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Histological and Immunohistochemical Analysis of a Nanobiomaterial in a Maxillary Sinus Lift Surgery: A Case Report

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ABSTRACT

The objective of this study was to histologically and immunologically analyze the level of bone substitution and the presence of new blood vessels in a nanobiomaterial composed of hydroxyapatite and BTCP in a maxillary sinus lift surgery. A case of a maxillary sinus lift was investigated. The patient had a 1.0 mm bone remnant on the left side and 2 grams of nanobiomaterial (80% hydroxyapatite and 20% β -tricalcium phosphate) were grafted into the pneumatized sinus. After 6 months, during dental implant placement surgery, grafted bone samples were collected with 4 mm trephines. These samples were sent to the laboratory for analysis where they were stained with masson's trichrome and immuno stained with osteonectin and osteopontin. After 6 months of bone regeneration a result of 14 mm of bone gain was reached, the analyzes in masson's trichrome showed an excellent gain of newly formed bone, in addition to a very high percentage of blood vessels. In the immunostaining, a very large number of osteoblasts and osteoclasts was observed, signaling an excellent osteoconduction and osteoinduction of the studied nanobiomaterial. The maxillary sinus lift surgery with nanobiomaterial provided a very favorable bone height and thickness gain, as well as the high vascularity and cellularity, which enabled the patient's oral rehabilitation with osseointegrated dental implants and permanent ceramic prostheses.

Keywords: Bone graft; Dental implants; Maxillary sinus lift surgery; hydroxyapatite.

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INTRODUCTION

Tooth loss results in horizontal and vertical resorption of the residual bone; thus, pneumatization of the maxillary sinus can develop simultaneously after tooth extraction in the posterior maxilla region.¹ To overcome this problem, several solutions have been proposed, such as the placement of angled implants in the anterior maxilla, short implants, zygomatic implants and maxillary sinus lift surgery combined with biomaterials or autogenous bone².

Maxillary sinus lift surgery followed by dental implant placement is considered a standard technique for the recovery of masticatory function in the posterior region of the atrophic maxilla³. However, the elevation of the Schneiderian membrane in the maxillary sinus lift surgery is a very delicate procedure; thus, the perforation of this membrane occurs frequently (10–55%).⁴

Tatum, in 1977⁵, presented the side-window osteotomy (or hinge osteotomy) for the first time. This approach was later modified and published by Boyne and James in 1980⁶. Later, Summers RB *et al*⁷ proposed an indirect sinus lift technique through the alveolar bone crest (the maxillary sinus floor) that is a minimally invasive approach. However, there must be at least 4 mm of the remaining bone.⁸

Many bone substitute materials have been used for maxillary sinus lift surgeries, including autografts, allografts, xenografts and alloplasts Brum, IS *et al*⁹. Although an autograft is considered the gold standard material for bone graft due to its osteoconductive, osteoinductive and osteogenic properties, local donor area morbidity and limited quantities are disadvantages of the usage of this material.¹⁰ In addition, allografts and xenografts have the risk of transmitting diseases and may cause religious concerns.¹¹ Many researchers have been looking for alternative biomaterials to overcome these drawbacks. Nanobiomaterials have been considered the best options because they have the same characteristics as the autogenous bone: osteoconductive and osteoinductive potential.¹²

Currently the use of L-PRF has been associated with biomaterials¹³, but so far, the combination of L-PRF with autologous bone and/or biomaterials does not seem to provide additional beneficial effects for maxillary sinus lift in terms of dental implant survival rate¹⁴ or in terms of dental implant stability, bone height, bone density, bone and tissue laminar volume, bone graft resorption, angiogenesis and tissue healing when compared to the use of only autologous bone or biomaterial¹⁵.

