

Effect of platelet-rich plasma on healing of class III furcation defects treated with autogenous bone grafting and guided tissue regeneration: a histomorphometric study in dogs

José Marcos Alves FERNANDES^a, Emilio BARBOSA E SILVA^a, Rodrigo Otávio Citó César RÊGO^a,
Rafael Scaf de MOLON^a, Daniela Leal ZANDIM-BARCELOS^a,
Luis Carlos SPOLIDÓRIO^b, Joni Augusto CIRELLI^a

^aDepartamento de Diagnóstico e Cirurgia, Faculdade de Odontologia de Araraquara,
UNESP – Univ Estadual Paulista, 14801-903 Araraquara - SP, Brazil

^bDepartamento de Fisiologia e Patologia, Faculdade de Odontologia de Araraquara,
UNESP – Univ Estadual Paulista, 14801-903 Araraquara - SP, Brazil

Fernandes JMA, Barbosa e Silva E, Rêgo ROCC, Molon RS, Zandim-Barcelos DL, Spolidório LC, Cirelli JA. Efeito do plasma rico em plaquetas na reparação de lesões de furca classe III tratadas com enxerto ósseo autógeno e regeneração tecidual guiada: estudo histomorfométrico em cães. Rev Odontol UNESP. 2011; 40(6): 310-316.

Resumo

Objetivo: O objetivo deste estudo foi avaliar histologicamente os efeitos do plasma rico em plaquetas (PRP), quando usado em combinação com enxerto ósseo autógeno e membrana bioabsorvível (Resolut[®]) no tratamento de defeitos de furca Classe III em cães. **Material e método:** Cinco cães foram usados neste estudo. Defeitos de furca classe III (5 mm de altura e de profundidade) foram criados cirurgicamente no terceiro pré-molar inferior de ambos os lados. Nove semanas após a primeira cirurgia, os terceiros pré-molares foram tratados com raspagem e alisamento radicular e cada defeito recebeu um dos seguintes tratamentos: Enxerto ósseo autógeno + membrana (grupo C) ou PRP + enxerto ósseo autógeno + membrana (grupo T). Após um período de cicatrização de 90 dias, os animais foram sacrificados. Processamento histológico de rotina e coloração com hematoxilina e eosina e tricrômico de Masson foram realizados para determinar o efeito dos tratamentos na regeneração dos tecidos periodontais. Os dados foram analisados pelo teste T² de Hotelling ($p < 0,05$). **Resultado:** A análise histomorfométrica da área de furca não mostrou nenhuma diferença estatisticamente significativa entre os grupos C e T. Os dois grupos de tratamento demonstraram resultados regenerativos semelhantes, com os defeitos de furca parcialmente preenchidos e a regeneração periodontal foi limitada à marca experimental apical das lesões. ($p > 0,05$). **Conclusão:** Dentro dos limites deste estudo, concluiu-se que o uso de PRP não melhorou a regeneração periodontal em defeitos de furca classe III tratados com enxerto ósseo autógeno e membrana bioabsorvível.

Palavras-chave: Defeitos de furca; plasma rico em plaquetas; regeneração tecidual guiada; membrana bioabsorvível

Abstract

Objective: The purpose of this study was to evaluate the effects of the platelet-rich plasma (PRP) when used in combination with autogenous bone graft and bioabsorbable membrane (Resolut[®]) in the treatment of Class III furcation defects in dogs. **Material and method:** Class III furcation defects (5 mm in height and in depth) were surgically created in the mandibular third premolars of five mongrel dogs. After nine weeks, the lesions were treated with scaling and root planning and each defect received one of the following treatments: autogenous bone graft + membrane (group C) or PRP + autogenous bone graft + membrane (group T). After a healing period of 90 days, the animals were sacrificed. Routine histological processing and staining with hematoxylin and eosin and Masson trichrome were performed and a histomorphometric analysis determined the effect of the treatments on periodontal tissue regeneration. Data were analyzed by Hotelling's T-squared ($p < 0.05$). **Result:** No statistically significant difference between C and T groups was observed by the histomorphometric analysis of the furcation area. Both treatment groups demonstrated similar regenerative results with the furcation defects partially filled and periodontal regeneration limited to the experimental notches of the lesions. ($p > 0.05$). **Conclusion:** According to the present results, PRP does not enhance the periodontal regeneration in class III furcation defects treated with autogenous bone graft and bioabsorbable membrane.

Keywords: Furcation defects; platelets-rich plasma; guided tissue regeneration; absorbable membrane.

