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Evaluation of L-PRF combined with deproteinized bovine bone mineral for early implant placement after maxillary sinus augmentation: A randomized clinical trial.

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L-PRF and DBBM for early implant placement

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All authors agree to be responsible for all aspects of the study in ensuring that questions related to the accuracy or integrity of any parts of the study are appropriately investigated and resolved.

ABSTRACT

Purpose: To investigate the effectiveness of adding leukocyte and platelet rich fibrin (L-PRF) to deproteinized bovine bone mineral (DBBM) for early implant placement after maxillary sinus augmentation.

Material and Methods: Twelve patients requiring two-stage bilateral maxillary sinus augmentation were enrolled to the study. The elevated sinus cavities were randomly grafted with DBBM + L-PRF (test) or DBBM alone (control) in a split-mouth design. Implants were placed in the augmented sites after 4 months in the test group and 8 months in the control group. Bone biopsies were collected during implant placement for histomorphometric evaluation. Resonance frequency analysis was performed immediately after implant placement in both groups. Cone-beam CT was obtained pre- and postoperatively for evaluation of graft volume changes.

Results: Both procedures were effective for maxillary sinus augmentation. CBCT analysis did not reveal differences in graft volume between test and control group at any of the evaluated time points ($P > 0.05$). Histological evaluation demonstrated increased percentage of newly formed bone for the test group (44.58 ± 13.9%) compared to the control group (30.02 ± 8.42%) ($P=0.0087$). The amount of residual graft in the control group was significantly higher (13.75 ± 9.99%) than in the test group (3.59 ± 4.22) ($P=0.0111$). Implant stability quotient (ISQ) immediately after implant placement was significantly higher in the control group (75.13 ± 5.69) compared to the test group (60.9 ± 9.35) ($P=0.0003$). The ISQ values at loading did not differ between the groups ($P=0.8587$). Implant survival rate was 100% for both groups.

Conclusion: The addition of L-PRF to the DBBM into the maxillary sinus allowed early implant placement (4 months) with increased new bone formation than DBBM alone after 8 months of healing.

KEYWORDS: Alveolar bone; bone substitutes, cone-beam computed tomography, dental implants, maxillary sinus; platelet-rich plasma; sinus floor augmentation.

1 INTRODUCTION

The posterior region of the maxilla is associated with several challenges regarding successful dental implant rehabilitation due to reduced bone quality and ridge resorption caused by sinus pneumatization after tooth loss.[1] Different approaches for treatment of severely resorbed posterior maxilla have been performed using onlay bone grafts,[2] interpositional grafts after maxillary osteotomy[3] and sinus augmentation procedures.[4] Sinus lifting with the lateral technique, initially described by Boyne and James[5] and established by Tatum,[6] is the most commonly used approach to augment the maxillary sinus, which allow the installation of dental implants in the severely resorbed posterior maxilla. The implant survival rate, placed immediately or in two-stage approach, after sinus lifting is higher than 95% according to a recent systematic review with meta-analysis[7] and with several retrospective studies,[8-11] over a period of at least 5-years of follow-up, and the complication rate are minimal. Furthermore, different graft materials, such as autogenous,[12] xenogenous,[13] alloplastic and the combination of bone grafts and growth factors can be safely used during maxillary sinus augmentation.[14, 15] Indeed, this technique has become a routine treatment modality over the years with highly predictability and effectiveness.

Demineralized bovine bone mineral (DBBM) is a biocompatible material with osteoconductive properties[16] that has been frequently used for maxillary sinus augmentation. This biomaterial possesses several advantages compared to the autogenous bone, such as unlimited availability, low resorption rate and reduced morbidity to the

patient. As well as the autogenous bone, the risk of immunological rejection of DBBM is minimum and a high clinical success rate has been observed with its use.[9] DBBM act as a scaffold allowing osteogenic cell migration from the maxillary sinus to the graft particles permitting the apposition of de novo bone formation.[14, 17, 18] However, DBBM compared to the autogenous bone, lacks the osteogenic and osteoinductive properties, and the maturation of this type of material may take up to 8 months[19] before implants can be safely installed in maxillary sinus, which might be considered a disadvantage of this material. To overcome this concern, several studies have investigated the addition of growth factors to grafting material aiming at enhance bone neoformation and accelerates graft maturation.[1, 18-32]

