Bisphosphonate-Related Osteonecrosis of the Jaws Treated with Platelet-Rich Plasma: Preliminary Results from a Case Series

Raffaello Viganò

Funding: No specific funding was received for this work.

Potential competing interests: No potential competing interests to declare.

Abstract

Objective: The aim of this case series was to evaluate the effectiveness of a new platelet-rich plasma preparation **B.P.F.C. Bio-Plasma® Rich in Pure Growth Factors®, for obtaining Biologically Guided Regeneration** (Bioplasma®, Dr. Raffaello Viganò, Varese Italy), for the treatment of Bisphosphonate-related osteonecrosis of the jaws (BRONJ).

Materials and Methods: Thirty-five patients (15 men, 20 women; aged between 51-78 years; mean age 64 years) were enrolled in this study and treated with a regenerative surgical procedure using a platelet-rich plasma preparation.

Results: All patients were treated successfully with regard to healing parameters.

Conclusion: This new preparation **B.P.F.C. Bio-Plasma® Rich in Pure Growth Factors®**, seems able to restore an intact mucosa in BRONJ-affected patients. Further studies are necessary to validate these results with a larger sample size and a longer follow-up time.

Introduction

Introduction

Bisphosphonates (BPs) are drugs characterized by two carbon-phosphate bonds able to reduce osteoclast activity. The mechanism of action of bisphosphonates is not yet well understood, but it involves a powerful inhibition of bone resorption. Furthermore, an antiangiogenic effect capable of decreasing bone resorption has been hypothesized. According to the presence of nitrogen bound to the chemical structure, BPs are classified as non-nitrogen BPs [etidronate, clodronate, etc.] and nitrogen BPs [risedronate, alendronate, pamidronate, and zoledronate]. [1][2]

BPs are widely used in the treatment of bone metabolism diseases, metastatic osteolytic lesions, multiple myeloma, Paget's disease, and osteoporosis. [3]

Adverse effects related to intravenous BPs include acute systemic inflammatory reaction, ocular inflammation, renal failure, nephrotic syndrome, and osteonecrosis of the jaws. [1]

Bisphosphonate-related osteonecrosis of the jaws (BRONJ) is defined as a side effect of the inhibition of osteoclasts, in which exposed and necrotic bone persisting for more than 8 weeks occurs in the maxillofacial region. This could be related to current or previous treatment with BPs, with no history of radiotherapy to the head and neck area. [4]

BRONJ lesions appear to be related to previous dental surgical procedures, like tooth extractions in the majority of cases. [5]

BRONJ occurs in patients undergoing treatment with intravenous BP. Recently, an increasing frequency of osteonecrosis of the jaw has been reported in patients receiving oral BPs. [6]

The real incidence of BRONJ is still undefined. It is more often localized in the mandible than in the maxilla (2:1 ratio). [7][8]

The etiopathogenic process has not been fully elucidated, and osteoclast inactivation doesn't explain the elective localization in the oral-maxillofacial region, supporting the theory that other mechanisms are involved in the development of BRONJ. Because of their long half-life, BPs stay in the bone for a long time, determining a risk of developing BRONJ even in patients who discontinue the use of these drugs. [9]

Some authors proposed sequestrectomy and coverage of the exposed area without the use of biomaterials.[6]

In recent years, the use of plasma rich in growth factors technology has shown successful results in many regenerative medical techniques. [10]

The emergence and application of these platelet-enriched preparations have revolutionized the field of regenerative medicine, in part due to the repair capacities of the growth factors and proteins secreted by the platelets, as well as the ease of preparation and the biosafety and biocompatibility. [11]

The aim of this case series study was to evaluate the ability of a platelet-rich plasma preparation BPFC (Bioplasma ricco in fattori di crescita) (Bioplasma®) to favor the healing process of patients affected by BRONJ.

Materials and Methods

Patient Selection

This study was based on data collected from 35 patients who were treated with a fibrin scaffold and a platelet-rich plasma preparation for the treatment of BRONJ. There were 35 patients in total (15 men, 20 women) aged between 51 and 78 years, with a mean age of 64 years. The characteristics of BRONJ are summarized in **Table 1**.

