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# Histologic and Histomorphometric Assessment of Sinus Floor Augmentation with Synthetic Hydroxyapatite (Nanobone®) Alone or in Combination with Platelet-rich Fibrin

## Clinical Trial

**Luís Filipe Santos Francisco**

**Dissertação** conducente ao **Grau de Mestre** em **Medicina Dentária (Ciclo Integrado)**

—

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**Clinical trial**

Trabalho realizado sob a Orientação do
**Professor Doutor Marco Paulo de Araújo Infante da Câmara**

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# Acknowledgements

Firstly, I would like to thank my parents Manuel Francisco and Natividade Santos for giving me a taste for dentistry and for always supporting me in my decisions and in the most challenging moments of the course. I would also like to thank my brothers for being always a family and a companion.

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To all the CESPU teaching staff and to CESPU, thank you for giving me the opportunity to obtain the knowledge I have today and to study at this University CAMPUS.

# Resumo

**Introdução:** Foram descritas várias técnicas para o aumento do enxerto do seio maxilar, incluindo a técnica da janela lateral e a abordagem da crista com osteótomos ou osseodensificação. A fibrina rica em plaquetas tem sido utilizada em procedimentos de elevação do seio maxilar devido à sua capacidade de acelerar a cicatrização de tecidos. **Objetivo:** O objetivo deste estudo foi avaliar o potencial da PRF em combinação com o Nanobone® para melhorar a regeneração óssea na elevação do assoalho do seio maxilar com a técnica da janela lateral. **Materiais e Métodos:** Dos 50 indivíduos triados numa consulta de avaliação pré-operatória e intervencionados entre janeiro e dezembro de 2023, apenas seis pacientes que cumpriam os critérios de inclusão do estudo consentiram em participar. Num estudo de boca dividida, foram realizadas doze cirurgias de enxerto sinusal. **Resultados:** As observações revelam que, para o grupo teste, há 27,5 ±4,9% de novo osso vital, 23,0 ±3,7% de partículas ósseas inertes e 49,4 ± 2,8% de tecido conjuntivo. Entretanto, no grupo de controlo, há 19,5 ± 3,0% de osso vital novo, 23,4 ± 5,7% de partículas ósseas inertes e 57,0 ± 3,5% de tecido conjuntivo. **Conclusões:** Os resultados indicam fortemente que a mistura de PRF líquido com Nanobone® não tem uma influência negativa na quantidade de formação óssea viável, e parece aumentar ligeiramente a quantidade de nova formação óssea e revascularização em comparação com o uso único de Nanobone®.

Palavras-chave: fibrina rica em plaquetas; biomateriais; aumento do seio maxilar; aumento do assoalho do seio maxilar.

# Abstract

**Background:** Several techniques have been described for maxillary sinus graft augmentation, including the lateral window technique and crestal approach with osteotomes or osseodensification. Platelet-rich fibrin has been used in maxillary sinus lift procedures due to its ability to accelerate soft and hard tissue healing. **Objective:** The aim of this study was to evaluate the potential of PRF in combination with the synthetic hydroxyapatite Nanobone® to enhance bone regeneration in sinus floor elevation with the lateral window technique. **Materials and Methods:** Out of 50 individuals screened in a preoperative assessment visit from the CESPU - Famalicão clinical unit and intervened upon between January 2023 and December 2023, only six patients who met the study's inclusion criteria consented to participate. In a split-mouth study, twelve sinus graft surgeries were carried out. **Results:** Observations reveal that, for the test group (Nanobone®/PRF), there is 27.5 ±4.9% new vital bone, 23.0 ±3.7% inert bone particles, and 49.4 ± 2.8% connective tissue. Meanwhile, for the control group (Nanobone®), there is 19.5 ± 3.0% new vital bone, 23.4 ± 5.7% inert bone particles, and 57.0 ± 3.5% connective tissue. **Conclusion:** The results strongly indicate that mixing liquid PRF with Nanobone® does not have a negative influence on the amount of viable bone formation, and it seems to slightly increase the amount of new bone formation and revascularization in sinus bone graft procedures with the lateral window technique compared to the single use of Nanobone®.

**Keywords:** platelet-rich fibrin; biomaterials; maxillary sinus augmentation; sinus floor augmentation.

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# List of abbreviations and acronyms

PRF – platelet-rich fibrin

DFDBA – demineralized freeze-dried bone allograft

M.I.C – Marco Infante da Câmara

M.F – Manuel Francisco

CBCT – Cone-beam computed tomography

# 1. Introduction

Dental implants are applied in oral rehabilitation of edentulous posterior maxillae. A prerequisite for implant placement is an adequate bone height and width (1).

Extraction of posterior maxillary teeth bone resorption can trigger significant bone loss in both vertical and horizontal dimensions, which may preclude implant placement.

Several techniques have been described for maxillary sinus graft augmentation such as the lateral window technique, and crestal approach with osteotomes or osseodensification (2–4). The main difference between the lateral window technique and crestal techniques is the type of bone graft used and immediate or delayed approach (1).

Knowledge of the anatomy of the maxillary sinus is essential for carrying out this surgical procedure, thus preventing possible complications from arising during sinus elevation.

Numerous grafting materials can be used in sinus graft procedures such as autogenous bone, demineralized freeze-dried bone allograft (DFDBA), synthetic alloplastic graft, xenograft, and growth factors (5). Although there is widespread debate on which is the ideal bone graft material, autologous bone is considered the gold standard due to its osteogenic, osteoinduction and osteoconduction properties (5). The healing period for maxillary sinus augmentation using autologous bone graft is approximately 6 months, which is the time needed for bone integration and creeping substitution (1).

An alternative to autogenous bone grafting is the use of alloplastic, xenograft or synthetic biomaterials. The bone maturation of these materials takes approximately 9 months (1).

Platelet-rich fibrin (PRF) has been used in maxillary sinus lift procedures due to its ability to repair and regenerate bone. PRF is an autologous platelet concentrate containing leukocyte (6). This procedure, described by Choukroun J, consists of centrifuging the patient's blood after venipuncture (1).

Blood-derived products obtained after the centrifugation of a blood sample is an autologous fibrin matrix that can be mixed with any bone graft substitutes or can replace bone graft materials entirely in sinus graft procedures (7,8).

The aim of this clinical-histological study was to evaluate the potential of PRF in combination with Nanobone® (Synthetic hydroxyapatite) to enhance bone regeneration in sinus floor elevation with the lateral window technique.