Leukocyte and platelet rich fibrin (L-PRF), first described by Dohan et al.[33] is an autogenous biomaterial containing several growth factors.[1, 18, 19, 25] L-PRF, a second-generation of platelet concentrates, is basically made of concentrated autologous platelets as well as leukocytes and cytokines.[34] It has been demonstrated that L-PRF activates the vascular system by promoting angiogenesis, and also by releasing several growth factors involved in soft and hard tissue healing.[30, 35] Additionally, previous studies demonstrated that L-PRF is capable of inducing bone regeneration[22, 36] and fibroblast proliferation,[37] thus improving and accelerating tissue healing,[35] and enhancing implant stability.[30] To obtain L-PRF, the patient's blood is collected through venipuncture without anticoagulant or additives, and is immediately centrifuged. After processing, a natural fibrin clot is localized in the middle of the tube. The fibrin clot can than be compressed to obtain a membrane. From each fibrin clot, one L-PRF membrane is obtained. The L-PRF membranes can either be cut into small pieces and mixed with DBBM or autogenous bone, or be used as a membrane[18] It is a safe, and cost-effective technique to improve repair following surgery.[24] Due to its dense fibrin fiber network with strong mechanical

characteristics,[8] it can act as a scaffold for a number of cell types and provide support for mesenchymal stem cells.[38]

Since growth factors play an important role in tissue regeneration, the present study aimed to investigate the effects of L-PRF to accelerate bone formation after maxillary sinus augmentation combined with DBBM as the graft material, and the outcomes of early placement of dental implants after sinus augmentation. The study was set up to test the hypothesis that the addition of L-PRF to DBBM would enhance new bone formation and graft maturation, thereby allowing faster implant placement. Graft volume dimensional changes, bone and soft tissue characteristics and implant stability were investigated using cone-beam computed tomography (CBCT), histology and RFA analysis, respectively.

2 MATERIALS AND METHODS

This prospective, double-blinded, randomized-controlled clinical trial was accompanied in accordance with the Consolidated Standards of Reporting Trials (CONSORT) Statement.[39] The study protocol was approved by the Ethical Committee on Human Research (CAAE #41357514.5.0000.5416) before patient enrolment and was registered in the Brazilian Registry of Clinical Trials (ReBEC - RBR-95m73t). All patients were informed about the surgical procedures and written informed consent was obtained from all subjects.

This randomized clinical trial was designed as a split-mouth study. Bilateral maxillary sinuses were randomly assigned to either the test (DBBM + L-PRF) or the control (DBBM) group.

2.1 Sample size calculation

The minimum sample size calculation for this study was calculated using G*Power 3.1.[40] Considering a standard deviation of 5.0% for the primary outcome (percentage of newly formed bone) and a mean difference of 5.5% between the test and control groups, an effect size of 1.1 was obtained. The effect size was calculated based on previous data.[41] Using this effect size with a given alpha level of 0.05, a power of 80% and an allocation ratio of 1, a sample size of 11 patients per group was calculated.[28]

2.2 Patients

Patients were recruited at the Department of Diagnosis and Surgery of the School of Dentistry at Araraquara – UNESP from December 2014 through May 2015. The inclusion criteria were as follows: patients who required bilaterally sinus floor augmentation for implant installation in the posterior maxillary region with residual bone height of < 4 mm (based on CBCT). The exclusion criteria were: compromised general healthy condition, smokers or ex-smokers, alcohol and drug abusers, irradiated patients, pregnancy, therapies with bisphosphonates and immunosupressives,[42] blood platelet disorders, chronic sinusitis, patients suffering from any pathology in the maxillary sinus, and uncontrolled diabetes.[43]

