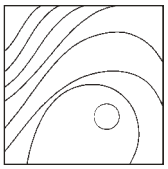
Platelet-Rich Plasma and Autogenous Bone Graft Combined with Guided Tissue Regeneration in Periodontal Fenestration Defects in Dogs



Ilker Keskiner, PhD[1]/Arzu Alkan, PhD[2]
Gokhan Acikgoz, PhD[3]/Nejat Arpak, PhD[4]
Suleyman Kaplan, PhD[5]/Huseyin Arslan, PhD[6]

The aim of this study was to evaluate the effects of platelet-rich plasma (PRP), autogenous bone (AB), and guided tissue regeneration (GTR) combination therapy compared to GTR therapy alone on healing of bone and cementum in fenestration-type periodontal defects in dogs. Six dogs were included in this study. Fenestration-type defects were created, and the following treatment groups were established: a control group treated with GTR alone and experimental groups treated with a combination of GTR + PRP, GTR + AB, and GTR + AB + PRP. The defects were evaluated by stereologic method and histomorphometric analysis, which were performed 4, 8, and 12 weeks postoperatively. The results showed a significant increase in trabecular bone area in the GTR + PRP group as compared with the control at 4 and 8 weeks (P < .05). The GTR + AB + PRP group showed significantly more trabecular bone area than both GTR and GTR + PRP groups at all time intervals (P < .05). The 8- and 12-week results in terms of cementum area revealed a significant difference between the GTR + AB + PRP group and the control in favor of the former (P < .05). Cementum area in the GTR + AB group was significantly greater than that in the GTR group at all time intervals (P < .05). Within the limitations of this study, PRP and AB, when used under barrier membrane, resulted in significant improvement in bone and cementum formation compared to GTR alone in periodontal fenestration defects; AB, rather than PRP, was responsible for this outcome. (Int J Periodontics Restorative Dent 2014;34:e112–e120. doi: 10.11607/prd.1997)

[1]Assistant Professor, Ondokuz Mayis University, Faculty of Dentistry, Department of Periodontology, Samsun, Turkey.
[2]Associate Professor, Erciyes University, Faculty of Dentistry, Department of Periodontology, Kayseri, Turkey.
[3]Professor, Ondokuz Mayis University, Faculty of Dentistry, Department of Periodontology, Samsun, Turkey.
[4]Professor, Ankara University, Faculty of Dentistry, Department of Periodontology, Ankara, Turkey.
[5]Professor, Ondokuz Mayis University, Faculty of Medicine, Department of Histology and Embryology, Samsun, Turkey.
[6]Associate Professor, Gaziosmanpasa University, Faculty of Medicine, Department of Histology and Embryology, Tokat, Turkey.

Correspondence to: Ilker Keskiner, Ondokuz Mayis University, Faculty of Dentistry, Department of Periodontology, Atakum, Samsun, Turkey; fax: 00903624576032; email: keskiner@omu.edu.tr.

The main rationale of periodontal therapy is regeneration of the periodontal attachment, including *cementum*, a functionally oriented periodontal ligament, and alveolar bone. The research to identify treatment modalities to promote regeneration of lost periodontal tissues has grown rapidly. Guided tissue regeneration (GTR) is a predictable way to restore periodontal structures. Epithelial cell exclusion, space provision, and clot establishment and stabilization are some of the basic characteristics of the GTR procedure. GTR has been used in periodontal defects solely[1] or in association with other biomaterials[2] and growth factors (GFs).[3] A recent systematic review indicated that the combination of barrier membranes and grafting materials may result in histologic evidence of periodontal regeneration, predominantly bone repair.[4]

Platelet-rich plasma (PRP), which can be regarded as an alternative approach to bone repair, is a concentration of platelets in a small volume of plasma. Platelets contain various GFs involved in wound repair and tissue regeneration, and it has been shown that activated

platelets release platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), insulinlike growth factor-I (IGF-I), epidermal growth factor (EGF), and others from α-granules. Because these concentrated platelets are suspended in a small volume of plasma, PRP also contains cell adhesion molecules (fibrin, fibronectin, and vitronectin) besides the GFs. It is believed that GFs have important effects on bone and cement, the components of periodontium, through cellular proliferation, migration, and differentiation.[5]