# 2. Materials and Methods

## 2.1. Study design

This clinical trial study is reported according to CONSORT guidelines (9). The interventions were approved by the Ethical Committee of the University Institute of Health Sciences (reference: CE/IUCS/CESPU-18/23) and performed according to the Declaration of Helsinki. The study has been registered in the ISRCTN registry (registration number: ISRCTN99349253).

## 2.2. Patient selection

Participants provided their informed consent after being thoroughly enlightened about the goal and methods of the study both orally and in writing.

A meticulous clinical examination, an assessment of oral hygiene, and a detailed analysis of the patients' medical and dental histories comprised the initial evaluation of each patient. The study's participants had to be at least eighteen years old and, have healed edentulous sites on the posterior maxillae region with a residual bone height of 5 mm or less to facilitate the placement of implants requiring sinus graft procedures. Alcoholism, smoking, drug abuse, diabetes, heart disease, bleeding disorders, weakened immune systems, radiation exposure, past or on-going use of steroids or bisphosphonates, sinus pathology, and prior bone augmentation were among the exclusion criteria.

Out of 50 individuals screened in a preoperative assessment visit from the CESPU-Famalicão clinical unit and intervened upon between January 2023 to December 2023, only six patients who met the study's inclusion criteria consented to participate. In a split-mouth study, twelve sinus graft surgeries were carried out.

Two experienced examiners (M.I.C and M.F.) carried out a full clinical examination of the mouth and the surgical procedure.

## 2.3. Pre-operative radiographic planning

The preoperative radiographic assessment involved the initial screening of patients using cone-beam computed tomography (CBCT, New Tom® Go 3D) and a panoramic X-ray. The condition of the Schneiderian membrane, the patency of the ostium, the existence of antral septa and other pathologies that could affect the alveolar bone, the level of pneumatization of the sinus, the thickness of the Schneiderian membrane, and other factors were assessed using CBCT images.

## 2.4. Presurgical phase

All patients underwent scaling 8 days prior to implant surgery. During this phase, preoperative instructions were given:

- To eat a light diet, avoiding fatty, fried, laxative and fermentable foods (milk, cheese, bananas) on the day of surgery;
- To not to wear jewellery or make-up ;
- To not to take medication based on acetylsalicylic acid (aspirin) in the 4 days before surgery;
- To begin using 0.12% chlorhexidine gluconate mouthwash (Bexident® Post Isdin, Spain,) 48 hours prior surgery (three times a day for two weeks) as well as a tongue scraper;
- To start antibiotic therapy 36 h before surgery (500 mg of levofloxacin) twice daily for 8 days.

## 2.5. PRF preparation

In order to obtain the PRF before the sinus elevation, blood samples from the patient were obtained in the operating room throughout the procedure. The dried monovettes without anticoagulant were inserted in an Intralock®

Centrifuge for 3 minutes at 2,700 rpm following the blood draw to obtain liquid fibrin.

Nanobone® particles were agglomerated in a sterile container with the liquid fibrin and applied to the surgical site in order to achieve the sinus augmentation.

A Straumann® Fex collagen membrane was applied in the lateral window access after the full packing of the maxillary sinus.

## 2.6. Surgical Procedure

The procedure was carried out under local anesthetic using Articaina Inibsa®. A crestal incision in keratinized gingiva as well as a posterior releasing incision were used to access the lateral maxillary sinus wall. A piezoelectric insert (Acteon Satelec® SL2), was used to outline a bone window measuring roughly 15-20 mm and was continuously irrigated with saline solution at a rate of 60ml/min. Subsequently, the Schneiderian membrane was lifted utilizing the piezoelectric inserts (Acteon Satelec® SL4 and SL5) continuously irrigated with saline solution at a rate of 60ml/min. To enhance the sinus floor augmentation, 1.2 ml Nanobone® with a particle size of 1.0 mm was introduced into the sinus cavity after the Schneiderian membrane was carefully elevated without being perforated.

Nanobone® alone hydrated with sterile saline was used to fill the sinus cavity in 6 sinuses (control group). Liquid PRF was added to the bone graft particles (test group) in the 6 contralateral sinuses.

Figure 1 illustrates the sinus lift procedure (liquid PRF/ Nanobone®).