2.3 PRF preparation

The L-PRF membranes were prepared according to the technique described by Dohan et al.[33] Venous blood samples were taken at the beginning of the procedure using vacutainers (BD, Franklin Lakes, NJ, USA) and centrifuged for 10 minutes at 300 g (3000 rpm) (Kasvi K14-0815, Curitiba, PR, Brazil). After centrifugation, a natural fibrin clot was present in the middle of the tube, between the acellular plasma at the top and the red

corpuscle at the bottom. Each fibrin clot was removed from the tube and placed in a metal box (Xpression, Intra-lock System, Sao Paulo, Brazil). The fluids present in the fibrin clots were squeezed out to obtain L-PRF membranes.

2.4 Sinus augmentation procedure

The surgical procedures were performed as described earlier.[12, 13, 44] Briefly, patients received local anesthesia (Articaine 4% and epinephrine 1:100,000; DFL, Rio de Janeiro, RJ, Brazil) followed by a mid-crestal and vertical releasing incisions along the residual alveolar bone to expose the lateral sinus wall. A lateral window approach was performed to access the sinus wall using diamond round bur. The surgical access respected the position of implant placement planning and the maxillary sinus anatomy.

After carefully sinus membrane elevation, the control side was filled with small particles (0.25-1 mm) of DBBM (Bio-Oss®, Geistlich Pharma AG, Wolhusen, Switzerland), while the test side was filled with a mixture of L-PRF membranes and DBBM (0.25-1 mm). For each membrane of L-PRF (4-5 mL) cut in few fragments, 0.5g of Bio-Oss® was mixed. The graft materials were gently compacted at the sinus cavity. A resorbable collagen membrane (Bio-Gide®, Geistlich Pharma AG, Wolhusen, Switzerland) was used to cover the lateral window after graft placement, and then the soft tissues were sutured.

The surgical procedures were performed by a single experienced surgeon (ECP). The bilateral maxillary sinuses were randomly assigned by means of a computer-generated randomization list to either the test (DBBM + L-PRF) or the control group (DBBM). The numbers were sealed in opaque envelopes by a person not involved in the study. The surgeon was blinded to the graft material applied to each sinus cavity before graft implantation. After sinus membrane elevation, the envelope containing the treatment

indication was opened by the surgeon assistant. The patients were not informed of the assigned materials.

After surgery, the patients received postoperative instructions for appropriate oral hygiene control and treatment with an oral antibiotic (amoxicillin, 500 mg three times a day for a week), an oral anti-inflammatory (nimesulide, 100 mg twice a day for 5 days), and an analgesic (paracetamol, 750 mg every 6 hours for 2 days). They were advised to rinse their mouth with chlorhexidine (0.2%) daily for 14 days. The sutures were removed seven days after the surgical procedure, and the area was not subjected to any direct loading during the entire bone regeneration phase.

2.5 Implant placement

After four months (test group) and eight months (control) of healing, dental implants were placed in both augmented maxillary sinuses. The implants (TitamaxTi EX ACQUA, Neodent, Curitiba, Brazil) were placed according to the manufacturer`s protocol.

2.6 Radiographic analysis

Each patient underwent four CBCT scans (SCANORA® 3Dx, Soredex, Tuusula, Finland): preoperative (T0), immediately after maxillary sinus augmentation (T1), after four months (T2 for test group) and eight months (T2 for control group) post-maxillary sinus augmentation. The following parameters were used for all scans: 10 mAs, 90 kVp and a 20 s scan time using a nine-inch field of view (FOV). The preoperative scan was used to evaluate the sinus anatomy and the residual alveolar ridge for implant placement in a two-stage surgery. The scan immediately after augmentation was performed seven days after the surgery. The four-month post augmentation scan was performed in the test group before implant placement. The eight-month scan was performed in the control

group prior to implant surgery. The raw data of the scans were reconstructed and exported in DICOM file format and imported into CBCT interpretation software (Planmeca Romexis 3D, Planmeca Oy, Helsinki, Finland).

