Horizontal Alveolar Ridge Augmentation in the Mandibular Posterior Region Using Biphasic Calcium Phosphate and Leukocyte- and Platelet rich fibrin (L-PRF): A case report.

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CASE REPORT

Abstract

In bone defects caused by tooth loss, tissue reconstructions are necessary to enable prosthetic rehabilitation with dental implants. Diverse techniques and materials of different origins are used for this purpose. Leukocyte- and Platelet-Rich Fibrin (L-PRF) has been used in association with osteoconductive biomaterials in procedures of bone regeneration and for covering grafted areas. The aim of this article was to demonstrate a clinical case of bone grafting in the posterior region of the mandible, performed with the use of synthetic biomaterial composed of biphasic calcium phosphate associated with Leukocyte- and Platelet-Rich Fibrin for performing bone augmentation in an alveolar ridge with a horizontal defect, thereby enabling later installation of dental implants and prosthetic rehabilitation in the region.

Keywords: bone graft, biomaterials, leukocyte- and platelet-rich fibrin, bone substitutes, dental implants.
Aumento horizontal de rebordo posterior de mandíbula utilizando fosfato de cálcio bifásico associado a fibrina rica em plaquetas e leucócitos (L-PRF): relato de caso.

Resumo

Nos defeitos ósseos causados pela perda dos dentes, as reconstruções teciduais são necessárias para possibilitar a reabilitação protética com implantes dentários. Diversas técnicas e materiais de diferentes origens são empregados para tal finalidade. A fibrina rica em plaquetas e leucócitos (L-PRF), tem sido associada a biomateriais osseocondutores nas regenerações ósseas e também no recobrimento de áreas enxertadas. O objetivo desse presente artigo é demonstrar um caso clínico de enxerto ósseo em região posterior de mandíbula, realizado com a utilização de biomaterial sintético de fosfato de cálcio bifásico associado a fibrina rica em plaquetas e leucócitos, para a realização de aumento ósseo em rebordo com um defeito horizontal, possibilitando a posterior instalação de implantes e a reabilitação protética na região. Palavras chaves: enxerto ósseo, biomateriais, fibrina rica em leucócitos e plaquetas, substitutos ósseos, implantes dentários.
Introduction

Tooth loss due to trauma, extractions or periodontal disease, leads to resorption of the alveolar ridge, resulting in vertical or horizontal bone defects or a combination of these. These often make it impossible to install osseointegrated implants [1, 2].

In regions of resorbed alveolar ridges, reconstruction of defects with bone grafts are required. These may be performed prior to or simultaneously with installation of the implants. Diverse techniques described in the literature have been successfully performed by clinicians, with the use of materials of different origins and types [3, 4]. In horizontal bone defects, reconstructions may be performed by means of fixation of bone blocks, techniques for division and expansion of the crest or by means of the guided bone regeneration (GBR) technique [1, 5-7].

Osteoconductive materials of synthetic origin have been widely used in bone regeneration procedures, particularly biphasic calcium phosphate, composed by the association of hydroxyapatite with tricalcium β-phosphate. They have characteristics of resorption to allow bone growth and maintain the volume of the graft area. Studies have shown high success and survival rates of implants installed in areas regenerated with this material [8-11].

Leukocyte- and platelet-rich fibrin (L-PRF), initially described by Choukroun in 2001 [12], is an autologous material that has been used in different regenerative procedures involving hard and soft tissues [13]. It is obtained by centrifugation of venous blood, leading to the occurrence of a natural and gradual process of polymerization. This results in a dense fibrin matrix, with a large number of platelets and leukocytes trapped within it. This product slowly releases cytokines and growth factors involved in angiogenesis and in the process of tissue regeneration in a period lasting from 7 to 14 days [14-16].
The aim of this study was to describe a clinical case of lateral augmentation of the alveolar ridge bone by means of the guided bone regeneration technique, with the use of biphasic calcium phosphate and L-PRF.

Case Report

The patient, a 61-year-old woman presented to the clinic of the specialization course in Implant Dentistry at the University of Salgado de Oliveira (Universo, Campus Niterói – RJ) for dental assessment. During anamnesis, the patient reported that she had no systemic problem. Her chief complaint was the absence of tooth 36 and presence of teeth with caries disease, and she inquired about the possibility of treatment and tooth replacement with an implant. During the clinical exam, the presence of an extensive caries lesion was verified in tooth 35 and absence of tooth 36. There was visible loss of tissue volume from the vestibular aspect (Figure 1). Therefore, Cone Beam computed tomography examination, laboratory blood tests (complete blood count, coagulogram, serum phosphorus and calcium, fasting glucose, alkaline phosphatase and creatinine) were requested. In addition, intra and extraoral photographs were taken for planning the case.