Table 1. BRONJ characteristics at the time of diagnosis

		Study Group
Location	Maxilla Mandible	25 10
Cause	Tooth extraction Periodontal Disease	30 5
Exposed/necrotic bone	+ -	28 7
Pain	+	35
Oral fistulae	+ -	29 7
Pus	+ -	32 3
Treatment result	+ -	35 0
Follow-up	12 months	35

All patients read and signed a written consent form. The study protocol was approved by the Ethical Committee for Human Studies of the “Ospedale di Circolo” - University of Varese, and it was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Preparation of Plasma Formulation

The preparation of the plasma was conducted in order to obtain a platelet-rich plasma preparation, a platelet-poor plasma preparation, and a fibrin scaffold. **The B.P.F.C. Bio-Plasma® Rich in Pure Growth Factors®, for obtaining Biologically Guided Regeneration**.

The protocol differs from the one originally described by Anitua[12] for the lack of sodium citrate and calcium chloride used as an anticoagulant and activator, respectively. Blood from patients was collected into 9 mL tubes **without sodium citrate**. After centrifugation with an EBA 270® centrifuge (Hettich, Tuttlingen, Germany) at room temperature, blood cells stratified according to a density gradient. The bottom fraction contains the erythrocytes, and the top fraction contains the platelets. The 2 mL above the buffy coat was separated and used in this study. The 1 mL above the buffy coat, which presents the highest platelet and growth factor concentrations, was identified as fraction 2 (F2), whereas the next 2 mL

above F1 was named fraction 1 (F1). Care was taken to avoid the buffy coat containing the leukocytes.

All the preparations were incubated at 37°C in glass pots for twenty minutes until **self-gelling (it is the only protocol that achieves this result)**, was obtained. Eventually, the gel preparation is ready to be used.

Surgical Procedure

A complete examination of the oral hard and soft tissues was carried out for each patient (Fig. 1). After local anesthesia was obtained by infiltrating articaine 4% containing 1:100,000 adrenaline (Ubistesin®, 3M Espe, St. Paul, MN, USA), surgery was performed. Necrotic and infected bone was removed when present. An osteoplasty was performed in order to regularize the bone profile.

Subsequently, the platelet-rich plasma gel preparation was inserted inside the surgical site, followed by the application of the platelet-poor plasma gel preparation in order to completely fill the surgical site.

The site was covered with the fibrin membrane and was secured in position by mattress sutures (Vycril®, Ethicon, Inc., Somerville, New Jersey, USA).

Post-operative instructions were given; all patients received oral antibiotics (AugmentinR, Glaxo-Smithkline Beecham, Brentford, UK) 2g per day for 7 days, starting the day before surgery. Post-operative pain was controlled by administering 550 mg naproxen sodium (Sinflex®, Recordati Almiral, Milan, Italy) every 12 hours for 5 days. Local application of hyaluronic acid gel (Gengigel®, Bracco, Milan, Italy) was done 3 times a day for 15 days. Dental probiotics (Periobalance™, Sunstar Americas Inc., Chicago, Illinois, USA) were taken 1 time a day for 30 days, and detailed instructions about oral hygiene were given. Suture removal was performed at 14 days.

Clinical Follow-Up Examination

All the patients were enrolled in an annual recall program to evaluate the absence of BRONJ or the remission of the clinical signs. Recall visits were set at 7 days, 14 days, 1 month, 3 months, 6 months, and 12 months postoperatively.

A clinical examination was performed, and the following parameters were investigated: pain, swallowing, non-complete healing, exposed bone, or oral fistulae. The absence of these clinical parameters was defined as clinical success. Moreover, an orthopanoramic and a Cone-Beam at the baseline (surgery) and at the 12-month recall were performed.

Results

After a BRONJ diagnosis, no patients discontinued the use of BPs solely because of surgery need. No intraoperative or postoperative complications were observed, and all 75 BRONJ patients were treated successfully.

Of the 35 sites treated, 25 were located in the maxilla and 10 were located in the mandible. All 35 patients showed an intact mucosa at the recall visits.

Resolution of the disease was defined as the maintenance of mucosal closure without clinical and radiographic signs of residual infection or exposed bone at the time of evaluation.

A success rate of 100.0% was found.

Discussion

BPs are drugs used for the prevention and treatment of secondary skeletal events (SREs) that cause bone metastases from solid tumors and multiple myeloma. The goal of treatment with BP is to improve the quality of life [13] of patients.