INTRODUCTION

The ultimate goal of the periodontal therapy is the restitution of the architecture and function of the original support structures (i.e. root cementum, periodontal ligament and alveolar bone) which have been destroyed during the course of periodontal disease¹. The use of bone grafts and bone substitutes, guided tissue regeneration (GTR) and, more recently, the application of polypeptide growth factors (PGFs) to the surgical wound are some of the commonly employed techniques used to promote periodontal regeneration²⁻⁷.

GTR therapy was introduced in 1980s and consists in the placement of a physical barrier over a periodontal osseous defect. This technique can prevent the faster proliferation of the gingival connective tissue and epithelial oral cells from growing into the bone defect, allowing the cells of the periodontal ligament to colonize the blood clot and regenerate the periodontal lost tissue⁸. However, the results obtained with this therapy in the treatment of large-size defects such as Class III furcation defects are very limited⁹⁻¹¹.

The amount of regenerated tissue and the course of soft tissue healing are dependent on the individual healing potential^{12,13}, which is significantly influenced by the presence and amount of polypeptide growth factors (PGFs) naturally available in the wound^{13,14}. Based on this knowledge, the application of certain growth factors has been used to promote periodontal regeneration. Findings from *in vitro* experiments^{15,16}, animals^{17,18} and clinical studies^{19,20} have indicated a potential influence of different growth factors on the regeneration of periodontal tissues. These proteins were used alone²¹, in combination with bone substitutes^{19,20}, or in addition to GTR therapy²².

Platelet-rich plasma (PRP), which is an autologous volume of plasma with a four to fivefold-increased platelet concentration above baseline, is a proven source of growth factors²³. The use of PRP is based on the potential of the plasma to release multiple wound-healing growth factors and cytokines²³ which are responsible for increasing collagen production, recruiting other cells to the site of injury, increasing cell mitosis, inducing cell differentiation and initiating vascular in-growth²⁴. It has also been verified the application of the PRP in addition to other regenerative procedures in intra-bony periodontal defects^{25,26} and class II furcation defects²⁷.

At present, it is still unknown an effective therapy that result in periodontal regeneration of Class III furcation defects. Thus, the aim of the present study was to evaluate histologically and histometrically the additional effect of PRP on the treatment of Class III furcation defects comparing the use of PRP associated to bone graft + GTR to bone graft + GTR only.

MATERIAL AND METHOD

1. Sample

Experimental group comprised five male mongrel dogs, weighing approximately 15 kg each and estimated ages of 1.5 to 3 years old, maintained in the animal facilities of the School

of Dentistry at Araraquara - UNESP. The study protocol was conducted according to the recommendations of the National Council for the Control of Animal Experimentation (CONCEA) and the protocol was approved by the local Institutional Experimentation Committee for Animal Care and Use (protocol 05/2003).

These animals were systemically healthy and showed no clinical and radiographic signs of destructive periodontal disease. A total of 10 third lower premolars were used and the teeth of the same animal were randomly distributed into 2 groups of treatment.

2. Creation of Class III Furcation Defects

The creation of class III furcation defects was performed as previously described⁷. Initially, the anesthetic procedure used throughout the study included a previous sedation of the animals with IM injection of cloridrate of chlorpromazine, 0.2 mL.kg⁻¹ (Bayer S.A. Saúde Animal, São Paulo, SP, Brasil) followed by induction of general anesthesia with IV sodium thiopental, 0.5 mL.kg⁻¹ (Abbott Laboratórios do Brasil Ltda., São Paulo, SP, Brasil). To control bleeding and ensure deep anesthesia, 2% lidocaine (Cristália Produtos Químicos Farmacêuticos Ltda., Itirapina, SP, Brasil) containing noradrenaline (1:100,000) was infiltrated into the mucosa. After, a full-thickness mucoperiosteal flap was reflected and the interradicular bone of each third premolar was removed with rotatory # 2 round burr (KG Sorensen, São Paulo, SP, Brasil) and hand micro Ochsenein chisels (Neumar, São Paulo, SP, Brasil) to create class III furcation defects. These defects had 5 mm of vertical height from cemento-enamel junction (CEJ) to the marginal bone and 5 mm of width on vestibular and lingual aspects (Figure 1). The roots were scaled with Gracey curettes (Neumar, São Paulo, SP, Brasil) to remove all periodontal ligament fibers, and filled with heat-softened gutta-percha (Odahcam, Herpe Produtos Dentários Ltda., Rio de Janeiro, RJ, Brasil) to avoid spontaneous regeneration.

