CHAPTER 6

Endoret® (PRGF®) Application in the Oral and Maxillofacial Field

AUTHORS

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SUMMARY

In 1995 the first intraosseous application of Endoret (PRGF) was reported in the regeneration of infected bone and soft tissue defect after tooth extraction. It is in the oral and maxillofacial surgery where the clinical use of Endoret (PRGF) was initiated and where its therapeutic formulations was designed. The oral and maxillofacial surgery (non-steril surgical field) also allows for the design of a split-mouth clinical trial. The safety, efficacy and predictability of this biological preparation trigged then its use in various medical fields including the orthopedics and sport medicine. In this chapter, we present a comprehensive review of the use of Endoret (PRGF) in oral and maxillofacial surgery. This review is evidence-based and provides guidelines for the use of the different therapeutic formulations of Endoret (PRGF). It will serve as a bridge of cross-talking between oral surgeons and orthopedic surgeons with the aim of approximating views and developing new ways of applying the various formulations in Endoret technology. In all its applications in oral and maxillofacial surgery, Endoret (PRGF) has improved the patient's quality of life, the healing of soft tissue and the regeneration of hard tissue. The preservation and handling of a grafting material has all improved once mixed with Endoret (PRGF).

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1. INTRODUCTION

The development of Endoret (PRGF) technology started by the meticulous study of platelets and the optimal conditions under which they can be concentrated and maintain their properties unaltered. As a consequence, a simple, dynamic and versatile protocol has been developed that enables the clinician to obtain formulations of great therapeutic potential. Some of the properties that are peculiar to Endoret (PRGF) are:

- Endoret (PRGF) is autologous and biocompatible.

- It is more precise to speak of Endoret (PRGF) technology, as it is not a unique preparation, but rather a set of formulations with a therapeutic action, easily obtained from the patient's own blood. The formulations that constitute Endoret (PRGF) technology are:

- Endoret (PRGF) supernatant: this is an excellent culture medium to maintain primary cells and autologous bone. It contains platelet- and plasma-derived proteins and factors (fig. 1a).

- Activated liquid Endoret (PRGF): The activation of Endoret (PRGF) using calcium chloride as an activator starts the release of contents of the platelets, giving rise to a liquid formulation rich in proteins and growth factors. This formulation is used to inject Endoret (PRGF) in tissues such as muscle, tendon, skin and joints. It also used to biofunctionalize implant surface (fig. 1b).

- Endoret (PRGF) clot: In 3-5 minutes, the activated liquid Endoret (PRGF) becomes a three dimensional matrix of fibrin rich in cellular components and growth factors. This formulation can be used in various applications, from the regeneration of extraction socket to the treatment of muscular, skeletal or vascular pathologies. (fig. 1c)

- Fibrin membrane: The retraction of the clot obtained by the activation of fraction 1 (F1) of Endoret (PRGF) results in a dense and elastic membrane. This fibrin membrane is biocompatible and is used as a biological membrane. (fig. 1d)

This chapter gives an overall view of Endoret (PRGF) applications in oral surgery. Oral surgery has been used as an experimental model where different aspects of wound healing with Endoret (PRGF) have been assessed. Many randomized clinical trials, some of them split-mouth designed, have been performed and published.

2. USE OF ENDORET® (PRGF®) IN THE TREATMENT OF EXTRACTION SOCKET

Extraction socket is the defect that remains in the alveolar ridge after tooth extraction. Endoret (PRGF) and autologous fibrin are optimal biomaterials for the treatment of extraction socket. They are autologous, easy to prepare, completely re-absorbable, and cost-effective. The biological technique of Endoret (PRGF) has no side effects or harmful consequences. All these characteristics are the basis on which Endoret (PRGF) is recommended even for the treatment of dry socket (inflammation of the extraction socket), the most common complication after tooth extraction. Various studies have reported that the use of platelet concentrates have been effective in reducing the occurrence of dry socket[1,2].

The protocol for the management of tooth extraction with Endoret (PRGF) include several important aspects[3,4]. Atraumatic tooth extraction minimizes trauma to the surrounding bone and gingival tissue. The socket is filled with a clot prepared from the fraction 2 (F2) of the Endoret (PRGF). Then, the fibrin membrane, prepared from the fraction 1 (F1) of the Endoret (PRGF), is placed to cover the socket. A suture is finally applied to retain the clot and membrane in the socket.

The outcomes of applying this protocol are less pain and inflammation after tooth extraction[4]. This has contributed to accelerate healing and regeneration of the extraction socket[4]. Figure 2 shows an example of an extraction socket that has been treated with Endoret (PRGF). Here we can observe the absence of inflammation, Endoret (PRGF) remodeling and the closure of the defect with a new keratinized mucosa.

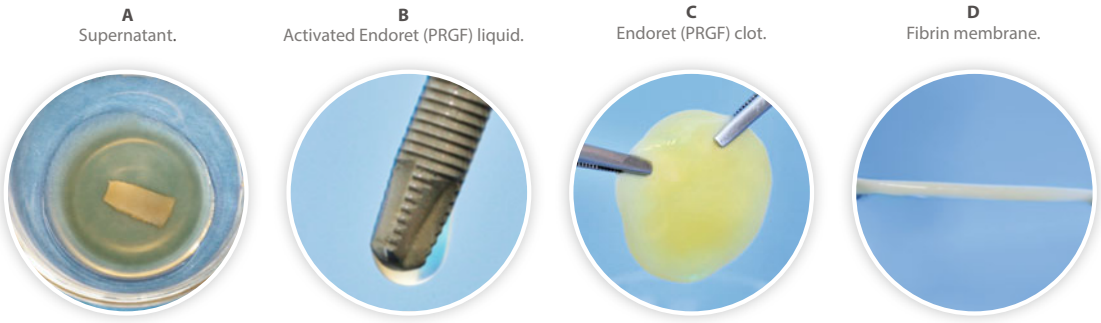


FIG. 1
Therapeutic formulations of Endoret (PRGF).

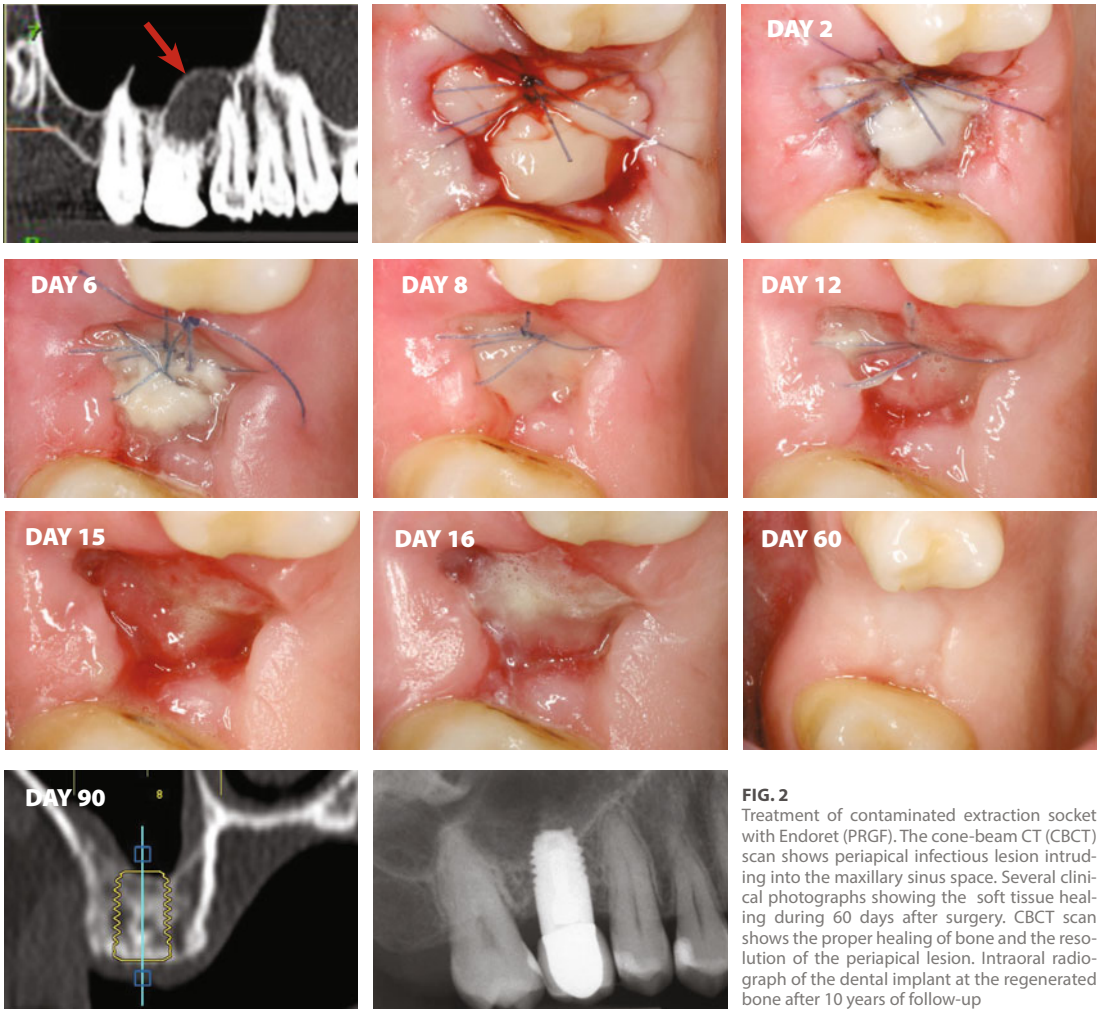


FIG. 2
Treatment of contaminated extraction socket with Endoret (PRGF). The cone-beam CT (CBCT) scan shows periapical infectious lesion intruding into the maxillary sinus space. Several clinical photographs showing the soft tissue healing during 60 days after surgery. CBCT scan shows the proper healing of bone and the resolution of the periapical lesion. Intraoral radiograph of the dental implant at the regenerated bone after 10 years of follow-up

Therapeutic potential of Endoret (PRGF) in the treatment of extraction socket: Experimental studies in animals

In 2000, we performed animal studies to investigate the regenerative potential of Endoret (PRGF)[4,5]. The experimental animal model was bone defect 5 mm in diameter produced in the tibia of goats. These defects were filled with Endoret (PRGF) in the experimental group and blood clot in the control group. After 8 weeks, bone samples were harvested and prepared for histological and histomorphometric analysis.

