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FRACTAL ANALYSIS IN CONE-BEAM COMPUTED TOMOGRAPHY AND PANORAMIC RADIOGRAPHY IMAGES OF NATIVE BONE BEFORE AND AFTER SINUS GRAFTS

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ABSTRACT

Objective: Compare the fractal analysis (FA) of the graft of a synthetic hydroxyapatite and its association with L-PRF, after elevation of the maxillary sinus floor, compared with native bone. **Materials and Methods:** A sample of 20 patients was included and cone-beam computed tomography (CBCT) and theFAwere performed in 10 (T1) and 180 days (T2) after the surgery. Panoramic radiographs were performed in T2. **Results:** In the CBCT, there was a statistically significant difference ($p < 0.05$) when comparing the mean values of the FA between Osteogen® + L-PRF in T1 and T2, also between T1 and T2 for Osteogen® and there was no statistically significant difference ($p > 0.05$) between Osteogen®+L-PRF in T1 with Osteogen® in T1, and also between Osteogen®+ L-PRF in T2 with Osteogen® in T2, and between Osteogen® and Osteogen® + L-PRF compared with native bone values. Between FA of the CBCT and panoramic radiography, the values of the graft area and native bone were higher in the CBCT; with statistically significant difference. **Conclusion:** The values of the FA showed no correlation of values between the tomographic and radiographic techniques presented. Thus, different bone reconstruction patterns obtained in grafts should be considered in relation to native bone.

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INTRODUCTION

Insufficient height of the residual bone in the edentulous posterior region of the maxilla is a common difficulty found when considering the installation of dental implants. Pneumatization of the maxillary sinus creates the need for gain in bone volume when it is insufficient in the posterior area.[1,2,3] The lateral window approach, alone or with simultaneous implant placement, is the maxillary sinus augmentation technique with more favorable prognosis and predictability.[4] Various grafting procedures have been described for restoring adequate bone volume and allowing the placement of endosseous implants in the posterior maxilla. These procedures include the use of autografts, allografts, xenografts, alloplastic bone and, recently, platelet concentrates either associated with grafts, or not.[5] Although satisfactory results have been achieved using various biomaterials for increasing the height of the alveolar bone (reduction of the maxillary sinus),[4] there are still analysis to be done to assess ideal combinations of materials and conditions for improving bone regeneration properties and long-term stability in the surgical site. Hydroxyapatite, the main constituent of the inorganic phase of bone, is used in several areas of Medicine and Dentistry due to its chemical and structural characteristics. The synthetic and pure forms of hydroxyapatite do not cause an exacerbated or unwanted inflammatory response.[6] Osteogen® is a synthetic hydroxyapatite with osteoconductive potential, and due to its physical characteristics, it is understood to be the property of graft materials thatacts as a basis around the ossification processes.[7] Another material used is platelet concentrates that are used with the idea of improving healing and bone formation, acting in the release of growth factors. The components of this fibrin and platelet network contain large amounts of key growth factors, such as platelet-derived growth factor, transforming growth factor ß1 and ß2 and vascular endothelial growth factor, capable of stimulating cell proliferation and improving angiogenesis.[8] At present, many techniques have been recommended for assessing bone quality and quantitatively characterizing structural changes in bone in sites for installing future implants, such as: histological evaluation,[1,4] computed tomography (CT),[7] fractal analysis (FA)[9] and Micro CT.[10] Among them, fractal analysis was introduced as a precise, easily available and low-cost method.[9] In this context, the present study analyzed by means of fractal analysis of cone-beam computed tomography (CBCT) images and panoramic radiographs of the sinus grafts of Osteogen® and Osteogen® + L-PRF in the 180-day repair period and compared it with native bone.

MATERIALS AND METHODS

A clinical, experimental, analytical, prospective, randomized, controlled and blind study was conducted. This study was approved by the institution's Research and Ethics Committee. Participating patients were properly informed about the content and objectives of the research and were supported by the right to non-identification and privacy. The inclusion criteria were: patients who had bone remnants with a height of less than 4 mm, requiring bone graft in the posterior region of the maxilla for future installation of implants, who agreed to participate in the research and with the terms of the present study. The exclusion criteria were: patients with systemic changes that indicate a surgical procedure or use of any medication that may interfere with bone metabolism, smokers, tests that did not show the full image of the maxillary sinus, tests that had the presence of technical artifacts that could hinder the evaluation of the maxillary sinus, maxillary mandibular relations unfavorable to the installation of implants, pathological conditions of the maxillary sinus or history of surgery of the maxillary sinus. For the sample calculation, the G-Power software (G* Power, version 3.1.9.2; Institute for Experimental Psychology®, Dusseldorf, Germany) was used. The level of significance considered was 5%; the test power was 80% and the minimum sample size required was 15 patients.The study involved the participation of 20 patients (10 men and 10 women) aged between 48 and 75 years (mean ± SD, 59.05 ± 8.77).