The volumetric measurements (in cm^3) of the grafts were taken using 1 mm sagittal sections by assessing the differential hyperdensity color of the images. The volumetric dimensions were automatically calculated by the Planmeca software and represented in cubic centimeters. One maxillofacial radiologist experienced with CBCT that had received prior training in Planmeca software performed all volumetric measurements in a standardized manner.

2.7 Histology and histomorphometric analysis

During implant site preparation, bone biopsies were harvested from the maxillary sinus with the aid of a trephine drill (3i Implant Innovations, Florida, FL, USA.) (3.0 mm in diameter and 15 mm in length). Two-bone biopsies cylinders were obtained per patient (one for each sinus) and the trephine sites were used for implant placement. The biopsy involved the residual maxillary bone and augmented sinus (bone graft), and preparation depth was defined from the planned implant length. After biopsies removal, the implants were placed in all patients.

Biopsies were immediately fixed in 10% buffered formaldehyde solution for 3 days and then processed, as described by de Molon et al. (2015).[12] Serial sections of 6-μm thickness were obtained from each specimen parallel to the long axis of the cylindrical core using an automatic microtome (Jung Supercut 2065, Leica Instruments GmbH, Heidelberg, Germany). The sections were mounted on slides and stained with hematoxylin and eosin (H/E). The histological evaluation was performed using an optical microscope (Diastar; Leica microsystems GmbH, Wetzlar, Germany) at 100-x

magnification. Images were selected and transferred to a computer display through a digital camera attached to an optical microscope (DFC-300-FX, Leica microsystems GmbH, Wetzlar, Germany) allowing for histomorphometric analysis.

Two blinded examiners performed the histomorphometric analysis. The digital images of histological slides were imported and analyzed using the system Image J for Windows (Image J 1.45; Wayne Rasband, National Institutes of Health, USA). The histomorphometric analysis was performed to measure newly formed bone, residual bone graft and fibrous tissue after four months (test group) or eight months (control group) of sinus augmentation. The measurements were also expressed as percentages of the total measured area.

2.8 Resonance frequency analysis

The implant stability quotient (ISQ) was measured with a RFA device (Osstell; Integration Diagnostics, Gothenburg, Sweden). SmartPegs were used to measure the implant stability immediately after implant placement, and at the moment of prosthetic loading in both groups. The measurements were performed in two directions, buccal-lingual and mesio-distal, and the mean values were used.[13] The ISQ measurements were performed in a standardized manner by one experienced examiner, who was masked to the treatment protocol.

2.9 Statistical analysis

Statistical analysis was performed using GraphPad Prism software (version 6.0, GraphPad Software, Inc., La Jolla, CA, USA). All data are expressed as the mean ± the standard error of the mean (SEM). All data were submitted to the D'Agostino & Pearson normality test to assess the normality of the data distribution. Histomorphometric, volumetric and ISQ measurements were compared within each patient (test versus

control) using paired t-tests. The differences between the graft volume at time T1 and T2 for both groups were also analyzed using paired t-tests. Differences were considered significant at $P < 0.05$.

3 RESULTS

3.1 Patient characteristics

A total of 12 patients were enrolled in this study (Fig. 1); 6 patients were male and 6 were female, and they ranged in age from 43 to 63 years (with mean age of 54.17 ± 6.95 years). Eight patients were partially edentulous and 4 patients were totally edentulous in the upper jaw (Table 1). 38 implants were placed in the augmented sites, 19 in the control group and 19 in the test group (Table 1). During the healing period, the patients did not wear any provisional removable dentures. All implants had the same diameter (4 mm) and length (11 mm). No complications were observed during or after the sinus augmentation procedure. No perforation of the sinus membrane was observed, and no complications such as migration of the graft material or opening of wound edges were observed in the test or control groups. The survival rate of the implants placed in the augmented maxillary sinus area 12 months after loading was 100% for both groups.