The defects treated with Endoret (PRGF) showed the presence of newly formed trabecular bone that was surrounded by a dense and highly vascularised connective tissue. The control defects were filled by a connective tissue with high cellularity and some small area of new bone formation (fig. 3).

Therapeutic potential of Endoret (PRGF) in the management of extraction socket: Preparation of future sites for dental implant and the first randomized controlled clinical study of a PRP in extraction socket

The first clinical study on the potential of Endoret (PRGF) to regenerate extraction socket for the future insertion of dental implants was reported in 1997, and published in 1999[4]. In that study, 20 patients in need of tooth extraction were recruited and randomized to receive Endoret (PRGF) or blood clot. The soft tissue healing of the defect was evaluated at day 3, day 7 and day 15 after tooth extraction. A dental implant was inserted at the site of extraction site after 10-16 weeks of tooth extraction. Before implant site preparation, a 6 mm long bone biopsy was obtained from the centre of the socket with a trephine bur.

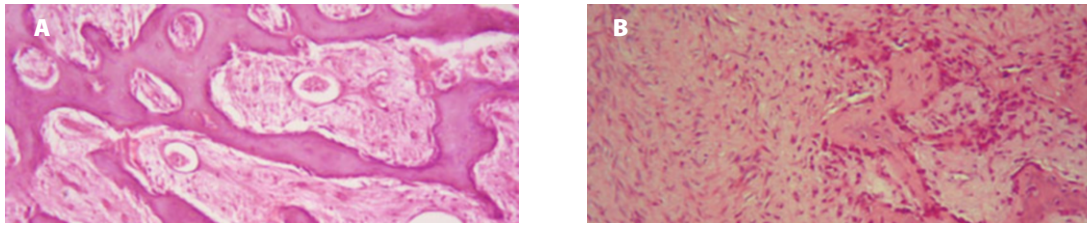


FIG. 3
The histological images of bone biopsy obtained from: A) The socket treated with Endoret (PRGF). B) The socket treated with blood clot.

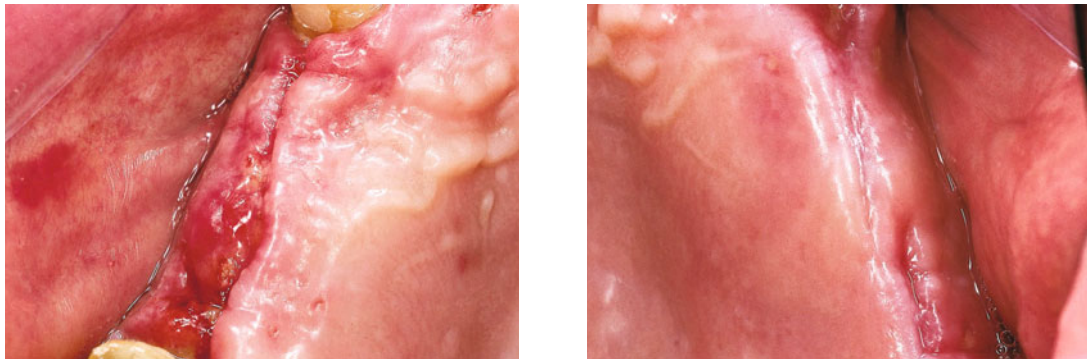


FIG. 4
Split-mouth design adopted to study the potential of Endoret (PRGF) in the treatment of extraction socket. Endoret (PRGF) was applied on the left side and the right side served as control. We can observe the differences in soft tissue healing between both groups after 14 days of surgery.

The soft tissue healing was excellent in the 10 patients treated with Endoret (PRGF). Three patients underwent bilateral tooth extractions, and thus a split-mouth study design was possible. This design permitted the comparison of the two treatments under the same biological conditions. Figure 4 shows the difference in soft tissue healing in the same patient who received Endoret (PRGF) on one side, and blood clot on the other side.

The histological analysis of the bone samples showed a difference in the degree of trabecular organization. More bone regeneration and better trabecular organization were observed at the sites treated with Endoret (PRGF) (fig. 5).

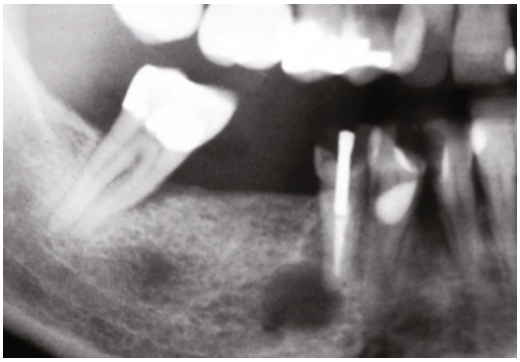


FIG. 5a
Radiographic image showing vertical fracture of the lower right first molar and the presence of periapical granuloma.

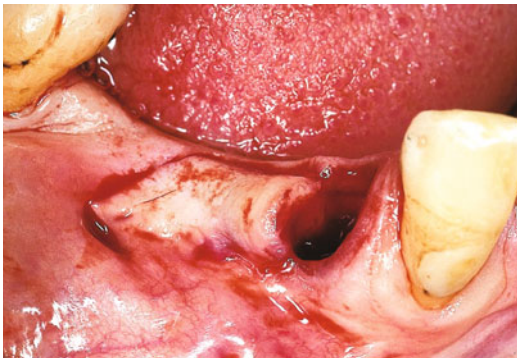


FIG. 5b
After flap elevation, atraumtic tooth extraction was performed and the socket was treated with Endoret (PRGF).

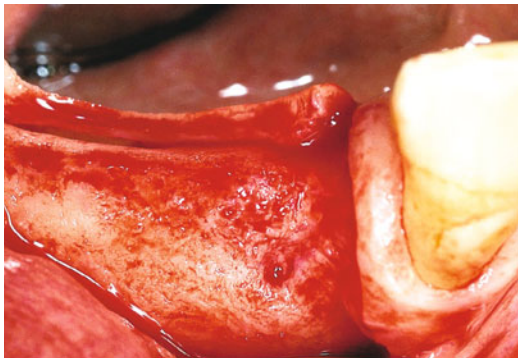


FIG. 5c
The Endoret (PRGF) stimulation of bone regeneration permitted the insertion of dental implant after 14 weeks of tooth extraction.

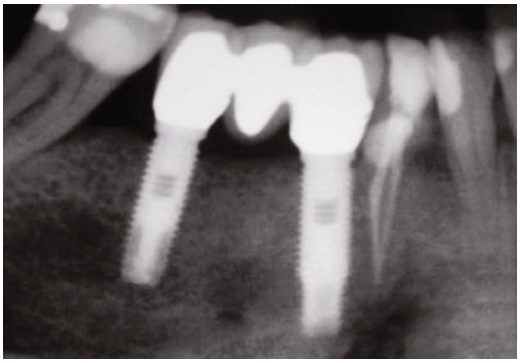


FIG. 5d
The clinical status of the dental implant after 4 years of insertion.

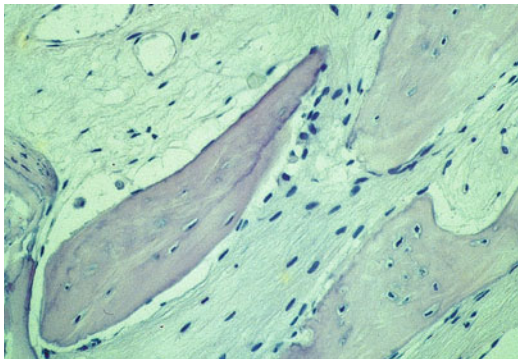


FIG. 5e
The histological analysis of bone biopsy obtained 14 weeks post-operatively. New formation of bone trabeculae with osteocytes was observed.

Therapeutic potential of Endoret® (PRGF®) in the treatment of extraction socket: Split-mouth clinical study to evaluate bone regeneration

In 2010, a split-mouth clinical study was performed to evaluate bone regeneration in the extraction socket treated with Endoret (PRGF)[6]. The study was performed in 14 patients and blood clot served as the control treatment.

A cone-beam CT (CBCT) scan was obtained for each patient at 11-14 weeks postoperatively. These scans were later analyzed to retrieve data about the volume of regenerated bone as well as the bone density expressed in Hounsfield units.

To measure the density, a cylinder resembling an implant was placed inside the defect. The visualizing software (BTI scan) gave two measurements of the density, one for the area located within the cylinder, and the second for area outside the limit of the cylinder.

The values of both densities and the one measured at the centre of the defect were all statistically higher in the Endoret (PRGF) group (fig. 6). Figure 7 shows a clinical case where complete soft tissue healing and bone regeneration were obtained in a patient in whom Endoret (PRGF) had been used.

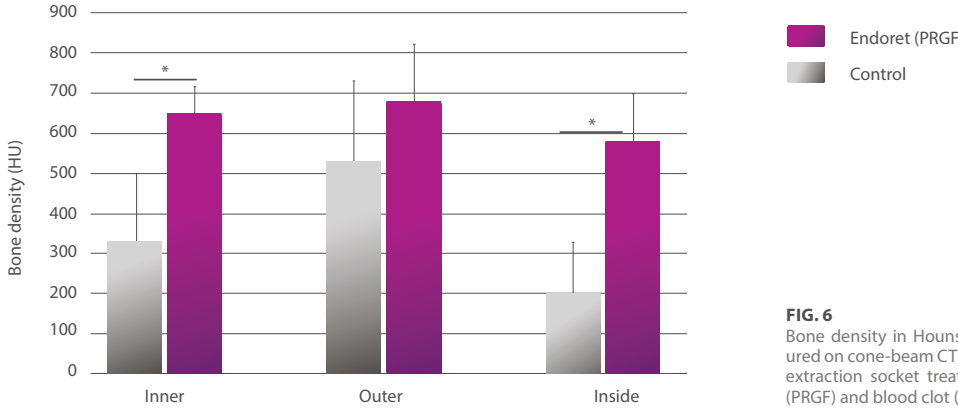


FIG. 6
Bone density in Hounsfield units measured on cone-beam CT scan obtained for extraction socket treated with Endoret (PRGF) and blood clot (control).