Osteocalcin (OC) and osteopontin (OPN) are the two main factors that regulate the biological and mechanical functions of bone.¹⁶ OC and OPN are produced during bone formation, at the end of the mineralization process, and control directly and / or indirectly the mass, mineral size and orientation of bone growth.¹⁷

Osteonectin, a component of the bone matrix, plays an important role on cell-matrix interaction in tissue remodeling. As well as osteoblasts, endothelial cells and fibroblasts secrete osteonectin.¹⁸ As active osteoblasts have osteonectin, this bone component was considered a marker for bone formation, but its function is still unclear.¹⁹ Osteocalcin is the most abundant non-collagen substance. It is a calcium-binding protein in mineralized tissues and is mainly synthesized by osteoblasts and fibroblasts in bone resorption and mineralization.²⁰ It is shown that serum osteocalcin level is a marker of bone turnover and a diagnostic marker of metabolic bone diseases. Osteopontin is also an important component of the bone. However, studies have shown that osteopontin is present during early bone formation and osteopontin levels are the highest in pre-osteoblastic cells. This protein plays an important role in bone resorption and mineralization²¹. In addition, OPN facilitates osteoclasts fixation and guides mineral deposition, influencing the shape and size of the mineral crystal.²²

The objective of this study was to clinically analyze the gain in height and thickness, and, in the laboratory, observe the histological and immunological behavior of a nanobiomaterial composed of 80% hydroxyapatite and 20% BTCP (Blue Bone®) that was grafted in a maxillary sinus lift surgery. The prosthetic rehabilitation in this case was performed with cone morse dental implants (Avantt®) and definitive porcelain crowns.

MATERIALS AND METHOD

Patient

This clinical case study followed a patient who needed a maxillary sinus lift surgery using biomaterial, posterior dental implant placement and prosthetic rehabilitation. There was a need to raise the maxillary sinus floor on the left side because the remaining bone was only 1.0 mm high (Figure 1).

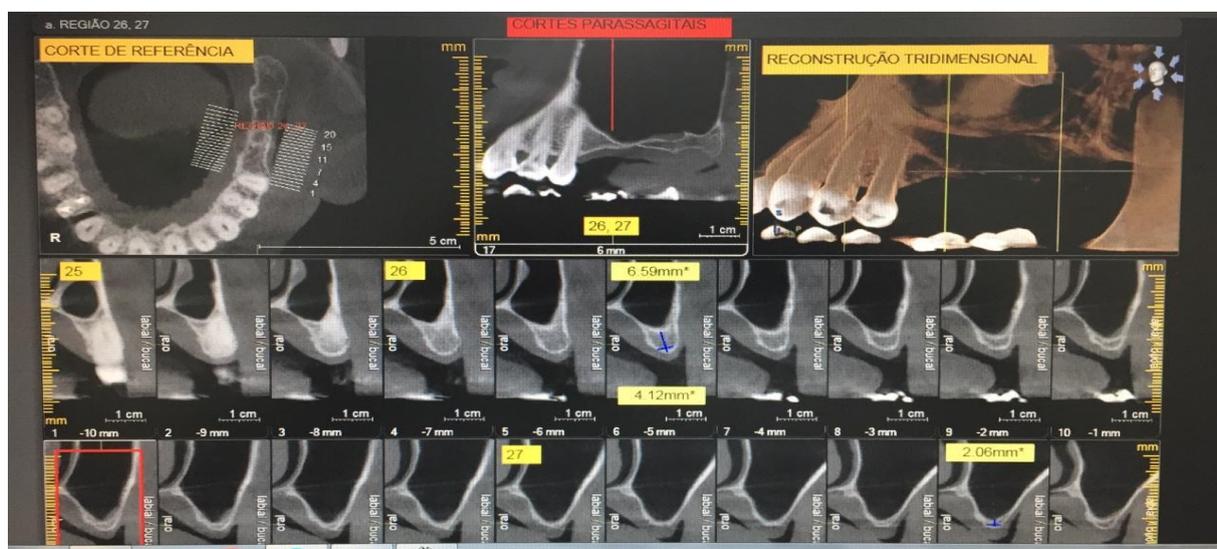


Figure 1: Pneumatized maxillary sinus with 1.0 mm of remaining bone.

The elevation of the maxillary sinus was performed through the lateral approach.

After the healing period, two dental implants were placed on the maxilla left side (where the sinus was lifted) and one on the maxilla right side. In the mandible, two dental implants were placed on the right side.

All of these dental implants were rehabilitated with porcelain prostheses. The procedures were performed at the Department of Oral and Maxillofacial Surgery of the Aeronautical Odontoclinic of Brasília (OABR) in January 2019. The total follow-up period was one year and five months.