Autogenous bone (AB) has been considered the gold standard among biomaterials.[6] It includes cells participating in osteogenesis, and tissue reaction is induced without inducing immunologic reactions. Moreover, it has a potential release of growth and differentiation factors sequestered within the grafts, which lead to predictable clinical results.[7] The main reason for adding PRP to bone grafts is that a higher number of activated platelets in a wound will increase the local concentration of secreted GFs and, subsequently, enhance tissue repair and regeneration. After Marx et al showed that a combination of bone grafts with PRP resulted in both faster radiographic maturation and higher bone density than in the control group, the effect of PRP on bone regeneration has been extensively investigated.[8] However, the results are conflicting or equivocal. Although some studies demonstrated the beneficial effects of PRP only in the initial phase of bone formation,[9,10] others did not reveal significant differences[11,12] or even show a lower level of bone formation and a delay in the remodeling of bone grafts loaded with PRP.[13]

Periodontal fenestration defects commonly have been used to study the healing of periodontal structures experimentally and to display the biologic potential of implant materials, root surface biomodification agents, and GFs to stimulate periodontal healing.[14,15] Periodontal fenestration defects enable investigators to study regeneration of alveolar bone, root cementum, and connective tissue ligament in a standardized, well-defined wound unrelated to bacterial plaque and wound trauma.[14] Consequently, this defect model is not comparable with the regular chronic periodontal defect, but it offers some advantages in investigating possible differences in the quality of the newly formed tissues by minimizing the negative influence of flap dehiscence or infection.[16,17] On the other hand, it has been shown that although critically sized, acute-type fenestration defects are more likely to develop periodontal regeneration, a complete closure of the defect does not occur spontaneously.[16–19]

In a review of the current literature, no study of the effects of a PRP and AB graft combination on GTR could be found. In addition, histologic animal studies that substantiated PRP's ability to form bone and cementum in periodontal defects are scarce. Therefore, the authors decided to examine the effects of PRP and AB alone and in combination on bone and cementum formation in barrier membrane–covered periodontal fenestration-type defects in the dog model.

Table 1 Surgical and histologic evaluation protocol

Dog no.	Operation site	Time of surgery
1 & 4	Right Left	4 wk 8 wk
2 & 5	Right Left	8 wk Day 0
3 & 6	Right Left	Day 0 4 wk

Method and materials

Animals

Six male, mixed-breed, 2-year-old dogs, each weighing 16 to 20 kg, were used in this experiment. The study was performed at Ondokuz Mayis University Medical and Surgical Research Centre, Samsun, Turkey, and the procedure was approved by the Experimental Animal Ethics Committee of the same university. The animals were fed a soft diet throughout the study. All animals initially had intact dentition with gingival status ranging from clinically healthy to mildly inflamed. No clinical evidence of periodontitis was detectable. The surgical protocols were performed on the right or left sides of the maxillary and mandibular jaws of each dog at two different periods, as shown in Table 1.

PRP preparation

Before the surgical procedures, 20 mL of blood was retrieved from each animal via the vena cephalica or the

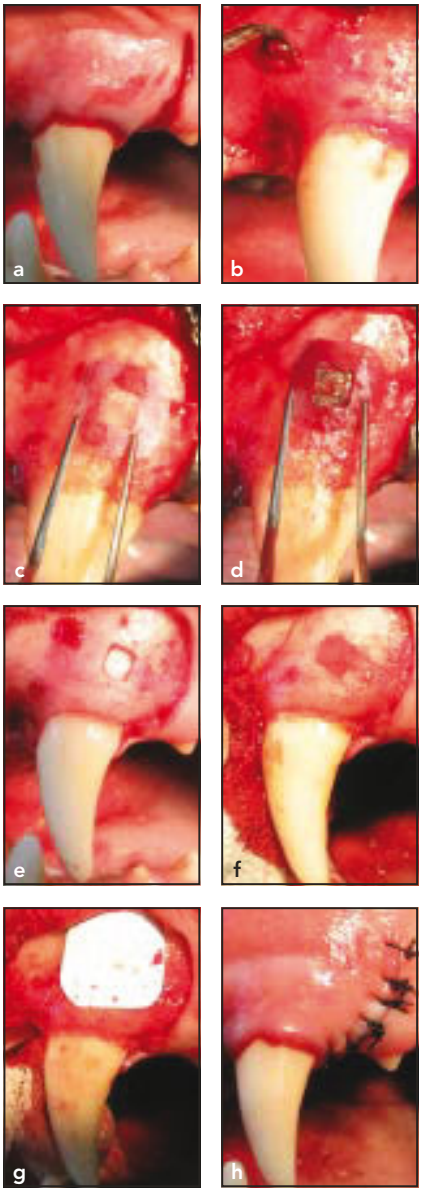


Fig 1 *Photographs demonstrating a surgical procedure on a dog's maxillary canine.* (a and b) *Incisions, elevation of the mucoperiosteal flap, and autogenous bone collection.* (c to e) *Template used for the preparation of the fenestration defect.* (f) *Autogenous bone in situ.* (g) *expanded polytetrafluoroethylene membrane covering fenestration defect.* (h) *Mucoperiosteal flap sutured.*

