case report

Stem cells for bone reconstruction in sinus lifting

Abstract / Introduction: *The demand for bone reconstruction in oral rehabilitation has been growing substantially. However, patients willing to undergo reconstructive surgery want less invasive procedures with less postoperative morbidity. Less invasive bone reconstruction techniques have used bone substitutes to achieve these objectives. Nevertheless, recent studies about tissue engineering have demonstrated that stem cells, in combination with bone grafts, may potentially improve the biological characteristics of grafting material.* Objective: *To describe a clinical case of sinus elevation using autologous bone marrow aspirate resulting from the isolation of a bone marrow mononuclear fraction combined with Bio-Oss.* Results: *Five months after the combined grafting procedure (Bio-Oss + bone marrow stem cells), bone biopsies were harvested during implant placement surgery. Histological images revealed a large amount of vital mineralized tissue for a 5-month postoperative time.* Conclusion: *The clinical use of bone marrow mononuclear fraction combined with Bio-Oss — a xenogeneic bone substitute — in maxillary sinus elevation seems to result in good bone repair and shorter healing time.*
Keywords: *Stem cells. Bone transplant. Dental implants. Osseointegration. Bone marrow.*

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» The authors report no commercial, proprietary or financial interest in the products or companies described in this article.

» The patient displayed in this article previously approved the use of her facial and intraoral photographs.

INTRODUCTION

Patients have increasingly sought treatments with implants to reconstruct their smile, but the correct positioning of osseointegrated implants often requires bone reconstructions to ensure the success of subsequent prosthetic rehabilitation.[1] Bone graft procedures have become more frequent, and a growing number of patients are willing to undergo reconstruction procedures. Autogenous bone grafting, although considered as the standard reference, has been increasingly avoided. In contrast, patients have sought less invasive reconstructions with less postoperative morbidity,[1,2] which has led to exponential increases in the number of studies about autogenous bone substitutes in the last years. Alloplastic (synthetic), xenogeneic and allogeneic grafts are among autogenous graft substitutes.[3-5] However, these bone substitutes do not have osteoconductive and osteogenic properties, and have little or no osteoinductive capacity. Therefore, graft healing and incorporation take from 6 to 8 months, a period that is considered too long. In addition, the areas that receive grafts with this type of biomaterial have greater amounts of remaining graft material in acomparison to those that receive autogenous bone grafts.[6-8]

In the last years, studies on tissue engineering have advanced in the knowledge about the capacity of mesenchymal stem cells to differentiate into a variety of specialized cells to produce fat, bone, cartilage and endothelial tissues. Thus, numerous studies have focused on the development of protocols for cell treatments that may be combined with bone substitutes[4,5,9] so as to maximize the results of bone repair[2,10,11,12] and restore tissues without the removal of large amounts of autograft. Additionally, these protocols also aim at allowing healing and osseointegration to occur within a shorter period of time.[13]

Based on the knowledge that the bone marrow is the source of mesenchymal stem cells with a potential for osteogenic differentiation, and that these cells are found, in large amounts, when mononuclear cell fraction is isolated from bone marrow, some studies have been conducted to develop a method for the concentration of bone marrow stem cells. The protocol for the use of bone marrow concentrate aspirate according to density gradients has been associated with bone substitutes that may be eventually used for guided tissue regeneration (GTR). In GTR, membranes or tissue barriers are used to prevent the interference of unwanted cells — from adjacent soft tissues — which may affect healing.[14-18] GTR has been conventionally used in maxillary sinus elevation in combination with autogenic, autologous, xenogeneic or synthetic grafting.[16,18] For maxillary sinus elevation, several authors recommend the use of Bio-Oss, a xenogeneic bovine bone graft, since its physical and mechanical characteristics are similar to those of human bone, which makes it a substitute with excellent osteoconductive properties.[19-22]

This study describes a clinical case of sinus elevation using an autologous bone marrow aspirate, obtained from the isolation of bone marrow mononuclear fraction by means of a density gradient method, in combination with Bio-Oss. It also evaluated the level of regeneration provided by this treatment.

CLINICAL CASE REPORT

A 55-year-old white male patient, with good oral hygiene, was seen at the Oral Rehabilitation Clinic of São Leopoldo Mandic School of Dentistry. Teeth #16 and #17 were

missing, and the patient expected to have them replaced with fixed implant-supported prostheses. After the first visit, tests were requested. CT scans revealed great bone volume loss due to right maxillary sinus pneumatization. The treatment plan consisted of maxillary sinus elevation using xenogeneic bone graft combined with bone marrow mononuclear fraction (Fig 1).