Biomaterial and Implant

The nanobiomaterial that was used to graft the maxillary sinus was the Blue Bone® from Regener® (Curitiba, Brazil). It is composed of 80% hydroxyapatite and 20% BTCP. The dental implants that were placed were the cone morse Avantt model from Systhex® (Curitiba, Brazil).

Operative, postoperative and prosthetic rehabilitation

One hour before the maxillary sinus lift surgery, the patient received antibiotics (4x 500 mg amoxicillin capsules, totaling 2 grams).

At the time of the surgery, the patient did a one minute mouthwash with a 0.12% chlorhexidine solution. After this, local anesthesia was administered (2% Lidocaine with 1: 100,000 adrenaline).

Subsequently, mid-level and relief incisions were performed for the release of the full thickness mucoperiosteal flap in order to expose the buccal alveolar bone where the lateral window was opened with a number 6 spherical bur.

The sinus membrane was lifted with a set of curettes for the maxillary sinus and 2 grams of Blue Bone® were grafted. To close and seal the lateral window a collagen membrane was utilized and the flap was repositioned and sutured. There was no immediate implant placement. After this procedure, the patient underwent periapical and panoramic radiographs and a cone beam computed tomography.

In the following 14 days, the patient took Clavulin® 785 mg every 12 hours, and recommendations such as "do not blow your nose" or "when sneezing, open your mouth" were given. There was no postoperative infection or maxillary sinusitis.

The dental implants were placed 6 months after the maxillary sinus lift surgery. At this time, during the drilling, samples from the grafted area were collected using 4 mm diameter trephines.

These samples were conditioned in 10% formaldehyde and sent to the anatomopathology department of the University of Pernambuco, Brazil where they were included in Paraplast® and analyzed. The ceramic prostheses over the implants were placed 6 months after an osseointegration period. (Figure 6)

Histology

For the histological analysis, the masson's trichrome staining was used, and the following protocol was followed: the heads were subjected to decalcification using 7% EDTA (ethylenediamine tetraacetic acid) and 0.1 M phosphate buffer (pH 7.4), for approximately 40 days. The samples were washed in distilled water, dehydrated with ethanol (70%, 95% and 100%), clarified with dimethylbenzene and embedded in paraffin blocks (Paraplast®) at 65 °C. Ten 7 µm thick serial coronal sections were obtained with a microtome (LEICA Co. Ltd., Nussloch, Germany). The sections were mounted on glass slides and subjected to the hematoxylin-eosin staining technique. The images were then obtained with objective lenses × 20 and × 40 of a microscopic device (Carl Zeiss - Axiolab) and recorded with a digital camera (JVC TK-1270 Color Video Camera).

Immunohistochemistry

A standard immunohistochemistry method with streptavidin-biotin was used for osteonectin and osteopontin. For immunohistochemical staining, 4 µm sections were cut from paraffin blocks and collected on glass slides previously silanized with the solution of 3-aminopropytriethoxy-silane (Sigma Chemical CO, St Louis, Mo / USA) at 20% in absolute alcohol. For the procedure, they were deparaffinized in xylo at 60 °C and then, when at room temperature, they were finally hydrated in decreasing concentrations of ethanol.

In order to remove the pigment from the fixative, the cuts were immersed in a solution prepared with 10% sodium hydroxide and absolute alcohol (1:1) for 5 minutes. Subsequently, they were washed in distilled water, then in a solution of 30% hydrogen peroxide and methanol (1:1) before the endogenous peroxidase block. The cuts passed through distilled water two times and, subsequently, were immersed in two pH 7.4 Tris solution baths for 2 minutes each. The sections were incubated in monoclonal antibody IgG1 (anti OPN Santa Cruz Biotechnology; 21742, CA, USA), in a concentration of 1:1000 for 3 hours at room temperature inside a humid chamber. Later they were washed in a pH 7,4 Tris solution.

Detection was achieved under similar conditions with goat antibodies (Dako, EnVision + Dual Link System-HRP, CA, USA), for 30 minutes. Then they were immersed in two pH 7.4 Tris solution baths for 5 minutes each. For development, the chromogen diaminobenzidine (DAB, 3,3'-diaminobenzidine, Dako) was used following the manufacturer's guidelines. After washing in a pH 7.4 Tris solution, the sections were stained with Mayer's hematoxylin. When finished the counterstaining, the cuts were dehydrated in increasing concentrations of ethanol and diaphanized in xylol. The slides were mounted on Entellan (EMS, Hatfield, PA, USA), analyzed with a microscopic device (Carl Zeiss - Axiolab) and recorded with a digital camera (JVC TK-1270 Color Video Camera).