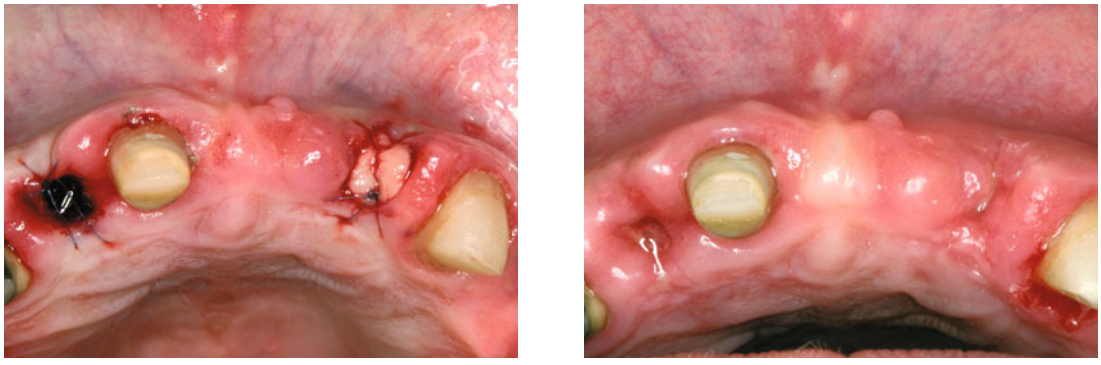


FIG. 7
The status of the extraction socket after application of the assigned treatment (Endoret (PRGF) or blood clot). A significant differences in soft tissue healing could be observed after 5 days of tooth extraction between both groups.

Therapeutic potential of Endoret (PRGF) in the treatment of extraction socket: assessor-blinded, parallel group, randomized controlled clinical trial

A single center, assessor-blinded, parallel group, randomized controlled clinical trial was conducted. Sixty patients with a simple one molar extraction in the mandible were randomized to receive Endoret (PRGF) or blood clot[7]. Thirty six patients were treated with Endoret (PRGF) and 24 with blood clot. A cross-stitch suture was placed in both groups. Clinical, radiographical and histological assessments were performed during 10-12 weeks of follow-up. The primary outcome was the percentage of patients having their sockets regenerated by ≥ 75% (from baseline to week 10-12). For measuring the primary outcome, CBCT scans at baseline and after 10-12 weeks of healing were imported into a software (BTI Scan II, BTI Biotechnology institute, Vitoria, Spain) and analyzed. The secondary variables were bone density, soft tissue healing index, thickness of regenerated keratinized mucosa, pain and inflammation. Additionally, histological analyses were performed for soft and hard tissue biopsies obtained before dental implant insertion.

After tooth extraction, the socket was examined for the presence/absence of bone septum. About 54% of the extraction sockets in the control group preserved the boney septum, while 39% of the experimental groups had the septum. The comparison of the extraction sockets showed that sockets in the experimental group had higher initial volume than in the control group (fig. 8).

Cone beam CT analysis has shown that the percentage of patients where the sockets are regenerated at ≥ 75% of the baseline volume was significantly higher in the Endoret (PRGF) group (96.7%) than the control group (45.5%) (fig. 9).

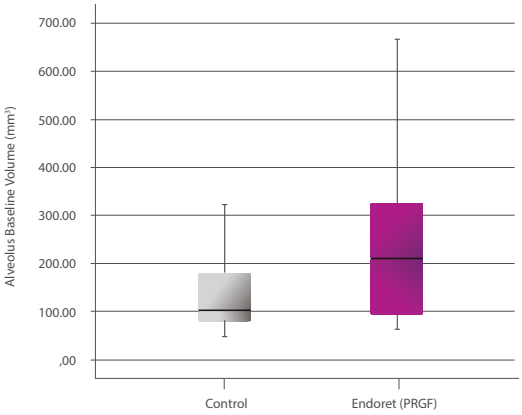


FIG. 8
Comparison of the volume of extraction socket between the Endoret (PRGF) group and blood clot group.

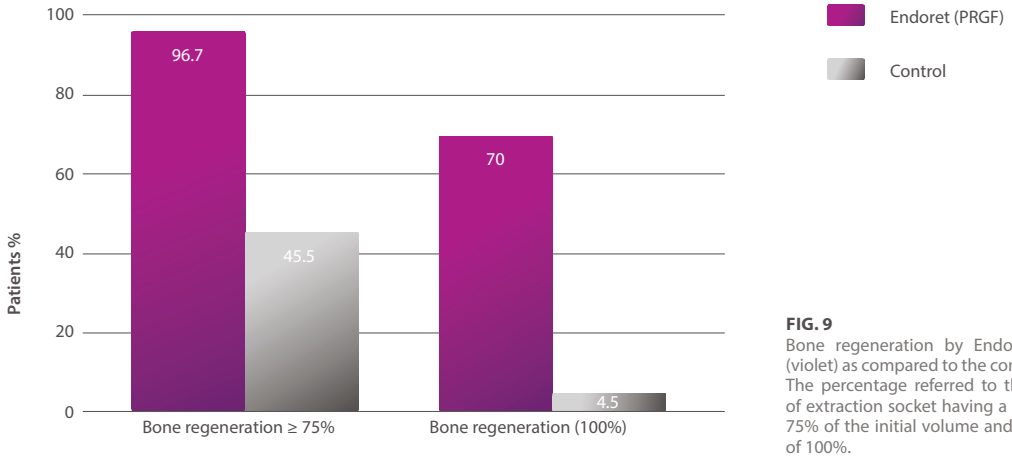
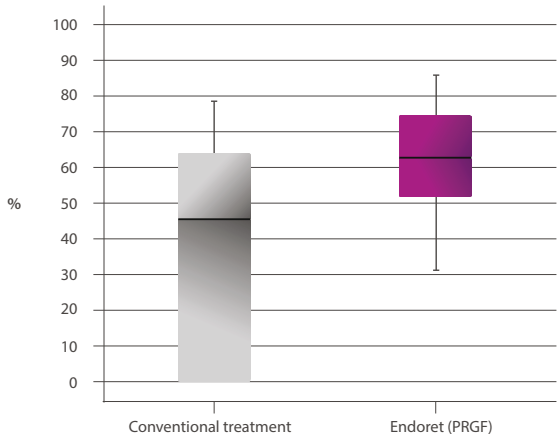


FIG. 9
Bone regeneration by Endoret (PRGF) (violet) as compared to the control (grey). The percentage referred to the fraction of extraction socket having a bone fill of 75% of the initial volume and a bone fill of 100%.

Histological analysis indicated the presence of significantly higher newly formed bone in the Endoret (PRGF) group (63.08% Vs. 35.56%) (fig. 10). No serious adverse events occurred in either group. Bone density was significantly higher in the Endoret (PRGF) group (450 HU Vs. 318 HU) (fig. 11).

Pain and inflammation (day 3 and day 7) were significantly lower in the PRGF group than the control group, but not at day 15 (figs. 12 and 13). Soft tissue healing scores were significantly higher in the test group at days 3, 7 and 15. Figure 14 showed the difference in tissue healing between the sockets treated with Endoret (PRGF) and blood clot.



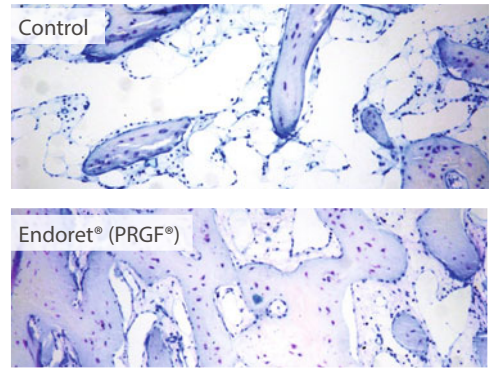


FIG. 10
Histological and histomorphometric analysis of newly formed bone in Endoret (PRGF) and control groups.

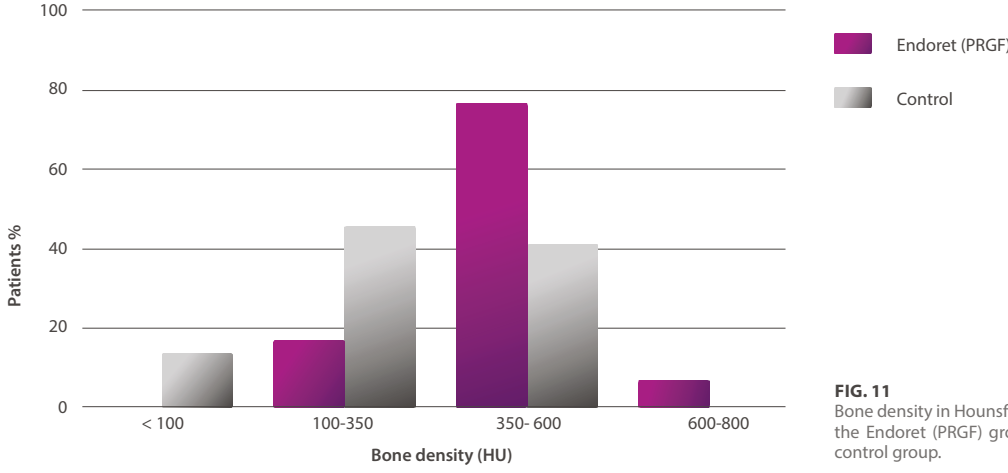


FIG. 11
Bone density in Hounsfield units for the Endoret (PRGF) group and the control group.

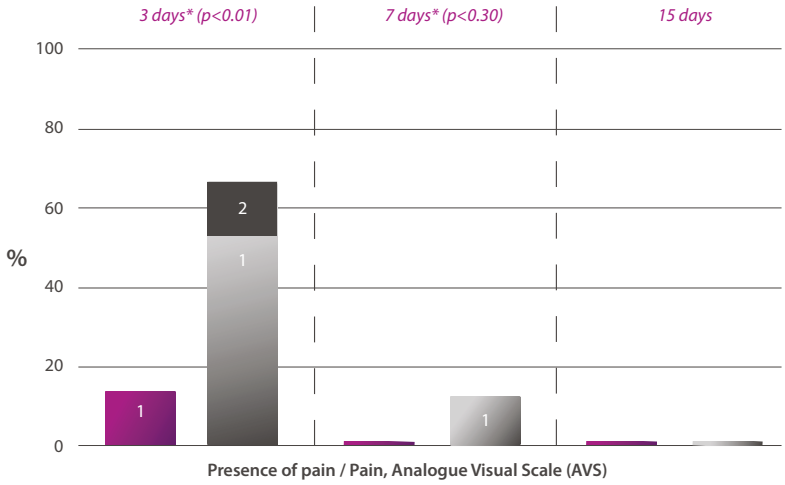


FIG. 12
The evolution of pain as measured on days 3, 7 and 15 after tooth extraction for Endoret (PRGF) and control groups.