In total there were 13 Partially edentulous patients, and a total of 7 edentulous patients. Patients with bilateral maxillary sinuses were randomly assigned to the Osteogen® group (Impladent, Ltd®, Holliswood, NY) + L-PRF and the Osteogen®V_Control Group (Impladent, Ltd®, Holliswood, NY) immediately before surgery, by a computer controlled draw. CARESTREAM 8100 Digital Panoramic and Cephalometric System©was used to acquire panoramic radiographs (East Carestream Company®, Rochester, New York, USA). In the CBCT images, the region of interest (ROI) was selected, delimiting the entire graft in the three planes (axial, sagittal and coronal). The areas were evaluated and standardized using a reference point located in the central region of the bone graft. To perform FA of panoramic radiography, ROI was selected in the central grafted region. In both the CBCT and in the panoramic radiography, the native bone the ROI of the maxillary tuber was selected in the central region of the posterior part of the maxilla, after the conclusion of the maxillary sinus graft procedure. In the canine pillar region, an area 3 mm above the canine root apex was selected, when this or the maxillary first premolar was present. In their absence, the area selected was in the central region between the end of the pyriform opening and the beginning of the maxillary sinus and residual bone base at the level of the bone crest. The calculation of fractal values was performed using the Box-counting method, using the open source software ImageJ™ made available by the National Institutes of Health®. The region selected was processed using the fractal analysis technique described by White and Rudolph (1999).[11] After calculating the fractal values in the CBCT, an averageof the axial, sagittal and coronal plane values was calculated.

Work protocol

Analysis of the maxillary sinus: Before the maxillary sinus lift surgery, the L-PRF was prepared. For centrifuging the blood collected from the patient, the Fibrin® surgical protocol was performed[12] using the Montserrat Fibrinfuge 25® centrifuge (Zenith Lab Co®, Changzhou Jiangsu, China). Blood was collectedin 8 glass tubes of 10ml, without clot activator inserted inside the adapter. Immediately, the filled tubes were taken to the centrifuge and positioned opposite each other on the centrifuge rotor, for vibrational stability of the system. The clots were removed from the tube, a slight debridement of the hemosedimentation was performed, and the membranes were placed on a perforated base, compressed, perforated and mixed with the Osteogen® biomaterial. Patients underwent preoperative CBCT performed with cone beam tomographs, Carestream® CS 8100 Digital Panoramic and Cephalometric System© (East Carestream Company®, Rochester, New York, USA) to assess possible previous local operations of the maxillary sinus. Bone reconstruction took place in two stages. Stage two involved bone reconstruction, and was performed in the following stages: a) The surgical procedure for maxillary sinus elevation was performed by the same surgeon who performed the procedure according to Zenóbioet al. (2018);[13] b) After the maxillary sinus floor membrane was elevated, the Osteogen® biomaterial (Impladent Ltd®, Holliswood, NY) was inserted into one of the randomly chosen sides (Figures 1 and 2), and the same biomaterial associated with L-PRF was inserted on the contralateral side (Figure 3); c) An L-PRF membrane was placed to close both sides of access to the maxillary sinus window; d) Suture was performed without tissue tension. The stages of Stage Three consisted of postoperative control and image acquisition: a) Postoperative clinical evaluation was performed at time intervals of 7 days and 10 days (suture removal period), postoperative tomography was performed (10 days); b) The clinical reassessment was performed at time intervals of 30, 60 and 90 days; c) Panoramic radiography, tomography and implant planning were performed 180 days postoperatively, and a second measurement was obtained.