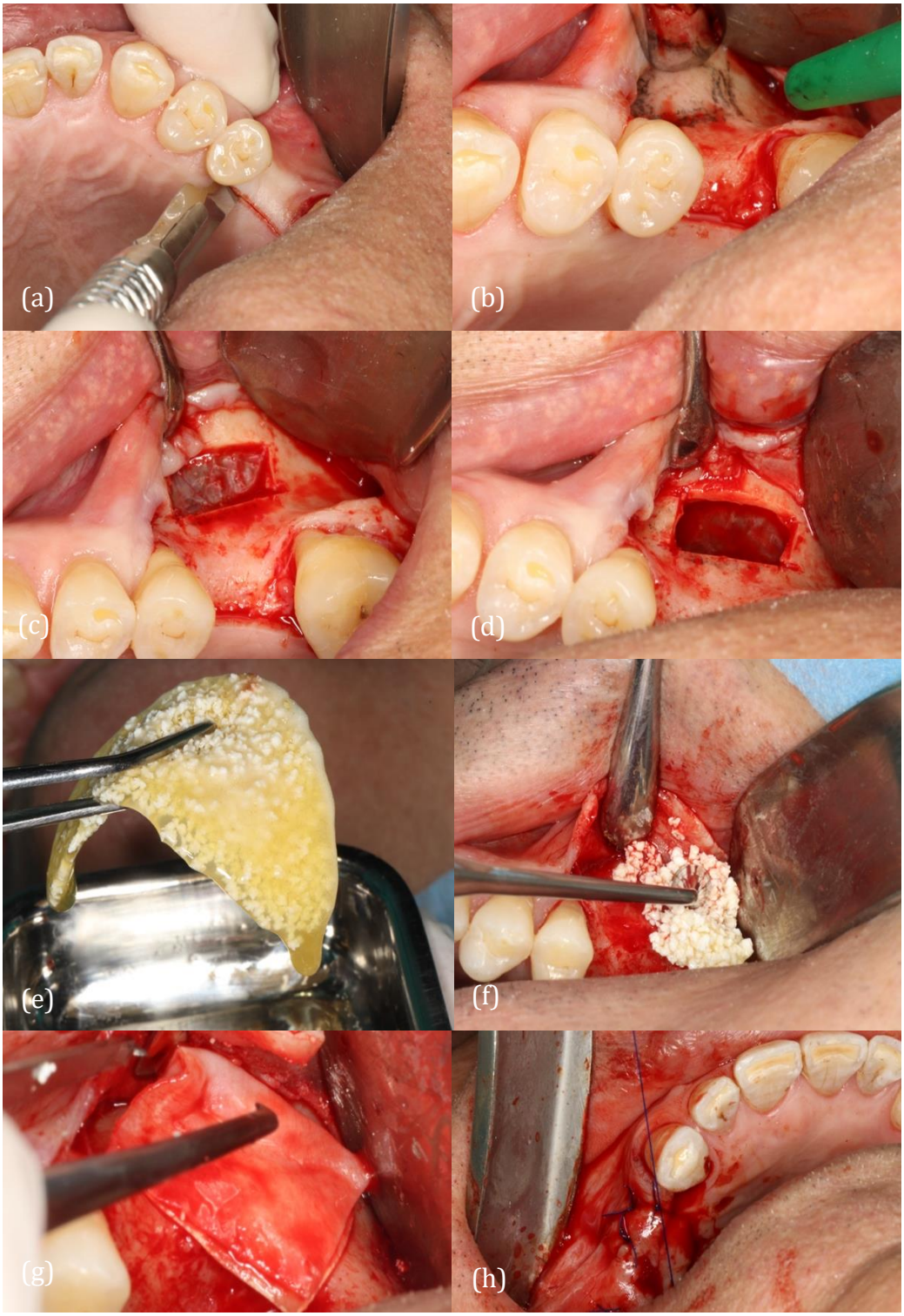


***Figure 1 -*** A visual representation of the surgery. **(a)** crestal incision in keratinized gingiva; **(b)** full-thickness flap elevation; **(c)** bony window with Acteon Satelec® piezoelectric device; **(d)** Schneiderian membrane elevation; **(e)** aggregation of Nanobone® with liquid fibrin; **(f)** biomaterial inserted in the sinus cavity; **(g)** Straumann® Fex collagen membrane over bony window; **(h)** the interrupted sutures using a monofilament suture (Nylon, Resorba®4.0).

## 2.7. Postoperative instructions

The following post operative instructions were given to avoid increased edema (swelling), pain, bleeding and infections:

- Avoid anything that creates pressure in the nasal cavity.

- For the four weeks after sinus graft surgery, do not sneeze or nose blow while holding the nose. If specified, this duration might be extended.

- Be careful to sneeze with your mouth open if necessary.

- Avoid using straws for drinking and avoid spitting.

- Avoid bearing down, which includes lifting heavy objects, blowing up balloons, playing musical instruments that require a blowing motion, and engaging in any other activity that raises nasal or oral pressure. Avoid scuba diving and flying in pressurized aircraft (as these activities will increase sinus pressure).

After surgery, bleeding usually ends a few hours later. For the first three days, some leaking or sporadic bleeding is typical.

- For thirty minutes, leave the gauze pad(s) immediately on the surgery site(s).

- Avoid biting onto the pad. Until the bleeding stops, apply hard biting pressure for 30 minutes and swap out the pad every 30 minutes.

- After attempting the treatments mentioned above, if bleeding persists, moisten a black tea bag, place it over the surgical site(s), cover it with gauze pads, and bite firmly for at least half an hour.

- Steer clear of demanding activities for a week. After surgery, intense physical activity may result in throbbing and bleeding.

Swelling is common after most oral surgeries. Usually, the swelling worsens for three days before starting to improve on the fifth day. For the first 36 hours, you should apply cold compresses to your face for 20 minutes and 5 minutes to help minimize swelling and pain. If sitting or sleeping, elevate the head with two or three pillows or in a reclining chair. Switch to low heat after 36 hours to help reduce swelling.

- After surgery, bruising may occur based on the procedure and the patient's propensity for bruising. Like any other bruise, bruises usually disappear within a few days to two weeks.

Regarding the oral hygiene procedure, you should start cleaning your teeth again, but more softly. If using toothpaste hurts, try brushing your teeth without it.

Restraining yourself from rinsing or spitting could cause the blood clot to come loose and cause discomfort or bleeding. The 0.12% chlorhexidine gluconate mouthwash should be continued to reduce plaque formation.

Alcohol should not be consumed for at least seven days after surgery. In addition to delaying wound healing, alcohol consumption is one of the main causes of infections.

Partial prostheses, including flippers, should not be used immediately after surgery until your post-operative appointment, unless there are specific instructions to the contrary. When it is placed, it must not touch the gums in the surgery area. Pressure from the partial denture can lead to bone graft loss.

A lot of fluids should be consumed following surgery, prefer-ably water. Drinks should not be sucked through a straw. Skip any carbonated beverages for a full 72 hours. As soon as it feels comfortable, (typically after seven days), go back to your regular diet, starting with softer foods.

Regarding medication, antibiotic therapy should be continued, and 1 g of paracetamol should also be taken 3 times a day for pain control management. The use of a Mometasone spray is also advised (1 application in each nostril twice daily for 3 days) due to the reduction in the activity of the cilia of the Schneiderian membrane and the thickening of mucous secretion.

After the post-operative indications were completed, patients were scheduled for sutures removal ten days after surgery.

## 2.8. Harvesting of the bone specimen

Dental implants can usually be inserted after the grafted bone has effectively merged, usually within 6 to 9 months. The dentist's assessment and each patient's unique recovery rate may influence the precise timing. In this

study, implants were placed six months after sinus floor augmentation in both groups. Using a 2.5 mm diameter trephine bur, a bone biopsy from the augmented site was obtained from both the control group and test group during this treatment. The bone samples were then stored in a sterile vial with 10% formaldehyde.

Figure 2 illustrates the collection of the specimen.

