the study procedures that are in line with the 2013 Helsinki protocols and ethical requirements.

A preoperative panoramic radiograph and a CBCT scan (at increasing depth of 1.0-mm intervals) of the maxilla were taken for each patient. The thickness of the sinus floor (≤ 2 mm) was evaluated using 3D radiographic software (3 Diagnosis 3.0, 3DIEMME).

Surgical sites were infiltrated by local anesthetic (Ultracain, Sanofi-Aventis Deutschland). CBCT was used to design the crestal window location and boundaries, and a full-thickness flap was reflected to expose the crest of the maxilla. A piezosurgical device (Mectron) was used to perform crestal window opening. The bony window was then elevated, without removal, at the first wall movement; then, the sinus membrane was gently elevated using the piezosurgical device and subsequently a sinus curette.

A mixture of cortical and cancellous (80/20) mineralized human bone allograft (Puros, Zimmer Dental), each consisting of a 50:50 mixture of 0.25 to 1.0 and 1.0 to 2.0-mm particles, moistened by saline solution, was used as grafting material. Depending on the sinus cavity and number of implants to place, up to 4 g of graft materials were gently packed on each sinus. Before soft tissue closure, an absorbable collagen membrane (BioMend, Zimmer/Biomet 3i) was placed over the window, and the buccal flap was repositioned using the ePTFE 5/0 suture (expanded polytetrafluoroethylene suture, W. L. Gore & Associates). Patients were then prescribed amoxicillin (Ratiopharm), 1 g, twice a day for 6 to 7 days, and Synflex forte 550 mg (Recordati) as analgesic, if needed, for pain control. Patients were directed to use a chlorhexidine mouthwash (0.2%), twice a day, and not to brush the surgical sites for 2 weeks. Sutures were removed 12 to 14 days after the surgery. Monthly follow-up was scheduled to check the healing until implant insertion.

Six months after surgery, CBCT scans were taken, and the bone core specimens were collected with the assistance of prosthetic-driven implant position surgical guides. A 3-mm-diameter trephine set at 600 rpm with saline irrigation was utilized (up to 10 mm) to obtain bone core specimens from the augmented areas. Each retrieved bone core sample was immediately labeled with black china ink on the crestal end and then fixed in 4% paraformaldehyde for the histologic study (Fig 1).

After 3 months, the implants were uncovered. Rehabilitation was completed by a fixed implant-supported prosthesis.

**Histotopography**

Fixed bone core biopsy specimens were dehydrated through ethanol series and embedded in polymethyl methacrylate (PMMA, all reagents: Fluka, Sigma-Aldrich Schweiz) as described. PMMA blocks were serially sectioned along the longitudinal axis of the cylindrical biopsy specimen to its center using a diamond saw microtome (SP1600, Leica Microsystems). A thick section (200 µm) was obtained, and then reduced to 100 µm by grinding, perfectly polished with emery paper and alumina. Each section was x-ray microradiographed (3K5, Italstructures) at 12 kV and 3 mA on high-resolution film (SO 343, Eastman Kodak). Microradiographs were photographed using a microscope (Eclipse Ni equipped with DS-U3 digital camera, Nikon) under ordinary light. Each microradiographed biopsy specimen was longitudinally split into 5 sectors (sector 1 = crestal sector; sector 5 = sinusal sector), each with 20% of the length of the biopsy specimen volume (Fig 2). The amount of bone tissue (bone volume [BV]), graft (graft volume [GV]), and fibrous tissue (fibrous tissue volume [FTV]) per tissue volume (BV/TV, GV/TV, and FTV/TV, respectively) was evaluated on the microradiographic sectors using an image analyzer and software (AnalySIS, Soft Imaging System).

**Statistical Analysis**

Bone, graft, and fibrous tissue data analysis was performed by Primer of Biostatistics, using the more-fitted regression to analyze trends. The null hypothesis...
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H₀ (no regression correlation) was rejected for a critical significance level of \( P < .05 \).

RESULTS

Surgery
Thirty-two patients completed the crestal window sinus augmentation procedure using human mineralized bone grafts. Primary wound closure was obtained in all surgeries without any complaint or adverse events observed during the follow-up. All implants achieved implant osseointegration (without clinical mobility) at the 3-month follow-up.

Histotopography Analyses and Statistics
A total of 32 bone core biopsy specimens were obtained for topographic analysis. The central section of the 32 PMMA-embedded core biopsy specimens had a length (mean ± SD) of 8.11 ± 1.86 mm and a diameter of 2.46 ± 0.45 mm. The histomorphometric evaluation of the percentage of residual graft, bone, and soft tissue of all biopsy specimens (Fig 2) returned a mean (± SD) value of 12.4% ± 6.85%, 32.5% ± 9.53%, and 55.1% ± 11.02%, respectively, 6 months after surgery (\( n = 32 \)). The distribution of those indices along the 5 sectors (from crestal to sinusal) of core-biopsy microradiographs is summarized in Fig 3. The graft shows the lower GV/TV amount in the first (crestal) sector; GV/TV increases up to the fourth sector, and then GV/TV slightly decreases in the fifth (sinusal) sector. The bone, contrarily, shows the greater BV/TV amount in the first (crestal) sector; BV/TV decreases up to the third and fourth sector, and then BV/TV increases in the fifth (sinusal) sector. The soft tissue shows the maximum FTV/TV amount in the middle of the core biopsy specimen (third sector); then, FTV/TV decreases going to both the crestal and sinusal sector.

