# EFFECT OF OZONE ACTIVATED PLATELET RICH PLASMA ON THE MANAGEMENT OF GRADE II FURCATION INVOLVEMENT (COMPARATIVE EXPERIMENTAL STUDY)

Moustafa S. Abouyoussef[1*] *BDS*, Ahmed M. Hommos[2] *PhD*, Samia S. Omar[3] *PhD*, GehanS.Kotry[2] *PhD*

## ABSTRACT

**INTRODUCTION:** Furcation defects are a frequent finding in periodontitis patients. Activated platelets release numerous proteins, among them adhesive glycoproteins and growth factors. Ozone ($O_3$) is a triatomic molecule, consisting of three oxygen atoms, and its application in medicine and dentistry has been indicated for the treatment of different pathologies.

**AIM OF THE STUDY:** The aim of this experimental study was to evaluate the effectiveness of combining Platelet Rich Plasma (PRP) and ozone therapy in the management of surgically created grade II furcation defects.

**MATERIALS AND METHODS:** A split mouth design was carried out using six healthy mongrel dogs. A total of eighteen critical sized grade II furcation defects were surgically created in the third and fourth mandibular premolars, bilaterally. After reflecting a mucoperiosteal flap, the defects in the experimental side were treated with PRP activated by ozone, β-TCP and collagen membranes, whereas, the control side defects were treated with PRP, β-TCP and collagen membrane. The dogs were sacrificed after 4 and 8 weeks. Samples were dissected and prepared for histological evaluation.

**RESULTS:** Histological results showed that PRP activated by ozone led to higher vascularization of the study samples at all time points; this was evident at four weeks in the form of numerous blood vessels entrapping RBCs and at eight weeks by the presence of red bone marrow and blood vessels entrapping RBCs within. Histomorphometry revealed that ozonated PRP accelerated bone regeneration at both time intervals, this acceleration was almost two –fold upon comparing test to controls.

**CONCLUSION:** Activation of PRP by ozone is effective in enhancing angiogenesis and bone formation during periodontal regenerative therapy in grade II critical –sized furcation defects in dogs.

**KEYWORDS:** Angiogenesis, β-TCP. Grade II furcation Periodontal regeneration, Platelet Rich Plasma.

---

1. Bachelor of Dental Science 2007, Faculty of dentistry, Misr International University, Egypt.
2. Professor of Periodontology, Faculty of Dentistry, Alexandria University, Egypt
3. Professor of Oral Biology, Faculty of Dentistry, Alexandria University, Egypt

**Corresponding author:*
*E-mail: moustafa.abouyoussef@gmail.com*

## INTRODUCTION

Furcation defects are a frequent finding in periodontitis patients. Molars affected with furcation involvement respond less favorably to periodontal therapy, they also exhibit a greater risk for further attachment loss than other teeth (1).

In the early 1980s Hunt and Neighlton (2) described the separation of a patient's blood by centrifugation into three layers; platelet-rich plasma (PRP), platelet poor plasma (PPP) and red and white blood cell layers. The authors observed that the PRP layer was of interest for use in wound healing, since this layer contains a concentrate of the patient's platelets. These platelets have granules that contain growth factors that affect every cell as well as the formation of tissue involved in wound healing and regeneration of soft tissue and bone. Marx et al. (3) described the use of PRP in dental surgery. They claimed that the addition of PRP to grafts showed a radiographic maturation rate 1.62 to 2.16 times than that of grafts without PRP.

The clinical efficacy of PRP depends mainly on the number of platelets and the concentration of their growth factors; these growth factors act as transmitters in most processes in tissues, particularly in healing, where they are responsible for proliferation, differentiation, chemotaxis and tissue morphogenesis (4). Growth factors are biologically active molecules used to achieve periodontal regeneration as they regulate cell proliferation, activity, chemotaxis and/or cell differentiation, alone or in combination with grafting materials. Among these are insulin-like growth factors (IGFs), fibroblast growth factors (FGFs), epidermal growth factor (EGF), platelet-derived growth factors (PDGFs), and vascular endothelial growth factor (VEGF), transforming growth factor-β (TGFβ) and bone morphogenetic proteins (BMPs) (5).