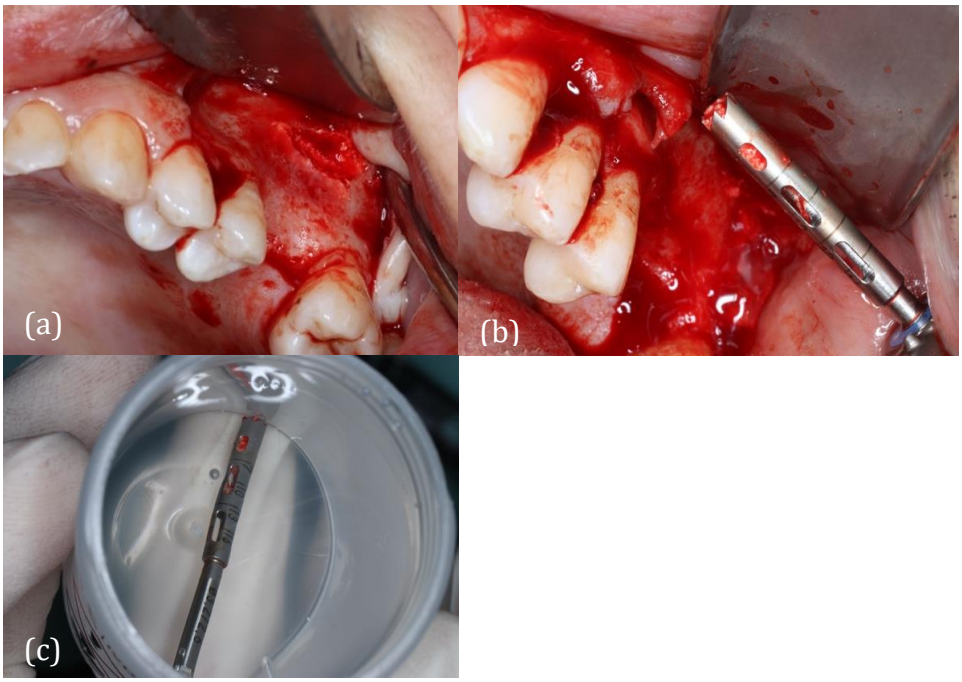


**Figure 2 -** Harvesting of the bone specimen with a 2.5 mm diameter trephine bur. **(a)** Surgical site; **(b)** trephine drill with collected bone; **(c)** trephine drill with bone specimen in a 10% formaldehyde sterile vial.

## 2.9. Sample Processing and Analysis

An analysis, both qualitative and quantitative, was conducted on the processed study material.

The hard tissue was carefully dissected before the material was extracted en bloc. Using high-precision Exakt® equipment (Exakt® Technologies, Oklahoma City, OK, USA), collected samples were processed using an undecalcified approach that produced high-quality histological pictures without morphological distortions of relevant structures or meaningful artifacts. In terms of staining techniques, toluidine blue was employed.

Histological processing was carried out following the protocol for non-decalcified techniques, recommended and described by Donath K (10): preparation of histologic sections using the cutting-grinding technique for hard tissue and other material not suitable for sectioning using routine methods - Equipment and Methodical Performance (Exakt® - Kulzer - Publication, Norderstedt.) without any attempt to remove the harvested tissue from inside the specimen. This preservation made it possible to maintain the proper orientation of the slides, making it possible to see all the tissue from lateral to medial.

Quantitative analysis was carried out by capturing images from the aforementioned video camera (Nikon® SMZ 1500, Tokyo, Japan) coupled with a stereomicroscope (Optronics" DEI 750D CE, Goleta, California, United States of America) and connected to a PC computer (Intel" Pentium" V).

Histomorphometry was carried out using image analysis software (Bioquant Nova, Bioquant - Image Analysis Corporation, Nashville, USA). This program is able to distinguish the different dye affinities of the tissues and components of the sample, converting this information into quantification of areas, three-dimensional reproductions, determination of densities, and other more specialized parameters. This evaluation system allows for greater objectivity and precision compared to other evaluation systems, such as radiomorphometry or systems with degree scales.

The images were assessed at a magnification of 10x0.5 for qualitative analysis.

Calibration of the program and analysis were always carried out by the same operator. All sessions were preceded by an intra-examiner calibration check.

Histomorphometry involved the use of the total area of the bone tissue sample as a reference, which was taken as the standard area for all defects in order to minimize measurement bias.

The following parameters were assessed in the histomorphometric analysis:

a) Quantification of the percentage of particles:

- Percentage of particles = Area occupied by particles / defect area x 100%

b) Quantification of the percentage of new bone tissue:

- Percentage of new bone tissue = (area of new bone tissue / area of defect) x 100%

c) Quantification of connective tissue:

- Connective tissue = (area of connective tissue / area of defect) x 100%

# 3. Results

Out of 50 individuals screened in a preoperative assessment visit from the CESPU - Famalicão clinical unit, only six patients who met the study's inclusion criteria consented to participate.

Figure 3 illustrates the design of the study in the form of a CONSORT diagram.