This study was approved by the Ethics in Research Committee of the São Leopoldo Mandic School of Dentistry under protocol number 2012/0317. The patient signed an informed consent form before the study. This case report is one among several others included in an experimental Masters research.

Innitially and immediately after the operation, a hematologist collected bone marrow from the patient. The area of the right iliac crest was cleaned with 2% chlorhexidine digluconate, followed by local anesthesia with 2% lidocaine hydrochloride and puncture of the posterior upper region of the iliac crest using a 40 x 12 mm needle with a reamer (Lee-Lok, Minneapolis, MN, USA) (Fig 2).

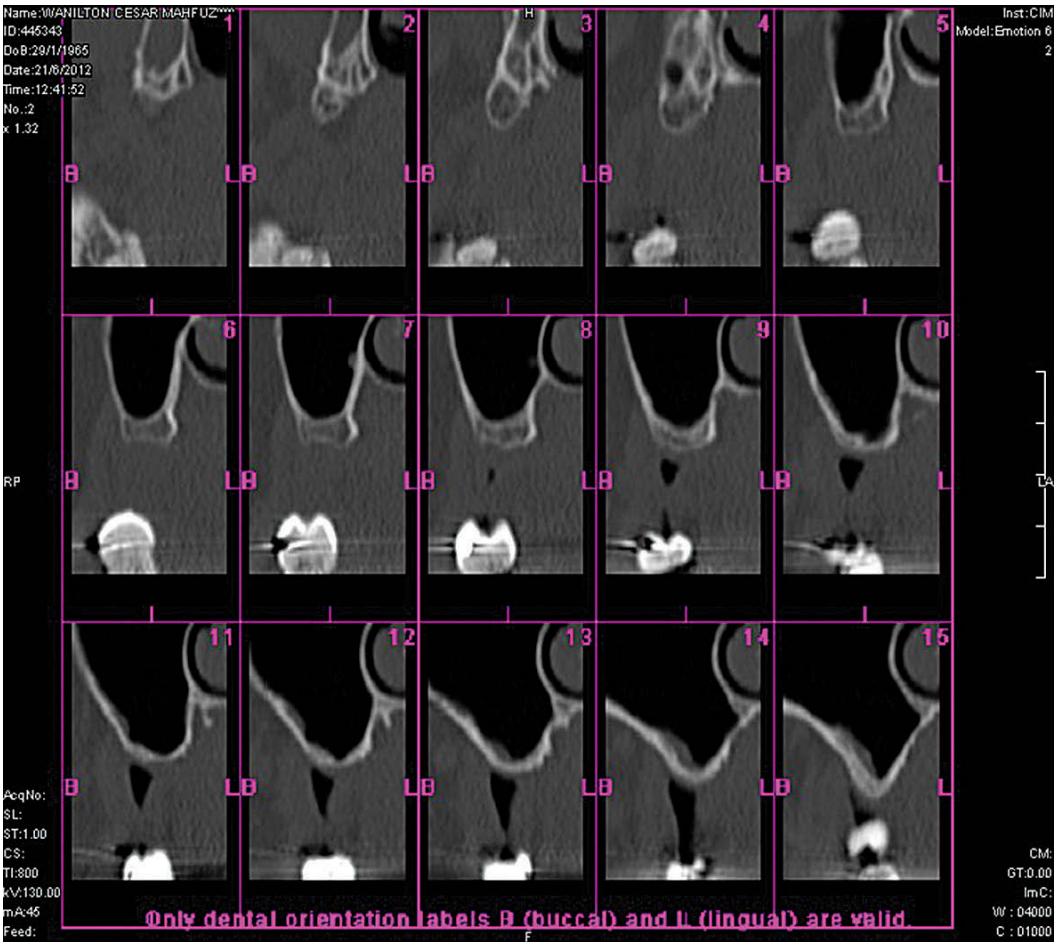


Figure 1. Sagittal CT scan reveals absence of bone tissue (maxillary sinus pneumatization) in right posterior maxilla.