The true incidence of BRONJ has not been established and in the literature ranges from 0.8% to 12% and 0.01% to 0.06% intravenously and orally administered, respectively [2]. BP

Potency of drugs, length of therapy, poor oral hygiene, periodontal disease, and ill-fitting dentures are risk factors for developing BRONJ [14]. Several studies have shown that BRONJ is often associated with oral surgeries such as tooth extraction [15]. The literature has shown that BRONJ incidence associated with tooth exodontias ranges from 36.7% to 73% [16]; [1].

Additionally, dental implants, abscesses, and removal of impacted teeth have been reported as possible causes of BRONJ development. Occasionally, a spontaneous presentation is observed and may presumably be attributable to chronic or latent infections [17].

BP's action is based on the ability to inhibit the activity of the osteoclasts while also being able to affect the normal bone turnover. BP-nitrogen is also thought to have antiangiogenic effects [18].

It is known that the bone of patients treated with BP is poorly vascularized and poorly supplied with all the substances necessary for wound healing [19].

The rationale for the use of platelet-rich plasma (PRP) technology lies in the ability to deliver growth factors necessary for wound healing in biological tissue devoid of endogenous growth factors.

PRP is capable of supplying various growth factors, such as platelet-derived growth factor, transforming growth factor-beta, endothelial growth factor, vascular endothelial growth factor, insulin-like growth factor-1, basic fibroblasts, and hepatocyte growth factor, all of which are capable of promoting healing and tissue regeneration [12].

A variety of treatment modalities have been proposed for BRONJ therapy, including topical conservative treatment, conservative surgical treatment or surgical resection, as well as hyperbaric or ozone therapy [4].

Several studies are present in the literature describing the use of PRP for BRONJ treatment[20][21][22][23][24][25][26].

Adornato et al. [24] proposed the use of PRP treatment in patients in whom topical treatment failed to achieve the resolution of BRONJ. In a sample of 12 patients who received cleansing therapies, 0.12% chlorhexidine rinses, and

intermittent antibiotic therapies, a margin resection was performed with suturing of the surgical site covered with PRP and an absorbable membrane. Patients had soft tissue ulcerations and bone exposure ranging in size from 5 to 25 mm before undergoing surgery. At the six-month follow-up, ten patients showed complete soft tissue healing, and one patient showed a recurrence of epithelial dehiscence.

One patient, with recovery by secondary intention, showed no regression of the bone exposure[24].

In a study of 32 patients treated with intravenous bisphosphonates, Mozzati et al.[25] proposed the use of PRGF after resective surgery of necrotic bone and osteoplasty and oxygenation of bleeding bone tissue.

This study, **The B.P.F.C. Bio-Plasma® Rich in Pure Growth Factors®, for obtaining Biologically Guided Regeneration, self-gelling, is the only protocol that achieves this result**, showed a 100.0% success rate with an observation period ranging from 48-50 months.

