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# COMPARATIVE STUDY OF FRACTAL IMAGES, OBTAINED FROM REGIONS OF MAXILLA NATIVE BONE AND MICRO CT OF SINUS GRAFTS

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**ABSTRACT**

***Objectives:*** To evaluate and compare the structure of bone organization of sinus grafts in the repair period of 180 days by means of cone-beam computed tomography (CBCT) of fractalimages and specimens evaluated by Micro CT. ***Materials and Methods:*** A prospective, randomized clinical study was conducted on a sample of 20 patients with 40 maxillary. After this period, a specimen from each graft (Osteogen® + L-PRF and Osteogen®) was obtained at the time of implant placement for analysis of the Micro CT, and fractal analysisof the images of the Micro CT and the CBCTs. These images were also compared to native bone. ***Results:*** In the comparison of the values of the fractal analysis between Osteogen® in the CBCT and in the analysis by Micro CT, a statistically significant difference was observed ($p < 0.05$), as well as between Osteogen® + L-PRF in the CBCT and in the analysis by Micro CT. When compared with native bone, a statistically significant difference was also observed ($p < 0.05$). ***Conclusion:*** The statistical difference between fractal analysis of tomographic images, grafts, as well as Micro CT images in relation to native bone presupposes different patterns of bone structures and sinus grafts.



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# INTRODUCTION

The loss of teeth and the subsequent lack of functional loading of the alveolar crest through the periodontal ligaments lead to alveolar atrophy, which combined with sinus pneumatization can result in a decrease in bone volume in the edentulous posterior maxilla to the point of preventing rehabilitation with prostheses supported by dental implants. Raising the floor of the maxillary sinus is a safe and predictable surgery to restore the bone volume necessary for implant placement (ARAÚJO; LINDHE, 2005; JOHNSON, 1969, TATUM *et al*., 1993; KIVOVICS *et al*., 2020). Different types of graft materials are used to increase bone volume in the maxillary sinus, for example, autografts, allografts, xenografts, alloplastic materials and growth factors. However, selecting the ideal graft material for bone augmentation in the maxillary sinus is still controversial (FILLINGHAM, Y.; JACOBS, 2016) The ideal graft material must be osteogenic, osteoinductive, osteoconductive and volumetrically stable to provide new bone formation that will allow for new bone formation the implant and implant osseointegration (OYAMA *et al*., 2004). Hydroxyapatite is a material of natural or synthetic origin, an osteoconductor whose constitution is based on the essential elements of human bone tissue: calcium and phosphate. Its use in surgeries that require bone neoformation, such as the lifting of paranasal sinuses, is already consolidated in the literature (MANGANO *et al*., 2007; KATTIMANI *et al*., 2014). There is no consensus in the literature as to the precise definition of the bone quality of the implant recipient bone. However, the term bone quality includes the degree of mineralization, cortical bone thickness and trabecular bone morphology (PAUWELS *et al*., 2015). The poor bone quality of the recipient bone of the dental implant is associated with less primary stability and higher rates of implant failure. The primary stability of the implant affects whether the physician opts for submerged or non-submerged implant placement protocols and determines the prosthetic loading time. Therefore, the evaluation of bone quality before placing the dental implant is essential (MERHEB *et al*., 2010; MISCH, 1990). Currently, many techniques are recommended to assess bone quality and characterize quantitatively structural changes in bone in places for future implant installation, such as: histological evaluation (ESPOSITO *et al*., 2014; CORBELLA *et al*., 2015), computed tomography (CT) (LÓPEZ VALENZUELA, C. *et al*. 2002), fractal analysis (FA) (MOLON *et al*., 2015) and Micro CT (FAJARDO RJ; MULLER R, 2001). Among them, fractal analysis was introduced as a precise, easily available and low-cost method (MOLON *et al*., 2015). According to Harris *et al.* (2012), computed tomography scans are the exams of choice for planning implant installation and among computed tomography scans, the Cone-Beam Computed Tomography (CBCT) is able to provide a three-dimensional image of the area, with low cost and employing low doses. radiation when compared to multslice tomography. Micro CT is capable of evaluating bone repair, the interface with biomaterial and the biocompatibility of bone substitutes, where the acquired images can be used for non-invasive quantitative morphometric analysis of regenerating bone (EFEOGLU *et al*., 2007), and it allows to evaluate the microstructure of biomaterial and neoformed bone tissue, in addition to quantifying the volume of the region of interest (CHAPPARD *et al*., 2010). The results of the analysis of bone formation after lifting the maxillary sinus using Micro CT, Kühl *et al*. (2010) indicated that Micro CT is a promising method for evaluating the three-dimensional system of grafts after maxillary sinus enlargement with autogenous bone and materials used as bone substitutes. Micro CT is a reproducible and efficient study method for high resolution hard tissue analysis. Kivovics *et al*., (2020) demonstrate that the morphological measurements by Micro CT correlate with the histomorphometric results, which is considered the gold standard for the evaluation of bone microarchitecture. Computed microtomography (Micro CT) is a reproducible and time-efficient method for studying high-resolution hard tissue specimens. Studies show that the morphological measurements by reconstruction with Micro CT correlate highly with the histomorphometric results, which is considered the gold standard for the evaluation of bone microarchitecture (CHAPPARD *et al*., 2005; MÜLLER *et al*., 1998). In comparison with conventional computed tomography (CT), cone-beam computed tomography (CBCT) allows the image of the jaw in high isotropic spatial resolution with low radiation dose (LUDLOW *et al*., 2015). Unlike conventional CTs, quantitative measurements of the gray value in the CBCT are unreliable and should generally be avoided (PAUWELS *et al*, 2015). Bone density measurements based on CBCT are inherently inaccurate because of beam configuration and flat panel detectors, artifacts and variations in scanning conditions. (KIVOVICS *et al*., 2020). In this context, the present study evaluated and compared fractal analysis by means of cone-beam computed tomography and Micro CT images of the sinus graft of Osteogen® and Osteogen® + L-PRF in the repair period of 180 days.