Medical ozone($O_3$) is used to disinfect and treat disease. Its mechanism of action is by the inactivation of bacteria, viruses, fungi, yeast and protozoa, stimulation of oxygen metabolism and activation of the immune system. $O_3$ can promote platelet aggregation particularly when heparin is used as an anticoagulant (6), Ebensberger et al. (7), evaluated the effect of irrigation with ozonated water on the proliferation of cells in the periodontal ligament adhering to the root surfaces of avulsed teeth.

They concluded that avulsed teeth when irrigated with ozonized water for 2 min. showed effective mechanical cleansing and root surface decontamination. In a study by Nagayoshi et al. (8) dental plaque samples were treated with 4 mL of ozonated water for 10 seconds. They observed that ozonated water was effective in killing gram-positive and gram-negative oral microorganisms and oral C. albicans which reflected its potential to control infectious micro-organisms in dental plaque. The aim of the work was to evaluate the effectiveness of ozone activated PRP therapy in the management of grade II furcation defects.

## MATERIAL AND METHODS

**Animal Selection:** A total of six healthy adult mongrel dogs *(Canis familiaris)* about 18 to 24 months old and weighing between 18 to 24 kg. were included in this study. The dogs had good systemic health with no gingival inflammation and showed intact maxillary and mandibular teeth. The animals were adapted to the housing conditions four weeks before the study.

All guidelines for the care and use of experimental animals according to Alexandria's University Ethics Committee were followed.

**Study design:** A split mouth design was carried out in this study. Grade II critical – sized furcation defects were surgically created in the mandibular third (PM3) and fourth (PM4) premolars on both sides of the jaws of each dog (9). The defects were divided into two groups, each comprising four defects:

- **Control group**: included defects treated by PRP, alloplast and then covered by a collagen membrane.
- **Study group:** included defects treated using ozonated PRP, alloplast, then covered by a collagen membrane.

**Materials**

**Bone substitute material (Alloplast)**: (Genesis BCP™) by Dio-implant, Korea). A biphasic calcium phosphate composed of hydroxyapatite (60%) and beta-Tri-Calcium- phosphate (β-TCP) (40%), with 70% macropores (100 to 500 µm) and 30% micropores (≤10 µm).

**Collagen Membrane:** Collagen membrane by BioTECK, Italy) Type 1 lyophilized equine collagen from Achilles tendon (25 x 25 x 0.2 mm).

**Platelet Rich Plasma (PRP):** 30 cc venous blood were drawn from each dog. Nine parts blood was anticoagulated with one-part saline containing heparin so that the final concentration was 30 IU/ml. Blood was centrifuged at 200 rpm for 20 min. An average platelet count of 3× 108 /ml plasma was used. A further centrifugation of PRP at 6000 rpm for 15 sec was applied to give a platelet-containing pellet and a supernatant of platelet-free plasma. A predetermined volume of the $O_2/O_3$ gas mixture at three $O_3$ concentrations (80 m g/ml per ml of PRP) was collected by a silicone coated disposable syringe and immediately introduced into a second syringe containing an equivalent volume of PRP via a 'y' connector. Final gas pressure remained at normal atmospheric pressure (10) (Figure 1).

**Surgical procedures:** All surgical procedures were performed under general anesthesia using intramuscular injection of 0.1 ml ketamine hydrochloride *(Ketamine10%® by Alfasan, Holland.)* and 0.05 ml xylazine hydrochloride *(Xyla-ject® by Adwia co. S.A.E., Egypt.)*, for each 100mg body weight.