Figures

Figure 5. Centrifuge B.P.F.C. Bio-Plasma®

Figure 6. Centrifuge Rotor

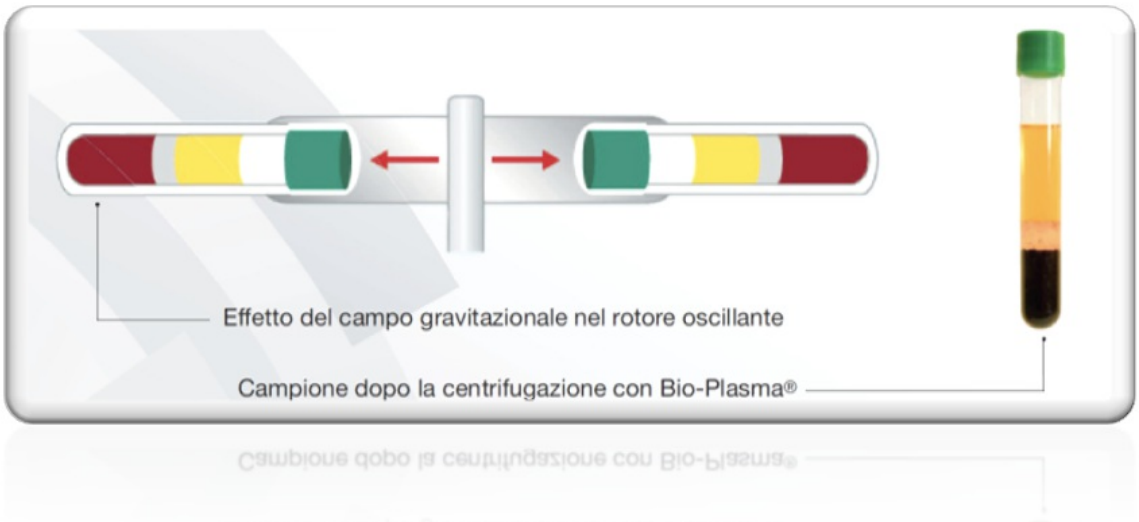

Figure 7. Oscillating Arms

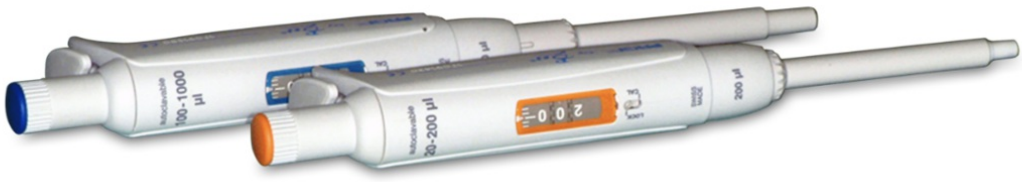

Figure 8. Pipette for Fractionation

Figure 9. Completed Collection

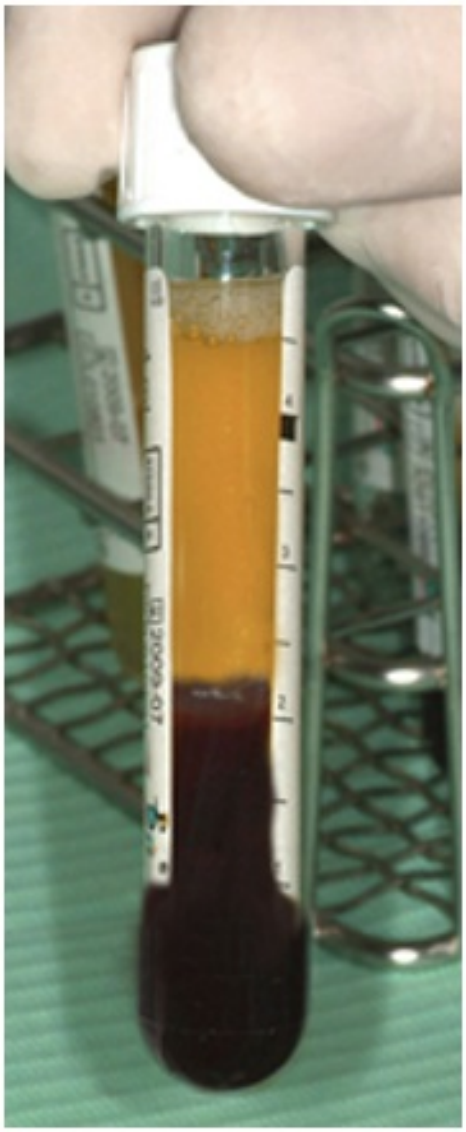

Figure 10. Centrifuged Plasma

Figure 11. B.P.F.C. Bio-Plasma® Components

Figure 12. B.P.F.C. Bio-Plasma® Fractionation

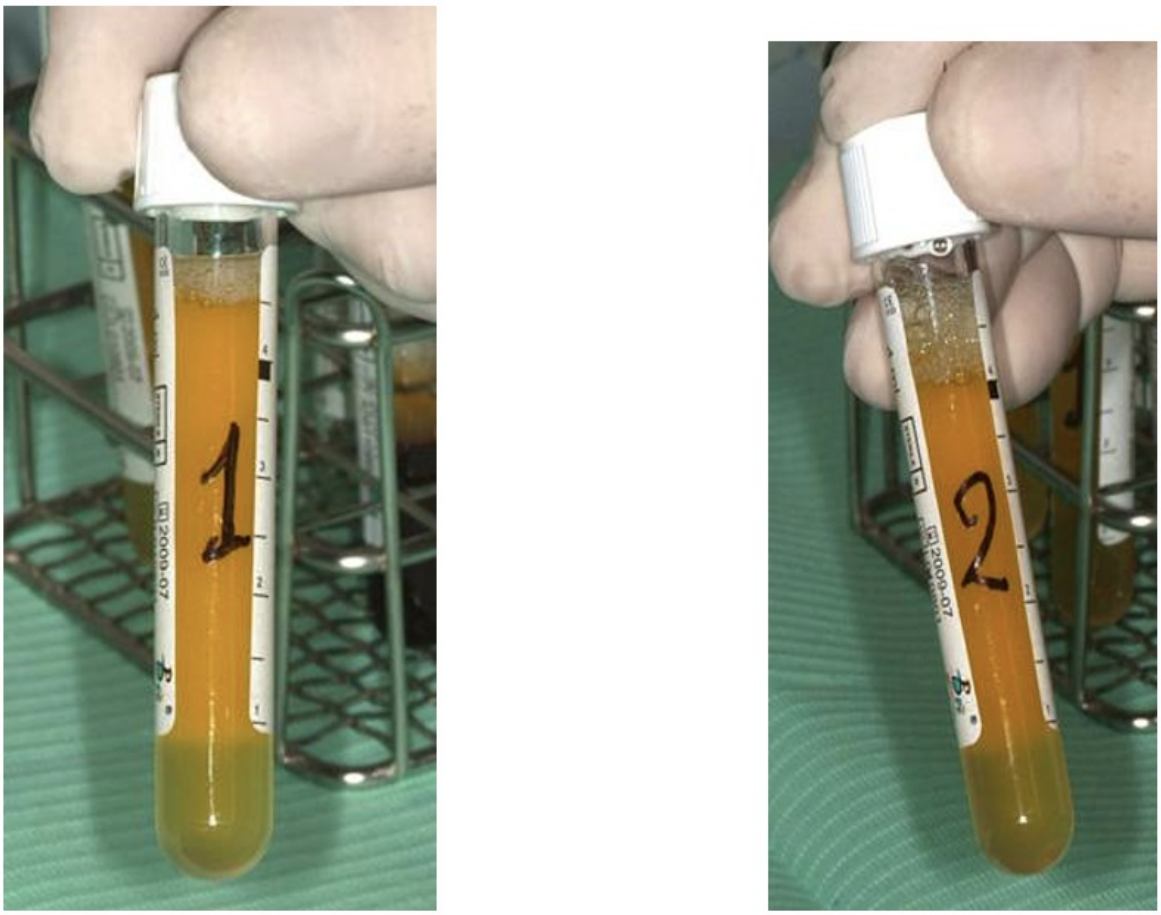

Figure 13. Fraction 1 - Fibrin with Poor Plasma. Fraction 2 - Rich Plasma

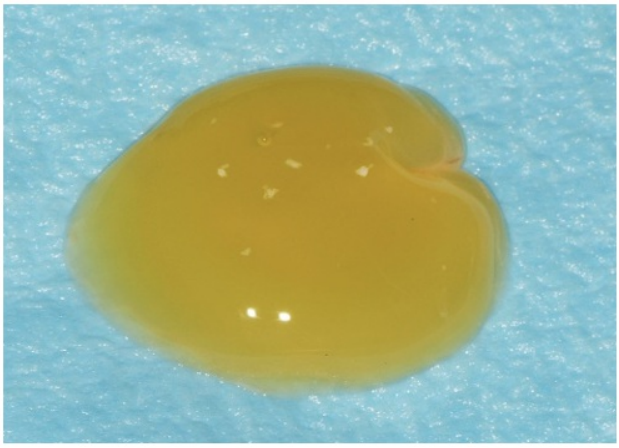

Figure 14. Enriched Fibrin Membrane

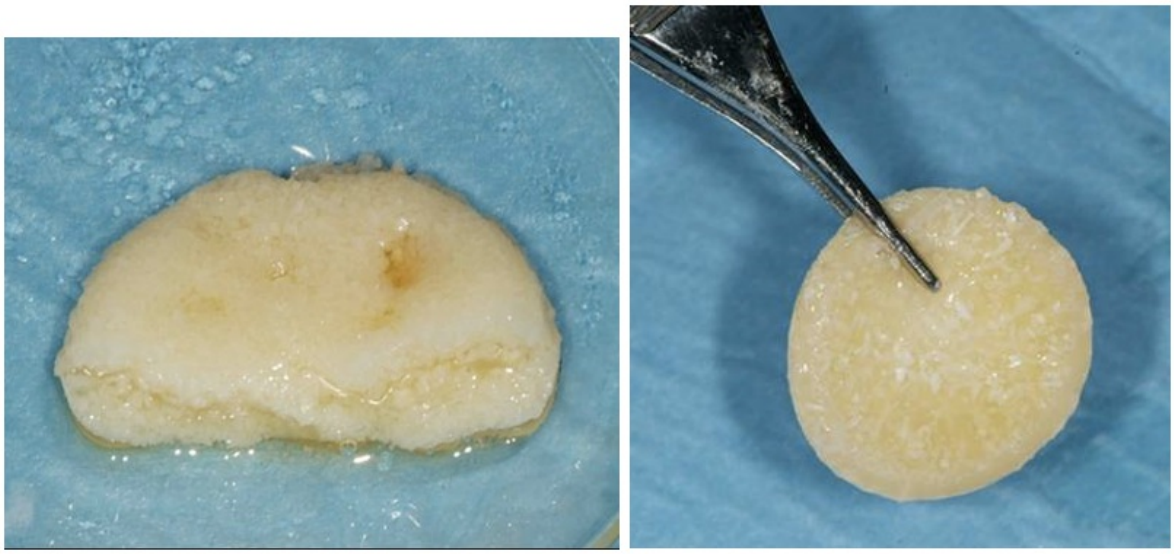

Figure 15. Rich Plasma with Biomaterial (G.B.R.) with Poor Plasma (G.T.R.)

Other References