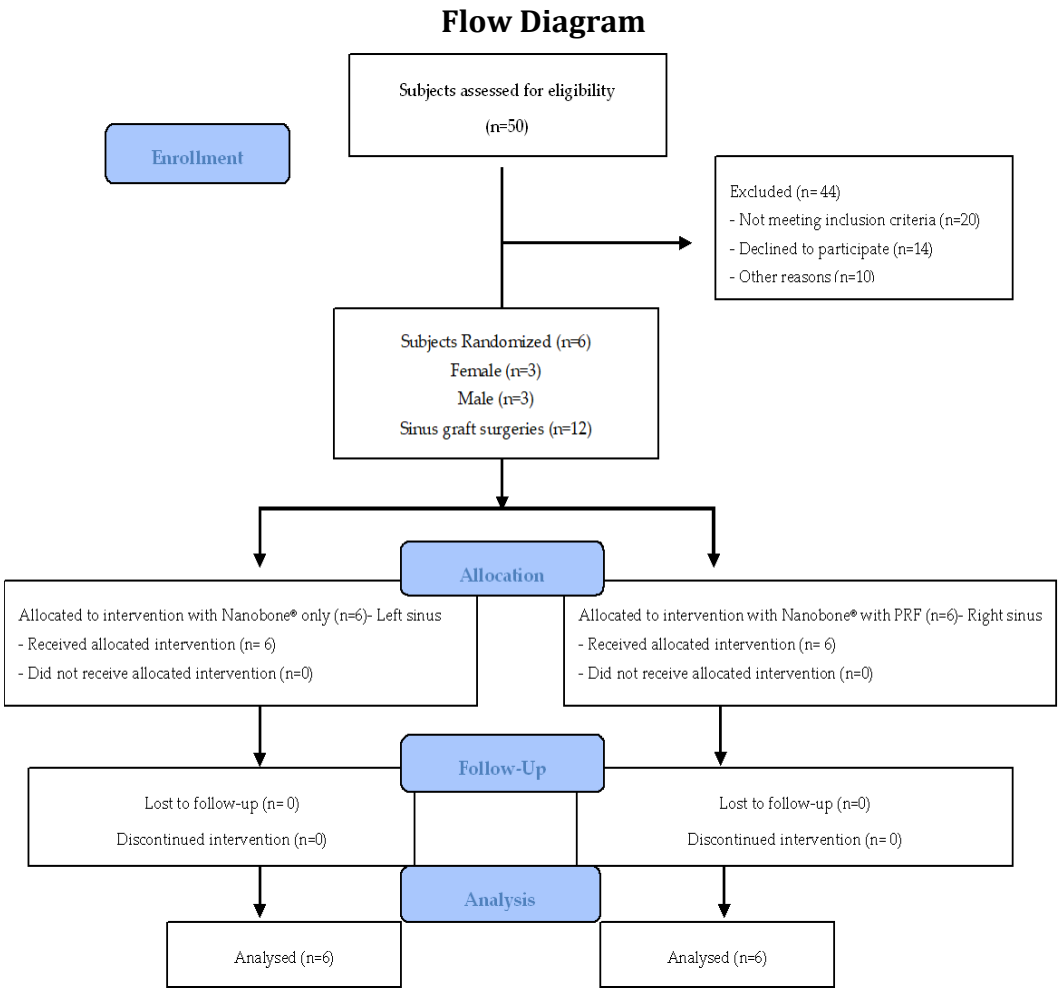


**Figure 3** - CONSORT flow chart

After bone sample collection, histomorphometric analysis was carried out on the samples from both the control group (NanoBone® alone) and test group (NanoBone®/ liquid fibrin).

Under light microscopy, and upon extensive evaluation of the histological lamellas of both groups, there seem to be differences between the two sets of results under analysis.

Table 1 illustrates the percentage of new bone formation in both groups.

| | % New Bone Tissue | % Particles | % Connective tissue |
|---|---|---|---|
| **Nanobone®/liquid fibrin** | 23,0 ± 3,7 | 27,5 ± 4,9 | 49,4 ± 2,8 |
| **Nanobone®** | 19,5 ± 3,0 | 23,4 ± 5,7 | 57,0 ± 3,5 |

Table 1 – Percentage of new bone in unfilled defects (mean ± SD)

The rate at which vital and inert bone is present in the bone trabecular sections allows one to assess the significance of turnover. Observations reveal that for the test group (Nanobone®/PRF), there is 27.5 ±4.9% new vital bone, 23.0 ±3.7% inert bone parti-cles, and 49.4 ± 2.8% connective tissue. Meanwhile, for the control group (Nanobone®), there is 19.5 ± 3.0% new vital bone, 23.4 ± 5.7% inert bone particles, and 57.0 ± 3.5% connective tissue. Osteoid tissue's significance in both group samples provides proof of significant turnover. After six months of bone healing, the histomorphometric results of the test group (Nanobone®/PRF) seem marginally better than those of the control group (Nanobone®).

Figure 4 illustrates the percentage of new bone formation in both groups (test and control group).

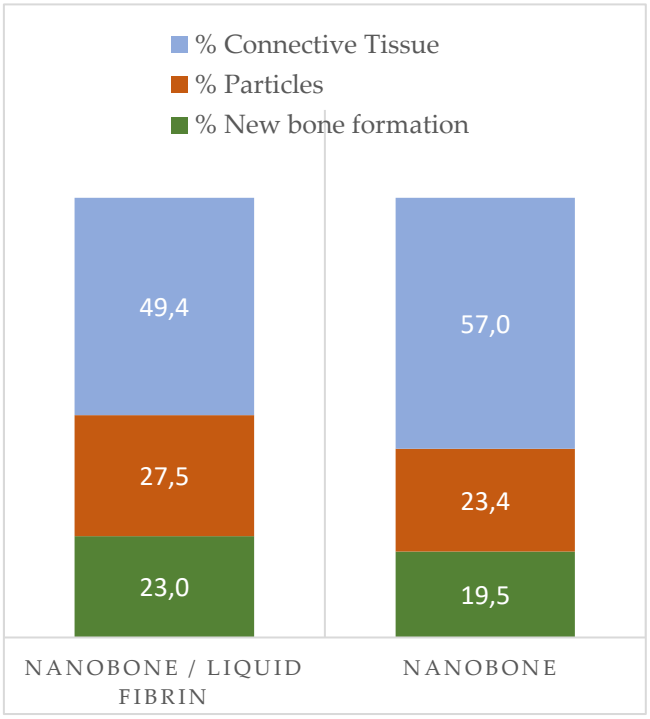


**Figure 4** - Histologic results in both groups (test and control group).

The most characteristic aspect of the histological images in the test and control group is the presence of a high density of particles of various sizes and shapes, many of them in a clear and intense process of disintegration/fragmentation (Figure 5 a, b).

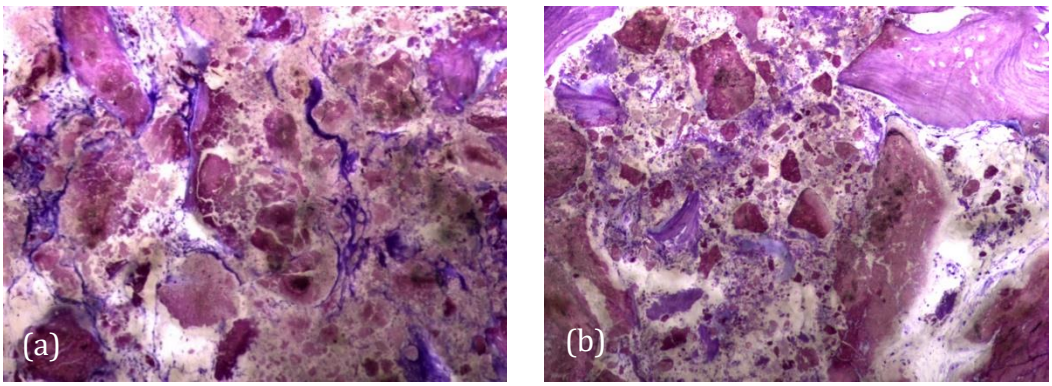


**Figure 5** - Histologic results in both groups: **(a)** test group; **(b)** control group.

The results, both in the test group and control group, also exhibited extensive areas occupied by a homogeneous/amorphous material (Figure 6 a, b, c), which, considering its colour characteristics, seems to originate from the

progressive dissolution of the particles under study. These aggregates generally contain multiple particles with very small dimensions.