This study followed a protocol to obtain bone marrow mononuclear fraction by density gradient isolation using Ficoll-Histopaque (Sigma-Aldrich, St Louis, MI, USA) according to the following method of cell layer separation: 1) collection of 4 mL of bone marrow (BM) aspirate from the posterior iliac crest; 2) in a laminar flow clean bench, BM aspirate was transferred to a 15-mL conic tube with 4 mL of buffer saline solution (PBSx1) and homogenized using a pipette; 3) the content was slowly transferred to another 15-mL conic tube containing 8 mL of Ficoll-Histopaque to avoid mixture of phases; 4) centrifugation at 400 g for 30 minutes; 5) division of

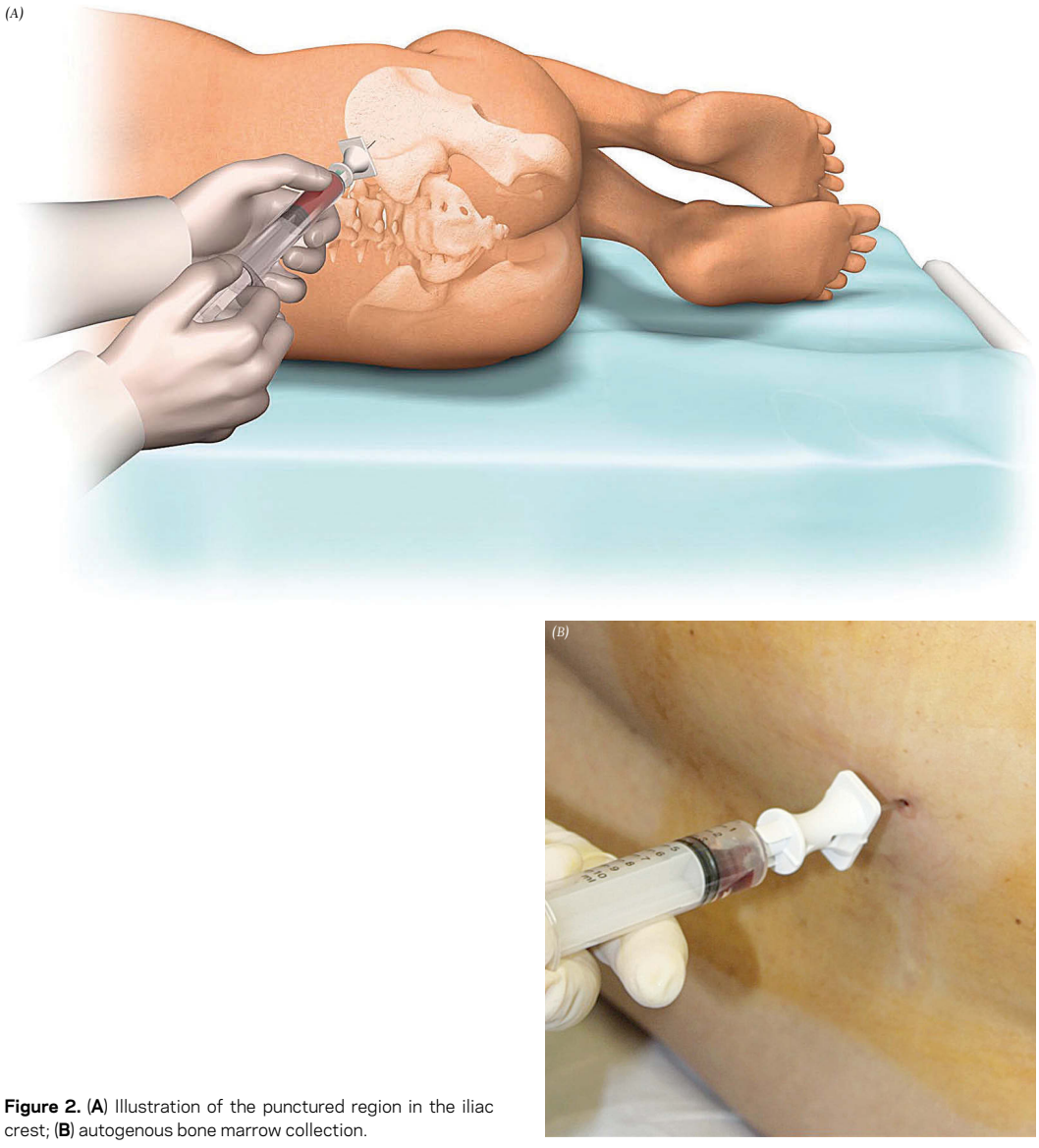


Figure 2. (**A**) Illustration of the punctured region in the iliac crest; (**B**) autogenous bone marrow collection.