Figure 1. Access to lateral wall of maxillary sinus

Figure 2. Biomaterial insertion in maxillary sinus

Obtaining and analyzing tomography data and comparison with panoramic radiography: Computed tomography scans were performed using cone beam scanners, Carestream® CS 8100 Digital Panoramic and Cephalometric System© (East Carestream Company®, Rochester, New York, USA), 0.3mm voxel size, with an exposure time of 40 seconds. The sections were 1mm thick with 1mm intervals and multiplanar reconstructions with reference to the occlusal plane. The 40 exams were saved in the Digital Imaging and Communication in Medicine (DICOM) format and imported into CS3D Image® software (Carestream Dental LLC®, Atlanta, Georgia, USA). The software wasmanipulated and the measurements were analyzed by an experienced and trained radiologist.

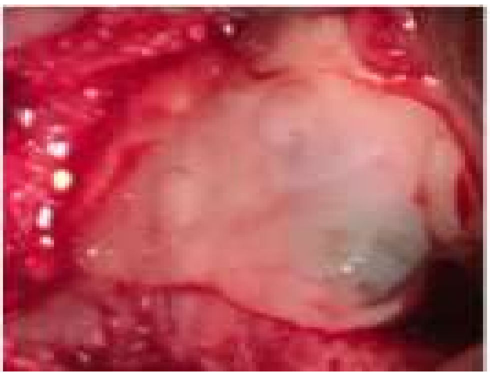

Figure 3. L-PRF membrane interposition in lateral access to sinus after placing the material

The observer manually delimited the areas that were cut and filled by the grafts in the initial and final images. To correlate the values of the fractal dimensions obtained in the T2-weighted CBCT, a fractal analysis was performed of the maxilla (maxillary tuber, canine pillar and graft areas bilaterally) on panoramic radiographs obtained by using the CARESTREAM 8100® Digital Panoramic and Cephalometric System© (East Carestream Company®, Rochester, New York, USA). In both the CBCT and panoramic radiography, in the native bone, the ROI of the maxillary tuber was selected in the central region of the posterior part of the maxilla, on conclusion of the maxillary sinus elevation procedure (Figures 4A, 4B, 4C and 7A).

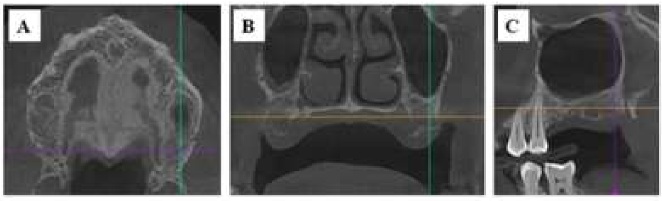

Figure 4. CBCT reference point for selection of ROI in region of maxillary tuber in axial (A), coronal (B) and sagittal (C) sections on the left side

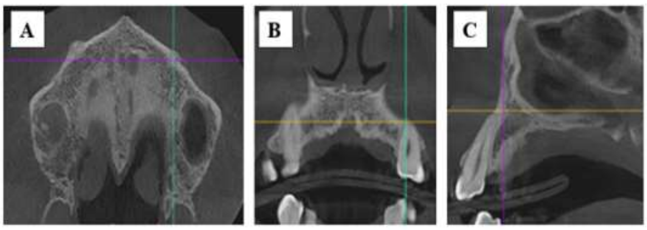

Figure 5. CBCT reference point for selection of ROI in canine pillar region in axial (A), coronal (B) and sagittal (C) sections on the left side

In the canine pillar region, an area 3 mm above the canine root apex was selected, when this or the maxillary first premolar was present. In their absence, the area was selected in the central region between the end of the pyriform opening and the beginning of the maxillary sinus and residual bone base at the level of the bone crest (Figures 5A, 5B, 5C and 7B). To perform FA by means of CBCT, the region of interest (ROI) was selected delimiting the entire graft in the three planes (axial, sagittal and coronal) (Figures 6A, 6B and 6C). The areas were evaluated and standardized using a reference point located in the central region of the bone graft. To perform FA on panoramic radiography, ROI was selected in the central grafted region (Figure 7C). The fractal values were calculated by means of the Box-counting method, using the open-source software ImageJ ™, made available by the National Institutes of Health®. The region selected was processed using the technique described by White and Rudolph (1999).[11] After calculating the fractal values in the CBCT, an average of the axial, sagittal and coronal plane values was calculated.

Measurement evaluation: The volume obtained in axial, sagittal and coronal sections was calculated using the ImageJ™ software (National Institutes of Health®, Bethesda, Maryland, USA) and fractal analysis was performed at time intervals T1 and T2. In the case of panoramic radiography, the graft area was delineated and analyzed using the same program as that used for cone beam tomography in T2.