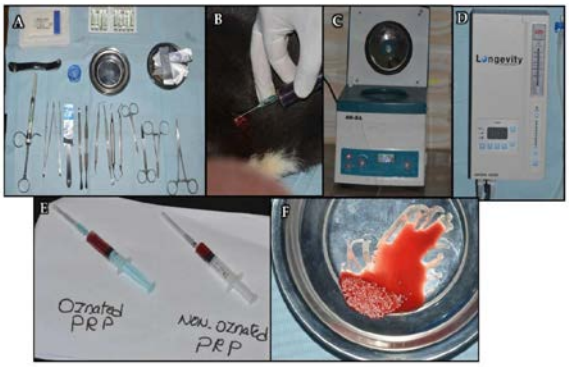


**Figure (1):** Clinical photographs showing PRP preparation. A) Armamentarium. B) blood collection from the cephalic venin. C) Centrifuge machine. D) Ozone machine. E) Samples of PRP ozonated and non-ozonated. F) mixing bone graft with PRP.C, PRF mixing with Alloplast.

**Creation of grade II critical sized furcation defects**

Infiltration anesthesia using 2% xylocaine/epinephrine (Artinibsa® by Inibsa, Spain.) was also administered at the surgical site. Sulcular incisions were performed using Bard Parker blade number 15, and then a mucoperiosteal flap extending from PM2 to M1 was elevated. The alveolar bone buccal to the furcation area was removed using carbide surgical burs on a low speed micro-motor under copious saline irrigation to expose the roots of the experimental teeth. Then grade II critical sized furcation defects were created in the furcations of PM3 and PM4 on either sides of the mandible. The dimensions of the defects were measured using a periodontal Michigan O probe (HU-FRIEDY, USA) with Williams' calibration. The dimensions of the resultant critical size created grade II furcations defects were as follows: 5 mm in the vertical component (distance from the furcation fornix to the base of the defect) and 4mm in the horizontal component (distance from the buccal surface to the internal wall of the furcation). Root planning was performed on all exposed root surface using manual curettes. After the creation of the defects was completed, reference notches were made on both roots (at the most apical part of each root in relation to the base of the defect) to act as histologic reference points later on.

Root conditioning was carried out using tetracycline (Tetracid® by Chemical industries development CID, Egypt) soaked cotton pellets in order to modify the root surface.

In the control side (left side), the defects were augmented with PRP, alloplast and then covered by collagen membrane. While in the study side (the right side) the defects were augmented with ozonated PRP, alloplast then covered by collagen membrane. (Figure 2)

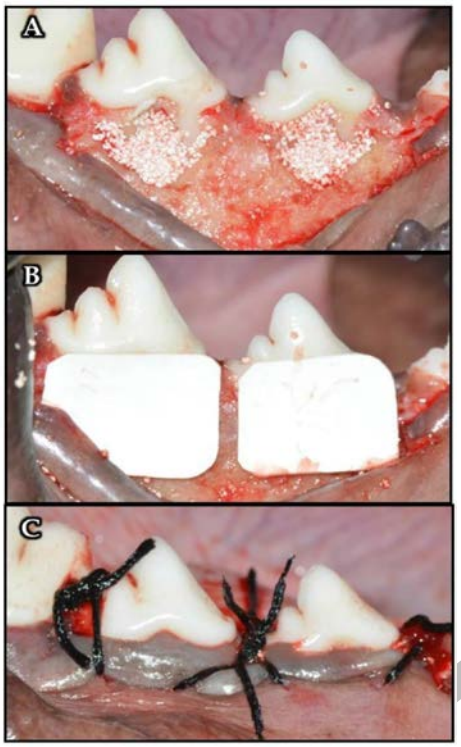


**Figure (2):** Clinical photographs showing the surgical procedures: A) Ozonated PRP + Alloplast mix. B) The collagen membrane covering the defect. C) Suturing of the surgical site.

**Post-surgical care:** All dogs received Amoxicillin 1gm IM (Amoxicillin® by Misr co. for pharmaceutical industries, Egypt) and diclofenac sodium 75mg IM (Declophen® by Pharco pharmaceuticals, Egypt), immediately after surgery. For the following 5 days, each dog received 500 mg amoxicillin and 25 mg of diclofenac sodium as analgesics in their diet three times daily. All dogs were put on a soft diet for the first 10 days and observed frequently for any signs of infection or inflammation.

**Animal sacrifice:** Dogs were sacrificed at two different time intervals; 4 and 8 weeks, post-operatively. Dogs were sacrificed by an overdose intra venous injection of thiopental sodium. The mandible was dissected out, divided into two, then preserved in 10% buffered neutral formalin for histological processing.