Sutures (Ethicon 4.0 Atralog, Johnson & Johnson, São Paulo, SP, Brasil) were removed after 7 days and the animals were maintained during 8 weeks with water-softened food to increase plaque accumulation, leading to root contamination as well as the development of a chronic inflammatory reaction. After this period, the dogs were anesthetized again to remove the gutta-percha with curettes and check the cronification of the defects. The animals also received supragingival scaling and dental prophylaxis. Chemical plaque control with daily (5 times/week) topical application of 0.2% chlorhexidine solution was initiated.

3. PRP Preparation

PRP was prepared according to Anitua²⁸ (1999). One 5 mL tube containing 0.5 mL of 3.2% sodium citrate solution as an anticoagulant (Vacutaine™, Becton Dickson UK Ltd., Belliver Industrial, State Plymouth, England) was drawn from each dog. The tubes were centrifuged (SIN - Sistema de Implante Nacional Ltda - Brasil) at 1200 rpm for 8 minutes at room temperature. The blood was then separated into three basic parts: red blood cells (at the bottom of the tube), PRP (a discrete gray line in the middle

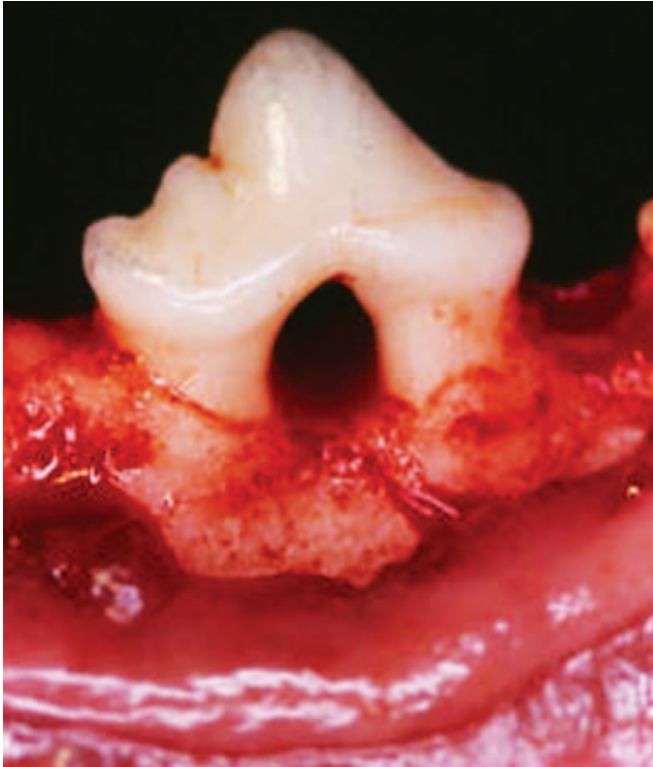


Figure 1. Class III furcation defect.

of the tube) and platelet-poor plasma (at the top of the tube). The portion corresponding to the PRP was pipetted out from each tube and stored in a sterile glass recipient. Subsequently, 10% calcium chloride was added to the preparation to activate platelets and to form the platelet gel. The autogenous bone graft was associated with the platelet gel and used to fill in the furcation defects.

4. Regenerative Treatment

The treatment of the defects was performed as previously described⁷. Briefly, after one week of the gutta-percha removal, full-thickness mucoperiosteal flaps were elevated, granulation tissue was removed and the root surfaces were thoroughly scaled with manual instruments (Gracey curettes, Neumar, São Paulo, SP, Brasil). Reference notches were done on the root surface at the marginal bone level both on mesial and distal aspect. After these procedures, the treatment varied according to the group in which the teeth were randomly assigned:

- Test group (group T): The defects of this group were filled with autogenous bone graft + PRP and bioabsorbable membranes Gore Resolut XT (W.L. Gore & Associates, Inc. Arizona) were trimmed according to the GRT principles and fitted to the vestibular and lingual surfaces (Figure 2). Resorbable sutures (Vycril Ethicon 6.0, Johnson & Johnson, São Paulo, SP, Brasil) placed around the cervical third of the tooth stabilized the membranes and flaps were coronally repositioned and secured with suspended and interrupted sutures;
- Control group (group C): The defects received the same treatment described for the test group, except the PRP application.