Statistical analysis: The data were initially submitted to the F normality test (to assess normality), which demonstrated their normal distribution. For comparative analysis between groups at time intervals (T1 and T2) and between materials, the Anova with Tukey tests were used. The Student's-ttest was used to assess whether there were differences between materials. The level of significance adopted was 5%. The analysis were performed using the GraphPad Prism 6.05 software (GraphPad Software, San Diego, California, USA).

RESULTS

No complications were observed during surgical procedures. In the CBCT, there was a statistically significant difference ($p < 0.05$) when the average values of the fractal analysis were compared between Osteogen® + L-PRF in T1 (1.347) and T2 (1.318). This was also foundin the comparison between T1 (1.353) and T2 (1.304) for Osteogen®. Furthermore, in the CBCT, there was no statistically significant difference ($p > 0.05$) when the mean values of fractal analysisbetween Osteogen® + L-PRF in T1 (1.347) were compared with Osteogen® in T1 (1.353).

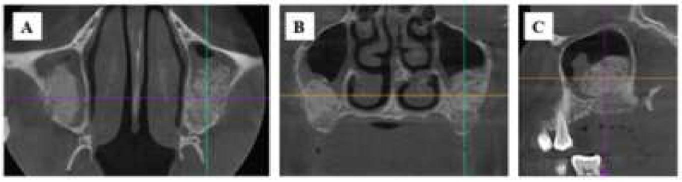

Figure 6. CBCT reference point for selection of ROI in graft region in axial (A), coronal (B) and sagittal (C) sections on the left side

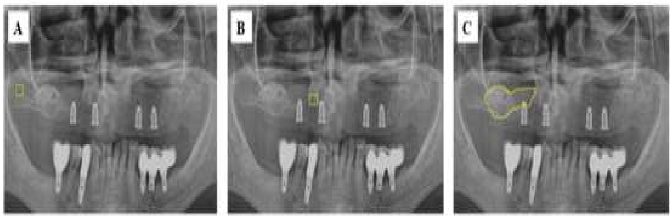

Figure 7. Reference point on panoramic radiography for selection of ROI in region of maxillary tuber (A), canine pillar (B) and graft (C) on the right side

This was also found when Osteogen® + L-PRF in T2 (1.318) were compared with Osteogen® in T2 (1.304) and between the values ofOsteogen® and Osteogen® + L-PRF with native bone. In the comparison between fractal analysis of the CBCT and panoramic radiography, the values obtained showed discrepancy, with the values of the graft area and native bone area being higher in the CBCT than in the panoramic radiography, and there was statistically significant difference ($p > 0.05$) (Table 1).

Table 1. Comparison of fractal analysis of grafts and native bone values between cone beam computed tomography and panoramic radiography

	T1	T2	p value
OSTEOGEN® CBCT	1.353 ± 0.105 a	1.304 ± 0.083 aB	< 0.05*
L-PRF + OSTEOGEN® CBCT	1.347 ± 0.078 a	1.318 ± 0.090 aB	< 0.05*
OSTEOGEN® CBCT	1.353 ± 0.105		> 0.05
L-PRF + OSTEOGEN® CBCT	1.347 ± 0.078		> 0.05
OSTEOGEN® CBCT		1.304 ± 0.083	
L-PRF + OSTEOGEN® CBCT		1.318 ± 0.090	
Canine Pillar CBCT		1.343 ± 0.081 B	
Maxillary Tuber CBCT		1.339 ± 0.062 B	
Native Bone CBCT		1.341± 0.053	
OSTEOGEN® panoramic		1.022 ± 0.096	
L-PRF + OSTEOGEN® panoramic		1.027 ± 0.084	< 0.05*
Canine Pillar panoramic		1.044 ± 0.067	
Maxillary Tuber panoramic		1.040 ± 0.059	< 0.05*
Native Bone panoramic		1.042 ± 0.045	

Means with standard deviations followed by (*) differed by Student's-*t* test, horizontally, in the comparison between the time intervals (T1 and T2).
Means with standard deviation followed by lowercase letters do not differ by the Student's-*t* test, vertically, at the time intervals T1 (p = 0.39) and the time interval T2 (p = 0.19).
Means with standard deviation followed by different letters differed by the ANOVA with Tukey post hoc tests, vertically, at T2, with a level of significance of p = 0.05.