**Histological procedures:** The specimens were decalcified in 8 % trichloroacetic acid and processed to obtain 5 microns thick sections. These sections were stained with Harris Hematoxylin and Eosin Stain (H&E) for general examination and evaluation of healing (11) and with Gomori's trichrome stain to examine new bone formation and collagen organization (12).

**Histological Results**

At one month, the study group showed newly formed bone trabeculae of moderate density spanning the whole width between the walls of the mesial and distal roots of the operated tooth and the bifurcation area coronally, while the control group showed less amount of formed bone, with persistence of greater amount of the scaffold material and less vascularity among the formed bone (Figure 3).

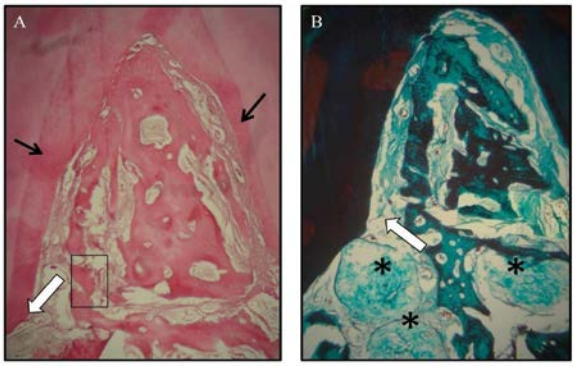


**Figure (3):** A) Light micrograph (LM), [study group, 4weeks] showing regenerating bone in the inter-radicular defect spanning most of its boundaries. Note the increased thickness of cementum (thin arrow). The thick arrow is demarcating the level of the created notch delimiting defect at the base of the defect. H&E, original magnification X40 B) LM, [control group, 4weeks] showing the less amount of the regenerating bone in the created defect than in the study group and the greater aggregates of the scaffold material (asterisk). The thick arrow is demarcating the level of the notch at the base of the defect. Trichrome stain, original magnification X40.

At two months, the study group exhibited an outstanding histological picture; noticeably, more bone formation could be seen in the different regions of the defect, centrally and peripherally where it formed the lateral walls adjacent to the PDL, and at the base of the defect. This was accompanied with excessive cementum deposition on the roots' surfaces facing the PDL. On the other hand, bone formation and defect fill in the control group was less than its appearance in the experimental group of the same observation period, but it appeared to differ than its appearance in the same group at the previous observation period, showing more bone formation. (Figure 4).

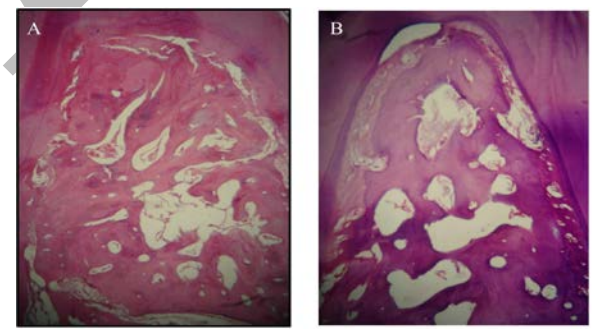


**Figure (4):** A) LMs (study group, 8weeks) showing the marked density of the formed bone in the regenerating defects and marked narrowing of the PDL especially adjacent to the bifurcation area. A: H&E, X: 40. B) LM, [control group, 8weeks] showing less bone formation in the defect, wider PDL, and wider bone marrow spaces between the interconnected formed trabeculae. H&E, X: 40.

**Histomorphometric results**

**1. The height of newly formed inter-radicular bone**

At 4 weeks: In the control group the mean height of newly formed inter-radicular bone was (40.17±2.64), while the study group showed a mean of (53.0 ±3.22) showing a highly significant difference (P <0.001).