- Marx, R., & Garg, A. (2005). Dental and craniofacial applications of platelet-rich plasma. Chicago: Quintessence Publishing.

References

1. [a, b, c]*Kato, G. F., Lopes, R. N., Jaguar, G. C., Silva, A. P., & Alves, F. A. (2013). Evaluation of socket healing in patients undergoing bisphosphonate therapy: Experience of a single institution. Medicina Oral Patología Oral y Cirugía Bucal. Advance online publication.*
2. [a, b]*Beninati, F., Pruneti, R., & Ficarra, G. (2013). Bisphosphonate-related osteonecrosis of the jaws (BRONJ). Medicina Oral Patología Oral y Cirugía Bucal. Advance online publication.*
3. [^]*Ruggiero, S. L., Fantasia, J., & Carlson, E. (2006). Bisphosphonate-related osteonecrosis of the jaw: background and guidelines for diagnosis, staging and management. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 102, 433-441.*
4. [a, b]*Ruggiero, S. L., Dodson, T. B., Assael, L. A., Landesberg, R., Marx, R. E., & Mehrotra, B. (2009). American Association of Oral and Maxillofacial Surgeons position paper on bisphosphonate-related osteonecrosis of the jaws. Journal of Oral and Maxillofacial Surgery, 67, 2-12.*
5. [^]*O'Ryan, F. S., & Lo, J. C. (2012). Bisphosphonate-related osteonecrosis of the jaw in patients with oral bisphosphonate exposure: clinical course and outcomes. Journal of Oral and Maxillofacial Surgery, 70, 1844-1853.*
6. [a, b]*Di Lorenzo, S., Trapassi, A., Corradino, B., & Cordova, A. (2013). Histology of the oral mucosa in patients with BRONJ at III stage: a microscopic study proves the unsuitability of local mucosal flaps. Journal of Clinical Medical Research, 5, 22-25.*