![Figure 1 – Residual root of tooth 35 and loss of vestibular tissue volume in the region of tooth 36.](image)

The laboratory blood exams showed no alteration. In the computed tomography assessment, loss of bone thickness in the region of tooth 35 was
verified (Figure 2), which required tissue regeneration therapy to enable implant installation. In addition, the need for extracting tooth 35 was shown. The patient was informed of the diagnosis and the treatment proposed for resolving the case. These were promptly accepted by the patient, who then signed the Term of Free and Informed Consent. Pre-operative medications were prescribed (2 g Amoxicillin, one hour before surgery; 4 mg Dexamethasone, 1 tablet 1 hour before surgery) [17].

In the first surgical stage, the patient was anesthetized with 2 tubes of 4% articaine hydrochloride with epinephrine (Articaine 4% 1:100,000 – Nova DFL, Rio de Janeiro, Brazil), with inferior alveolar, lingual and buccal nerve blocks. Initially, tooth 35 was extracted. Subsequently, a scalpel fitted with a 15C blade (Swann-Morton®, Sheffield, England) was used to perform a full-thickness supracrestal incision, starting from the distal region of tooth 34 to the mesial region of tooth 37. Intrasulcular incisions around these teeth, on the buccal and lingual sides were also made. A Molt 2-4 Surgical Curette (Quinelato ®, Rio Claro-SP) was used to detach a mucoperiosteal flap to expose the bone region (Figure 3A). A 701 carbide drill (Komet, Santo André – SP) was used to perform perforations in the buccal cortical bone to promote means of vascularization in
the region to be grafted (Figure 3B). After this, venous blood was collected from the patient using eight 9ml plastic vacuum tubes, without the addition of anticoagulant (Vacuette®, Greiner Bio-One, Americana, SP, Brazil). For the purpose of obtaining the leukocyte and platelet-rich fibrin membranes (L-PRF), six tubes sprayed with serum clot activator were used. Two tubes without clot activator were used to obtain leukocyte and platelet-rich fibrin membranes (L-PRF) in the liquid phase. The tubes were centrifuged in a bench centrifuge (Daiki DT4000, Hunan Labwe Scientific Instruments Co., Ltd., Hunan, China), using a centrifugation protocol of 408 g (G force) for 3 minutes to obtain fibrin in the liquid phase and 408 g for 12 minutes to obtain fibrin clots. The clots were removed from the tubes with sterile forceps and pressed into a metal case to make the L-PRF membranes. To preserve the alveolar ridge in the region of tooth 35 and perform horizontal reconstruction in the region corresponding to tooth 36, 0.5 cc of the alloplastic bone substitute, biphasic calcium phosphate (Nanosynt ® - FGM, Joinville-SC, Brazil) with granulation ranging from 500 to 1000 µm was used and was mixed with platelet-rich fibrin and leukocytes in the liquid phase. After approximately 15 minutes of mixing, the material was agglutinated, and was taken to the alveolus of tooth 35, placed in the bone bed in the region corresponding to tooth 36 (Figures 4A, B,C and D). After this, the entire grafted region was covered with 4 L-PRF membranes, two of them arranged horizontally and the other two arranged vertically, enveloping the lingual and buccal regions of the flap (Figure 5). On conclusion of coating with L-PRF membranes, suturing was performed with a 5.0 mononylon thread (Ethicon® – Johnson and Johnson – São Paulo-SP) (Figure 6).
Figure 3 – A – Detachment of the mucoperiosteal flap, showing evidence of the area of bone defect. B – Preparation of the bone bed with perforations made with the use of Carbide burr 701 (buccal view).

Figure 4 – A – Leukocyte- and platelet-rich fibrin (L-PRF) in the liquid phase added to the alloplastic substitute. B- Agglutinated particulate graft. C and D – Filling the socket of tooth 35 and bone graft positioned in the region of the bone defect of tooth 36.
The patient received postoperative care instructions and prescriptions (500 mg Amoxicillin, 1 tablet every 8 hours for 7 days; 500 mg dipyrone, 1 tablet every 6 hours for 2 days; 00 mg Nimesulide, 1 tablet every 12 hours for 3 days; 0.12% Chlorhexidine for washing the region 2 times a day for 7 days).

Six months later, the patient returned for consultation and underwent a new Cone Beam CT scan. The tomographic images showed areas compatible with an increase in bone volume in the grafted region (Figure 7). In the intraoral clinical
examination, the increase in tissue volume in the grafted region was evident (Figures 8A and B).

Figure 7 - CBCT six months after the bone regeneration procedure.

Figure 8 – A- Initial clinical appearance. B – Post-bone regeneration view (6 months later).

After this, the second surgical step was performed in the grafted region for the installation of osseointegrated implants and for extraction of tooth 37 that was without the prosthetic crown and had an extensive carious lesion. Two external hexagon implants of the brand Strong SW (S.I.N. Implant System® – São Paulo, Brazil), platform 4.1 (3.75 x 10 mm) were installed in the region of teeth 35 and 36 (Figures 9A, B, C and D). The socket of tooth 37 was filled with alloplastic bone substitute (Nanosynt® - FGM, Joinville-SC, Brazil) and the region was
covered with two L-PRF membranes. Subsequently, suturing was performed in the region (Figure 10).