phases was monitored: in the upper phase, there is plasma and its soluble contents; in the interface, there are mononuclear cells; in the layer immediately below, there is Ficoll-Histopaque; and at the lowest point, there is a layer of cell sediment with erythrocytes and granulocytes; 6) using a precision pipette, the interface of mononuclear cells was removed and transferred to another conic tube containing 4 mL of PBS and homogenized; 7) centrifugation at 200 g for 10 minutes at room temperature to obtain a new pellet at the bottom of the tube; 8) removal of supernatant; 9) re-suspension of the pellet in 1 mL of PBS to obtain final cell suspension (Figs 3 to 6).

During the laboratory procedures, the patient received 1 g amoxicillin and 4 mg dexamethasone, extra oral asepsis with 2% chlorhexidine digluconate and intraoral asepsis with 0.12% chlorhexidine digluconate mouthwash before the beginning of the surgery.

Local anesthesia with 2% mepivacaine hydrochloride and 1:100.00 epinephrine was injected into the sulcus and the palatine region. An incision was made over the crest, slightly lingual, and a single vertical

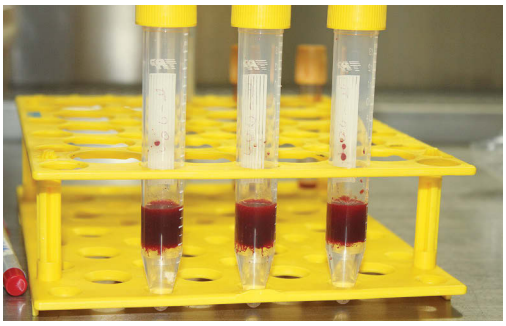


Figure 3. Conic tubes with autogenous bone marrow aspirate and Ficoll-Histopaque.

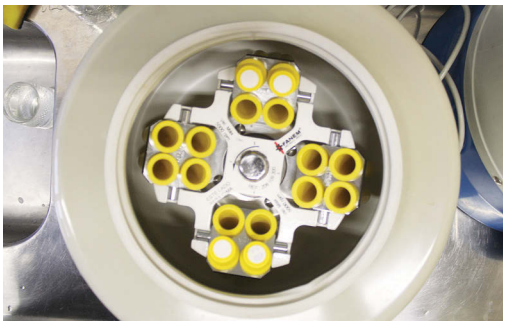


Figure 4. Centrifugation at 400 g for 10 minutes.

Figure 5. Pipetting the supernatant.

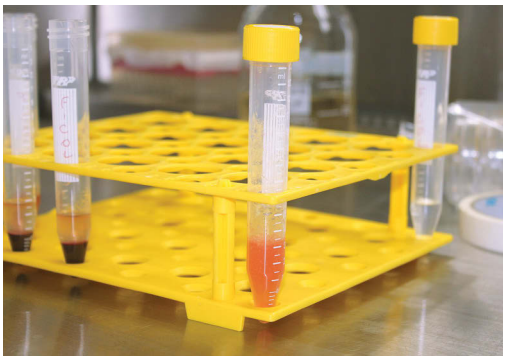


Figure 6. Bone marrow mononuclear fraction.

incision with a #15 scalpel was made to raise a total flap and provide access to the region. Using large diameter diamond and spherical steel burs, an ovoid bone cavity was created with total stripping to access the floor of the maxillary sinus. This cavity received the graft after the Schneiderian membrane was raised and displaced. After this surgical approach, the grafting material was prepared in combination with the bone marrow mononuclear fraction (BMMF) obtained according to the method described above. A sterile dappen dish with a lid was used for homogenization. The dish was filled with the content of one vial of xenogeneic bone substitute (Bio-Oss 2g Large Particles 1.0-2.0 mm, Geistlich, Switzerland) for BMMF addition and homogenization. After complete filling and composite graft accommodation, an absorbable collagen membrane (Bio-Gide, Geistlich, Switzerland) of adequate size was positioned to fully cover the surgical cavity that received the graft. Mononylon 5.0 was used for suture. The following postoperative medications were prescribed: 500 mg amoxicillin every 8 hours for 3 days and 35 drops of 500 mg/mL metamizole sodium every 6 hours while the patient felt pain. Ten days after the operation, the suture was removed with no further complications (Figs 7 to 11).