7. ^American Association of Oral and Maxillofacial Surgeons. (2009). Position Paper on Bisphosphonate-Related Osteonecrosis of the Jaw – 2009 Update. Australian Endodontic Journal, 35, 119–130.
8. ^Mavrokokki, T., Cheng, A., Stein, B., & Goss, A. (2007). Nature and frequency of bisphosphonate-associated osteonecrosis of the jaws in Australia. Journal of Oral and Maxillofacial Surgery, 65, 415-423.
9. ^Reid, I. R., Bolland, M. J., & Grey, A. B. (2007). Is bisphosphonate-associated osteonecrosis of the jaw caused by soft tissue toxicity? Bone, 41, 318-320.
10. ^Anitua, E., Sanchez, M., Orive, G., & Andia, I. (2007). The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. Biomaterials, 28, 4551-4560.
11. ^Anitua, E., Sanchez, M., Nurden, A. T., Nurden, P., Orive, G., & Andia, I. (2006). New insights into and novel applications for platelet-rich fibrin therapies. Trends in Biotechnology, 24, 227-234.
12. a, b Anitua, E. (1999). Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. International Journal of Oral and Maxillofacial Implants, 14, 529-535.
13. ^American Association of Oral and Maxillofacial Surgeons. (2007). Position paper on bisphosphonate-related osteonecrosis of the jaws. Journal of Oral and Maxillofacial Surgery, 65, 369-376.
14. ^Rayman, S., Almas, K., & Dincer, E. (2009). Bisphosphonate-related jaw necrosis: a team approach management and prevention. International Journal of Dental Hygiene, 7, 90-9.
15. ^Ruggiero, S. L. (2009). Bisphosphonate-related osteonecrosis of the jaw (BRONJ): initial discovery and subsequent development. Journal of Oral and Maxillofacial Surgery, 67, 13-18.
16. ^Marx, R. E., Cillo, J. E., & Ulloa, J. J. (2007). Oral bisphosphonate-induced osteonecrosis: risk factors, prediction of risk using serum CTX testing, prevention, and treatment. Journal of Oral and Maxillofacial Surgery, 65, 2397-410.
17. ^Ficarra, G., Beninati, F., Rubino, I., Vannucchi, A., Longo, G., Tonelli, P., et al. (2005). Osteonecrosis of the jaws in periodontal patients with a history of bisphosphonates treatment. Journal of Clinical Periodontology, 32, 1123-1128.
18. ^Rodan, G. A., & Fleisch, H. A. (1996). Bisphosphonates: mechanisms of action. Journal of Clinical Investigation, 97, 2692-2696.
19. ^Favia, G., Pilolli, G. P., & Maiorano, E. (2009). Histologic and histomorphometric features of bisphosphonate-related osteonecrosis of the jaws: An analysis of 31 cases with confocal laser scanning microscopy. Bone, 45, 406-413.
20. ^Cetiner, S., Sucak, G. T., Kahraman, S. A., Aki, S. Z., Kocakahyaoglu, B., Gultekin, S. E., Cetiner, M., & Haznedar, R. (2009). Osteonecrosis of the jaw in patients with multiple myeloma treated with zoledronic acid. Journal of Bone and Mineral Metabolism, 27, 435-443.
21. ^Curi, M. M., Cossolin, G. S., Koga, D. H., Araújo, S. R., Feher, O., dos Santos, M. O., Zardetto, C. (2007). Treatment of avascular osteonecrosis of the jaw in cancer patients with a history of bisphosphonate therapy by combining bone resection and autologous platelet-rich plasma: report of 3 cases. Journal of Oral and Maxillofacial Surgery, 65, 349-355.
22. ^Lee, C. Y., David, T., & Nishime, M. (2007). Use of platelet-rich plasma in the management of oral bisphosphonate-associated osteonecrosis of the jaw: A report of 2 cases. Journal of Oral Implantology, 33, 371–382.
23. ^Bocanegra-Perez, S., Vicente-Barrero, M., Knezevic, M., Castellano-Navarro, J. M., Rodríguez-Bocanegra, E., Rodríguez-Millares, J., Perez-Plasencia, D., & Ramos-Macías, A. (2012). Use of platelet-rich plasma in the treatment

of bisphosphonate-related osteonecrosis of the jaw. International Journal of Oral and Maxillofacial Surgery, 41, 1410–1415.

24. [a, b, c] *Adornato, M. C., Morcos, I., & Rozanski, J. (2007). The treatment of bisphosphonate-associated osteonecrosis of the jaws with bone resection and autologous platelet-derived growth factors. Journal of the American Dental Association, 138, 971–977.*
25. [a, b] *Mozzati, M., Gallesio, G., Arata, V., Pol, R., & Scoletta, M. (in press). Platelet-rich therapies in the treatment of intravenous bisphosphonate-related osteonecrosis of the jaw: A report of 32 cases. Oral Oncology.*
26. [^] *Coviello, V., Peluso, F., Dehkhargani, S. Z., Verdugo, F., Raffaelli, L., Manicone, P. F., & D'Addona, A. (2012). Platelet-rich plasma improves wound healing in multiple myeloma bisphosphonate-associated osteonecrosis of the jaw patients. Journal of Biological Regulatory and Homeostatic Agents, 26, 151–155.*