RESULTS AND DISCUSSION

After the 6 months period of graft healing in the left maxillary sinus region, a gain of 14 mm in bone height and an average of 5 mm in thickness were observed through a CBCT (figure 2).

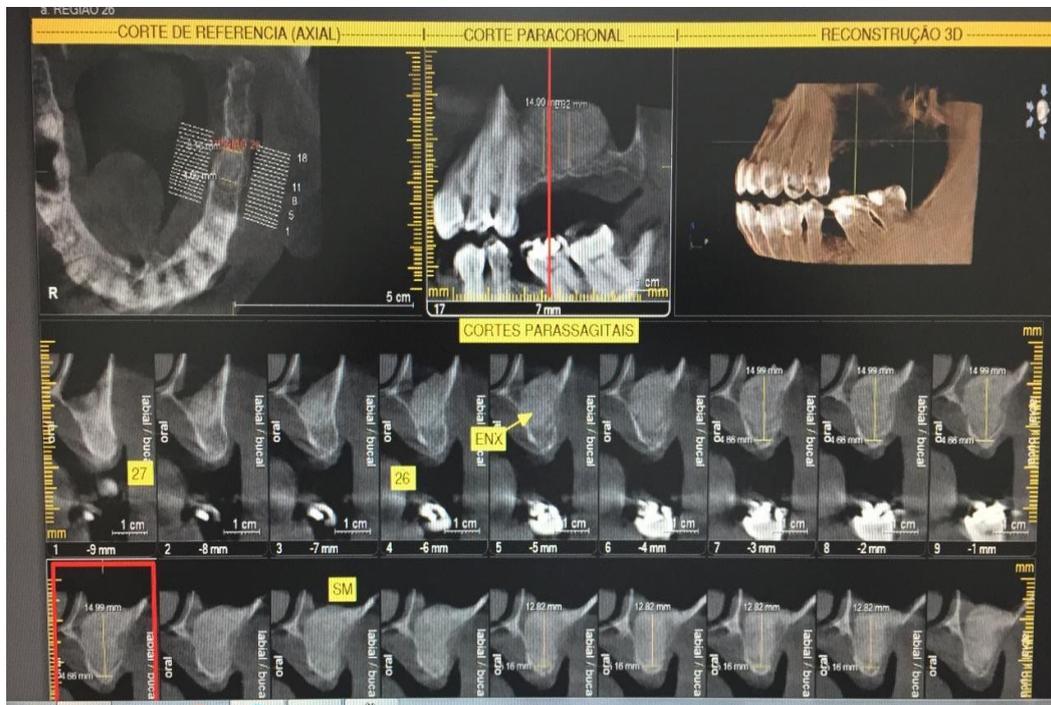


Figure 2: We can observe a 14 mm gain in bone in the region grafted with Blue Bone. Using a trephine, 4mm thick fragments were removed for histological and immunological analysis (figures 3 and 4).



Figure 3: It is possible to observe a large amount of bleeding flowing from the areas of collection.

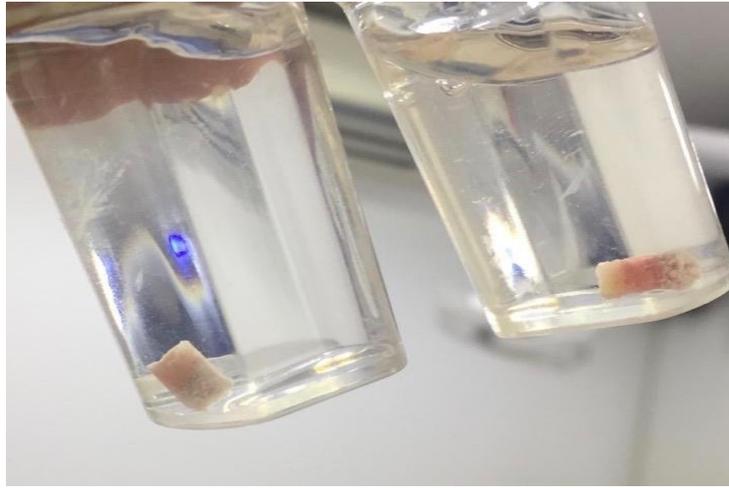


Figure 4: Samples conditioned in formalin 10%.