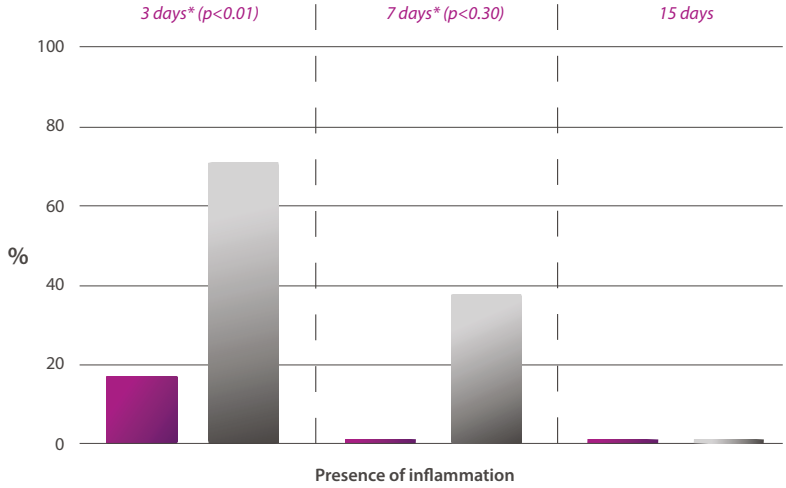


FIG. 13
The evolution of inflammation as measured on days 3, 7 and 15 after tooth extraction for Endoret (PRGF) and control groups.

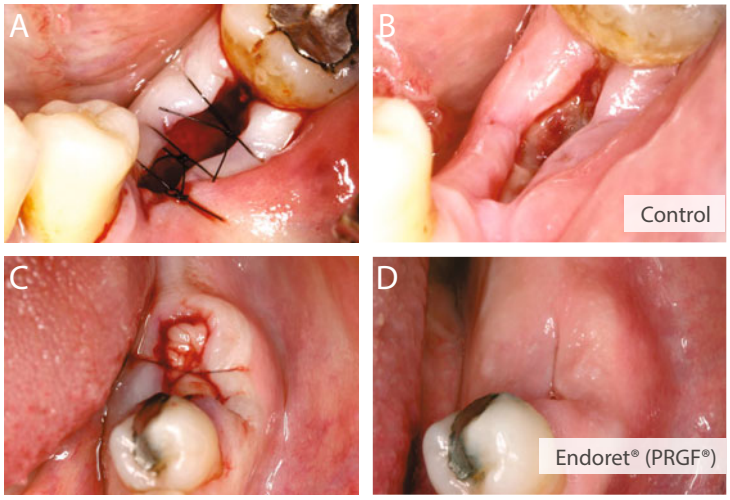


FIG. 14
A) Tooth extraction in the control group. B) Socket regeneration in the control group after 15 days. C) Tooth extraction in the Endoret (PRGF) group. D) Socket regeneration in the Endoret (PRGF) group after 15 days.

Histological analysis indicated the presence of significantly thicker keratinized epithelium in the Endoret (PRGF) group. The thickness was almost double in the Endoret (PRGF) group in comparison the control group (fig. 15).

The main conclusions we obtained from this study are:

1. The use of Endoret (PRGF) resulted in statistical significant differences for the following variables:
   - Defect fill by ≥ 75% of the initial volume.
   - Bone density
   - Histomorphometric analysis
2. Endoret (PRGF) produced statistically significant better soft tissue healing and higher thickness of the regenerated keratinized mucosa.
3. The use of Endoret (PRGF) produced statistically significant lower pain and inflammation.
4. The absence of surgical and post-operative complications indicates that Endoret (PRGF) is safe and effective.

3. THE USE OF ENDORET® (PRGF®) IN MAXILLARY SINUS FLOOR AUGMENTATION

In 1970 Tatum[8] described the first technique for sinus floor elevation through the removal of bone block from the lateral wall of the maxillary sinus. The Schneiderian membrane is then carefully elevated to create space above the alveolar ridge. This space was then filled with a bone graft. The objective of this technique was to obtain vertical bone augmentation in the posterior regions of the maxillary alveolar ridge. In 1980, the technique was modified by Boyne and James[9] to extend their use to permit the insertion of dental implants. In 1996, Anitua[10] described the use of Endoret (PRGF) in combination with bone grafts obtaining very good results for graft integration and reduced post-operative inflammation.

Lateral sinus floor elevation with Endoret® (PRGF®)

In this technique, a bone block is removed from the lateral wall of the maxillary sinus to produce a window and access the Schneiderian membrane.

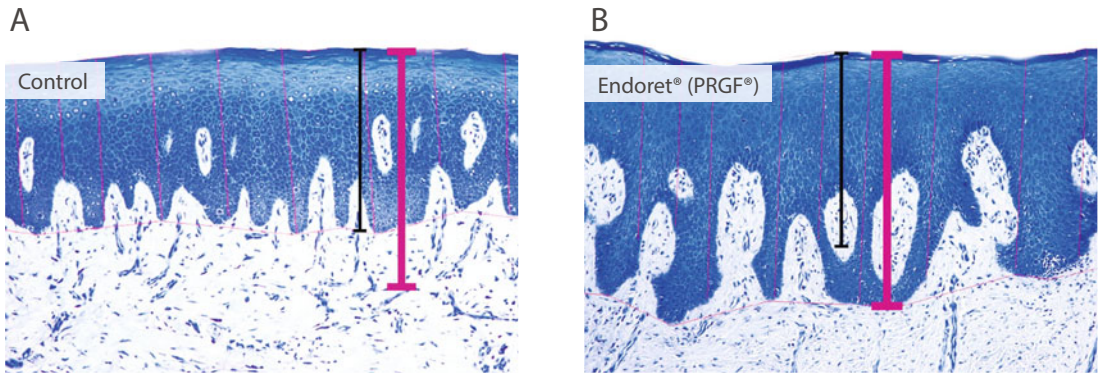


FIG. 15
Histological images of biopsies of the regenerated gingiva harvested at the centre of socket of the control (A) and Endoret (PRGF) (B) groups. The thickness was almost the double in the Endoret (PRGF) group.

Conventionally, this window is produced using a tungsten carbide or diamond bur connected to a hand piece. Heating and vibration of the drilling bur frequently resulted in perforations of the Schneiderian membrane that impaired the performance of sinus floor elevation. We have pioneered in 1994-1995 the use of ultrasonic surgery to create this window and avoid such complication. This new technique also avoided injury to the soft tissues during cutting.

Once the bone block is removed, it is conserved in the Endoret (PRGF) liquid to increase the possibility of cell survival. This is motivated by the fact that after completing the sinus elevation procedure, the block is repositioned to cover the lateral window and enhance bone regeneration.

The Schneiderian membrane is then carefully elevated by angled periotomes. The angulation of these periotomes is specifically designed to permit access to all borders of the opened window. Once the membrane is elevated, bone graft mixed with activated F2 of the Endoret (PRGF) is implanted to fill the created space below the membrane. The graft mixture with the Endoret (PRGF) will result in the formation of cohesive mass, facilitating its application to the sinus. The procedure is completed by repositioning the bone block. For this, the block is rotated 30º. Endoret (PRGF) membrane prepared from F1 is then applied to cover the surgical area, and the flap is repositioned and sutured (fig. 16).

Clinical outcomes of the use of Endoret® (PRGF®) in sinus floor elevation: A split-mouth clinical trial[11]

To study the advantages of Endoret (PRGF) use in lateral sinus floor elevation, 5 patients who needed bilateral sinus floor elevation were recruited. The procedure on one side was performed using Endoret (PRGF) and on the other was performed without Endoret (PRGF). Endoret (PRGF) was mixed with bovine bone graft only on the experimental side. The survival of the implants placed after sinus floor elevation was 100% during the observation period.

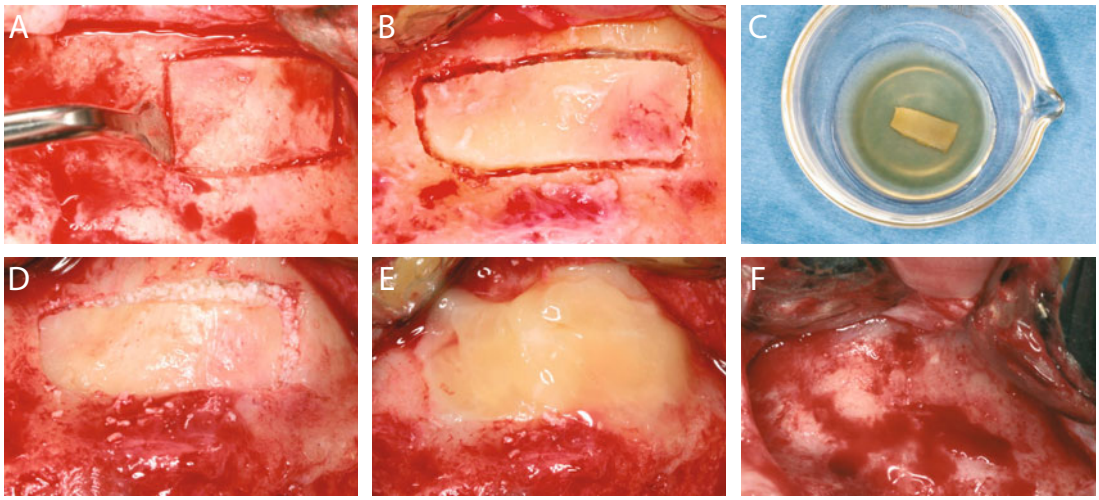


FIG. 16
Lateral sinus floor augmentation: a) Creating a window to access the sinus floor. b) After finishing bone cutting. c) The removed bone block maintained in Endoret (PRGF) liquid. d) the bone block was repositioned after grafting the sinus floor. e) Fibrin membrane prepared from F1 of Endoret (PRGF) covering the surgical area. f) The osseointegration of the bone block at the surgical re-entry.