3.2 Cone-beam computed tomography analysis

To evaluate the repeatability of the measurements, the Pearson correlation coefficient (r) was calculated in the differences of the three measurements in five samples with an r-value of 0.995 ($P<0.001$). A total of 24 CBCT scans were taken from 12 patients to evaluate graft volume changes after maxillary sinus augmentation.

The results of the CBCT analysis are described in Table 2. The mean graft volume observed immediately after sinus augmentation (T1) for the test (1.68 ± 0.42 cm^3) and

control (1.46 ± 0.53 cm^3) groups was not statistically different. After four months of healing (T2), the mean graft volume in the test group lowered to 1.10 ± 0.25 cm^3. A reduction in the mean graft volume was also observed in the control group after eight months of healing (T2=0.91 ± 0.35 cm^3). Differences in graft volume between the two time points in each group were statistically significant (P<0.0001 for the test and P=0.0002 for the control group). After four months of healing, a significant reduction in the graft volume could be observed for the test group (33.14 ± 10.74%). Similar result was noted for the control group after eight months of healing (36.71 ± 15.81%). The comparison of the rate of resorption between the groups did not show statistically significant difference. In both groups, the augmented bone presented adequate volume for implant placement.

3.3 Bone histomorphometry analysis

Immediately before implant placement, bone biopsies were collected from the maxillary sinus on both sides for a descriptive and histomorphometric analysis (Fig. 2). Our findings revealed a statistically significant increase (P=0.0083) in the amount of newly formed bone between the test group (2.35 ± 0.73 mm^2) and the control group (1.58 ± 0.44 mm^2). Consequently, the percentage of new bone formation was significantly increased (P=0.0087) in the test group (44.58 ± 13.9%) compared to the control group (30.02 ± 8.42%) (Table 3).

A significant higher (P=0.0104) amount of residual graft material was found in the control group (0.71 ± 0.51 mm^2) than in the test group (0.18 ± 0.22 mm^2). As expected, the percentage of bone graft in the control group (13.75 ± 9.99%) was also greater (P=0.0111) than in the test group (3.59 ± 4.22%). Regarding the amount of fibrous tissue in the maxillary sinus cavity, the data demonstrated no significant differences between

groups. The control group showed a slight increase in the amount of fibrous tissue (1.61 ± 0.65 mm^2) compared to the test group (1.40 ± 0.59 mm^2). Similarly, the percentage of fibrous tissue was also not different between groups (30.64 ± 12.46% and 26.60 ± 11.13% for the control and test groups, respectively) (Table 3).

3.4 Resonance frequency analysis

ISQ was measured immediately after implant placement four and eight months after sinus augmentation for the test and control group, respectively. Moreover, at the time of implant loading, RFA was measured again. All implants evaluated in both groups demonstrated high ISQ values. The results showed significantly higher (P=0.0003) ISQ values after implant placement in the control group (75.13 ± 5.69) than in the test group (60.90 ± 9.35) (Table 4). However, at the time of implant loading a significant increase in ISQ was observed in the test group (60.9 ± 9.35 to 76.08 ± 5.86, P=0.0014). No differences were observed between groups at loading (Table 4).

4 DISCUSSION

In this study, we hypothesized that the addition of L-PRF to DBBM would enhance new bone formation and regeneration, allowing faster implant placement after maxillary sinus augmentation. We used different methodologies to evaluate graft volume changes, bone characteristics and implant stability, i.e. CBCT, histology and RFA analyses. Our results demonstrate that there was no differences related to graft volume changes between groups immediately after graft placement and after the healing period of four and eight months for the test and control group, respectively. However, a significant decrease in graft volume between the two time points (baseline and after healing time) was observed for both groups. Interestingly, the amount of newly formed bone was significantly increased

when L-PRF was added to the graft, compared to the control group. As expected, the amount of residual graft was significantly higher in the control group compared to the test group, and no differences were observed between groups regarding the amount of fibrous tissue. Finally, ISQ values were significantly higher in the control group compared to the test group; however for both groups, safe ISQ values were found, which enabled implant osseointegration even as soon as four months after surgery in the L-PRF group. Moreover, ISQ at loading for the test group showed significant increase, which was similar to the control group.