Five months after grafting, new CT scans were obtained, the region was reopened and surgical cavities were prepared for the placement of implants using a 2-mm trephine bur. At the same time, two bone samples were removed and fixed in 10% formaldehyde immediately after removal. After that, two osseointegrated morse

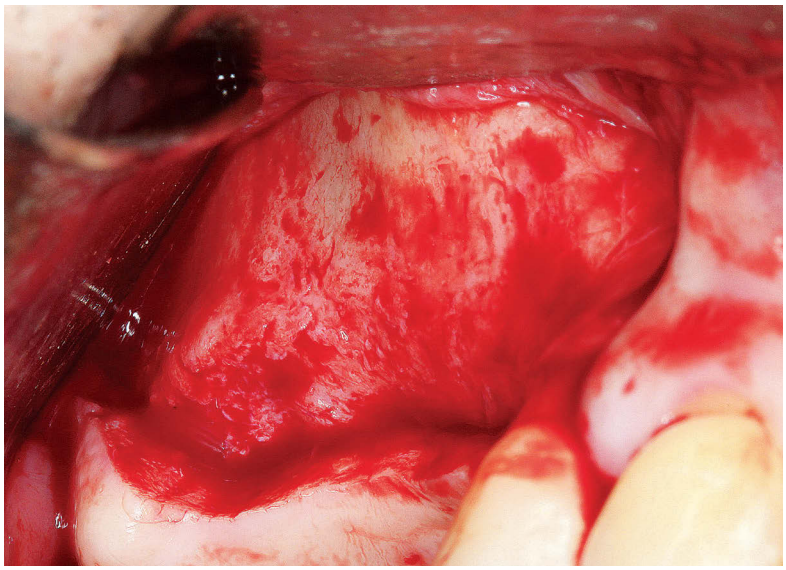
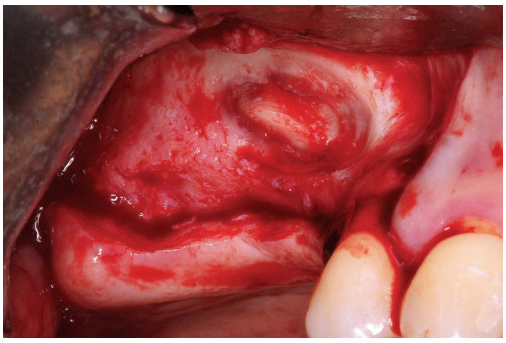


Figure 7. Total flap raising and bone exposure.

(A)



(B)

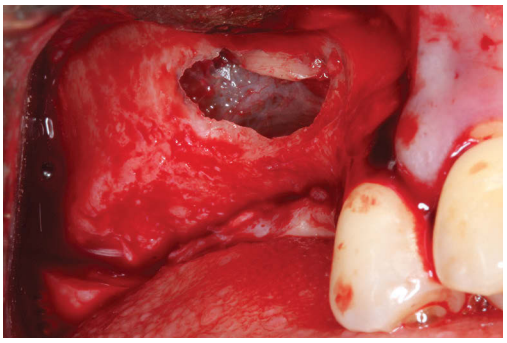


Figure 8. (A) Opening access to the maxillary sinus floor; **(B)** Schneiderian membrane raising and access to the maxillary sinus floor.

Figure 9. Xenogeneic bone substitute combined with BMMF.

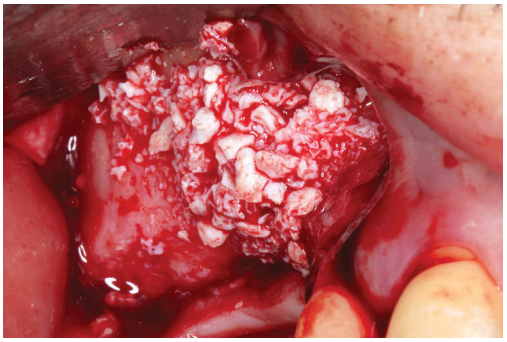


Figure 10. Maxillary sinus floor filled with xenogeneic graft combined with BMMF.