At clinical examination, there was greater tissue inflammation and pain in the control side. Histological analysis of bone biopsies obtained before implant insertion showed more new bone formation and more blood vessels in the Endoret (PRGF) group (fig. 17).

Thus, the association of lateral sinus floor elevation with Endoret (PRGF) is safe and efficient for vertical bone augmentation. Endoret (PRGF) resulted in greater new bone formation and better vascularization of the graft.

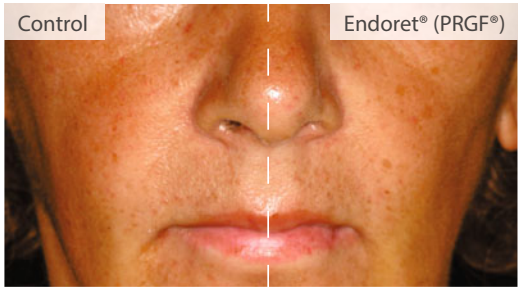


FIG. 17a
Clinical picture of split-mouth case showing more inflammation in the control group than the Endoret (PRGF) group.

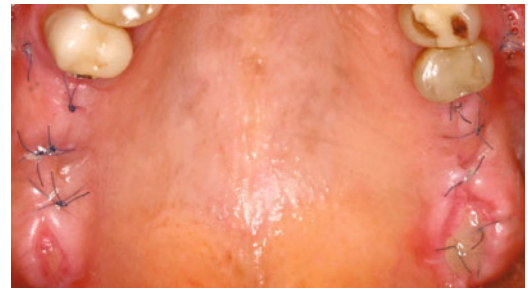


FIG. 17b
Comparison of soft tissue healing between Endoret (PRGF) and control groups.

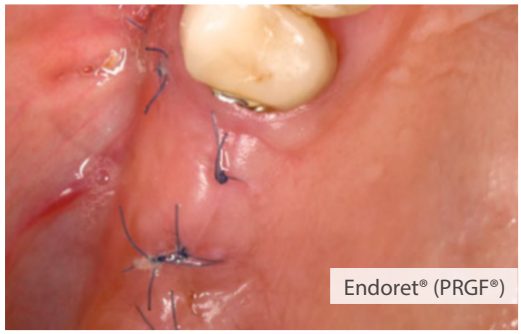


FIG. 17c
Image at higher magnification of extraction socket treated with Endoret (PRGF). The image was taken after 7 days of surgery to show the complete healing of the gingival tissue.

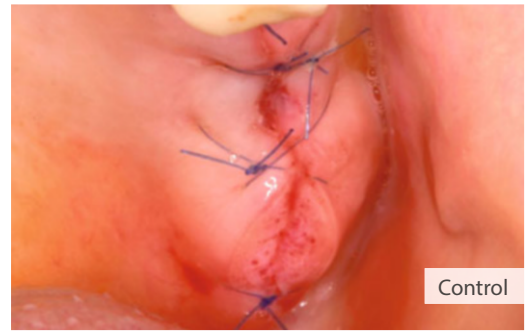


FIG. 17d
Image at higher magnification of tissue healing in the control side after 7 days of surgery. The healing was not complete due to the presence of dehiscence in the posterior region.

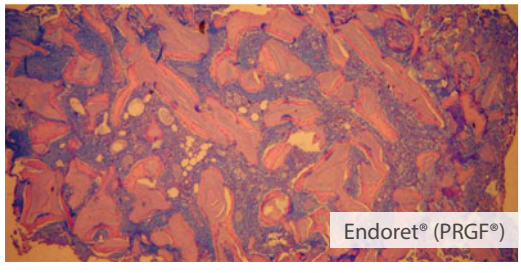


FIG. 17e
Histological image showing the formation of more bone tissue in the Endoret (PRGF) group. The goof quality of the tissue preserved the biopsy during processing.

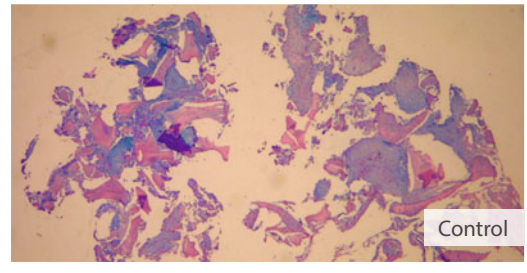


FIG. 17f
Histological image showing the formation of less bone tissue in the control group. The low quality of the tissue could not preserve the biopsy during processing.

Use of Endoret (PRGF) in the management of temperomandibular joint osteoarthritis and TMJ chronic pain

Temperomandibular joint (TMJ) disorders are a principal aetiological factor for chronic facial pain. TMJ disorders affect one third of adolescents and young adults and thus are not limited to older patients[12]. TMJ chronic pain would influence the patient's quality of life by limiting daily activities such as talking, smiling, and mastication. Generally, treatment of pain in such cases will be manifested in improving the functionality of TMJ and the patient's quality of life.

Several randomized clinical trials have shown the efficacy of Endoret (PRGF) in the treatment of knee osteoarthritis and has been better than hyaluronic acid[12-15]. In a recent publication, the use of Endoret (PRGF) has been shown to be efficient in the treatment of osteoarthritis of TMJ associated to chronic pain16. The visual analogue scale score of pain was 7.69 ± 1.9 at baseline, 1.54 ± 1.74 at 1 months and 0.23 ± 0.65 at 6 months. These differences in the results are statistically highly significant. In terms of maximum mouth opening, it was 30.15 ± 4.44 mm at baseline, 37.54 mm ± 5.10 at 1 month, and 39.54 ± 4.55 mm at 6 months. These differences were statistically significant ($P < 0.0001$ and $P < 0.01$, respectively). For that, articular injections of Endoret (PRGF) in the TMJ represent a an effective tool to control pain and to improve TMJ mobility.

A recent randomized clinical trial[17] analyzed the adjuvant use of Endoret (PRGF), in comparison to hyaluronic acid, in the surgical treatment of patients with anterior disc displacement without reduction who did not respond well to conservative treatment. These patients suffer pain and TMJ mobility limitations. The aims of the surgical treatment were to improve pain and TMJ function. Treatment success in this pathology (arthroscopic lavage and lysis and an anterior disc insertion coblation and part of the lateral pterygoid muscle) is based on reducing pain below 20 (on a visual analog scale from 0 to 100) and to get a maximum mouth opening of ≥ 35 mm. Based on these criteria, the results of this clinical trial show that the use of Endoret (PRGF) has significantly shortened the time required to achieve these fundamental goals.

- Patients in the control group still feel pain with a VAS ≥ 5 about a year after treatment and it took 18 months to get a VAS value close to 1, while patients in the Endoret (PRGF) group already have a pain VAS value ≤ 2 at 6 months after surgery.
- Considering the maximum opening, patients in the control group have taken 12 months to have a maximum opening ≥ 35 mm while patients treated with Endoret (PRGF) have achieved it in 6 months.
- Another interesting aspect of the study is that the technique used has resulted in a significant improvement in osteoarthritis, since 12 patients no longer show signs of osteoarthritis in the MRI performed 2 years after the treatment. But 10 of these 12 patients have been treated with Endoret (PRGF).

In another RCT[18], the effectiveness of the injection of Endoret (PRGF) was compared to hyaluronic acid (HA) following arthroscopic surgery in patients diagnosed with internal derangement of the temporomandibular joint (TMJ) with osteoarthritis (OA). In the study, 50 patients received an injection of Endoret (PRGF), and 50 received an injection of HA. The pain intensity and the maximum mouth opening before and after the procedure were compared. Endoret (PRGF) resulted in better results than hyaluronic acid, with a significant reduction in pain at 18 months, compared with HA treatment. The study concluded that the injection of Endoret (PRGF) following arthroscopy is more effective than the injection of HA with respect to pain in patients with advanced internal derangement of the TMJ.

4. ENDORET® (PRGF®) IN MEDICALLY COMPROMIZED PATIENTS

Hematological diseases and coagulopathies

In a randomized controlled clinical trial[19], Mozzati et al. have compared the effectiveness of autologous Endoret (PRGF) to that of a fibrin glue (Tisseel) as local measure in the management of patients with bleeding disorders and in need of dental extractions. The study was motivated by the risk of secondary bleeding and the possible need of repeated surgical and hematological interventions in these patients. Moreover, fibrin glue, with recognized efficacy, has the risk of viral infection transmission.

The study sample included 120 patients with different blood disorders who needed dental extraction without hospitalization. All patients received systemic hematological treatment. The main outcome was the secondary bleeding after the 7-day follow-up period or protracting after the repair procedure. The systemic treatment included tranexamic acid as oral antifibrinolytic agents. The treatment was complemented with desmopressin (a dose of 0.3 mg/kg in single subcutaneous injection about 30-60 minutes before the dental procedure) in patients with mild/moderate hemophilia A or type I von Willebrand's disease with a prior favorable response. However, in severe congenital bleeding disorders, desmopressin was replaced by a specific replacement therapy with a plasma-derived or recombinant form of the deficient factor. The dosage of the deficient factor was calculated to ensure a peak value greater than 50% in the peri-operative period and in the 3 days following tooth extraction. In patients with hemophilia A with inhibitors, recombinant activated factor VII (rFVIIa) was administered as a bolus dose of 90 mg/kg in close temporal proximity with respect to the single dental extraction.

In the group managed with fibrin glue, 106 extractions (7 retained third molars) were performed. Secondary bleeding occurred 3/60 patients (5%) on the third day after extraction. These patients necessitated additional surgery and systemic treatment (in one case the procedure had to be repeated on the seventh day) to control bleeding.

In the group managed with Endoret (PRGF), 98 extractions (23 retained third molars) were performed. Secondary bleeding occurred in two patients (3.3%) on the first day after extraction. Bleeding was effectively controlled with surgery but without systemic treatment.