The ideal time for implant placement is dependent of several aspects mainly related to the recipient area, socket dimension, bone quality and quantity, and time required for partial or complete tissue healing.[45] Autogenous bone possesses several advantages because of its osteogenic, osteoinductive and osteoconductive properties.[13] However, the limited availability of this tissue and morbidity during graft removal are drawbacks related to this approach.[46] Consequently, the use of biomaterials to fill the maxillary sinus is necessary. In this context, DBBM is a widely used biomaterial due to its similarity to the human bone, and high rate of clinical success.[47] Nevertheless, DBBM lacks the osteogenic properties acting mainly as a scaffold for new bone formation. Importantly, the maturation of this type of material may take up to 8 months[19] before implants can be safely placed in maxillary sinus, which might be considered a disadvantage of this material. To mitigate this shortcoming, the addition of growth factors, particularly L-PRF, to the graft material has been suggested as an alternative approach to increase bone formation, enhance implant stability, favor osseointegration, and accelerate tissue maturation and healing.[1, 18, 21, 22, 28-30, 34, 35, 44, 45, 47-52]

CBCT is considered a reliable technique for the 3D visualization of graft volume changes after sinus augmentation. In this study, we performed three different CBCTs at

different time points: immediately after the bone graft, and after four and eight months of healing. Our results did not reveal any statistically significant differences between the groups regarding graft volume changes. Comparing the measured volumes one week after sinus augmentation with those measures after four (test group) or eight (control group) months of healing, a significant volume loss was observed in both groups. The mean percentage of volume loss was 33.14% for the test group (DBBM + L-PRF) and 36.71% for the control group (DBBM only). These variations in the volumetric changes were also observed in previous studies.[53-55] Accordingly, previous studies have demonstrated that the mean volume reduction of DBBM in the maxillary sinus ranges from 15.2 to 21.5%.[37] The disparities found in the literature in regard to the dimensional changes could be influenced by the residual alveolar ridge, maxillary sinus anatomy, amount of grafted material into the sinus, and compression force during graft placement.[53, 56, 57]

The maturation time for implant placement after sinus augmentation with DBBM may take several weeks to form new bone, or close to eight months of healing for safe implant installation.[19, 58] This fact could be closely related, despite of the graft characteristics, to the graft volume. Consequently, a larger DBBM graft volume requires more time before implant loading.[59] In the current study, the amount of DBBM was reduced in the test group since L-PRF was added to the graft material. The area (0.71 ± 0.51 mm^2 control group; 0.18 ± 0.22 mm^2 test group) and percentage (13.75 ± 9.99% control group and 3.59 ± 4.22% for test group) of graft material was statistically higher in the control group compared to the test group. Furthermore, L-PRF contains dense fibrin fiber network that helps to avoid the small particles of DBBM from dispersing. This means that less amount of graft material is needed to fill the maxillary sinus to obtain a sufficient vertical bone height for appropriate implant length installation.[59] Moreover, the fibrin fiber has a positive impact on handling and facilitates adhesion to the bone defect

walls.[60] Paralleling recent observations,[59] our findings indicate that L-PRF can act as a delivery system for DBBM particles during sinus lifting. On the other hand, Nizan et al.[28] did not observe any additional benefit of L-PRF on bone formation after six months of sinus augmentation. Corroborating these clinical findings, recent studies showed similar results when adding L-PRF to the graft materials into the maxillary sinus, i.e., no beneficial effect on regeneration and new bone formation.[61, 62] In these studies, the long graft-healing time did not allow them to verify potential effect of L-PRF in accelerating bone formation.