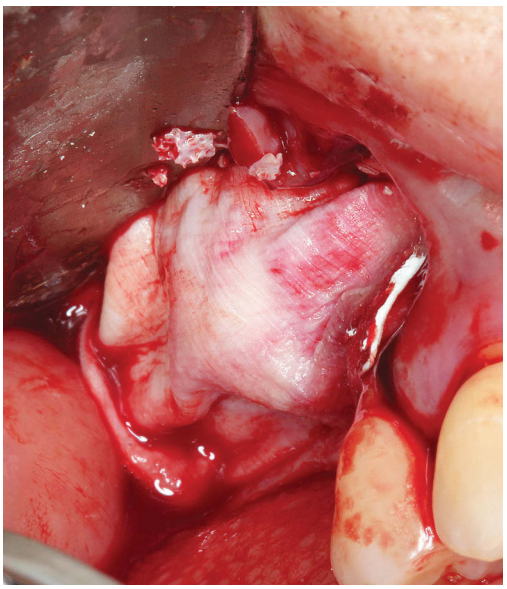


Figure 11. Placement of membrane (Bio-Gide) as a barrier over the grafted region.

taper implants were placed (4.0 x 10 mm, morse taper Black Fix, Titanium Fix, Brazil) (Figs 12,13,14).

Later on, histological hematoxylin-eosin (HE) stained slides were prepared and examined under light microscopy at 100x magnification.

RESULTS

A large amount of vital mineralized tissue was found five months after the surgery, which is less than recommended for grafts without any combination with cells. An histological image of another case that received only the Bio-Oss graft, with no cell

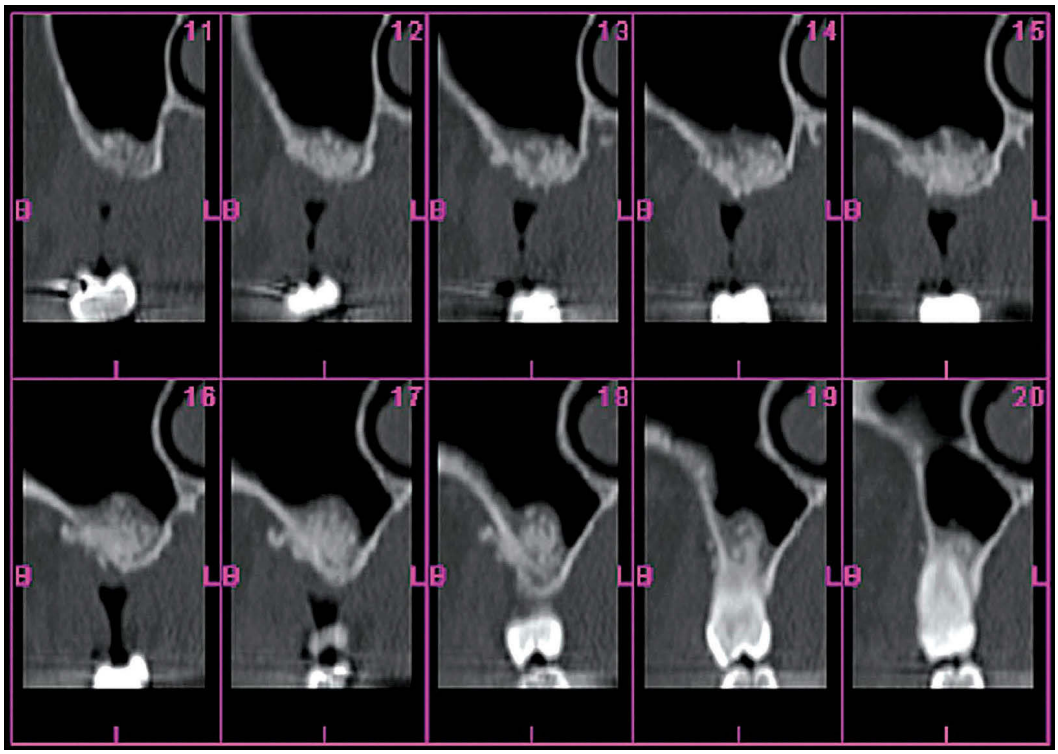


Figure 12. Sagittal CT scan reveals bone tissue in right posterior maxillary sinus previously pneumatized.