Notably, 4 out of the five secondary bleeds occurred in patients with hemophilia A. Concomitant diabetes or liver disease significantly increased the bleeding risk.

Endoret (PRGF) was as effective as the fibrin glue as a local haemostatic measure. Endoret (PRGF) has the advantages of the autologous origin, no need for additional systemic treatment in post-extraction repair surgery, earlier onset of neo-angiogenesis and, overall, reducing patients' distress and costs to the health system.

Patients with insulin-dependent diabetes mellitus

Endoret (PRGF) has been tested in a RCT that recruited patients affected by insulin-dependent diabetes mellitus[20]. The study was conducted in a split-mouth design and evaluated the healing Index, residual socket volume, visual analogue scale, postsurgical complications, and outcome of a patient questionnaire. During follow-up period of 21 days, patients were evaluated at 4 time points. Finally, 34 patients affected by insulin-dependent diabetes mellitus were included. Endoret (PRGF) resulted in significantly smaller residual socket volumes and better Healing Indices from days 3 to 14. The patients' questionnaire outcomes were unanimously in favor of PRGF treatment. The study also reported that small sample of patients with glycemia values of at least 240 mg/dL showed worse healing index. The study concluded that Endoret (PRGF) is efficient in improving tissue healing (closure of the extraction socket) and

the differences between PRGF and control groups have been statistically significant.

5. PREVENETION AND TREATMENT OF MEDICATION-RELATED OSTEONECROSIS OF THE JAW

Bisphosphonates

Bisphosphonates are analogues of pyrophosphate, and are used as medication to inhibit bone resorption in numerous pathologies such as osteoporosis, Paget disease, multiple myeloma, malignant hypercalcemia, and metastasis related to breast and prostate cancers[21,22]. They are seekers of bone, the tissue where they act and accumulate. Bisphosphonates are not metabolized, and are eliminated in the urine.

The clinical use of bisphosphonates is based on the fact that the dose necessary to have an antiresorptive effect is several times lower than the dose needed to inhibit bone mineralization. Several studies have reported that bisphosphonates improve the mechanical properties of osteoporotic bone and reduce the risk of bone fracture[21,22].

The potency of bisphosphonates depends on their affinity to hydroxyapatite and their potency to inhibit the enzyme farnesyl diphosphate synthase[21]. The inhibition of bone remodeling also varies between bone sites. This inhibition is higher in the trabecular bone than the cortical bone. It also seems that these drugs do not inhibit the bone remodeling at the periosteal surface of bone. The recovery of the bone remodeling rate is not immediate after discontinuing the treatment with bisphosphonates.

Bisphosphonates-related osteonecrosis of the jaw (BRONJ) is the most relevant side effect of bisphosphonates in the oral and maxillofacial field. For the diagnosis of BRONJ, the following criteria should be fulfilled: the presence of exposed necrotic bone for more than 8 weeks, an evidence of treatment with bisphosphonates, and the absence of a history of radiotherapy.

The first cases of BRONJ were described by Marx and Ruggiero[23,24]. Since then, the management of patients positive for treatment with bisphosphonates is a question of debate given the absence of effective protocol for the prevention and treatment of BRONJ[24]. For this reason, dentists and maxillofacial surgeons have a great interest in having such an effective protocol. An important reference in this aspect is the guide of the American Association of Oral and Maxillofacial surgeons (AAOM)[25]. The main objective of this guide is to minimize the risk of development of BRONJ, alleviate the symptoms of BRONJ (pain and infection) and minimize the disease progression[26].

Several longitudinal studies have identified risk factors associated with the occurrence of BRONJ. Of the most important is dentoalveolar trauma (tooth extraction), prolonged time of treatment with bisphosphonates, and the use of intravenous potent bisphosphonates (0.8%-12%)[27]. Although the risk is low, BRONJ has been described in patients who received oral bisphosphonates (0.01%-0.04%).

Why Endoret® (PRGF®)?

Several theories have been suggested to explain the occurrence of BRONJ. Of these are the bacterial infection, anti-angiogenic effect of bisphosphonates, accumulation of micro-fractures within the bone, and the effect of bisphosphonates on bone remodeling.

The Endoret (PRGF) family presents several properties that could reduce the risk of MRONJ and be useful in its treatment. Endoret (PRGF) stimulates neo-angiogenesis, cellular proliferation and migration, and inhibits inflammation[28]. Endoret (PRGF) also has antimicrobial properties against Candida albicans, Entrecoccus Faecalis, Streptococcus aga-

lactiae, Streptococcus oralis, Staphylococcus aureus and Staphylococcus epidermis[29,30]. The biodegradation of Endoret (PRGF) does not require osteoclastic activity but it is resorbed by circulating macrophages.

6. ENDORET® (PRGF®) IN THE PREVENTION OF BRONJ

Tooth extraction is the main risk factor for the occurrence of BRONJ. This has motivated Mozzati et al. to study the efficacy of Endoret (PRGF) in the prevention of BRONJ in patients treated with zoledronic acid[31]. One hundred seventy six patients were recruited and randomized to Endoret (PRGF) group and control (blood clot) group. The Endoret (PRGF) group had 91 patients and the control group had 85. The Endoret (PRGF) clot prepared from the F2 of Endoret (PRGF) was first introduced into the socket after atraumatic tooth extraction. The socket was then covered by a fibrin membrane prepared from F1 of Endoret (PRGF).

The results showed the occurrence of BRONJ in 5 patients in the control group where 267 tooth extractions were performed. Endoret (PRGF) was effective in preventing the occurrence of BRONJ in all patients where 542 extractions had been performed[31].

Scoletta et al. performed another clinical study where Endoret (PRGF) was used to manage the extraction socket in patients treated with intravenous bisphosphonates[32]. The results showed correct soft tissue healing in 62 out of 63 patients. The radiographical analysis showed normal bone regeneration after 6 months of tooth extraction[32].

Treatment of medication-related osteonecrosis of the jaw by Endoret® (PRGF®)

We recently reported the outcomes of patient treatment with BRONJ by Endoret (PRGF)[26]. The patient was 50 years old and attended the clinic for the first time in April, 2008. Upon clinical examination, the presence of exposed necrotic bone was observed in the fourth quadrant. The lesion was associated with a history of tooth extraction and treatment with intravenous bisphosphonates. The patient was suffering from severe pain, halitosis, and difficulty to masticate and to open the mouth (fig. 18).

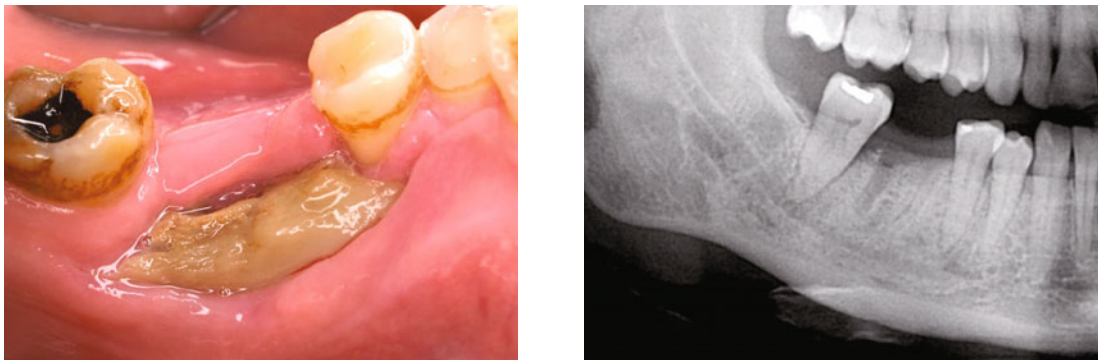


FIG. 18
The presence of soft tissue ulcer and bone exposure on the lower right posterior sector of the mouth. The lesion was associated with a recent tooth extraction. The diagnosis was bisphosphonates-related osteonecorsis of the jaw.

Following the AAMOS guide, conservative treatment that included lesion cleaning with chlorhexidine, analgesics and antibiotics was undertaken. The patient was seen in April, June, and September 2009. Hyperbaric oxygen treatment was also performed.

However, the progression of the lesion did not stop, with increase in the size of the ulcer and the occurrence of bone sequestration (fig. 19). The patient started to suffer from hemiparesthesia of the mandible at the side of the lesion[26].

Facing this situation, we developed a protocol to resolve the problem of the patient. This protocol consisted of the resection of the necrotic bone area and the bio-stimulation of tissue regeneration with Endoret (PRGF). This protocol was applied using piezoelectric surgery to remove the necrotic bone. The defect was then filled by Endoret (PRGF) clot prepared from F2 and the surgical area was then covered by a fibrin membrane prepared from F1 of the Endoret (PRGF). The flap was repositioned and sutured. Activated F2 of Endoret (PRGF) was then injected at the incision borders (fig. 20).

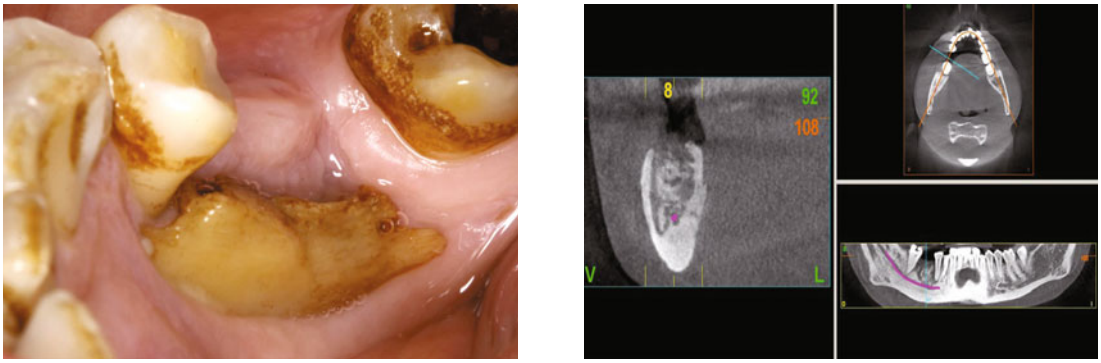


FIG. 19
The progression of the lesion of bisphosphonates-related osteonecorsis of the jaw. The lesion increased in size although a conservative approach and hyperbaric oxygen therapy were applied to control the pain and infection.