Then, Avantt® model cone morse dental implants were placed in the maxilla and in the mandible (figure 5).



Figure 5: Panoramic radiograph after implant placement.



Figure 6: Porcelain prosthesis final photo

Histology

Histological analysis were stained with the masson's trichrome. This technique has the characteristic of staining the presence of collagen in blue, which refers to the presence of newly formed bone, and in red the presence of loose connective tissue (figures 7 and 8).

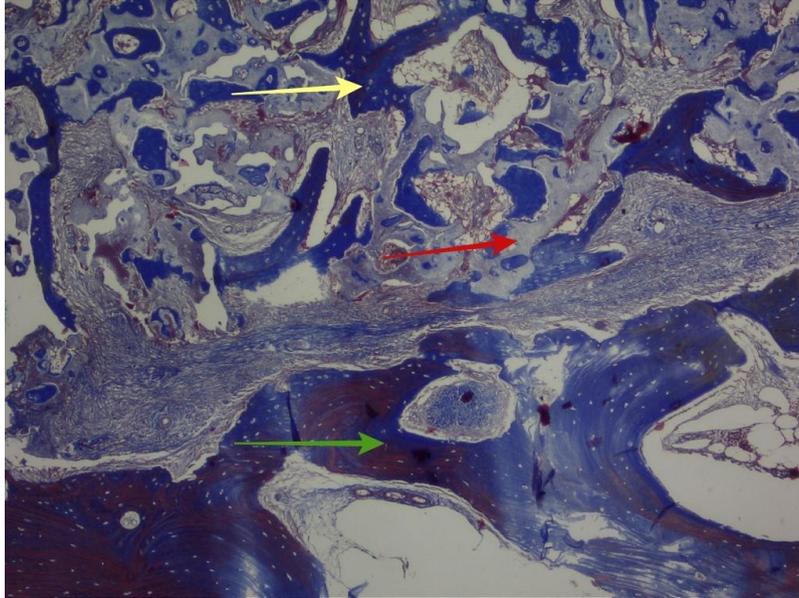


Figure 7: The green arrow points at the bone beam of 1mm of the remaining bone of the maxillary sinus. The red arrow points at a transition tissue with small areas of newly formed bone. The yellow arrow points at newly formed bone with viable osteocytes at the region of the maxillary sinus.

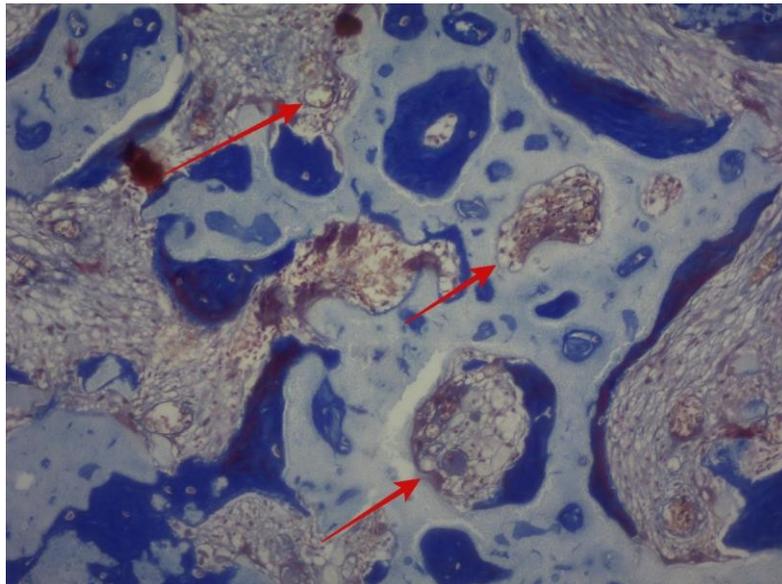


Figure 8: The red arrows point at the formation of blood vessels in the graft region.