DISCUSSION

One of the most important factors for achieving clinical success of dental implants is bone quality, and in the field of dentistry, several methods are used to assess bone quality and quantity, among them: histological analysis, computed tomography and fractal analysis.[9] Histological examinations are considered the gold standard for the evaluation of bone microarchitecture, however they are considered very invasive.[14,15]Due to the lack of an efficient protocol for assessing the quality of bone tissue after sinus grafts, the possibility of FA associated with the images of CBCT and panoramic radiography led us to guidelines with regard to the grafted bone tissue after the period of repair at 180 days. Servais et al. (2018)[16] reported that in terms of FA, an area with 0% bone would correspond to a FD equal to 1, and a region with 100% bone would correspond to a FD value equal to 2. Amer et al. (2012),[17] reported that the standard FD value for healthy bone was 1.5, and in our sample the highest value was 1.353 for Osteogen® in T1 on the CBCT image; and 1.044 on the panoramic radiography image, in the canine pillar region. It is worth mentioning that in the study by Amer et al. (2012), [17]intraoral periapical radiographs were used, while in the present study,CBCT and panoramic radiographs were used, which could lead to different results, given the overlapping images of anatomical structures and distortion of bone structures present on radiographs, which could lead to outliers of FD.

According to the analysis of the FD of panoramic radiographs and CBCT in the present study, the values obtained showed discrepancies; in the CBCT there was greater tissue organization shown by the mean value obtained, which was 1.341 for native bone, and the panoramic radiography exhibited an average value of 1.042 for the same area. In the analysis of the panoramic radiographs of the FD in the graft areas, the average value on the Osteogen® side was 1.022, and on the L-PRF + Osteogen® side, it was 1.027. These results were divergent and showed statistically significant difference (p <0.05) when compared with the analysis of CBCT in T2, where the values were 1.304 on the Osteogen®side, and 1.318on the LPRF + Osteogen® side According to Kiljunenet al. (2015)[18] panoramic radiographs have the limitations of a two-dimensional examination with anatomical overlapping and geometric distortion. The limitations of the radiographs were believed to have led to differences in the results, in addition to the difficulty of correctly designing the panoramic radiograph image in comparison with the CBCT. In the present study, on an average, the FD values analyzed on panoramic radiography for native bone were 1,042. Gumussoyet al. (2016)[19] compared FD between patients with and without chronic kidney disease, and reported that the value found on panoramic radiographs for dental apexes of the second premolar and mandibular left first molar was 1.411 in the healthy group. This value was established in regions that differed from those chosen in the present study, which may justify this difference between the values. In a study to determine the pattern of bone remodeling after lifting the maxillary sinus in humans, using the fractal dimension(FD) and panoramic radiographs, Molonet al. (2015),[9] found the value of FD 1.750 at 180 days after the procedure. Whereas in our study, the values were also obtained after 180 days, and also using panoramic radiographs, the values found forOsteogen® andOsteogen® + L-PRF were 1.022 and 1.027, respectively. When we analyzed and compared the average values of fractal analysis of panoramic radiographs, images and materials tested in our study with those of Molonet al. (2015),[9] we were able to report a discrepancy in the values obtained for graft areas. This could suggest that these differences between the values found were due to the different regions analyzed. Likewise, when the average values of fractal analysis of panoramic radiographs,and images of native bone were analyzed and compared with those ofGumussoyet al. (2016),[19]this suggested that the divergence of values also occurred due to the different regions analyzed. It was also observed that the mean values obtained between the materials tested. i.e., Osteogen®, Osteogen® + L-PRF and native bone values, by means of fractal analysis in panoramic radiographs and CBCT (Table 1), demonstrated divergence between the values obtained, which may be related to the imaging technique used or to the area of study selected. Torres et al. (2011)[20] used CBCT to compare FD between patients with osteonecrosis associated with bisphosphonate and a healthy control group. The results showed higher FD values of 1.67-1.72 in the study group, than in the control group, of 1.65-1.67. This result was similar to those obtained by Demiralpet al. (2019),[21] who used panoramic radiographs in their study to assess the trabecular pattern of cancer patients taking bisphosphonates.