At the end of surgical procedures, the dogs received IM injections of antibiotics (penicilin G benzatine, 40,000 $\mu\text{L.kg}^{-1}$) and

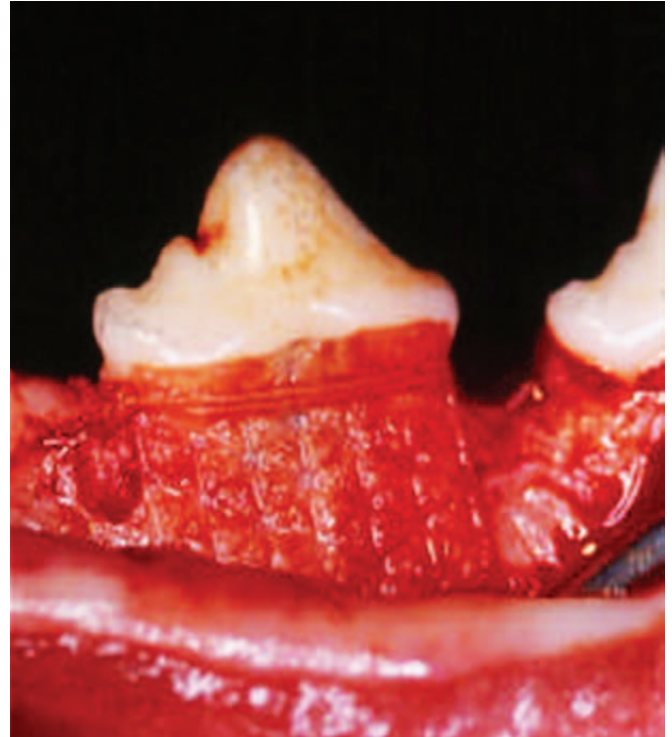


Figure 2. Absorbable membrane positioned according to GTR protocol.

analgesics (dipirone, 2 mL.10 kg^{-1}) as post-operative medications. The animals were then introduced into a reparative period of 90 days. During the first 4 weeks, the wounds were protected by feeding the dogs a soft diet. Chemical plaque control with daily (5 times/week) topical application of 0.2% chlorhexidine solution was maintained.

5. Sacrifice and Histological Processing

The animals were sacrificed by an overdose of sodium thiopental (Abbott Laboratórios do Brasil Ltda., São Paulo, SP, Brasil) 3 months after the regenerative treatment. The jaws were removed, dissected and the blocks containing the experimental specimens were fixed in formalin 10% and decalcified in Morse solution.

After decalcification, routine histological processing and paraffin embedding were done, and 5 μm thick tissue slices were obtained throughout the blocks on the mesiodistal plane. For each tooth, five sections were selected, including the first and last sections that showed the reference notches on both mesial and distal roots and three sections equally spaced between those two representing the vestibular, central and lingual portions of the furcations. These selected sections were stained with hematoxylin and eosin (H/E) and Masson Trichrome.

6. Histometric Analysis

The histometric analysis was performed as previously described⁷ and was done by another examiner that was also blind to the treatment groups. A computerized image analysis system (Diasar - Cambridge Instruments, Buffalo, NY, USA) consisting of

a light microscope coupled to a video camera DXC-107AP/107AP - (Sony Electronics Inc., Japan) and connected to a microcomputer with image analysis software Jandel Sigma Scan Pro, Version 2.0 (Jandel Corporation-San Rafael, CA, USA).was used to obtain linear and area measurements. The following variables were analyzed: 1) Free: linear extension of root surface not covered by any tissue and in contact with dental plaque; 2) EP (epithelium): linear root surface extension covered by epithelial tissue plus the extension of epithelial tissue present within the lesion that did not contact dental plaque; 3) CE (cementum): root surface extension with newly formed cementum; 4) CT (connective tissue): root surface extension with attached connective tissue fibers without any cementum; 5) LPR (Linear periodontal regeneration): linear root surface extension covered with new cementum and new alveolar bone; 6) ES (empty space): furcation area without any tissue; 7) STA (soft tissue area): defect area filled by connective and epithelial tissues; 8) MTA (mineralized tissue area): defect area filled by mineralized tissue (Figure 3).

7. Descriptive Analyses

An experienced pathologist (LCS) that was blind to the treatment groups performed the histological descriptive analysis. The type and quality of tissues formed within the defect as well as the presence of any unusual tissue reactions such as resorption and anquilosis were evaluated.

8. Statistical Analysis

To analyze differences between T and C groups in all area and linear histometric measurements, T2 Hotelling multivariate statistical test with 0.05 of significant level and Bonferroni test were used.

RESULT

1. Descriptive Analysis

The healing was uneventful on the experiment and control sides in all dogs; no suppuration or abscess formation was observed. The results of the descriptive histological analysis for both treatment groups were similar. The furcation lesions were predominantly filled out by a dense connective tissue covered by an epithelium of variable thickness. This epithelium demonstrated normal characteristics with pronounced projections for the subjacent connective tissue which showed an inflammatory infiltrate with different intensity, probably in response to the presence of dental plaque in the furcation roof. Newly formed cementum was observed at the root notch and eventually extending coronally until the furcation roof (Figures 4 and 5). This new cellular cementum had variable thickness without the presence of incremental lines, denoting disorganized apposition. In some areas of the notches, suggestive image of fibers collagen from the connective tissue imbedded inside the cementum was observed. Bone regeneration was negligible, extending only to the base of the furcation defect.