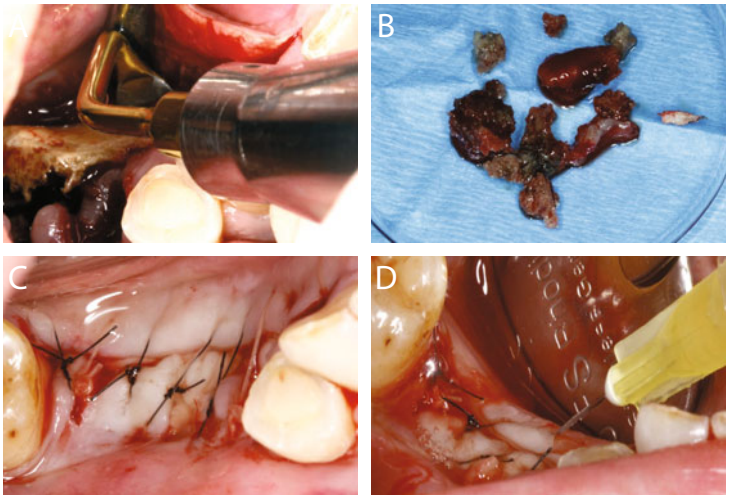


FIG. 20
The biological protocol developed to treat the bisphosphonates-related osteonecorsis of the jaw include the resection of necrotic bone, filling the defect with Endoret (PRGF) clot and covering the area with fibrin membrane. Activated Endoret (PRGF) liquid were injected at the borders of the flap after suturing.

At follow-up, the patient reported suffering from less pain, and the recovery of inferior alveolar nerve function was apparent[26]. The application of Endoret (PRGF) was effective in the stimulation of soft tissue healing and the closure of the defect, although bone filling is not evident at that time (fig. 21). Recovery of nerve function and no pain was complete after one month of treatment.

Bone regeneration was favorably progressing as evidenced by the recovery of tissue volume at the central area of the defect after 20 months of treatment (fig. 22).

Cone-beam CT scan was obtained at 1, 6, 12, 18 and 32 months postoperatively to assess the bone regeneration (fig. 23). Reduction of the residual volume of the defect and bone regeneration were observed after 6 months of treatment. The volume of the residual bone defect was 25% and 10% after 12 and 18 months of treatment (fig. 23). The bone healing started from the borders of the defect and continued toward its centre as evidenced from serial radiographs. Bone regeneration facilitated the elimination of tissue invagination initially seen at the centre of the defect. Figures 21 and 22 show the evolution of the soft tissue healing.

After 32 months, the CBCT showed complete bone regeneration indicating the viability of bone defect regeneration in patients having BRONJ (fig. 23). Thus, the bio-stimulation of tissue healing with Endoret (PRGF) is effective in the regeneration of hard and soft tissues lost from drug-related osteonecrosis of the jaw.

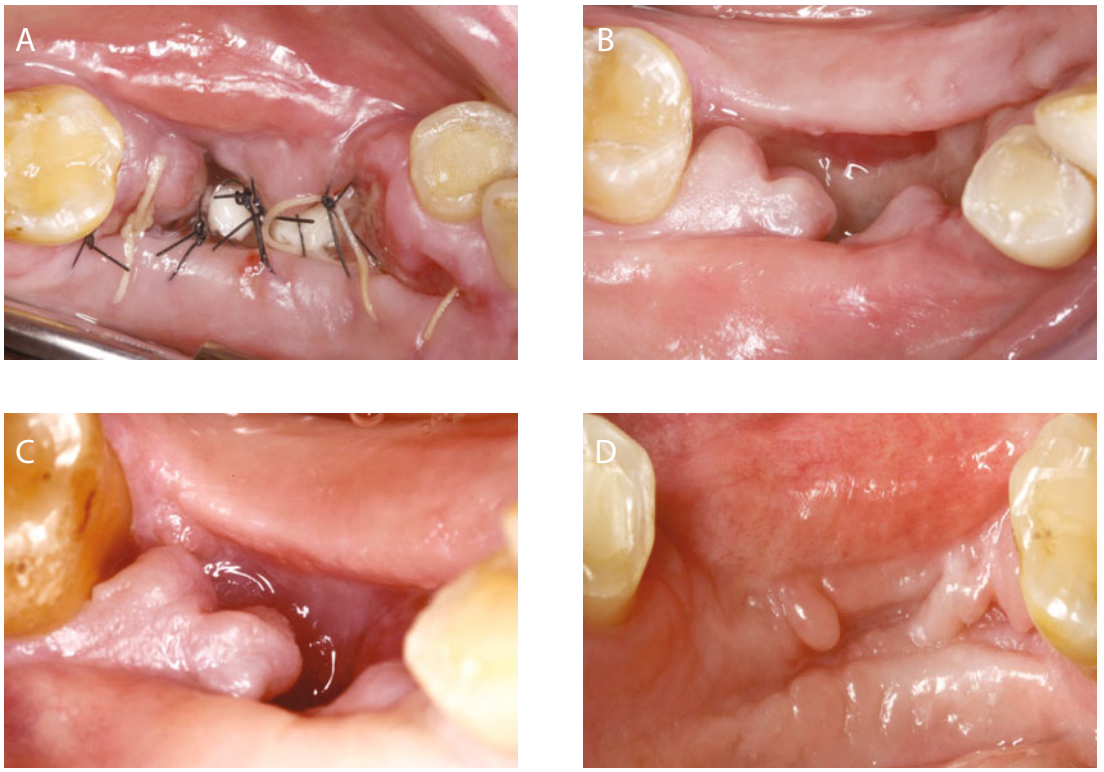


FIG. 21
The healing of the soft tissues after the application of Endoret (PRGF) treatment and the achievement of complete closure the ulcer. A) immediately after surgery.
B) one week postoperatively.
C) 3-4 weeks postoperatively.
D) 6 months postoperatively.

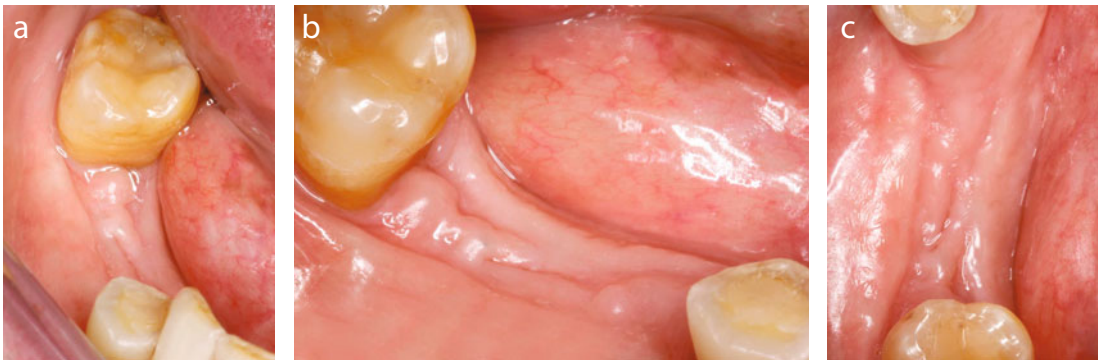


FIG. 22
Recovery of the volume of the residual alveolar ridge after 20 months of receiving PRGF (Enodret) therapy.

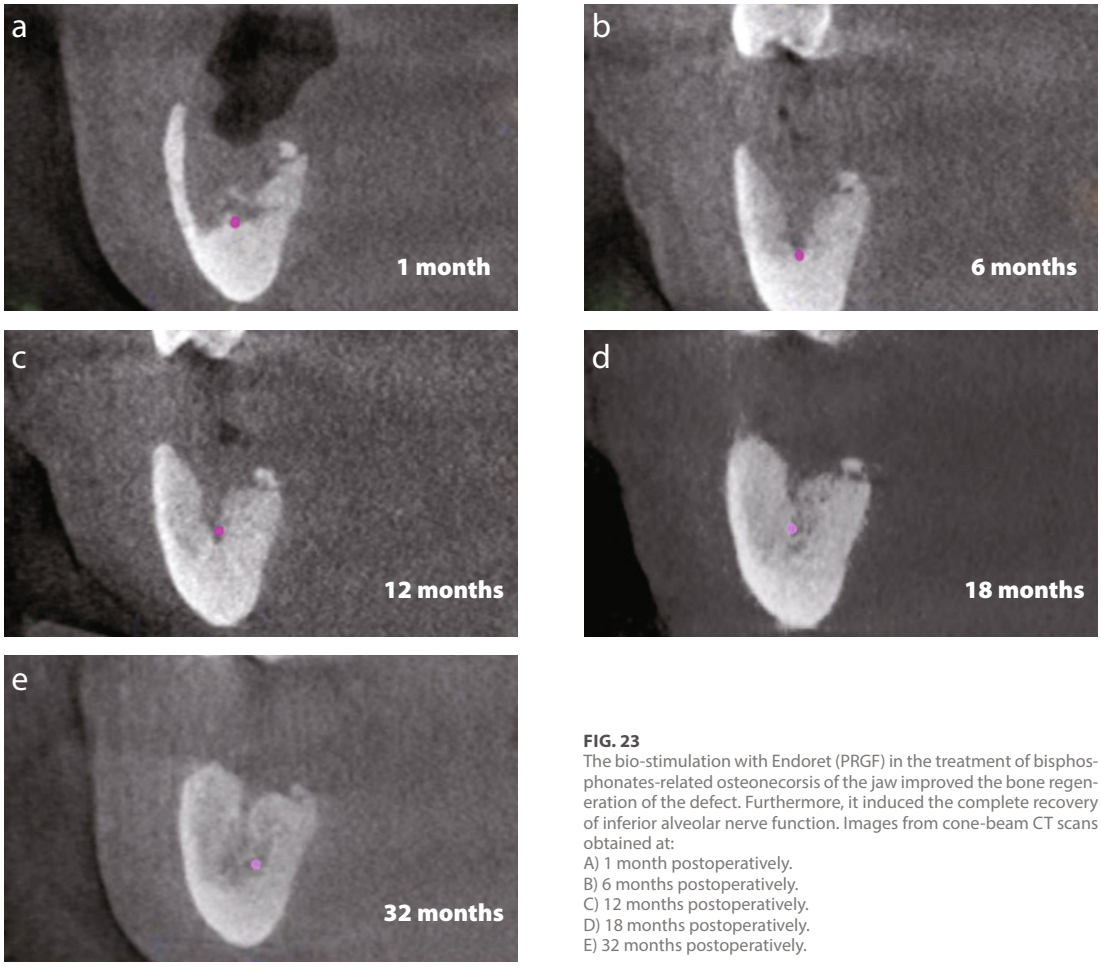


FIG. 23
The bio-stimulation with Endoret (PRGF) in the treatment of bisphosphonates-related osteonecorsis of the jaw improved the bone regeneration of the defect. Furthermore, it induced the complete recovery of inferior alveolar nerve function. Images from cone-beam CT scans obtained at:
A) 1 month postoperatively.
B) 6 months postoperatively.
C) 12 months postoperatively.
D) 18 months postoperatively.
E) 32 months postoperatively.