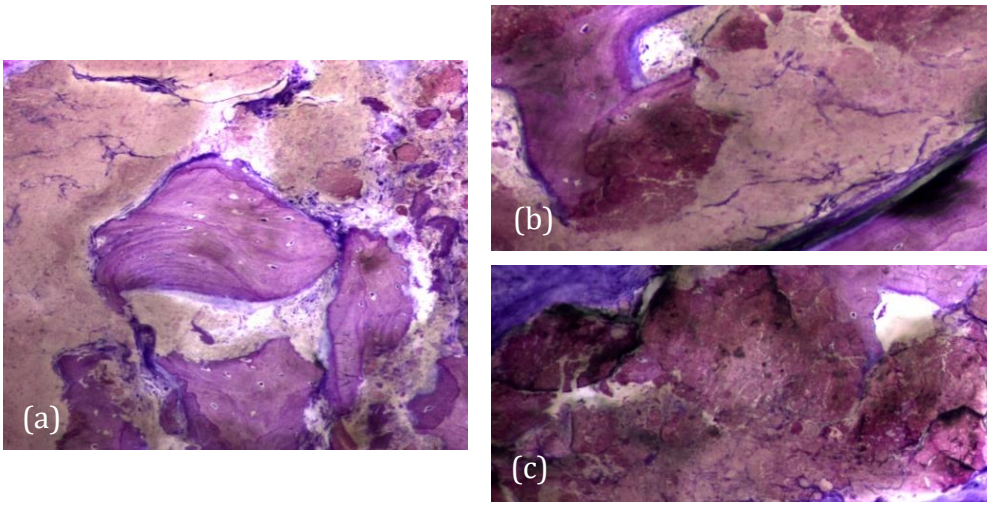


**Figure 6** - Histologic results in both groups: **(a)** test group; **(b, c)** control group.

It is possible to observe, in both groups, the presence of numerous bone trabeculae formed by immature bone tissue containing numerous irregularly arranged osteocytes. It is worth noting the existence of many areas of osteoid tissue, reflecting an active process of osteogenesis (Figure 7 a, b).

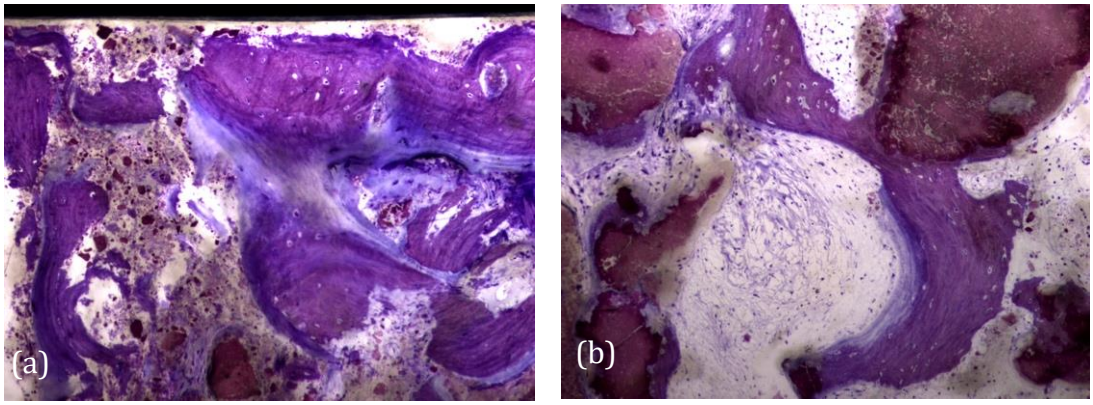


**Figure 7** - Histologic results in both groups: **(a)** test group; **(b)** control group.

In this respect, recently formed bone tissue is often seen in close apposition to the particles, although it is not common to find particles completely surrounded by bone tissue. It is interesting to note the perfect continuity between the bone tissue and the particles, with no border/interface between them. It should also be noted that even the particles where bone

tissue is directly attached are themselves in the process of disintegration in both the control group and test group (Figure 8 a, b).

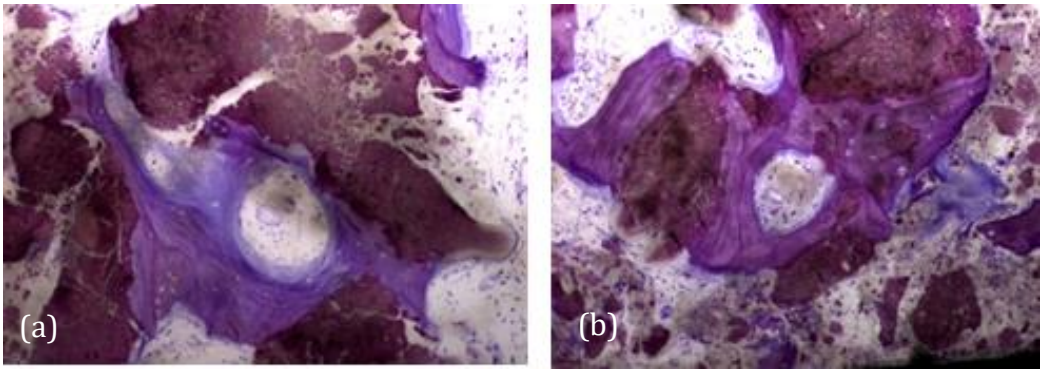


**Figure 8** - Histologic results in both groups: **(a)** test group; **(b)** control group.

It was also possible to identify numerous osteoclasts, both on the surface of areas of immature bone tissue and on the surface of the particles (Figure 9), demonstrating the presence of active resorption phenomena.

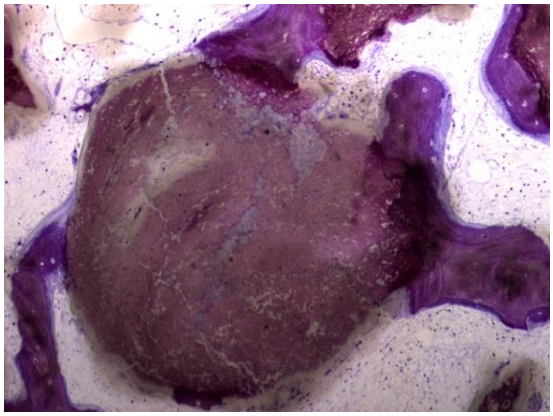

**Figure 9** - Histologic results on the test group.

In this context, it should be noted that most of the particles have a peripheral rim with lower dye density, suggesting a process of surface demineralization in both groups (Figure 10).