# MATERIALS AND METHODS

A clinical, experimental, analytical, prospective, randomized, controlled and blind study was performed for the analysis of the images, being approved by the Ethics and Research Committee of the institution (number 29277014.5.0000.5137). Patients were duly informed about the content and objectives of the research and were supported by the right to non-identification and privacy. Inclusion criteria were: patients aged ≥21 years who had bone remnants less than 4 mm in height, requiring bone graft for future implantation and who agreed 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, smoker, tests that did not show the full image of the maxillary sinus, tests that had the presence of technical artifacts that hinder the evaluation of the maxillary sinus, pathologies of the maxillary sinus or history of surgery of the maxillary sinus. To calculate the sample, 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). Partial edentulous patients totaled 13 and edentulous patients 7. Bilateral maxillary sinuses were randomly assigned to Osteogen® (Impladent, Ltd, Holliswood, NY) + L-PRF or the control (Osteogen®) immediately before surgery by computer draw.

**Work protocol**

**Analysis of the maxillary sinus:** Before the maxillary sinus lifting surgery, the L-PRF was prepared, for centrifuging the blood collected from the patient, the Fibrin® surgical protocol was used (OLIVEIRA LA *et al*., 2018) using the Montserrat Fibrinfuge 25® centrifuge (Zenith Lab Co®, Changzhou Jiangsu, China). Blood collection was performed with 8 glass tubes with 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 on cone beam tomographs, Carestream® CS 8100 Digital Panoramic and Cephalometric System© (East Carestream Company®, Rochester, New York, USA) to assess possible pre-local operatives of the maxillary sinus. Bone reconstruction took place in two stages. Stage two involved bone reconstruction, and was performed in the following steps: a) The surgical procedure for elevation of the maxillary sinus was performed by the same surgeon who acted according to Zenóbio *et al*. 2018; 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 were postoperative control and image acquisition: a) Postoperative clinical evaluation was performed at 7 days and 10 days (suture removal period), postoperative tomography was performed (10 days); b) The clinical reassessment was performed at 30, 60 and 90 days; c) Tomography and implant planning were done 180 days postoperatively, and a second measurement was obtained; d) The installation of an osseointegrated implant was performed in the area pre-defined by the pre-prosthetic surgical planning together with the collection of the specimen for analysis of the Micro CT (Figure 4).