Figure 9 – A – Detachment of the mucoperiosteal flap, showing evidence of the increase in horizontal bone. B – Extraction of tooth 37. C – Preparation of the bone bed for installation of implants in the region of teeth 35 and 36. D – Implants placed.

Figure 10 - A - Suture. B and C – Healing caps installed (7 days after reopening).
Four months after implants were placed, the sites were reopened, and healing caps were inserted (Figures 10B and C). Two months after the reopening procedure, the patient received a fixed splinted dental prosthesis relative to teeth 35 and 36.

Figure 11 – A – Implant supported dental prosthesis in region of teeth 35 and 36. B - Final panoramic radiograph.

Discussion

Guided bone regeneration, a procedure widely used for alveolar bone ridge augmentation, is a technique that has been well-documented in the literature. Moreover, a high survival rate has been shown for implants installed in the areas of defects treated with this safe and predictable therapy. In GBR, the role played by resorbable or non-resorbable membranes used as a barrier, refers to creating and maintaining space, preventing soft tissue growth, and providing the graft with stability [4,5,18,19].

In the present report, alveolar preservation and horizontal augmentation were performed with the use of a particulate biphasic calcium phosphate graft associated with leukocyte- and platelet-rich fibrin (L-PRF) in the liquid phase. The entire grafted area was covered with L-PRF membranes, without association of resorbable or non-resorbable membrane barriers as described in the GBR technique [20]. One of the advantages of using L-PRF membranes was that, due to their highly dense structure, they can be sutured onto the soft tissues, thereby promoting enhanced stability in the region. Furthermore, in case of suture dehiscence in the operated area, the L-PRF membrane does not cause problems, as it remains exposed to the oral environment, and continues with its function of...
covering the graft. Another advantage of using L-PRF membranes is that they are an autogenous biomaterial, easy to obtain, and favor cost reduction when compared with the use of resorbable or non-resorbable membranes.

Several studies have evaluated the use of L-PRF membranes as a barrier and have shown positive results in bone regeneration. One in vivo study, using a canine animal model evaluated the effect of L-PRF membrane on bone regeneration in lateral [alveolar] ridge augmentation. Bone defects were created, filled with xenograft (Bio-Oss) and alloplastic graft (Cerasorb), and were covered with non-resorbable PTFE membranes (Cytoplast TXT 200), resorbable collagen membranes (Bio-Gide) and L-PRF membranes. The results of the histological analyses of the group that received the L-PRF membrane for covering the defects, showed a larger quantity of neoformed bone and smaller amount of soft tissue formation, when compared with the other groups, with statistically significant values. With regard to analysis of the volumetric parameters by means of micro-CT, the L-PRF membrane group had a higher volume of newly formed bone (mm) and a higher proportion (%) of volume of neoformed bone in relation to volume [21].

Another in vivo study evaluated the effect of resorbable membranes and platelet-rich fibrin membrane (PRF) on bone healing in critical defects in rat tibiae. The rats were divided into three experimental groups using one layer of collagen membrane, two layers of collagen membranes and platelet-rich fibrin (PRF) membranes and a control group without any covering membrane. After 7 and 28 days, the histomorphometric analysis showed that the group that received PRF membranes had higher level of bone neoformation and a lower level of fibrosis when compared with the other groups [22].

Clinical studies with humans, with the use of L-PRF membrane for covering grafted areas have been published in the literature. These have shown positive results in several situations, and may be an alternative material for use in the bone regeneration process [23-26].

By means of a modification made in the centrifugation protocol to obtain leukocyte- and platelet-rich fibrin clots, it was possible to develop leukocyte- and platelet-rich fibrin in the liquid phase. This concerned platelet concentrate shows an abundant quantity of activated fibrinogen in the liquid phase containing growth factors responsible for tissue regeneration [27]. One of the clinical applications in
bone regeneration is the possibility of agglutinating particulate biomaterials [2,28].

In the present clinical case, the use of leukocyte- and platelet rich fibrin in the liquid phase, associated with particulate synthetic biomaterial, made it possible to agglutinate the biomaterial particles, thereby favoring their accommodation and stability in the bone defect of the grafted region. This was also applied on the L-PRF membranes, promoting better adhesion between them in the surgical bed. This characteristic of agglutination and adhesiveness was advantageous when the grafting technique was being performed.

In the second surgical stage of the implants placement, it could be noted that the regenerated area had a bone density that allowed good primary stability of the implants at the time of their placement.

**Conclusion**

In the present case report, the use of L-PRF membranes for covering the biphasic calcium phosphate particulate graft proved to be an effective therapeutic approach to guided bone regeneration for the purpose of horizontal bone augmentation. However, further controlled studies are necessary to evaluate the success of this therapeutic approach.

**Consent**

Written informed consent was obtained from the patient for publication of his clinical details and clinical images.

**Conflicts of Interest**

The author(s) declare(s) that they have no conflicts of interest.
References