At 8 weeks: The control group showed a mean of (30.83±5.27), while the study group showed a mean of (52.50±5.79). This difference was also statistically significant. (P <0.001). (Table 1) & (Figure 5)

**Table (1):** Comparison between the two studied groups according to the height of newly formed inter-radicular bone.

| Bone Height | Study (n = 7) | Control (n = 7) | t | p |
|---|---|---|---|---|
| **4 weeks** | | | | |
| Min. – Max. | 42.0 – 58.0 | 22.0 – 36.0 | 6.780* | <0.001* |
| Mean ± SD. | 52.50±5.79 | 30.83±5.27 | | |
| Median | 54.50 | 31.50 | | |
| **8 weeks** | | | | |
| Min. – Max. | 49.0 – 56.0 | 37.0 – 44.0 | 7.543* | <0.001* |
| Mean ± SD. | 53.0 ±3.22 | 40.17±2.64 | | |
| Median | 54.0 | 40.0 | | |

*: Statistically significant at p ≤ 0.05

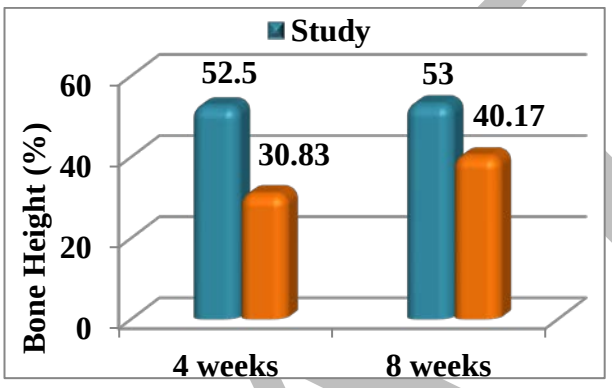


**Figure (5):** Comparison between the different groups according to the height of newly formed inter-radicular bone at 4 and at 8 weeks.

**2. The percentage of the formed bone surface**

At 4 weeks: In the control group the mean percentage of the formed bone surface was (279.50%±44.36), while the study group it was (350.0 %±40.84) showing a highly significant difference (P <0.001).

At 8 weeks: The control group showed a mean of (178.17%±35.03), while the study group showed a mean of (262.50%±27.27) showing a significant difference (P <0.001). (Table 2) & (Figure 6)

**Table (2):** Comparison between the two studied groups according to the percentage of the formed bone surface area

| Bone Surface Area (%) | Study (n =7) | Control (n = 7) | t | p |
|---|---|---|---|---|
| **4 weeks** | | | | |
| Min. – Max. | 56.20–68.38 | 31.06–52.94 | 6.16* | 0.000* |
| Mean ± SD. | 61.39±4.29 | 40.93±7.67 | | |
| Median | 59.91 | 43.71 | | |
| **8 weeks** | | | | |
| Min. – Max. | 66.96 –79.37 | 54.58- 68.60 | 4.75* | 0.001* |
| Mean ± SD. | 74.93±4.12 | 62.91±5.27 | | |
| Median | 75.17 | 62.83 | | |

t, p: t and p values for **Student t-test**
*: Statistically significant at p ≤ 0.05

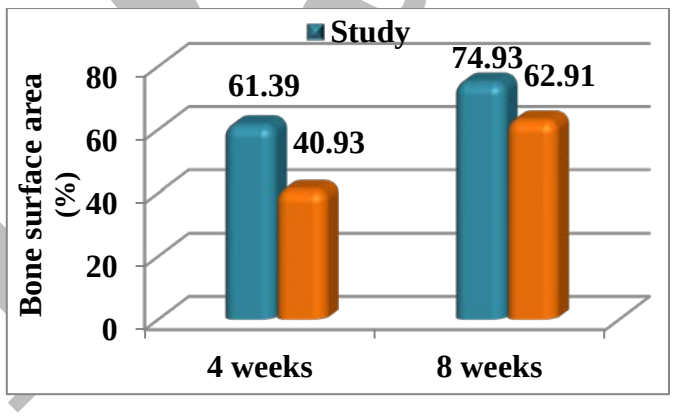


**Figure (6):** Comparison between the two studied groups according to the percentage of the formed bone surface area

## DISCUSSION

In the current study, grade II critical sized furcation defects were created in six mongrel dogs. A split mouth design was carried out in which the defects of the control side of each dog were augmented with PRP,β-TCP alloplast material and covered with a collagen membrane, while in the study side of the same dog the defects were augmented with ozonated PRP and β-TCP covered with a collagen membrane. After one and two months, tissue blocks containing the studied teeth with the surrounding hard and soft tissues were obtained and examined histologically and histomorphometrically.