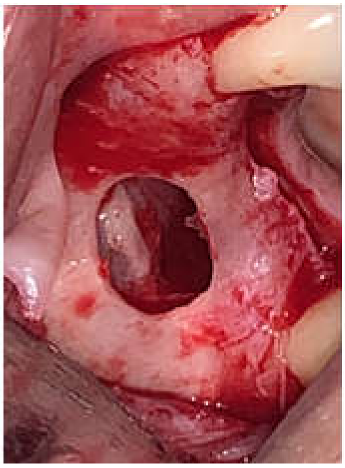

**Figure 1. Access to the maxillary sinus lateral wall**

**Micro CT analysis material:** For Micro CT analysis, samples were scanned using a compact Micro CT scanner (SkyScan 1174, Bruker Micro CT®, Belgium), with 50kV source voltage, 800µA source current and 10 pixel pixel size, 03µm. A 0.5mm Al filter was used. The samples were fixed on a stage that rotated 180 ° with images acquired every 0.7 °. The acquired shadow projections (16-bit TIFF format) were subsequently reconstructed into 2D slices using the NRecon® software interface (v.1.7.4.6, Skyscan, Bruker Micro CT®, Belgium). Quantitative analysis were performed using the CTAn® software (v.1.18.8.0, Bruker Micro CT®, Belgium) and the CTVox® software (v.3.3.0, Skyscan, Bruker Micro CT®, Belgium) was used for volumetric visualization 3D.

**Figure 2: Insertion of biomaterials in the maxillary sinus**

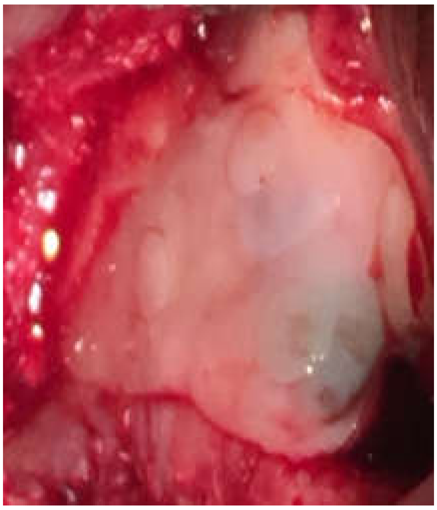

**Figure 3. L-PRF membrane interposition in the lateral access of the sinus after placing the material**

**Figure 4. Sample of the material sent for reading from the Micro CT**

After the 180-day period of bone repair of sinus grafts, and at the time of installation of the osseointegrated implants, a specimen was removed for analysis (Figures 4 and 5) on the Bruker® micro tomography (Kartuizersweg 3B 2550 Kontich, Belgium) and collected with a 3mm Herta® (RibeirãoPreto - SP - Brazil) trephine drill on the side where the Osteogen® + L-PRF graft was performed and the control side of the Osteogen®, a cross-section was performed on the specimen to perform the analysis (Figure 6). The material was analyzed according to the following guidelines:

a-) analysis of the central region in sections of 0.7 mm x 0.7 mm x 0.7 mm
b-) analysis of the central region with a larger volume of 2 mm x 2 mm x 2 mm

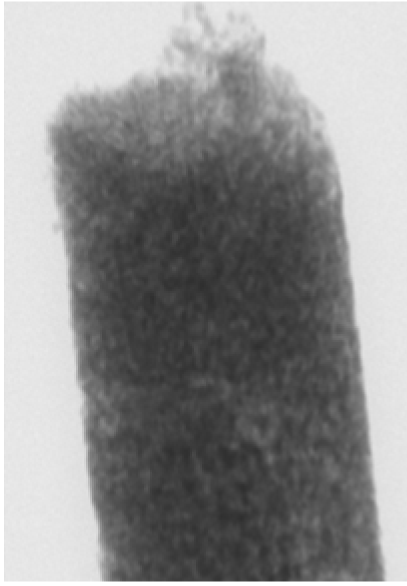

**Figure 5. Specimen analysis by Micro CT**

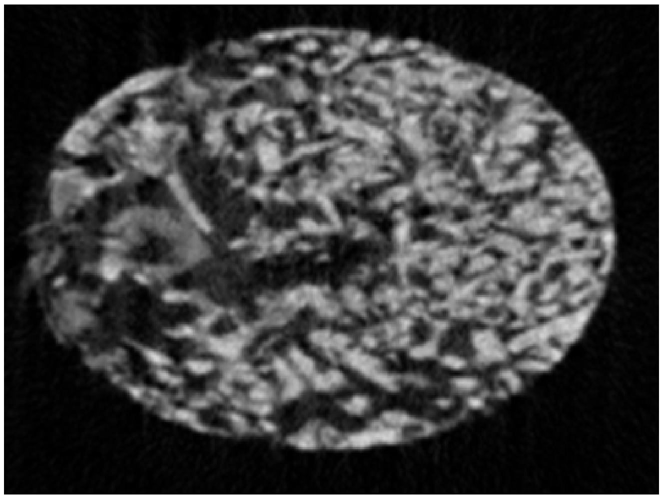

**Figure 6. Cross section for specimen evaluation**

**Table 1. Comparison of the mean values fractal analysis of L-PRF + OSTEOGEN®in cone bean computed tomography and Micro CT**

| | L-PRF + OSTEOGEN® CBCT | L-PRF + OSTEOGEN® Micro CT |
|---|---|---|
| Mean | 1.318* (a) | 1.638* (b) |
| Standard deviation | 0.090 | 0.140 |