Immunohistochemistry

In osteonectin, we can see large areas stained in brown that determine the presence of osteoblastic and fibroblast activity which justifies the great presence of bone matrix found in the nichrome trichrome. (Figure 9)

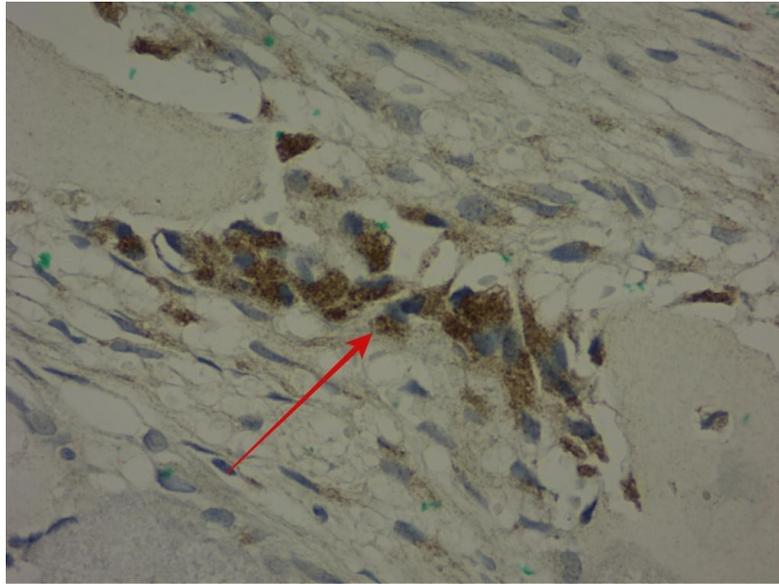


Figure 9: Osteoblastic and fibroblast activity

In osteopontin, we can see large areas stained in brown that determine the presence of osteoclastic activity which well is represented in masson's trichrome, where we can observe a large presence of newly formed bone matrix. Therefore, areas with little osteopontin marking certainly did not present large mineralized areas. (Figure 10)

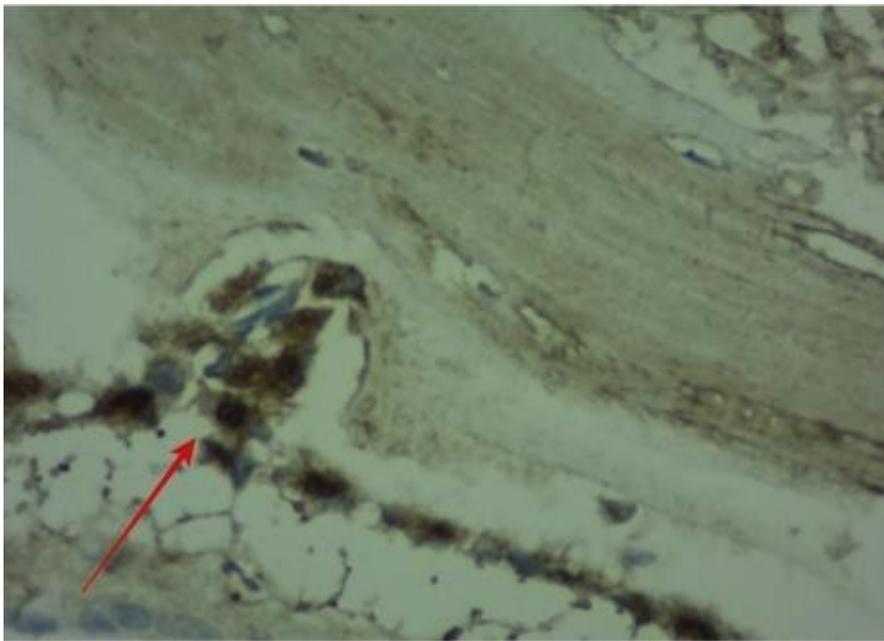


Figure 10: Osteoclastic activity

DISCUSSION

Helfrich, M et al²³ randomized and controlled prospective trial of divided mouth compared the increase in percentage of newly formed bone in the sinus floor with deproteinized bovine bone mineral with or without the addition of enamel matrix derivative after 6 months of healing. After this 6 month graft healing period, at the same time of implant placement, bone biopsies were collected for histomorphometric analysis that revealed a significantly greater amount of newly formed bone in the test group compared to the control group (22.6% and 15.5%, $p =$

0.033, respectively)²⁴. No significant differences were found in the remaining amount of graft or connective tissue. In the study here presented, we can also observe a gain in newly formed bone above the percentage found in the literature.