The present study evaluated whether the application of PRP in grade II furcation lesions would enhance periodontal regeneration when activated by ozone. Clinical reports have demonstrated that the association of GTR, bone substitutes and PRP could

provide good results in the treatment of intrabony defects and grade II furcation lesions (13-15).

The use of PRP for periodontal regeneration has been based on the idea that this preparation contains polypeptide growth factors (PGFs). Some specific PGFs, like PDGF and TGFβ, could promote the growth and differentiation of periodontal ligament and alveolar bone cells and could be responsible for the clinical improvement observed in the experimental sites (16,17). Another interesting feature of PRP is its sticky consistency due to its high fibrin content. The fibrin component of PRP may work as a hemostatic agent aiding in stabilizing the graft material and blood clot (18).

The positive effect of PRP on bone formation and maturation was demonstrated in previous studies (19,20) and was evidenced in the present study by the large area of mineralized bone observed in both groups.

The current histological results proved the effect of ozone on PRP especially its effect in increasing the bone density and surface area in the study group. This can be interpreted acby the fact that ozone ($O_3$) can promote platelet aggregation particularly when heparin is used as an anticoagulant (21).

PRP treated with $O_3$ significantly increases the amount of platelet-derived growth factor (PDGF), TGFβ and IL-8. These factors were found to be released in a dose- dependent manner after ozonation of heparinised PRP samples (22).

In this study the histological results showed a distinct difference between the control and the study groups, regarding vascularization and bone formation. The study group showed numerous blood vessels rich in RBCs in the granulation tissue of the specimens at 4 weeks and also at 8 weeks more blood vessels and red bone marrow were observed.

In the current study histomorphometric analysis was performed using Image J software. The height of newly formed inter-radicular bone and the percentage of the formed bone surface, in both study and control groups at four and eight weeks were analyzed. The histomorphometric results showed that PRP activated by ozone accelerated bone regeneration at four and eight weeks showing great difference between the study and the control groups in all parameters.

## CONCLUSION

Within the limitations of the present study and the associated findings, it could be concluded that activation of PRP by ozone together with guided tissue regeneration procedures yields a favorable effect of accelerating bone formation and vascularization.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