vena jugularis, if the former was unsuccessful, using vacutainer tubes containing 10% sodium citrate anticoagulant. The whole blood was first centrifuged (ALC PK130, DBJ Labcare) at 200 g for 7 minutes to separate the red blood cells from the plasma. The plasma was then taken to a second centrifugation at 250 g for 8 minutes, so the platelet-poor plasma (PPP) was separated from the PRP. The PPP was discarded and a 3 mL volume of PRP, together with the top 1 mm of the red blood cell layer, was taken to a sterile recipient in which 0.3 mL of 10% calcium chloride (STA, Diagnostica Stago), a citrate inhibitor that allows the plasma to coagulate, and surgical blood from the operation area were added. At an average room temperature of 25°C, the gel consistency was achieved in 3 to 4 minutes. Operative blood included thrombin, an activator that allows polymerization of the fibrin into an insoluble gel and causes platelets to degranulate and release GF into the surgical area. Platelet counts were performed to confirm the concentration of platelets in each dog's blood and PRP.

Surgical prepping and anesthesia

Food was withheld the night preceding the surgery. All animals were preanesthetized with atropine (Drogsan; 0.04 mg/kg) and ksilazin hydrochloride (Rompun, Bayer; 2.2 mg/kg); the systemic anesthesia was obtained with intramuscular (IM) injections of ketamine hydrochloride (Ketalar, Pfizer; 11 mg/kg).

Surgical procedure

Preparation of fenestration defects

Fenestration defects were performed in maxillary and mandibular canines and first molars and maxillary third incisors. The two roots of molars were used. This provided 14 fenestration defects per dog.

All visible plaque and calculus were removed with curettes. Following intracrevicular incisions and vertical incisions extending to the mucogingival junctions at the mesial and distal ends of the predetermined surgical sites (Fig 1a), full-thickness mucoperiosteal flaps were elevated at the vestibular aspects of the teeth (Fig 1b). The authors prepared a template, and, using a lead pencil, 4 x 4 mm areas were outlined on the bone as described by Wang et al approximately 5 mm apical to the alveolar crest to indicate the location of the fenestrations (Figs 1c and 1d).[20] Standardized fenestration-type defects were produced by surgically removing the bone plates using saline-cooled diamond burs and curetting the root to completely remove the periodontal ligament and cementum (Fig 1e). Using a small round bur, reference notches indicating the apical and coronal borders of the defect were prepared on the root surfaces. The defects were then treated by one of the following treatment modalities: group 1: GTR alone (control [C]); group 2: GTR + PRP; group 3: GTR + AB; and group 4: GTR + AB + PRP.

Autogenous bone collection and application

AB was harvested from the cortical bone plate approximately 7 mm away from the planned fenestration defects by scraping with a back action chisel (see Fig 1b). AB and PRP were mixed at a 1:1 ratio in volume. Graft with or without PRP was applied to the defects immediately according to a preestablished protocol so that each grafted defect was filled to exactly the level of the surrounding bone (Fig 1f).

An expanded polytetrafluoroethylene (e-PTFE) barrier membrane (Cytoflex, Unicare Biomedical) was trimmed and placed over each defect, extending approximately 3 mm beyond the defect margins (Fig 1g). The membranes were trimmed in such a manner that no collapse should occur into the defects. Mucoperiosteal flaps were then repositioned and sutured with 3-0 silk sutures (Fig 1h).

Postsurgical procedures

A broad-spectrum antibiotic (sefozin sodium; 10 to 12 mg/kg, IM) was administered immediately postsurgery and redosed for 3 days. A nonsteroidal anti-inflammatory analgesic (Caprofen-Rimadyl, Pfizer; 2 to 4 mg/kg) was administered for pain relief. Sutures were removed under sedation, approximately 10 days postsurgery.

The animals were anesthetized and euthanized after 4, 8, and 12 weeks postsurgery by an intravenous injection of concentrated sodium pentobarbital (Euthasols, Delmarva Laboratories).