2. Histometric Analysis

Histometric results are shows in Table 1. No connective tissue was found in contact with the root surface in all specimens.

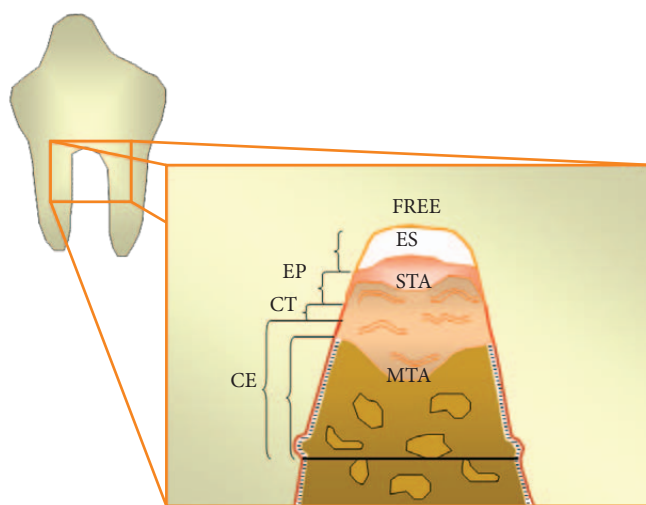


Figure 3. Schematic representation of linear and area variables evaluated in the histometric analysis.

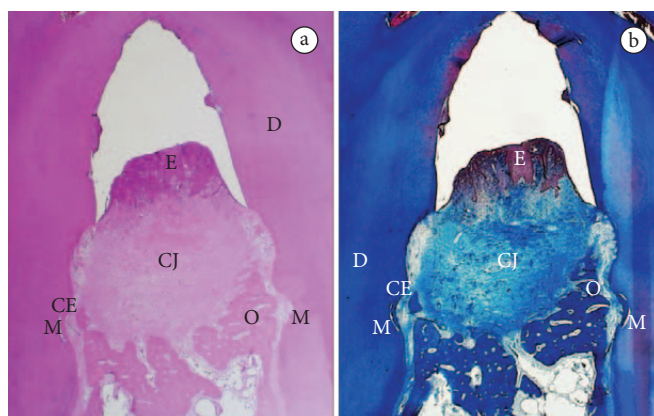


Figure 4. a) Panoramic view of the class III furcation in the Test group, showing poor bone regeneration within the defect (Hematoxylin and Eosin, original magnification $\times 20$); b) Masson Trichrome, original magnification $\times 20$).

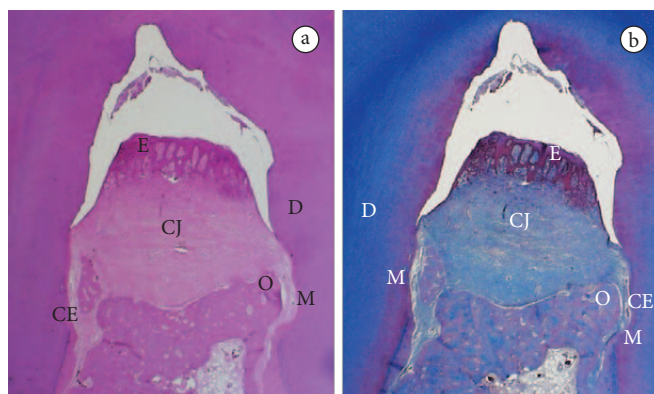


Figure 5. a) Panoramic view of the class III furcation in the Control group, showing poor bone regeneration within the defect (Hematoxylin and Eosin, original magnification $\times 20$); b) Masson Trichrome, original magnification $\times 20$).