## REFERENCES


1. Raja S, Nath G, Emmadi P, Ramakrishnan, Ahathya. Treatment of an isolated furcation involved endodontically treated tooth - a case report. J Conserv Dent.2007; 10:129-33.
2. Albanese A, Licata ME, Polizzi B, Campisi G. Platelet-rich plasma (PRP) in dental and oral surgery: from the wound healing to bone regeneration. Immun Ageing.2013; 10: 23.
3. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet Rich Plasma growth factors enhancement for bonegrafts. Oral Surg Oral Med Oral Pathol Oral RadiolEndod.1998; 85:638-46.
4. Kaigler D, Avila G, Wisner-Lynch L, Nevins ML, Nevins M, Rasperini G, et al. Platelet-Derived Growth Factor Applications in Periodontal and Peri-Implant Bone Regeneration. Expert OpinBiolTher.2011; 11:375-85.
5. Retzepi M, Donos N. Guided Bone Regeneration: biological principle and therapeutic applications. Clin Oral Implants Res.2010;21:567-76.
6. Iuliano L, Colavita AR, Leo R, Pratic`o D, Violi F. Oxygen free radicals and platelet activation. Free Rad Biol Med.1997; 22:999-1006.
7. Ebensberger U, Pohl Y, Filippi A. PCNA-expression of cementoblasts and fibroblasts on the root surface after extraoral rinsing for decontamination. Dent Traumatol.2002; 18:262-6.
8. Nagayoshi M, Kitamura C, Fukuizumi T, Nishihara T, Terashita M. Antimicrobial effect of ozonated water on bacteria invading dentinal tubules. J Endod. 2004; 30:778- 81.
9. Ghaitth HM, Lotfy M, Soliman S, Fahmy RA. Quantitative Analysis of Interradicular Bone Regeneration after Use of Modified Perforated Collagen Membrane versus Occlusive Membrane in the Management of Grade II Furcation Defects in Dogs. Impact Factor.2015; 6:391.
10. Toffler M, Toscano N, Holtzclaw D, Dds D, Ehrenfest DD, Bullet N. Introducing Choukroun's platelet rich fibrin (PRF) to the reconstructive surgery Milieu. JIACD. 2009; 1: 21-32.
11. Su CY, Kuo YP, Tseng YH, Su CH, Burnouf T. In vitro release of growth factors from platelet-rich fibrin (PRF): a proposal to optimize the clinical applications of PRF. Oral Surg Oral Med Oral Pathol Oral RadiolEndod.2009; 108:56e61.
12. Yilmaz D, Dogan N, Ozkan A, Sencimen M, Oral BE, Mutlu I. Effect of platelet rich fibrin and beta tricalcium phosphate on bone healing.A histological study in pigs. ActaCirúrgicaBrasileira.2014;29:59-68.
13. Suaid FF, Carvalho MD, Ambrosano GM, Nociti FH Jr, Casati MZ, Sallum EA. Platelet-rich plasma in the treatment of Class II furcation defects: a histometrical study in dogs. J Appl Oral Sci.2012;20:162-9.
14. Mansouri S, Ghasemi M, Darmian S, Pourseyediyan T. Treatment of Mandibular Molar Class II Furcation Defects in Humans With Bovine Porous Bone Mineral in Combination With Plasma Rich in Growth Factors. J Dent (Tehran). 2012;9:41-9.
15. Suaid FF, Carvalho MD, Ambrosano GMB, Junior FHN, Casati MZ, Sallum EA. Platelet-rich plasma in the treatment of Class II furcation defects: a histometrical study in dogs. J Appl Oral Sci. 2012; 20:162-9.
16. Giannobile WV, Hernandez RA, Finkelman RD, Ryan S, Kiritsy CP, D'Andrea M, et al. Comparative effects of platelet-derived growth factor-BB and insulin-like growth factor-I, individually and in combination, on periodontal regeneration in Macaca-fascicularis. J Periodontal

Res.1996;31:301-12.
17. Sigurdsson TJ, Lee MB, Kubota K, Turek TJ, Wozney JM, Wikesjö UM. Periodontal repair in dogs: recombinant human bone morphogenetic protein-2 significantly enhances periodontal regeneration. J Periodontol.1995;66:131-8.
18. Polson AM, Garrett S, Stoller NH, Greenstein G, Polson AP, Harrold CQ, et al. Guided tissue regeneration in human furcation defects after using a biodegradable barrier: a multi-center feasibility study. J Periodontol.1995;66:377-85.
19. Jensen TB, Rahbek O, Overgaard S, Søballe K. No effect of platelet-rich plasma with frozen or processed bone allograft around noncemented implants. IntOrthop. 2005;29:67-72.
20. Ray AK, Jones AC, Carnes DL, Cochran DL, Mellonig JT, Oates TW., Jr Platelet- derived growth factor-BB stimulated cell migration mediated through p38 signal transduction pathway in periodontal cells. J Periodontol.2003;74:1320-28.
21. Bocci V, Valacchi G, Rossi R, Giustarini D, Paccagnini E, Pucci AM, et al. Studies on the biological effects of ozone: 9. Effects of ozone on human platelets. Platelets.1999;10:110-6.
22. Weglein A. Effect of autologous fibrin gel and platelet rich plasma activated by ozone versus those activated by calcium chloride on wound healing and prevention of infection in high risk cesarean sections: randomized controlled study. 2017. Available at: http://www.houstonsportsdoctor.com/blog/effect-of-autologous-fibrin-gel-platelet- rich-plasma-activated-by-ozone-versus-those-activated-by-calcium-chloride-on-wound-healing-prevention-of-infection-in-high-risk-cesarean-sections-randomized-controlled-study-10338.html.