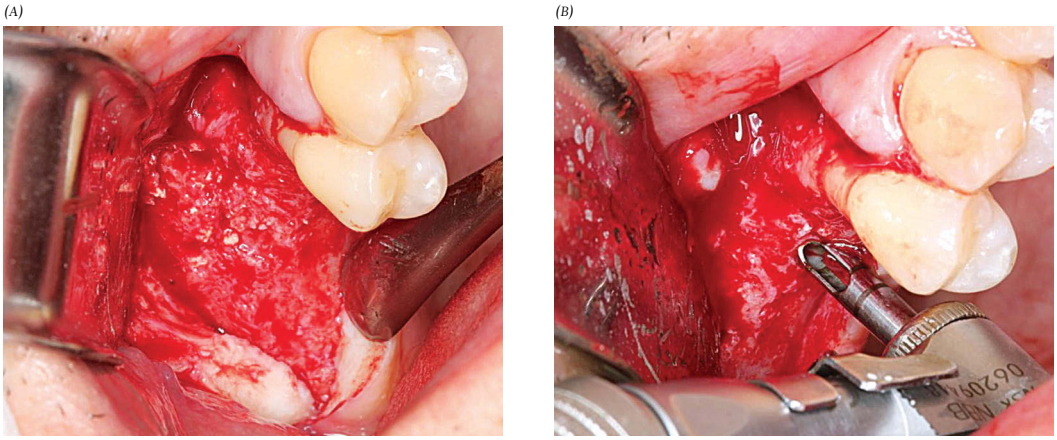


Figure 13. (A) Full-thickness flap raised; **(B)** bone perforation using a 2-mm trephine bur.

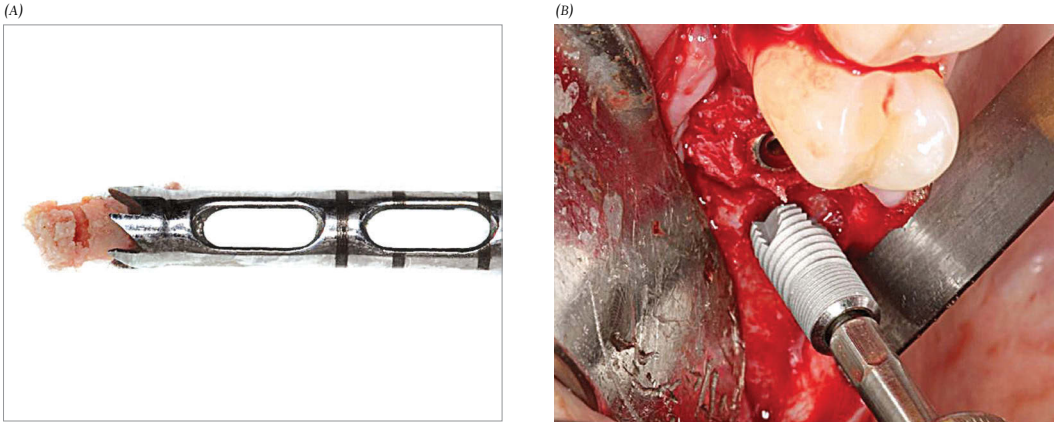


Figura 14. (A) Specimen for histological examination, collected using a 2-mm trephine bur; **(B)** placement of osseointegrated implants.

treatment, is provided for comparison and shows results at 6 months after conventional sinus elevation surgery using a lateral bone access and sinus cavity that received a xenogeneic graft (Bio-Oss 2 g large particles 1.0-2.0 mm, Geistlich, Switzerland), as recommended by the manufacturers (Fig 15B).

DISCUSSION

This clinical report described the use of Bio-Oss, a xenogeneic bone substitute, in combination with bone marrow mononuclear fraction isolated by density gradient for maxillary sinus elevation using lateral access. Analyses confirmed tissue