Table 1. Histometric parameters: mean, standard deviation and range (mm for linear or mm² for area measurements) for each regenerative treatment

	C group (n = 5) (range)	T group (n = 5) (range)	P
EP	3.16 ± 1.34 (2.09 – 5.51)	3.70 ± 1.64 (2.25 - 6.53)	>0.05
CE	2.97 ± 1.19 (1.41 – 4.04)	2.23 ± 1.01 (0.72 – 3.13)	>0.05
LPR	0.74 ± 0.84 (0.00 – 1.63)	0.51 ± 0.60 (0.00 – 1.43)	>0.05
ES	1.62 ± 0.52 (1.01 – 2.41)	1.88 ± 0.47 (1.38 – 2.45)	>0.05
STA	7.71 ± 1.09 (6.45 – 8.88)	7.39 ± 1.00 (6.31 – 8.90)	>0.05
MTA	0.44 ± 0.33 (0.00 – 0.77)	0.09 ± 0.1 (0.00 – 0.22)	>0.05

DISCUSSION

The present study evaluated the effect of platelet-rich plasma (PRP) associated to GTR and autogenous bone graft in bone regeneration of class III experimental furcation defects created in dogs. After 3 months of healing, no additional benefits were observed in that type of defect when PRP was added to the regenerative treatment.

The PRP is derived from a preparation of an autologous platelet concentrate from the patient blood serum. It is activated by calcium chloride, and the result of this activation is the release of a cascade of growth factors present in the granules of platelets²⁹. The mechanism of this technique is based on the release of growth factors from the clot fibrin, rich in these components, during a natural healing event. The PRP is also known by its osteoinductive properties. However, in our results, those properties were not demonstrated and no additional benefits on the periodontal regeneration were observed when PRP was associated to autogenous bone graft and absorbable membrane. In addition, bone regeneration in the furcation of both groups was poorly achieved. This result can be attributed to the critical size of the defects. In the present study, the class III furcation lesions had a cervical to apical height and width of 5 mm and a limited amount of periodontal tissue was left only at the apical area of the defect, reducing its regenerative potential. According

to Park et al.³⁰ (1995), periodontal regeneration of class III furcation requires longer healing period (more than 11 weeks) and the result seems to be more variable and unpredictable. These defects tend to be more vulnerable to bacterial infection and bone healing is less predictable and reliable on them.

Previous studies^{7,22,30,31} reported in the literature have described positive results for class III furcation defects in dogs after regenerative treatments with growth factors. Methodological variation may explain divergent results observed in the present study in comparison to those, e.g. defect size (as previously described) and the use of mongrel dogs. This fact can result in significant genetic variability that leads alteration in the metabolism and physiology of the tissues, such as inflammatory reaction on the defect. Also, adequate biofilm control play an important role in the regeneration treatment success, since the presence of biofilm in the defect area during the healing process is described as one of the failure causes of periodontal regeneration therapies. In our study, even though daily chlorhexidine application was performed, biofilm was observed attached to the root surface of the furcation roof in various histological cuts. This may be justified by the difficulty of adequate biofilm control in the class III furcation after gingival tissue recession and furcation exposure, which usually happens at the beginning of the healing process.

Some of the previously cited studies used specific growth factors instead of PRP. The PRP is a pool of different growth factors with unknown concentration, which may have significant variability and, consequently, unpredictable effects. Additional studies are necessary to better understand the real regenerative potential of PCR, especially in critical size defects.

According to Mellonig³² (1999), it is important to note that the type of regenerative material to be used is only one of the determinants for successful outcome. Other important issues to be considered is: patient selection, the morphology and size of the defect, occlusal considerations, tooth mobility, root preparation, suture closure of the flap, antibiotic coverage, plaque control, wound stabilization. All these variables are considered important when seeking a true periodontal regeneration.

CONCLUSION

The association of platelet-rich plasma with autogenous bone graft and absorbable membrane did not show additional benefits on the periodontal regeneration in class III furcation in dogs when compared to autogenous bone graft and resorbable membrane only.

REFERENCES

1. Karring T, Lindhe J, Cortellini P. Regenerative periodontal therapy. In: Lindhe J, Karring T, Lang NP. Clinical periodontology and implant dentistry. 4th ed. Copenhagen: Blackwell-Munksgaard; 2003.
2. Fernandes JM, Rego RO, Spolidorio LC, Marcantonio RA, Marcantonio Júnior E, Cirelli JA. Enamel matrix proteins associated with GTR and bioactive glass in the treatment of class III furcation in dogs. *Braz Oral Res.* 2005;19:169-75. <http://dx.doi.org/10.1590/S1806-83242005000300003>