Histologic tissue preparation and stereologic analysis

Block sections, including teeth and surrounding structures, were fixed in 10% buffered formalin and decalcified in 5% formic acid. The specimens were washed with tap water, dehydrated with ascending concentrations of ethyl alcohol, cleared in xylene, and embedded in paraplast. Tissue blocks were sectioned on a microtome (Leica-RM 2145, Leica Microsystems) in 8-µm thickness by the end of tissue. A pair of sampled sections in each step was taken from these sections at 80-µm intervals in a systematic random sampling manner. From each tissue block, approximately 50 pairs of sections were sampled. Each pair of sections was stained with hematoxylin and eosin. These sections were analyzed in a systematic random manner at 800 × 1000 µm and 200 × 277 µm steps for bone and cementum, respectively. The digitizing setup consisted of a modified light microscope (SOIF, oil objective; NA = 1.25; ×1,000) set at ×10 for bone and ×40 for cementum. The images of the histologic sections were transferred to the monitor of a computer via a camera (MD130 electronic eyepiece, 1.3 megapixels; OME-TOP Systems). A digital image processing program (Image J, National Institutes of Health) was used to constitute a cross grid that was used to estimate the area fraction of the mineralized bone matrix and cementum. The area of mineralized bone matrix was recorded as trabecular bone area. The cross-shaped grids were randomly placed on the defect with distances of 107 and 19 µm in bone and cementum, respectively. A systematic random sampling of the area on the section was conducted by means of the dial indicators. This ensured that all locations within a defect's cross section were equally represented and that the profile of the fenestration defect in the section was sampled with an equal probability, regardless of shape, size, orientation, or location of its part.

During each step of sampling, the points that were superimposed on the tissue region of interest, ie, bone and cementum, were counted. The area fraction of each tissue was estimated by dividing the total point number superimposed on the interested tissue by the total point that was posed on the whole cross section of the defect (sampling areas [SAs]). Trabecular bone and cementum area fractions were estimated by the formula given below, where B is bone, C is cementum, and SA is the sampling area.

$$\text{Area fraction of B} - \text{C} / \text{SA} = \frac{\text{Total points superimposed on bone or cementum}}{\text{Total points superimposed on the sampled areas}}$$

The unit of the area fraction is a percentage. Each of the histologic parameters was estimated separately by histologists unaware of the experimental group.

Statistical analysis

One-way analysis of variance was performed to test whether there were any differences among the four

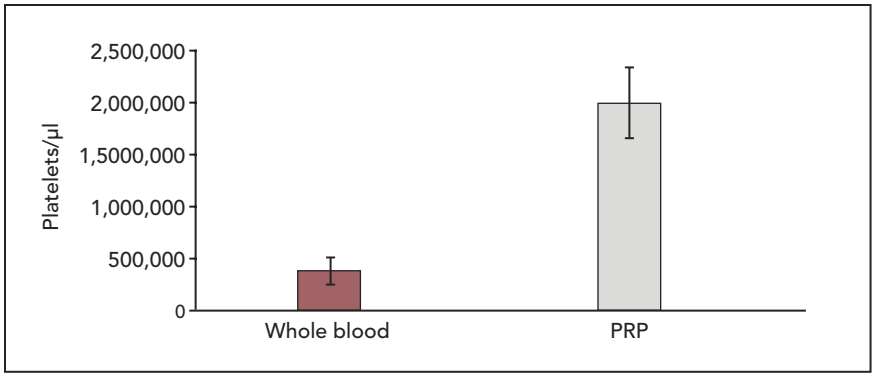


Fig 2 *Mean platelet numbers with SD in whole blood and PRP samples.*

Table 2 Intra- and intergroup comparisons of trabecular bone area (%, mean ± standard error) with regard to time intervals*

	4 wk	8 wk	12 wk
GTR	36.86 ± 1.07 Cb	45.65 ± 1.40 Ca	48.69 ± 1.98 Ca
GTR + PRP	43.91 ± 0.61 Bb	51.25 ± 0.78 Ba	51.38 ± 1.02 BCa
GTR + AB	48.05 ± 0.88 Ac	53.09 ± 0.31 ABb	54.50 ± 0.40 ABa
GTR + AB + PRP	50.99 ± 0.42 Ab	56.02 ± 0.90 Aa	58.62 ± 0.79 Aa

*Different letters (A, B, C) within column differ significantly ($P < .05$). Different letters (a, b) within row differ significantly ($P < .05$).

Table 3 Intra- and intergroup comparisons of cementum area (%, mean ± standard error) with regard to time intervals*

	4 wk	8 wk	12 wk
GTR	2.14 ± 0.12 Bb	7.79 ± 0.06 Ba	7.87 ± 0.12 Ba
GTR + PRP	2.69 ± 0.16 ABb	7.91 ± 0.11 ABa	8.00 ± 0.05 ABa
GTR + AB	2.85 ± 0.21 Ab	8.12 ± 0.03 Aa	8.20 ± 0.08 Ba
GTR + AB + PRP	2.81 ± 0.20 ABb	8.13 ± 0.06 Aa	8.32 ± 0.08 Aa

*Different letters (A, B, C) within column differ significantly ($P < .05$). Different letters (a, b) within row differ significantly ($P < .05$).