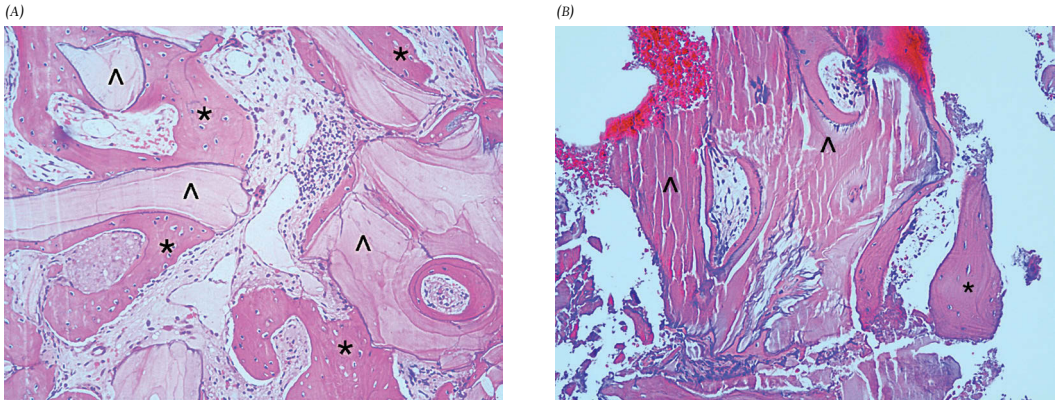


Figure 15. A) Histological image (HE staining) under 100x magnification of the specimen obtained from the clinical case described herein, in which the region of the maxillary sinus received a Bio-Oss (^) graft combined with mononuclear fraction (five months after surgery). There is a large amount of vital mineralized tissue (*) around the Bio-Oss (^) particles. **B**) Histological image (HE staining) under 100x magnification shows another clinical case of the maxillary sinus that received a Bio-Oss (^) graft without any combination with mononuclear fraction (six months after surgery). There is a smaller amount of vital mineralized tissue (*) around the Bio-Oss (^) particles.

quality after a short period of time in comparison with the time required to perform the conventional technique. This has also been reported by other scientific studies employing methods of use of fresh or processed bone marrow.[13,23]

Autogenous bone grafting is considered as a standard reference due to its osteoinductive, osteoconductive and osteogenic properties. Nevertheless, it presents greater surgical morbidity, since it requires two of more surgical sites in cases of greater amount of donor tissue. Extra-oral sites may have to be used, which increases the operative risk as well as the surgical costs, and generates postoperative discomfort. For this reason, a growing number of patients avoid this technique.[1,2] This problem has led to a search for bone substitutes that may replace autogenous bone. However, such substitutes do not have the osteogenic and osteoinductive qualities that are inherent to autogenous grafts.[8]

Choosing biomaterial with physical, chemical and mechanical characteristics as close as possible to autogenous bone has become increasingly necessary due to the need to use the area that received the graft for the placement of osseointegrated implants. International studies report that Bio-Oss, a xenogeneic bovine bone substitute, is a material with characteristics that are very similar to those of human bone, which is associated with its good osteoconductive properties.[24-28] The main disadvantage of lyophilized xenogeneic bone, or any other bone substitute, is the lack of factors that promote osteogenesis and osteoinduction. Deficiency in these factors require longer healing and osseointegration time (from 6 to 8 months) in comparison to the use of autogenous bone of which cellularity and growth factors provide it with a high osteogenic and osteoinductive potential. This potential, inherent to autogenous grafts, reduces the time necessary for bone healing down to 4 to 6 months.[13,23]

The literature has reached a consensus regarding the aforementioned fact. For this reason, methods to enrich bone substitutes have been investigated using cells from the bone marrow of the recipients. Different studies have described collection techniques and the use of fresh bone marrow directly inserted into the surgical sites,[1,2] as well as the culture of mesenchymal stem cells obtained from the bone marrow, as well as bone marrow concentration techniques. The study reported herein used the method described in another study published by the same group of authors, which confirmed that the use of Bio-Oss, a lyophilized xenogeneic bone graft, combined with autogenous bone marrow mononuclear fraction increases the amount of vital bone and reduces the time of graft healing, as confirmed in this clinical report.[23] This method has also been recently published in a book.[29]

Cell culture techniques employed in humans have disadvantages over the use of fresh or concentrate marrow, such as the cost of laboratory processing and the waiting time between collection and graft surgery, because of the large number of cells necessary to perform the procedure and the risks of contamination,[30] as well as the ethical principles involved in the duplication of cells for which there are still no markers. For this reason, the use of a protocol of autogenous bone marrow aspiration and concentration of its mononuclear fraction using density gradient may be a feasible method to improve the quality of the graft material, substantially reduce graft healing time and increase bone quality in the area that receives the graft and that, later on, receives the osseointegrated implants with sufficient torque to achieve adequate primary stability. Moreover, surgical time is not longer, and bone marrow harvesting generates minimal discomfort in the donor area.

CONCLUSION

The clinical use of a bone marrow mononuclear fraction concentrate combined with Bio-Oss, a xenogeneic bone substitute, in maxillary sinus elevation seems to result in good bone repair and shorter healing time.

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