Maxillary sinus lift surgery has proven to be an effective methodology for gaining height and thickness in a deficient maxillary sinus²⁵. However, there are many techniques that a professional can choose from. Historically, lateral window or ridge osteotome approaches have been the most discussed techniques²⁶

In the study here presented, the lateral window technique used did not present any surgical complications. This success rate is well described in the literature.

In a systematic review²⁷ only randomized controlled trials and controlled clinical trials were selected. These studies have shown considerable variation of biomaterials and cellular techniques that were used. Only a few studies have demonstrated the potential for regeneration in sinus lift grafting and claim that more randomized clinical trials are needed to obtain more accurate results. This corroborates with other authors who claim that biomaterials do not currently have osteoinductive potential. However, many other articles have been studying the osteoinductive characteristics of biomaterials of different origins, and claim that it is possible that biomaterials present this osteoinductive potential.^{28,29,30}

Liu R *et al*³¹ In a meta-analysis, the effectiveness of L-PRF in maxillary sinus lift surgery were evaluated. For this study, only randomized controlled studies: clinical, radiographic and histomorphometric results were considered.³² The percentage of contact area between the newly formed bone with only the bone substitute did not show any statistically significant difference in comparison with the newly formed bone with the bone substitute plus the L-PRF.³³ The percentages of new bone formed in relation to the soft tissue area were higher in the L-PRF group but were not significantly different. It was concluded that there were no statistical differences in the rate of survival and formation of new bone.^{34, 35}

Wallace SS *et al*³⁶ In a analysis of forty-three studies, 3 randomized controlled clinical trials, 5 controlled trials, 12 case reports and 23 retrospective analyzes were identified. The meta-regression was performed to determine the variable effect of block graft versus particulate graft techniques, implant surface, graft material and the use of a membrane over the lateral window. The survival rate of implants placed on grafted sinus by the lateral window technique varied between 61.7% and 100%, with an average survival rate of 91.8%. For the lateral window technique, the implant survival rates reported in this systematic review, favorably backs up the reported survival rates for implants placed in non-grafted posterior maxillas.

M Esposito *et al*³⁷ have concluded that short implants (5 mm) can be successfully loaded into the jawbone with a residual height of 4 to 6 mm but their long-term prognosis is unknown. Bone substitutes can be used successfully as substitutes for autogenous bone. Even though the

ideal is not to use the lateral window technique if the sinus floor bone remnant is less than 3mm.³⁸ This comes in contrast to our case where the bone remnant was 1mm (Fig 1) and did not present any complications. However more clinical cases will certainly be needed in order to have a satisfactory statistical basis.

Osteopontin is directly related to the presence of a more resistant bone matrix, although this mechanism is not well documented in the literature.³⁹ In another study, new information was presented on the cellular behavior of osteopontin.

Foster BL *et al*⁴⁰ in *in vitro* studies revealed that Osteopontin regulates the initiation and growth of hydroxyapatite crystals, and that this inhibitory capacity depends on the calcium binding properties of the polysaccharide sequence and serine phosphorylation. It is important to note that *in vivo* studies have confirmed that osteopontin regulates mineral growth in physiological and pathological situations.⁴¹

Immunocytochemically, osteonectin can be observed in active osteoblasts, osteoprogenitor cells, young or aged osteocytes. Inactive osteocytes also contain this protein, suggesting that osteonectin is a marker of osteoblastic functional differentiation of bone cells.⁴² In another study that evaluated the metabolic rate of osteonectics in contact with nanohydroxyapatite, reported that there was no statistical difference in the rate of bone growth, indicating that ON had no antiproliferative effect on cell culture, as opposed to that observed by other authors.^{43,44}

CONCLUSION:

We can conclude that the maxillary sinus lifting technique was a successful alternative for the oral rehabilitation of this case. Histologically we can observe the presence of newly formed bone at a very satisfactory level, and great presence of blood vessels. In relation to **immunohistochemical**, we can see osteoclast and osteoblast activities showing that the grafting material has the necessary characteristics to be indicated as a substitute for autogenous bone.

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