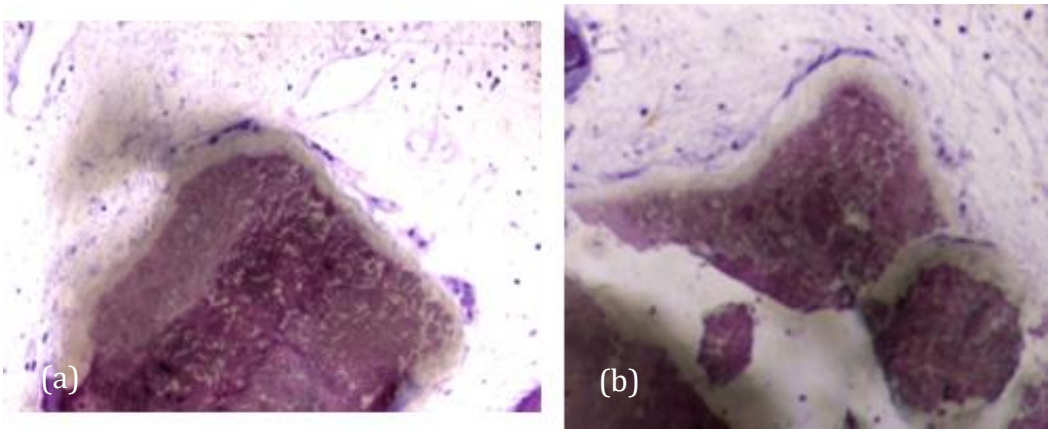


**Figure 10** - Histologic results in both groups: **(a)** test group; **(b)** control group.

Also noteworthy is the observation of areas of lamellar/mature bone tissue formed by thick trabeculae in the test group (Figure 11 a), showing the existence of Havers systems that are already completely formed (Figure 11 c) or in formation process in both groups (Figure 11 b).

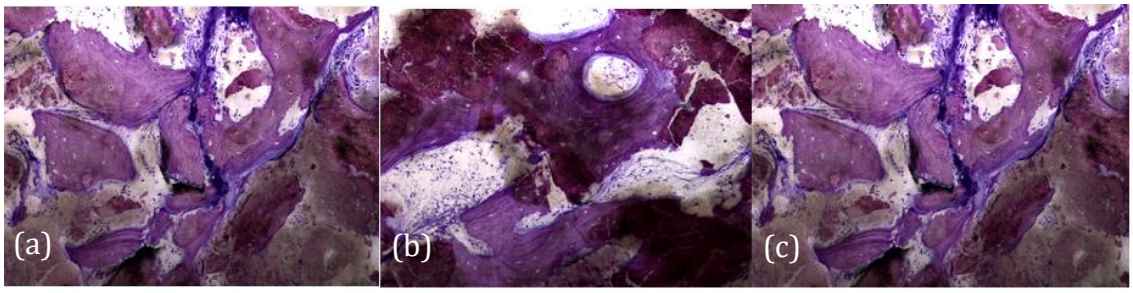


**Figure 11** - Histologic results in both groups: **(a, b)** test group; **(c)** control group.

The fact that they contain numerous perfectly osteointegrated particles is characteristic of these trabeculae (Figure 12).

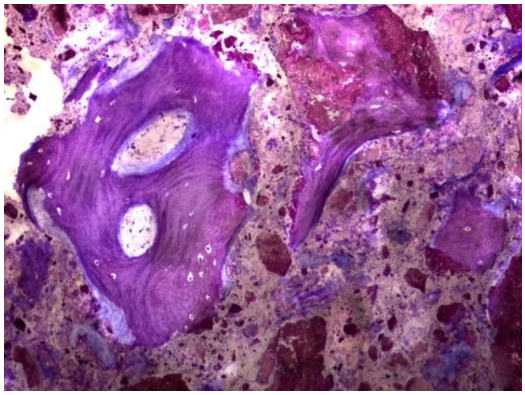

**Figure 12** - Histologic results in control group.

In the longitudinal sections, it is possible to appreciate the biomaterial granules presented along the entire length of the section. There are also areas of cancellous bone tissue made up of bone trabeculae, areas of lamellar bone and others, possibly in smaller quantities, of immature bone in which the characteristic irregularly arranged osteocytes are visible. Bone tissue formation activity at the site where the maxillary sinus floor elevation intervention was carried out was evident, as evidenced by the presence of bone tissue in contact with some of the biomaterial granules along the entire length of the cut, apparently in greater quantity near the lateral third and, above all, at its medial limit. In fact, the trabeculae were sparser in the middle third. However, it should be noted that there may be some individual physiological variability in the anatomical design of the sinus. It was also possible to see the existence of bone tissue between the surroundings and between some particles forming bridges between them, demonstrating the osteoconductive effect of the biomaterial's inorganic mineral granules.

The ossification processes found are of intramembranous origin.

In some histologic images, clot remnants are visible, more prevalent in the lateral and medial thirds, certainly related to the collection method and consequent rupture of blood vessels.

The entire length of the section also shows many granules surrounded only by loose connective tissue, with no signs of bone formation activity. The implanted material did not reveal any type of histological image compatible with a local adverse reaction, and no foreign body giant cells or other inflammatory cells were detected.

Where it was identified that bone formation activity had occurred, the formation of bone trabeculae was visible, with organized lamellar bone tissue, bone ap-position on the particles and the formation of bone tissue bridges between them, sur-rounded by medullary spaces filled with loose connective tissue, with fibroblast-like cellular elements and blood vessels.

On the other hand, some aspects related to the degradation of the biomaterial granules can be observed, namely what appears to be their disintegration into smaller particles, the difficulty in defining the limits of granules, and the presence of osteoclastic activity on its surface. When there is

bone- tissue-forming activity on the surface of the granules, most of the time, it only occurs on part of the surface, and it is very rare to find granules completely surrounded by bone apposition.

# 4. Discussion

The purpose of this clinical trial was to evaluate the potential of PRF in combination with synthetic hydroxyapatite Nanobone® to enhance bone regeneration in sinus floor elevation with the lateral window technique.

Due to pneumatization of the maxillary sinus and atrophy of the alveolar bone ridge, the edentulous posterior maxilla often provides a limited bone volume (11). The remaining bone is often of type IV quality, which makes implant rehabilitation more difficult in this region (11).

Tooth loss is often followed by a complex biophysical process known as residual ridge resorption. This process reaches its highest point in the first year after tooth loss and then resorption continues at a slower but steady pace in the following years (12,13). All edentulous patients suffer from bone resorption, which is a chronic, gradual, and irreversible process (14).