To statistically analyze the trend of GV/TV%, BV/TV%, and FTV/TV% in the 32 biopsy specimens, polynomial (degree 2) regression analysis was performed to better model outcomes and give the greater correlation coefficient. The best polynomial equation (Fig 4a) relating graft percentage to sector was GV/TV% = 0.30 + 7.65s – s² (\( r² = 0.092 \)), with a maximum noted at sector 4 (pre-sinusal). The best polynomial equation (Fig 4b) relating bone percentage to sector was BV/TV% = 60.78 – 18.9s + 2.63s² (\( r² = 0.198 \)), with a minimum between sectors 3 and 4. The best polynomial equation (Fig 6c) relating fibrous tissue percentage to sector was FTV/TV% = 38.92 + 11.25s – 1.63s² (\( r² = 0.055 \)), with a maximum between sectors 3 and 4.

DISCUSSION
Maxillary sinus floor augmentation has been regarded as one of the ideal locations for graft material evaluation in dentistry. It has fewer variables related to the

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Fig 2  Representative image showing (left) the microradiograph of a thick central section of bone sample of maxillary sinus floor augmented by mineralized human bone allograft, 6 months after surgery. (Right) Note the five longitudinal sectors (sector 1 = crestal sector; sector 5 = sinusal sector; each containing the 20% of the biopsy specimen volume) of biopsy specimen splitting for the topographic analysis. Bar = 2 mm.

Fig 3  Graph showing the relative amount of graft, bone, and fibrous tissue in the five sectors of the 32 analyzed biopsy specimens.
surgeon’s ability, and it is easier to measure the starting defects as well as outcomes. Several studies have examined the outcomes of vertical augmentation of the sinus floor. However, these works are primarily focused on the volume changes with time, the blood supply, the surgical technique, or, above all, the type of graft to be optimally used.

In all works concerning graft outcomes, executed by histologic, microradiographic, CBCT, or microcomputed tomography (micro-CT) analyses, the main goal was the single or comparative evaluation of one or more used grafts. Most of the works did not even consider differentiating the preexisting (basal or native) bone from newly formed bone. Only a few authors attempted a topographic analysis of the regenerated skeletal segment and differentiated the analysis in sectors that did or did not contain the basal bone. Therefore, the present study is one of the first to apply 3D topographically to assess the outcome of sinus floor augmentation by mineralized allograft at different sectors (five longitudinal sectors) of obtained bone core biopsy specimens after 6 months of healing.

De Lange et al (2014) by lateral access and using both histology and micro-CT, performed a topographic analysis, but their goal was the comparison of the two grafts (deproteinized bovine bone and biphasic calcium phosphate) used and not a study examining the material displacement inside biopsy specimens. In the micro-CT analysis, they found the preexisting bone at the crestal end, followed by the regenerated bone up to the sinusal end of the biopsy specimens. In the latter, they found an increase of the bone up to the first crestal sectors, bone decreases up to the third quarter, and then a small increase. The graft increases up to the half of the biopsy specimen, then remains unchanged. The authors did not present the exact result of the fibrous tissue content, but a calculation by difference indicated the maximum amount of fibrous tissue between the third and the fourth (sinusal) quarter of the regenerated part of the biopsy specimens.

Soardi et al (2014) conducted a study aiming to compare CBCT and microradiographic outcomes, in which they performed topographic analyses. In 21 long core biopsy specimens (> 11 mm) of mineralized human bone allograft sinus floor that was augmented, which was laterally accessed, they analyzed transverse sections taken 6, 8, and 10 mm from the crestal surface. The histomorphometric analyses of microradiographs showed that the maximum mineralized content was at 8 mm, and it decreased at both 6 and 10 mm.

The results of this study showed that bone had maximum content at the crestal end and the minimum between the third and fourth sector, but then it increased at the sinusal end. The graft had the minimum content at the crestal end and the maximum at the fourth sector, and then it decreased at the sinusal end. The fibrous tissue had maximum content between the third and fourth sector and decreased toward both the crestal and sinusal ends, with a mirrored trend of bone. The bone and graft trends (and the corresponding mirrored trends of fibrous tissue) were likely ascribed to the sinus floor vascularization. The decreasing amount of graft and fibrous tissue at the sinusal end was likely due to the sinus membrane blood supply. On the contrary, the great amount of bone at the crestal end and the corresponding low amount of graft and fibrous tissue) had to be due to the greater vascularization of the sinus floor, but also to the re-formed periosteal vessels.

The outcomes of the present study were largely in line with both of the two previous works. Due to the crestal access, the present study had no preexisting bone, and the newly formed bone decreased up to the third and fourth sector, then increased as found by de Lange et al (2014). The graft had its maximum at the fourth sector.
and decreased toward the two extremities, particularly the crestal one. The fibrous tissue had maximum content between the third and fourth sector and cannot be correlated with the outcomes of works since no authors attended to its topographic distribution. The differences with the Soardi et al (2014) work can be attributed to the different surgical approach (lateral window, the same used by de Lange et al (2014)), as well as to the insufficiently performed topographic analysis.

The limitations of this study include, but are not limited to, the following. First, only human allograft was tested in this study; hence, it is difficult to compare with other bone substitute materials. Future studies will be needed to assess if other graft materials behave similarly as reported in this study. Second, this study did not examine the effect of influence of implant types (eg, surface treatment) on the bone maturation after 6 months of healing. Again, future studies in this area will be beneficial to understand the effect of different implant types (eg, surface coating) on the graft maturation.

CONCLUSIONS

Based upon topographic analysis, mineralized human bone allograft is capable of achieving adequate vertical bone height for implant placement. The need for a topographic analysis to assess the outcomes of sinus augmentation is emphasized.

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REFERENCES