(ab* $p<0.05$)

**Image acquisition and analysis of CBCT and Micro CT tomographs data:** Computed tomography scans were performed on cone-beam tomographs, 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 cuts 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. In the CBCT, the region of interest (ROI) was selected, delimiting the entire graft in the three planes (axial, sagittal and coronal).

**Table 2. Comparison of mean values fractal analysis of grafts and native bone in cone bean computed tomography and Micro CT**

| | L-PRF + OSTEOGEN® CBCT | L-PRF + OSTEOGEN® Micro CT | CANINE CBCT | TUBER CBCT |
|---|---|---|---|---|
| Mean | 1.318 * (a) | 1.638 * (b) | 1.343 *(c) | 1.339 *(d) |
| Standard deviation | 0.090 | 0.140 | 0.081 | 0.062 |

(abcd* p<0.05)

**Table 3. Comparison of mean values fractal graft analysis with Osteogen® and native bone in cone bean computed tomography and Micro CT)**

| | OSTEOGEN® CBCT | OSTEOGEN® Micro CT | CANINE CBCT | TUBER CBCT |
|---|---|---|---|---|
| Mean | 1.304 * (a) | 1.638 * (b) | 1.343 *(c) | 1.339 *(d) |
| Standard deviation | 0.083 | 0.123 | 0.081 | 0.062 |

(abcd* p<0.05)

**Table 4. Micro CT histomorphometric analysis values**

| | TV [$mm^3$] | BV [$mm^3$] | BV/TV [%] | BS [$mm^2$] | Tb.Th [μm] |
|---|---|---|---|---|---|
| LPRF + OSTEOGEN® | 0,248 | 0,122 | 44,865 | 6,014 | 85,209 |
| OSTEOGEN® | 0,307 | 0,123 | 40,598 | 5,258 | 101,110 |

TV Tissue volume, BV Bone volume, BV/TV Percent bone volume, BS Bone surface, Tb.Th Trabecular thickness

The areas were evaluated and standardized using a reference point located in the central region of the bone graft. To perform the native bone FA in the CBCT, the ROI of the maxilla tuber was selected in the central region of the posterior part of the maxilla, after the end of the maxillary sinus. In the canine pillar region, an area 3 mm above the canine's root apex was selected, when this or the first upper premolar was present. In their absence, the selected area 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. After calculating the fractal values in the CBCT, an average was performed between the values of the axial, sagittal and coronal planes. The manipulation of the software and the analysis of the CBCT and Micro CT measurements were performed by an experienced and trained radiologist. The observer manually delimited the cut areas filled by the grafts in the initial and final images of the CBCT and the final image of the Micro CT. Different parameters were examined for sample bone evaluation, which included tissue volume (TV), bone volume (BV), ratio of sample bone volume to sample volume (BV / TV), bone surface (BS), thickness trabecular (Tb.Th) and fractal dimension (FD) were analyzed.

**Statistical analysis:** The data were initially submitted to the F normality test (to assess normality), which demonstrated its normal distribution. For the comparative analysis between groups and between materials, after the 180-day period of CBCT and Micro CT, the *Anova* test with *Tukey* was used. *Student's t* test was used to assess the existence of differences in materials. The level of significance adopted was 5%. The analyzes were performed using the *GraphPad Prism 6.05 software (GraphPad Software*, San Diego, California, USA).

# RESULTS

In the comparison of the fractal analysis of the CBCT between Osteogen®+ L-PRF (1.318) and evaluated by Micro CT (1.638), a statistically significant difference was observed (p <0.05) (Table 1). When comparing the fractal analysis of the CBCT between Osteogen® + L-PRF (1.318), Micro CT (1.638) and native bone (canine pillar: 1.343 and maxillary tuber: 1.339), there is a statistically significant difference (p <0.05) (Table 2) and when used alone in the CBCT, Osteogen® (1.304) also showed a statistically significant difference (p <0.05) compared with Osteogen® from Micro CT (1.638) (Table 3). The mean values of the morphometric results of the Micro CT were Osteogen® Tissue Volume (TV) 0.248$mm^3$ and the Osteogen® side 0.307$mm^3$.