3. Casati MZ, de Vasconcelos Gurgel BC, Gonçalves PF, Pimentel SP, da Rocha Nogueira Filho G, Nociti FH Jr, et al. Platelet-rich plasma does not improve bone regeneration around peri-implant bone defects--a pilot study in dogs. *Int J Oral Maxillofac Surg*. 2007;36:132-6. PMID:16890407. <http://dx.doi.org/10.1016/j.ijom.2006.06.004>
4. Demir B, Sengün D, Berberoğlu A. Clinical evaluation of platelet-rich plasma and bioactive glass in the treatment of intra-bony defects. *J Clin Periodontol*. 2007;34:709-15. PMID:17635247. <http://dx.doi.org/10.1111/j.1600-051X.2007.01108.x>
5. Rosetti EP, Marcantonio RA, Cirelli JA, Zuza EP, Marcantonio E Jr. Treatment of gingival recession with collagen membrane and DFDBA: a histometric study in dogs. *Braz Oral Res*. 2009;23:307-12. PMID:19893967. <http://dx.doi.org/10.1590/S1806-83242009000300014>
6. de Oliveira CA, Spolidório LC, Cirelli JA, Marcantonio RA. Acellular dermal matrix allograft used alone and in combination with enamel matrix protein in gingival recession: histologic study in dogs. *Int J Periodontics Restorative Dent*. 2005;25:595-603. PMID:16353534
7. Rossa C Jr, Marcantonio E Jr, Cirelli JA, Marcantonio RA, Spolidorio LC, Fogo JC. Regeneration of Class III furcation defects with basic fibroblast growth factor (b-FGF) associated with GTR. A descriptive and histometric study in dogs. *J Periodontol*. 2000;71:775-84. PMID:10872959. <http://dx.doi.org/10.1902/jop.2000.71.5.775>
8. Dahlin C, Linde A, Gottlow J, Nyman S. Healing of bone defects by guided tissue regeneration. *Plast Reconstr Surg*. 1988;81:672-6. PMID:3362985. <http://dx.doi.org/10.1097/00006534-198805000-00004>
9. Becker W, Becker BE, Berg L, Prichard J, Caffesse R, Rosenberg E. New attachment after treatment with root isolation procedures: report for treated Class III and Class II furcations and vertical osseous defects. *Int J Periodontics Restorative Dent*. 1988;8(3):8-23.
10. Pontoriero R, Lindhe J, Nyman S, Karring T, Rosenberg E, Sanavi F. Guided tissue regeneration in the treatment of furcation defects in mandibular molars. A clinical study of degree III involvements. *J Clin Periodontol*. 1989;16:170-4. PMID:2723098. <http://dx.doi.org/10.1111/j.1600-051X.1989.tb01635.x>
11. Pontoriero R, Nyman S, Ericsson I, Lindhe J. Guided tissue regeneration in surgically-produced furcation defects. An experimental study in the beagle dog. *J Clin Periodontol*. 1992;19:159-63. PMID:1556243. <http://dx.doi.org/10.1111/j.1600-051X.1992.tb00632.x>
12. Cortellini P, Tonetti MS. Focus on intrabony defects: guided tissue regeneration. *Periodontol 2000*. 2000;22:104-32. PMID:11276509. <http://dx.doi.org/10.1034/j.1600-0757.2000.2220108.x>
13. Kornman KS, Robertson PB. Fundamental principles affecting the outcomes of therapy for osseous lesions. *Periodontol 2000*. 2000; 22:22-43. PMID:11276514. <http://dx.doi.org/10.1034/j.1600-0757.2000.2220103.x>
14. Wikesjö UM, Selvig KA. Periodontal wound healing and regeneration. *Periodontol 2000*. 1999;19:21-39. <http://dx.doi.org/10.1111/j.1600-0757.1999.tb00145.x>
15. Marcopoulou CE, Vavouraki HN, Dereka XE, Vrotsos IA. Proliferative effect of growth factors TGF-beta1, PDGF-BB and rhBMP-2 on human gingival fibroblasts and periodontal ligament cells. *J Int Acad Periodontol*. 2003;5:63-70. PMID:12887144
16. Papadopoulou CE, Dereka XE, Vavouraki EN, Vrotsos IA. In vitro evaluation of the mitogenic effect of platelet-derived growth factor-BB on human periodontal ligament cells cultured with various bone allografts. *J Periodontol*. 2003;74:451-7. PMID:12747449. <http://dx.doi.org/10.1902/jop.2003.74.4.451>
17. Wikesjö UM, Razi SS, Sigurdsson TJ, Tatakis DN, Lee MB, Ongpipattanakul B, et al. Periodontal repair in dogs: effect of recombinant human transforming growth factor-beta1 on guided tissue regeneration. *J Clin Periodontol*. 1998;25:475-81. PMID:9667481. <http://dx.doi.org/10.1111/j.1600-051X.1998.tb02476.x>
18. Cochran DL, Wozney JM. Biological mediators for periodontal regeneration. *Periodontol 2000*. 1999;19:40-58. PMID:10321215. <http://dx.doi.org/10.1111/j.1600-0757.1999.tb00146.x>
19. Sarment DP, Cooke JW, Miller SE, Jin Q, McGuire MK, Kao RT, et al. Effect of rhPDGF-BB on bone turnover during periodontal repair. *J Clin Periodontol*. 2006;33:135-40. PMID:16441739 PMID:2579262. <http://dx.doi.org/10.1111/j.1600-051X.2005.00870.x>
20. Nevins M, Giannobile WV, McGuire MK, Kao RT, Mellonig JT, Hinrichs JE, et al. Platelet-derived growth factor stimulates bone fill and rate of attachment level gain: results of a large multicenter randomized controlled trial. *J Periodontol*. 2005;76:2205-15. PMID:16332231. <http://dx.doi.org/10.1902/jop.2005.76.12.2205>
21. Howell TH, Fiorellini JP, Paquette DW, Offenbacher S, Giannobile WV, Lynch SE. A phase I/II clinical trial to evaluate a combination of recombinant human platelet-derived growth factor-BB and recombinant human insulin-like growth factor-I in patients with periodontal disease. *J Periodontol*. 1997;68:1186-93. PMID:9444594
22. Cho MI, Lin WL, Genco RJ. Platelet-derived growth factor-modulated guided tissue regenerative therapy. *J Periodontol*. 1995;66:522-30. Review. PMID:7562342
23. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1998;85:638-46. [http://dx.doi.org/10.1016/S1079-2104\(98\)90029-4](http://dx.doi.org/10.1016/S1079-2104(98)90029-4)
24. Freymiller EG, Aghaloo TL. Platelet-rich plasma: ready or not? *J Oral Maxillofac Surg*. 2004;62: 484-488. PMID:15085518. <http://dx.doi.org/10.1016/j.joms.2003.08.021>
25. Camargo PM, Lekovic V, Weinlaender M, Vasilic N, Madzarevic M, Kenney EB. Platelet-rich plasma and bovine porous bone mineral combined with guided tissue regeneration in the treatment of intrabony defects in humans. *J Periodontol Res*. 2002;37:300-6. PMID:12200975. <http://dx.doi.org/10.1034/j.1600-0765.2002.01001.x>
26. Camargo PM, Lekovic V, Weinlaender M, Vasilic N, Madzarevic M, Kenney EB. A reentry study on the use of bovine porous bone mineral, GTR, and platelet-rich plasma in the regenerative treatment of intrabony defects in humans. *Int J Periodontics Restorative Dent*. 2005;25:49-59. PMID:15736778