They usedFD analysis and compared their results with those of healthy individuals. Mean FD values were higher in the study group (1.39) than in the control group (1.38) without statisticallysignificant difference ($p \geq 0.05$). These findings corroborated the values of the present study, in which the value of 1.318 for FD in the CBCT of the study group (Osteogen® + L-PRF) in T2 washigher than the value of 1.304 found in the control group (Osteogen®) in T2. The differences in the FD values found in these studies may have been due to the differences in methodologyof previous studies. In CBCT, the data obtained did not indicate an increase in FD in the time interval from T1 to T2 when the values of Osteogen®were compared, which could lead to determining better cellular organization with a longer repair time (T1: 1.353 and T2: 1.304), with statistically significant difference (p <0.05). The FD values were also lower in T2 when Osteogen® + L-PRF (T1: 1.347 and T2: 1.318) was compared in the CBCT, with this difference being statistically significant (p <0.05). These findings differed from those obtained in the study by Heoet al.

(2002),[22]in which the FD gradually increased according to the time interval of the analysis. Molonet al. (2015),[9] reported that there was an increase in FD in the groups analyzed. However, in the present study, the FD value in T1 and T2 was obtained from an allogeneic graft and with the use ofCBCT, whereas in the study by Molonet al. (2015),[9] autogenous bone grafts were usedand the FD values were obtained by means of panoramic radiography. Unlike panoramic radiography, CBCT is more accurate in portraying the bone microstructure of the grafts, because it captures three-dimensional images. Fazzalariet al. (1996)[23] showed that certain values obtained by fractal analysis may be subject to interference from some constant factors occurring in images captured close to the area selected for evaluation, which is a factor to be considered when fractal analysis is performed in panoramic radiographs, which may show image overlapping.

Trindade-Suedamet al. (2010)[24] reported the testing of various materials by fractal analysis in rabbits after maxillary sinus grafting, in which they obtained higher mean values than those reported in our study, however the species evaluated by these authors differedfrom those in the present study, which may justify the divergence between the values found. Kozakiewiczet al. (2013)[25] used FD of periapical radiographs to evaluate the effectiveness of different bone replacement materials used for filling alveolar defects, the authors observed that there was variation in the repair patterns of different materials, over the course of the time interval of observation, showing that there is an individual pattern of tissue remodeling. The fractal analysis of this study showed higher values for native bone compared with biomaterials used in bone defects, the data being similar to those found in our work, when we obtained higher FD values in panoramic radiographs in the region of native bone when compared with those of the materials tested. Several authors[26,27]have reported that there was no statistically significant difference between several biomaterials associated with L-PRF. Moreover, they reported that the combination of materials with L-PRF provided an improvement in healing, in a shorter time of bone maturation. These findings corroborated those of our study, because when the use of Osteogen® + L-PRF in T1 was compared with Osteogen®by CBCT in T1, there was no statistically significant difference; moreover, there was no statistically significant difference when these biomaterials were compared in T2. According to Rodrigues (2016),[28] the FD values were between 1 and 2, with 1 corresponding to the value of a structure that fills nothing or practically nothing of a predetermined fixed space. When the fractal dimension corresponds to 2, this biological structure occupies the entire available space of a region within a predetermined area. High values of fractal dimension, close to 2, indicate more complex bone structures; values closer to 1 represent less complex structures. In our study, the FA values in the CBCT of the biomaterials evaluated were closer to 1 in T2 than in T1, suggesting that in T2, the structures were less complex and that the graft was replaced by bone, with repair and healing.

In the study by Kato et al. (2019),[29] the authors reported the lack of standardization for the selection of ROI and definition of FA. These studies should be better delineated so that, as was done in our study, the following data are provided: image details, such as resolution, size, location and selection of bone tissue evaluation standards. In the ImageJ™ program, which was used in the present study to calculate fractal analysis, there is no possibility of calibrating the removal of noise from images by using the despeckle tool, which can reduce the quality of the image and the analysis made, especially in panoramic radiographs, which already have the limitation of a two-dimensional examination with low resolution and greater overlapping of structures.[18] In the imaging techniques tested in the present study to assess bone structure in sinus graft materials, it was assumed that there were different patterns of bone remodeling after maxillary sinus elevation surgery. Fractal analysis proved to be an easy method to perform and of the type that allowed evaluation of the bone structure. However, standardization of the fractal analysis methodology is necessary, in order to allow reliable results to be obtained, and make it possible to obtain an effective preoperative analysis.

Conflict of Interest: The authors declares that there is no conflict of interest regarding the publication of this article.

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