In our study, the bone volume (BV) on the Osteogen® + L-PRF side was 0.122$mm^3$ and on the Osteogen® side 0.123mm3. The percentage of bone volume / tissue volume (BV / TV) found in our study was on average 44.865% on the Osteogen® + L-PRF side and on the Osteogen® side 40.598%. The mean bone surface value (BS) was 6.014$mm^2$ for the Osteogen® + L-PRF side and 5.258$mm^2$ for the Osteogen® side. The trabecular thickness value (Tb.Th) was on average 85.209μm and 101.110μm for the Osteogen® + L-PRF and Osteogen® side, respectively (Table 4).

# DISCUSSION

The use of non-invasive methods to assess and design the best time for the installation of osseointegrated implants is still not well understood in the current literature, so in the present study we made the comparison via fractal analysis of the CBCT and Micro CT images of sinus grafts and CBCT images of native bone to determine the possibility of using these methods as preoperative parameters for surgical planning in implantology. Gauthier *et al*. (2005) concluded in their studies that Micro CT allows obtaining an accurate qualitative description of internal bone growth and performing quantitative analysis. Thus, in the present study, the choice of using the images of the Micro CT analysis is justified by the advantage in determining the structural characteristics and bone growth measures by means of images in three dimensions of this evaluation (GAUTHIER, 2005; HO, 2006), being non-invasive and fast, obtaining high-resolution three-dimensional images, which characterizes and measures the three-dimensional properties of biomaterial and bone tissue regeneration (JONES *et al*., 2004; PORTER *et al*., 2005). According to Panmekiate *et al*. (2015) very high or very low values obtained for analysis of bone microarchitecture using CBCT may not be correct, due to the way they are acquired and analyzed, this being one of the variables observed in the present study, where the values were different when comparing the fractal analysis of CBCT and Micro CT, with values of 1.318 being found in Osteogen® + L-PRF in CBCT and 1.638 in Micro CT, with a statistically significant difference (p <0.05) and 1.304 in Osteogen® at CBCT and 1.638 at Micro CT, also with a statistically significant difference (p <0.05). Thomsen *et al*. (2005) after comparing morphometric data from classical histology and Micro CT for spongybone, concluded that Micro CT could be used as a substitute for histological analysis in the evaluation of bone structures due to the high correlation of the evaluation of morphometric data. The present study obtained comparative results of the fractal analysis of Micro CT between the materials Osteogen® + L-PRF (1.638) and Osteogen® (1.638) in the reparational period of 180 days and comparing with native bone structure of Tuber - spongy bone - (1.339) and Canine - cortical bone - (1.343) of the CBCT, the results demonstrate a greater organization in the area with the graft materials in relation to the native bone. Fazzalari *et al*. (1996) demonstrated that certain values obtained by fractal analysis may suffer interference from some constant factors in images close to the area (ROI) selected for evaluation, being a factor to be considered when fractal analysis in CBCT. The area determined in the present study for CBCT and Micro CT was defined as the total area (volume) of the grafted material in an attempt to avoid this type of interference. Trisi *et al*. (2006) compared Micro CT images with classic histological samples of autogenous bone and bioactive glass bone substitute (Biogran®) after maxillary sinus enlargement in three patients. The reported results were that the bone and the substitute material were clearly distinguishable in all samples due to the lower density of the bone versus the substitute material, thus, the values of the Micro CT of the total bone volume were reliable in comparison with the histomorphometry. In the images obtained in the present study by Micro CT, changes in the materials were observed at the level of fractal analysis, where it can be seen that at 180 days in the tested biomaterials Osteogen® and Osteogen® + L-PRF we obtained values of 1.638 in both, being these values differ from those of the native bone of the tuber (1.339) and area of the canine (1.343) of the CBCT.