In 1988, to simplify the description of the residual ridge and thus aid communication between clinicians, Cawood *et al.* (15) developed a classification of edentulous jaws based on a randomized cross-sectional study. According to this classification, the residual ridge is classified into six classes (Class I to Class VI) according to the type of bone loss in height and width (15). In the posterior maxillary region, bone loss is both vertical and horizontal (from the buccal aspect).

The pneumatization of the sinus combined with alveolar bone resorption leads to insufficient bone quantity for implant placement (16,17).

For this reason, sinus mucosa is required to be lifted from the sinus floor (sinus lift augmentation), and new bone formation is achieved by using bone substitute materials that are accommodated below the Schneiderian membrane (16,17).

A complete and ideal graft material has not yet been found; autogenous bone possesses several advantages such as osteogenic, osteoinductive, and osteoconductive properties, but it presents some difficulty in obtaining autogenous bone graft in sufficient quantities and requires some more complex expertise from the surgeon to address donor areas (18).

In terms of new bone formation, the use of autogenous bone in maxillary sinus augmentation is predictable and successful; however, donor site morbidity is inevitable (19). The osteoconductive properties of biomaterials and allogeneic sources have enabled them to be widely used in maxillary sinus augmentation procedures to replace autogenous bone, which harbours osteogenic cells that induce direct bone regeneration (20). With the advent of blood matrix technology, biomaterials extracted from the patient's blood, such as platelet-rich fibrin (PRF), have recently been more frequently reported in sinus floor augmentation treatments. PRF has been used in dental practices and is simple to handle and prepare (20). This natural and optimized blood clot could be used during a sinus lift for protection of the sinus membrane or to improve bone graft maturation. PRF promotes the growth and differentiation of osteoblasts, among many other types of bone cells (21).

In the last few years, PRF has been used alone or combined with different grafting materials (22). Evidence has shown that the use of PRF alone or its association with various grafting materials in maxillary sinus floor augmentation demonstrated successful bone regeneration (23,24). According to our results, the use of PRF mixed with synthetic hydroxyapatite (test group) exhibited increased vital bone formation in comparison with synthetic hydroxyapatite (control group): 23.0% versus 19.5%, respectively. This can be explained by two positive factors: the osteoconductive capacity of Nanobone® and the positive influence of liquid fibrin on enhancing bone cells differentiation.

A study by Ortega-Mejia *et al.* (7), showed a slightly higher percentage of new bone formation in the PRF group in comparison to the use of grafting biomaterials alone. These results could be explained by its osteoinductive qualities and better revascularization process, accelerating the healing period of the bone tissue (25).

A study by Tanaka *et al*. (26) also showed a higher percentage of new bone formation in the histological evaluation of sinus lift using deproteinized bovine mixed with PRF.

In their study, Choukroun *et al*. (1) revealed that, after 4 and 8 months of healing, respectively, there were no changes in the newly created bone

between the PRF mixed with freeze-dried bone allograft (DFDBA) and the DFDBA alone. This suggests that the addition of PRF could shorten the healing period prior to implant placement.

A study by Zhang *et al*. (27) showed that PRF combined with Bio-Oss® had no significantly synergistic effect on new bone formation or the graft volume. These different results could be due to different bioactive properties between Bio-Oss® and Nanobone®. This study's findings are consistent with the study by Choukroun *et al.* (1) in that poor bone growth was primarily located farther from the sinus floor. They suggest that, as the source of precursor cells, the sinus floor is crucial to bone repair. Precursor cell migration to the site may be less stimulated by PRF mixed with Bio-Oss® than by PRF combined with FDBA (27). In our study, we harvested biopsies from the site furthest from the sinus floor; even so, there was an increase in cell activity in the Nanobone® and liquid PRF group. The results showed in the study by Zhang *et al.* (27) could be explained by the lack of precursor cells in the PRF group combined with Bio-Oss®. Furthermore, according to several studies, the slow resorption property of this bone substitute slows the replacement of new bone formation (28,29).

Liu *et al.* (30) carried out a study to compare the in vitro biocompatibility of Bio-Oss® versus Nanobone® and their ability to support and promote the proliferation of human osteoblasts. According to their study, both materials showed low cytotoxicity and excellent biocompatibility. However, the test for cell proliferation was superior for Nanobone®, which may explain the difference between our results and those found in the study by Zhang *et al*. (27).

During bone remodelling, osteoclasts' degradation of biomaterials is a crucial and desired process. It has been shown that osteoclasts' biodegradation rate of Nanobone® appears to have more similarity to the body's natural process of remodelling bone (31). Targeted vascularization facilitates the migration of osteoblasts to vascularized sites and can accelerate the creation of new bone (31). Prior studies showed that when placed in vascularized connective tissue, several synthetic bone graft materials, such as Nanobone® replacements, were covered and degraded by osteoclasts,

resulting in resorption lacunae and resorptive trails (31,32). Our results are in accordance with this; histologically, we observed numerous osteoclasts, both on the surface of areas of immature bone tissue and on the surface of the particles.

The fact that NanoBone® is made of nanocrystalline hydroxyapatite, which resembles the crystalline phase of native bone, may be the reason for this material's biocompatibility. In addition to these characteristics, when PRF is added to this biomaterial, the fibrin clot acts as a biological binder between the various components of the graft and a matrix that allows the migration of osteoprogenitor cells to the centre of the graft, the retention of stem cells, and neoangiogenesis when mixed with the graft material. This can be explained by the fact that PRF promotes an increase in bone mass and greater revascularization of the produced bone.

A significant limitation of the present study was the small sample size. To truly understand the extent of healing time shortening gained using PRF, it would be interesting to harvest bone specimens three different times after sinus augmentation (after 4, 6 and 9 months).

# 5. Conclusion

According to this study, the nanoporous hydroxyapatite used to raise the sinus floor in humans is osteoconductive, promotes the production of new bone in a similar manner to that of most other bone substitute materials, and appears to be degraded, at least partially, by cells that resemble osteoclasts.

The results strongly indicate that mixing liquid PRF with Nanobone® does not have a negative influence on the amount of viable bone formation; it seems to slightly increase the amount of new bone formation and revascularization in sinus bone graft procedures with the lateral window technique compared to the single use of Nanobone®.

PRF seems to be a reliable method for incorporating bone substitutes and, due to its osteoconductive characteristics, it seems to help in shortening the bone healing period.

Nevertheless, to validate the current findings and assess the long-term effectiveness of PRF mixed with nanoporous hydroxyapatite, prospective investigations involving larger patient groups are required.

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