To investigate the implant stability, RFA analysis was performed immediately after implant placement for both groups by measuring the ISQ as a function of stiffness of the bone-implant interface.[13, 63] This measurement is affected by innumerous factors, such as the healing time, bone quality and density, firmness of the fixation, degree of osseointegration, hardness of the bone, and the implant height above the alveolar crest.[64-66] It was previously pointed out that ISQ values ranging from 57 to 82 denote appropriate implant stability and a complete process of osseointegration.[67] In the current study, the control group showed statistically higher ISQ values compared to the test group (75.13 ± 5.69; and 60.90 ± 9.35 for the control and test group, respectively). This outcome might be attributed to the difference in the healing time between both groups. According to a previous study,[31] ISQ values after sinus augmentation utilizing L-PRF progressively increase over time, meaning that the time for implant healing play a crucial role for increased secondary implant stability. This was confirmed in our studies because the ISQ values at loading demonstrated a significant increase in the test group compared to the initial value immediately at implant placement (60.90 ± 9.35 and 76.08 ± 5.86). Recent observations have demonstrated that the addition of L-PRF improves implant stability and allows for faster osseointegration.[52] The difference between our study and their study

might be accounted by the implant site (posterior vs. anterior), differences in bone quality, and healing period (baseline, one and four weeks of healing). The present data suggest that a site undergoing sinus augmentation with DBBM + L-PRF can offer sufficient implant stability, decreasing the necessary time for bone graft maturation and allowing earlier implant placement.

The outcomes of the current investigation proved the null hypothesis. It was demonstrated that the addition of L-PRF into the maxillary sinus resulted in increased amount of newly formed bone compared to the control group. Moreover, L-PRF seems to accelerate bone graft maturation allowing early implant placement after sinus augmentation.

An important consideration should be mentioned when interpreting the present outcomes. One limitation of this investigation is that the groups (test and control) were compared to a different time points (eight months for the control and four months for the test group). With this experimental design, it was not possible to confirm if there would be more new bone in the control group if we had gone in earlier to place the implants. Current literature[28, 62] shown that a healing period of at least six months is necessary before implants can be safely placed in the grafted sinus with Bio-Oss, and for this reason our studies have focused in two different times points of evaluation. The platelet quantification in total blood was not performed. Therefore, we could not determine whether the patient variability platelets concentration may have influenced our findings.

In addition, an interesting caveat that our studies did not address is whether the bone graft maturation would have changed if the dimensions of the maxillary sinus and the amount of biomaterial used were measured during the surgical procedures. We believe that the amount of biomaterial in the control group was higher compared to the test group, since the L-PRF membranes increase the graft volume. Thus, less bone graft material was

placed in the test group compared to the control. For all those reasons, further randomized clinical trials are warranted before definitive conclusions about the use of L-PRF can be drawn. Our data, despite the inherent limitations related, pointed out to important aspects and encourage earlier intervention in the maxillary sinus grafted with DBBM + L-PRF allowing fast graft maturation and implant placement.

5 CONCLUSION

Taken together, our data demonstrated that the addition of L-PRF to the DBBM graft increased the newly formed bone after 4 months of healing. The residual graft material was statistically lower in the test group, which might have influenced the early maturation of the bone graft. Collectively, our findings suggest that L-PRF lead to faster bone graft maturation, and this outcome might suggest sinus augmentation with a shorter healing time before implant placement.

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FIGURE LEGENDS

Figure 1. Flowchart of the experimental design.

Figure 2. Representative histological section from group DBBM + L-PRF **(a)** and only DBBM **(b)**. NB is native bone; NFL corresponds to the newly formed bone; B is biomaterial; and ST is soft tissue. Yellow color corresponds to the newly formed bone; red is biomaterial; Blue is native bone.

TABLE LEGENDS

Table 1. Patient demographics and clinical data.

Table 2. Graft volume measurements immediately after sinus augmentation (T1) and after a healing period of four months for the test (DBBM + L-PRF) and 8 months for the control (DBBM) group (T2).
the control group).

Table 3. Histomorphometric results after four months of healing for the test (DBBM + L-PRF) and 8 months for the control (DBBM) group.

Table 4. Implant stability quotient (ISQ) measured by means of resonance frequency analysis immediately after implant placement and at implant loading in both groups.