In the present study, a statistically significant difference was observed between the biomaterials tested, in the FA of the Micro CT images at 180 days, which presented the value of 1.638 in the Micro CT and the native bone in the CBCT 1.343 for canine abutment and 1.339 for the tuber of the jaw. This result can be justified by the finding in the study by Iida *et al*. (2020) who compared bone neoformation data measured histologically and microtomographically in maxillary sinuses enlarged with a xenograft of greater density and greater mineral content compared to natural bone. Statistically significant differences between the two measurement methods were observed after 2 and 8 weeks of healing, the new bone increased by about 21% in histological analyzes while, in Micro CT, it increased by only about 4%. In the same period, the proportion of the xenograft decreased from 51.6 ± 4.9 to 45.3 ± 3.3% in histological analyzes whereas in Micro CT the xenograft increased in percentages. Thus, he reported in his results that histological analyzes and Micro CT produced different results when a xenograft with higher density and higher mineral content compared to natural bone was used. According to Sener; Cinarcik and Baksi (2015), the FD determined from CBCT and Micro CT have greater potential to evaluate bone microarchitecture and allow the complete characterization of the trabecular network, this being our comparison factor, in our study, between the native bone structure . In the present study, fractal analysis using CBCT and Micro CT images of the sinus graft of Osteogen® and Osteogen® + L-PRF obtained values of 1.638 in Micro CT for both biomaterials and in CBCT obtained for Osteogen® + L-PRF 1.318 and for Osteogen® 1.304. Chappard *et al*. (2005), when studying bone measurements comparing histomorphometry and Micro CT, reported that Micro CT provided similar results at the 3D level than those obtained by histomorphometry performed on histological sections of the sample. In addition, they observed that Micro CT provides reliable morphometric data and in less time than histomorphometry, allowing a non-destructive bone examination before pathological analysis. One of the factors that should be considered as a limitation and next research stage of the present study is to compare histomorphometric data and bone tissue evaluation through histological analysis with results from fractal analysis.

One of the most studied parameters in studies on osseointegration in maxillary edges is BV / TV (Nkenke *et al*., 2003; Bodic *et al*., 2012), which represents the percentage of BV in relation to TV within the bone nucleus. Tb.Th represents the average thickness of individual trabecula and Tb.Sp shows the space between the trabecula within a sample. Nakata *et al*. (2016) evaluated bone remodeling histologically after maxillary sinus enlargement with porous hydroxyapatite alloplasts in 3 non-smoking patients, and bone architecture and graft residues were assessed by Micro CT. The results found by Nakata *et al*. (2016) in relation to TV, both native bone + hydroxyapatite and only hydroxyapatite were on average 10.944mm$^3$, while in our study the L-PRF + Osteogen® side was 0.248mm$^3$ and the Osteogen® side 0.307mm$^3$. The BV found by Nakata *et al*. (2016) averaged 3.148 mm$^3$ for native bone + hydroxyapatite and 0.115mm$^3$ only hydroxyapatite, while in our study the L-PRF + Osteogen® side was 0.122mm$^3$ and the Osteogen® side 0.123mm$^3$. The BV / TV found in our study was on average 44.865% on the L-PRF + Osteogen® side and on the Osteogen® side 40.598%, while Nakata *et al*. (2016) found an average of 29.433% and 1.233%, for native bone + hydroxyapatite and only hydroxyapatite, respectively. The average BS of our study was 6.014mm$^2$ for the L-PRF + Osteogen® side and 5.258mm$^2$ for Osteogen®, and by Nakata *et al*. (2016) was on average 80.349mm$^2$ for native bone + hydroxyapatite and 10.009mm$^2$ only hydroxyapatite, and its Tb.Th was on average 80.35urn and 22.481urn for native bone + hydroxyapatite and only hydroxyapatite, respectively, and our study found in mean values of 85.209μm and 101.110μm for the L-PRF + Osteogen® and Osteogen® side, respectively.As in the present study, Nakata *et al*. (2016) also performed bone remodeling analysis 180 days after maxillary sinus lifting and grafting placement surgery, but there were differences between the values found, which can be justified by the difference in the size of the selected ROI, which was 3mm in the study by Nakata *et al*. (2016), and 2 mm in our study. 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 using the despeckle tool, which can reduce the quality of the image and the analysis performed.

# CONCLUSION

The average values of the fractal analysis provided by the sinus grafts tested in the CBCT and in the Micro CT in comparison with native bone determined patterns of bone organization different from the values found in the literature, in radiographic and non-tomographic methods, even when compared to native bone. Thus, studies should refer to new fractal values regarding the use of tomographic images and Micro CT for real accuracy to determine the pattern of bone tissue remodeling in sinus grafts after the 180-day repair period.

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