groups in a completely randomized design:

$$\hat{Y}_{ij} = \mu + \alpha_i + e_{ij}$$

where $\hat{Y}_{ij}$ is observation values (the values of trabecular bone and cementum areas), μ is the overall mean, α_i is the effect of the i^{th} treatment (i = 1, 2, 3, 4: 1 = C; 2 = GTR + PRP; 3 = GTR + AB; 4 = GTR + AB + PRP), and e_{ij} = residual error. Means were separated by using Tukey multiple comparison. Significance was evaluated at $P < .05$ for all tests. In addition to this analysis, paired sample t test analysis was performed to determine the differences between time intervals. This test was also used to evaluate the differences between platelet counts from the whole blood and PRP samples. All of the computational work was performed by means of SPSS version 10.0 (SPSS).

Results

There was no problem with healing in the animals and no membrane exposure was observed. Each group was supposed to have 7 defects, for a total of 84; however, 3 of them were discarded, 2 from the 8-week control group and 1 from the 8-week GTR + AB group, because of problems in the histologic preparation step and the extension of the defect base beyond the root surface to the bone during surgery, respectively.

The average whole blood and PRP platelet counts were 384.88 ± 131.16 × 103/µl and 1,995.69 ± 349.36 × 103/µl, respectively. Platelet counts in PRP were 5.19 times higher than found in whole blood (P = .000; Fig 2).

The mean (%) and standard errors of trabecular bone and cementum areas for all groups (C, GTR + PRP, GTR + AB, and GTR + AB + PRP) and intra- and intergroup comparisons with regard to time intervals are presented in Tables 2 and 3. Intragroup comparisons of trabecular bone areas in all groups revealed statistically significant differences between 4 and 8 weeks but failed to

show any difference between 8 and 12 weeks, except for the GTR + AB group. Although intergroup comparisons showed that GTR + PRP at 4 and 8 weeks demonstrated better outcomes in terms of the trabecular bone area compared with the control group ($P < .05$), no statistically significant difference was observed at 12 weeks. There was no significant difference between GTR + AB and GTR + AB + PRP groups with regard to the trabecular bone area in all time intervals. Although the difference between the GTR + PRP and GTR + AB groups was significant at 4 weeks, this difference disappeared at 8 and 12 weeks. PRP, AB, and GTR combination therapy resulted in better healing compared to GTR therapy alone in terms of new bone formation.

Intragroup cementum area comparisons showed that the cementum area significantly increased at 8 and 12 weeks compared with 4 weeks. However, the cementum area remained unchanged between 8 and 12 weeks. Intergroup comparisons revealed no statistical significance between GTR + PRP and GTR groups in all time intervals. An increase in the cementum area in the GTR + AB + PRP group was significantly greater than that observed in the GTR group at 8 and 12 weeks. A histologic view of a fenestration defect at 8 weeks that belongs to GTR + PRP group is shown in Fig 3a. The newly formed cementum (Fig 3b) and trabecular bone (Fig 3c) are noted in the magnified views.

Discussion

This was a histomorphometric and stereologic study in which the additional benefits of a PRP and AB combination in a GTR procedure was investigated in terms of bone and cementum formation.

The therapeutic outcome of an individual technique or agent may be improved by the addition of one or more. At the time of this study, investigators had already evaluated the combined effects of various graft materials, barrier membranes, and GF on periodontal regeneration. The literature review revealed that the combination of PRP with different graft materials was used by several authors[21,22] in periodontal defects, either using GTR in both test and control groups, as was done in the current study, or not using any membranes at all.[23,24] Taking these studies into account, a recent systematic review and meta-analysis showed that PRP may exert a positive adjunctive effect when used in combination with graft materials, but not with GTR, for the treatment of intrabony defects.[25]

The finding that PRP + AB + GTR offered better results than GTR membranes alone was consistent with the findings of Camargo et al.[26] As Del Fabbro et al pointed out, the design of the earlier studies precluded making an exact distinction of the role played by each agent in the regenerative process.[25] The presence of only PRP + GTR and AB + GTR groups in the current study design allowed the authors to maintain that the better outcome was related to the graft material beginning from the earliest phases of wound healing as demonstrated by a histomorphometric evaluation. In other words, the addition of PRP to AB in the presence of a barrier membrane did not improve the healing outcome in terms of the trabecular bone area, as evidenced by the finding that the trabecular bone areas in the GTR + AB + PRP and GTR + AB groups were similar for all of the time intervals examined. The explanation for this outcome may reside in the type of graft material used in this study. The ongoing inherent effects that the AB possessed may have masked the effects of PRP. The gold standard AB graft has two particularly important properties: containing cells that participate in osteogenesis[27] and releasing growth and differentiation factors.[7] To the best of the authors' knowledge, this study also was the first one conducted comparing the role of AB with those of PRP and GTR alone and investigating its effect in combination therapy in periodontal regeneration.