A surgery was planned to place 2 dental implants in the regenerated alveolar bone. Figure 24 shows the placed implants which were immediately loaded. Two biopsies were obtained at the implant sites before drilling with a trephine bur. The histological analysis confirmed the neoformation of bone tissue in the area previously affected by BRONJ (fig. 25). In the same surgery, the lower right second molar was extracted due to a vertical fracture. The extraction sockets were successfully regenerated by PRGF (Endoret). The patient follow-up indicated the absence of any adverse effects and the correct function and integration of the implants a year after the placement of the definite prosthesis (fig. 26).

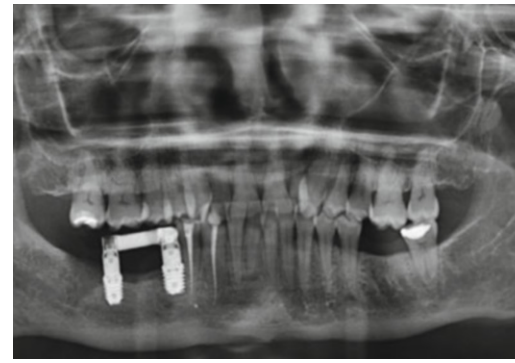


FIG. 24
Panoramic radiograph after 24 hours of surgery showing the placed dental implants and the prosthesis for immediate loading. Also the extraction socket of the lower right second molar that was treated with Endoret (PRGF) without any complication.

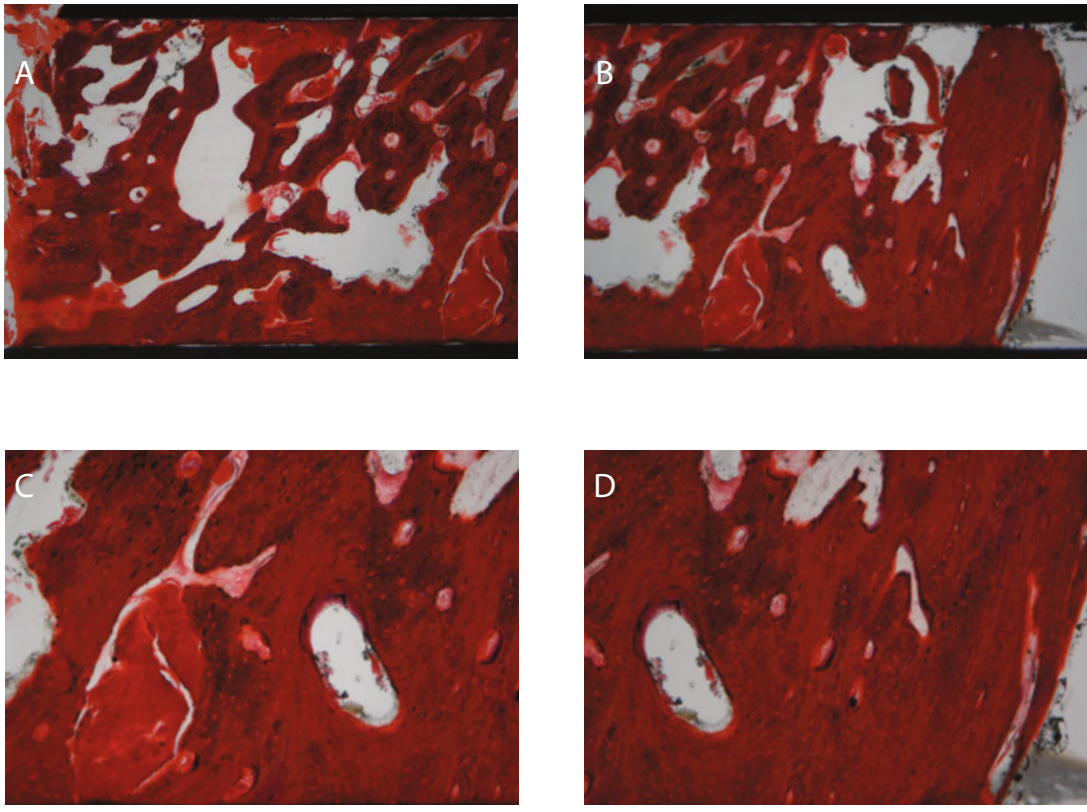


FIG. 25
Histological analysis of the two biopsies from regenerated bone at the area previously affected by BRONJ (C and D at 10x; A and B at 5x).

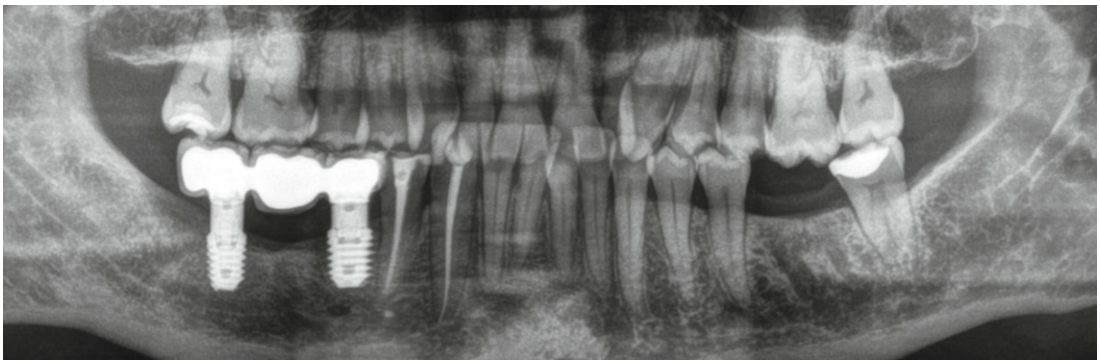


FIG. 26
Panoramic radiograph after 1 year of the placement of the prosthesis showing the proper healing of the extraction socket of the lower right second premolar and the well-osseointegration of the dental implants.

The efficacy of Endoret (PRGF) in the treatment of Medication-related osteonecrosis of the jaw from intravenous bisphosphonates has been evaluated in 32 patients[33]. All patients had received intravenous bisphosphonates for an average of 37 months when the BRONJ lesion had been diagnosed.

The treatment protocol included the surgical resection of the necrotic bone and the bio-stimulation of tissue regeneration with Endoret (PRGF)[33]. The patients had been followed for 48-50 months, confirming the efficacy of Endoret (PRGF) to stimulate the closure of the defect and the absence of intra- and post-operative complications[33].

Prevention and treatment of osteoradionecrosis

In a controlled split-mouth clinical trial[34], the capacity of Endoret (PRGF) in the prevention of osteoradionecrosis due to tooth extraction in patients who received radiotherpay.

In the study, 20 patients in need of bilateral paired dental extraction were treated as following: On the side directly impacted by radiation, the experimental side, extraction sockets were treated with Endoret (PRGF), whereas, on the other side (control), sockets were left to heal naturally (blood clot). To measure the effectiveness, the following variables were assesed: residual socket volume (RSV), healing index (HI), pain, and postsurgical complications. The study period was 30 days after surgery and included 4 evaluation sessions.

Endoret (PRGF) resulted in better values for RSV and HI at all checkups without any postoperative complications. However, in the control side, slower healing and 2 cases of bone exposure were observed. Interstingly, these two cases were then effectively treated with Endoret (PRGF) application. In this light, Endoret (PRGF) could be effective in the management of patients with a history of head and neck radiotherapy, accelerating and fostering mucosal healing and avoiding postextraction bone exposures.

In another study[35], osteoradionecrosis (ORN) defined as exposed necrotic bone, that not heal for at least 3 months. Ten patients with ORN were treated by debridement of necrotic bone using an ultrasound device followed by application of Endoret (PRGF) to improve and accelerate soft-tissue healing. Patients were followed up to 1 year clinically and radiographically. Visual analogue scale (VAS) was used to evaluate pain in the first week after surgery. A modified healing index assessed the clinical evolution of the cases.

Endoret (PRGF) was effective in the treatment of all patients with ORN without any intraoperative or postoperative complications. The authors have stated that the clinical and radiographic evaluations showed no signs of persistent infection or exposed bone up to 12 months of follow-up. The maturity and quality of the regenerated tissues was excellent, surgical wounds always achieving complete closure. VAS scores and trismus were very low in all patients, who did not require analgesics after the third day post-surgery.

These clinical findings recommend Endoret (PRGF) as effective tool not only for the treatment of osteoradionecrosis but also for its prevention.

7. CONCLUSIONS

The plasma rich in growth factors is an autologous, versatile, leukocyte-free platelet concentrate. The Endoret (PRGF) results in the formation of a fibrin scaffold and the release of growth factors that pave the way for tissue regeneration. More than 200 scientific papers evidence the safety and predictability of the Endoret (PRGF). Since its first intraosseous application in 1995, many randomized controlled clinical trials and observational studies have confirmed the properties of Endoret (PRGF) as antimicrobial and anti-inflammatory agent. Endoret (PRGF) improves the postoperative healing as patients experienced lesser pain, swelling, and hematoma than the control treatment (a blood clot). This has great impact on the patient's quality of life and the healing of medically-compromised patients. Clinical and histological evaluation of soft tissue healing indicates an accelerated defect closure by the formation of a thicker gingival tissue than the control treatment. Endoret (PRGF) alone or in combination with bone graft and/or bone substitutes has enhanced the bone tissue regeneration and results in a more mature bone tissue.

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