27. Lekovic V, Camargo PM, Weinlaender M, Vasilic N, Aleksic Z, Kenney EB. Effectiveness of a combination of platelet-rich plasma, bovine porous bone mineral and guided tissue regeneration in the treatment of mandibular grade II molar furcations in humans. *J Clin Periodontol.* 2003;30:746-51. PMID:12887344. <http://dx.doi.org/10.1034/j.1600-051X.2003.00368.x>
28. Anitua E. Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. *Int J Oral Maxillofac Implants.* 1999;14:529-35. PMID:10453668
29. Sánchez AR, Sheridan PJ, Kupp LI. Is platelet-rich plasma the perfect enhancement factor? A current review. *Int J Oral Maxillofac Implants.* 2003;18:93-103. PMID:12608674
30. Park, JB, Matsuura M, Han KY, Norderyd O, Lin WL, Genco RJ, et al. Periodontal regeneration in class III furcation defects of beagle dogs using guided tissue regenerative therapy with platelet-derived growth factor. *J. Periodontol.* 1995;66:462-77. PMID:7562336
31. Lynch SE, de Castilla GR, Williams RC, Kiritsy CP, Howell TH, Reddy MS, et al. The effects of short-term application of a combination of platelet-derived and insulin-like growth factors on periodontal wound healing. *J Periodontol.* 1991;62:458-67. PMID:1920013. <http://dx.doi.org/10.1902/jop.1991.62.7.458>
32. Mellonig JT. Enamel matrix derivative for periodontal reconstructive surgery: technique and clinical and histologic case report. *Int. J. Periodontics Restorative Dent.* 1999;19:9-19.

CORRESPONDING AUTHOR

Joni Augusto Cirelli

Departamento de Diagnóstico e Cirurgia, Faculdade de Odontologia de Araraquara, UNESP – Univ Estadual Paulista,

Rua Humaitá, 1680, 14801-903 Araraquara, SP, Brazil

e-mail:cirelli@foar.unesp.br

Recebido: 03/12/2011

Aceito: 26/12/2011