Fenestration-type periodontal defects were used instead of intrabony periodontal defects because they provide an optimal healing environment and wound stability that excludes factors interfering with healing, such as bacterial plaque, epithelial migration, and wound trauma. Fenestration-type defects with a dimension of 4 × 4 mm in this study were consistent with those used in other studies of potential alveolar bone and cementum regeneration.[14,16,18,20,28] Incomplete bone regeneration in membrane-covered control defects presented histologic

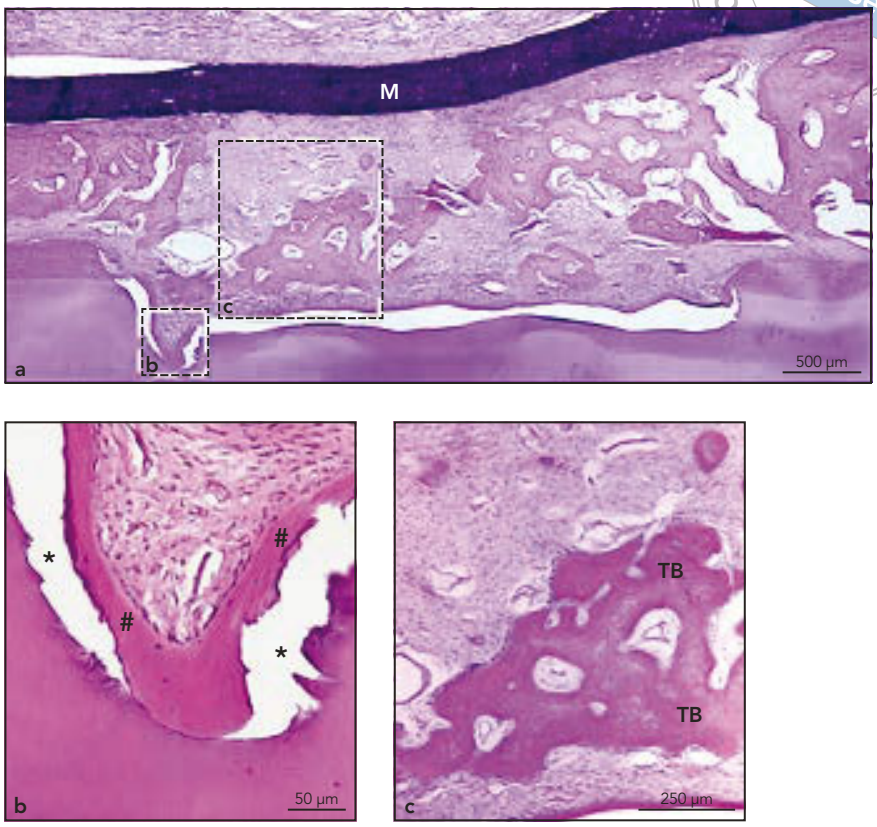


Fig 3 (a) *Panoramic view of a fenestration defect at 8 weeks covered with an e-PTFE membrane (M) that belongs to the GTR + PRP group. (Hematoxylin and eosin stain)* (b) *A magnified view shows the newly formed thick cementum (#) was close to the border of the defect and was partially detached (*) from the underlying dentin.* (c) *The trabecular bone was noted in the magnified view.*

evidence that fenestration defects in the current study were not too small to regenerate by themselves.

Weibrich et al[29] studied the effects of PRP alone in different concentrations on peri-implant bone regeneration and showed significant accelerated results only in PRP with an intermediate concentration of platelets (2- to 6-fold from native donor blood), whereas in lower (0.5- to 1.5-fold) or higher (9- to 11-fold) concentrations, a beneficial effect could not be observed. It was reported that the proliferation of adult mesenchymal stem cells and their differentiation were directly related to platelet concentration. They showed a dose-response curve that indicated that a sufficient cellular response to platelet concentrations first began when a 4- to 5-fold increase over baseline platelet number was achieved.[29] A 5.19-fold increase in platelet concentration, compared to the baseline in this study, met the concentration Weibrich et al recommended.

The GTR + PRP group induced significantly greater new bone formation at 4 and 8 weeks compared with GTR alone. Within the limits of this study, it may be concluded that PRP alone improved early bone healing, but this effect no longer existed at 12 weeks. Some studies have suggested that platelets and PDGF are more active during the early stage of bone graft healing because the life span of platelets is short.[15] However, Marx[30] opposed the idea and suggested that after the initial burst of PRP-related GFs, the platelets synthesize and secrete additional GFs for the remaining days of their lifespan. When the platelet expires, the macrophage stimulated by the platelets carries on the function of wound-healing regulation by secreting some of the same GFs as well as other GFs.[30] Indeed, an alternative explanation to the decrease in the PRP effect at 12 weeks may be the

positive ongoing influence of the barrier membrane masking the effects of the platelet concentrate, as was also stated by Del Fabbro et al.[25]

PRP used under barrier membranes showed no beneficial effect on cementum formation in any of the time intervals compared with GTR alone. However, the GTR + AB and GTR + AB + PRP groups showed an increase in cementum area compared to the control group at 4 and 8 weeks and throughout the study period, respectively. This finding may have indicated that AB rather than PRP increased cementogenesis. This fact is also supported by a very early study[31] and later studies,[32,33] which pointed to the effects of autogenous grafts in the regeneration of complete attachment apparatus including cementum.

In the present study, newly formed cementum was found to be thicker in the borders than near the center of the defect (Fig 3b). The origin of cementoblasts and the molecular factors regulating their recruitment and differentiation are not fully understood. It has been suggested, however, that new cementum is formed by cells that migrate from an existing layer of cementum or periodontal ligament.[34] This has also been reported in previous studies conducted on fenestration-type defects adjacent to the periodontal ligament and bone surrounding the defect; it probably represented the potential of cells from both tissues to migrate and produce a limited amount of regeneration.[15,17,28] The newly formed cementum was partially detached from the underlying dentin. This separation may be explained by the weak attachment between cementum and dentin. As also stated by Bosshardt et al, a residual smear layer on the dentin surface after surgical instrumentation might have precluded appropriate anchorage for the new cementum to the underlying dentin.[35]

Conclusions

Using PRP with GTR accelerated bone formation in the early healing period but had no beneficial effects on cementum formation compared to GTR alone. A procedure combining PRP, AB graft, and GTR resulted in better healing compared to GTR alone in terms of both new bone and cementum formation. AB rather than PRP was responsible for this outcome.

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References

1. Araújo MG, Berglundh T, Lindhe J. GTR treatment of degree III defects with 2 different resorbable barriers. An experimental study in dogs. J Clin Periodontol 1998;25:253–259.
2. Araújo MG, Lindhe J. GTR treatment of degree III furcation defects following application of enamel matrix proteins. J Clin Periodontol 1998;25:524–530.
3. Stavropoulos A, Wikesjö UM. Growth and differentiation factors for periodontal regeneration: A review on factors with clinical testing. J Periodontal Res 2012;47:545–553.
4. Sculean A, Nikolidakis D, Schwarz F. Regeneration of periodontal tissues: Combinations of barrier membranes and grafting materials—biological foundation and preclinical evidence: A systematic review. J Clin Periodontol 2008;35(Suppl 8): 106–116.
5. Gao J, Symons AL, Bartold PM. Expression of transforming growth factor–beta 1 (TGF-beta1) in the developing periodontium of rats. J Dent Res 1998;77:1708–1716.
6. Rosenberg E, Rose LF. Biologic and clinical considerations for autografts and allografts in periodontal regeneration therapy. Dent Clin North Am 1998;42:467–490.
7. Marx RE. Clinical application of bone biology to mandibular and maxillary reconstruction. Clin Plast Surg 1994;21:377–392.
8. 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; 85:638–646.
9. Schlegel KA, Donath K, Rupprecht S, et al. De novo bone formation using bovine collagen and platelet-rich plasma. Biomaterials 2004;25:5387–5393.
10. Wiltfang J, Kloss FR, Kessler P, et al. Effects of platelet-rich plasma on bone healing in combination with autogenous bone and bone substitutes in critical-size defects. An animal experiment. Clin Oral Implants Res 2004;15:187–193.
11. Aghaloo TL, Moy PK, Freymiller EG. Investigation of platelet-rich plasma in rabbit cranial defects: A pilot study. J Oral Maxillofac Surg 2001;60:1176–1181.
12. Jakse N, Tangl S, Gilli R, et al. Influence of PRP on autogenous sinus grafts. An experimental study on sheep. Clin Oral Implants Res 2003;14:578–583.
13. Choi BH, Im CJ, Huh JY, Suh JJ, Lee SH. Effect of platelet-rich plasma on bone regeneration in autogenous bone graft. Int J Oral Maxillofac Surg 2004;33:56–59.
14. Selvig KA, Bogle GC, Sigurdsson TJ, Wikesjö UME. Does root surface conditioning delay healing? J Clin Periodontol 1996;23:119–127.

15. Choi SY, Nilvéus RE, Minutello RD, Zimmerman GJ, Wikesjö UM. Effect of a collagen matrix on healing in periodontal fenestration defects in dogs. J Periodontol 1993;64:878–882.
16. Petterson EC, Aukhil I. Citric acid conditioning of roots affects guided tissue regeneration in experimental periodontal wounds. J Periodont Res 1986;21: 543–552.
17. Tal H, Pitaru S, Moses O, Kozlovsky A. Collagen gel and membrane in guided tissue regeneration in periodontal fenestration defects in dogs. J Clin Periodontol 1996;23:1–6.
18. Caton JG, De Furia EL, Polson AM, Nyman S. Periodontal regeneration via selective cell repopulation. J Periodontol 1987; 58:546–552.
19. Claffey N, Mostinger S, Ambruster J, Egelberg J. Placement of a porous membrane underneath the mucoperiosteal flap and its effect on periodontal wound healing in dogs. J Clin Periodontol 1989;16:12–16.
20. Wang HL, Hamilton RL, Castelli WA, Chiego DJ, Smith BA. Effect of root conditioning on periodontal wound healing with and without guided tissue regeneration: A pilot study. 1. Histologic evaluation. Int J Periodontics Restorative Dent 1993;13:551–561.
21. Christgau M, Moder D, Wagner J, et al. Influence of autologous platelet concentrate on healing in intrabony defects following guided tissue regeneration therapy: A randomized prospective clinical split-mouth study. J Clin Periodontol 2006;33:908–921.
22. Döri F, Huszár T, Nikolidakis D, Arweiler NB, Gera I, Sculean A. Effect of platelet-rich plasma on the healing of intrabony defects treated with an anorganic bovine bone mineral and expanded polytetrafluoroethylene membranes. J Periodontol 2007;78:983–990.
23. Okuda K, Tai H, Tanabe K, et al. Platelet-rich plasma combined with a porous hydroxyapatite graft for the treatment of intrabony periodontal defects in humans: A comparative controlled clinical study. J Periodontol 2005;76:890–898.
24. Piemontese M, Aspriello SD, Rubini C, Ferrante L, Procaccini M. Treatment of periodontal intrabony defects with demineralized freeze-dried bone allograft in combination with platelet-rich plasma: A comparative clinical trial. J Periodontol 2008;79:802–810.
25. Del Fabbro M, Bortolin M, Taschieri S, Weinstein R. Is platelet concentrate advantageous for the surgical treatment of periodontal diseases? A systematic review and meta-analysis J Periodontol 2011;82: 1100–1111.
26. 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 Periodontal Res 2002;37:300–306.
27. Carranza FA, Takei HH, Cochran DL. Reconstructive periodontal surgery. In: Newman MG, Takei HH, Klokkevold PR, Carranza FA, eds. Clinical Periodontology. 10th ed. St Louis: Saunders; 2006: 968–990.
28. Aukhil I, Simpson DM, Suggs C, Pettersson E. In vivo differentiation of progenitor cells of the periodontal ligament. An experimental study using physical barriers. J Clin Periodontol 1986;13:862–868.
29. Weibrich G, Hansen T, Kleis W, Buch R, Hitzler WE. Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration. Bone 2004;34: 665–671.
30. Marx RE. Platelet-rich plasma: Evidence to support its use. J Oral Maxillofac Surg 2004;62:489–496.
31. Hiatt WH, Schallhorn RG, Aaronian AJ. The induction of new bone and cementum formation. IV. Microscopic examination of the periodontium following human bone and marrow allograft, autograft and nongraft periodontal regenerative procedures. J Periodontol 1978;49: 495–512.
32. Camelo M, Nevins ML, Lynch SE, Schenk RK, Simion M, Nevins M. Periodontal regeneration with an autogenous bone-Bio-Oss composite graft and a Bio-Gide membrane. Int J Periodontics Restorative Dent 2001;21:109–119.
33. Verdugo F, D'Addona A. Long-term stable periodontal regeneration by means of autologous bone grafting in patients with severe periodontitis. Int J Periodontics Restorative Dent 2012;32:157–164.
34. Park JC, Um YJ, Jung UW, Kim CS, Choi SH, Kim CK. Histological characteristics of newly formed cementum in surgically created one-wall intrabony defects in a canine model. J Periodontal Implant Sci 2010;40:3–10.
35. Bosshardt DD, Sculean A, Windisch P, Pjetursson BE, Lang NP. Effects of enamel matrix proteins on tissue formation along the roots of human teeth. J Periodontal Res 2005;40:158–167.