

Original Article

Guided tissue regeneration and platelet rich growth factor for the treatment of Grade II furcation defects: A randomized double-blinded clinical trial - A pilot study

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ABSTRACT

Background: The treatment of furcation area defects remained as a challenging issue in periodontal treatments. Regeneration treatment of furcation defects is the most discussed periodontal treatment. Although not completely hopeless in prognosis, the presence of the furcation involvement significantly increases the chance of tooth loss. The current research was conducted to compare the additive effect of combined guided tissue regeneration (GTR) and platelet-rich growth factor (PRGF) on the treatment of furcation bony defects.

Materials and Methods: A randomized, triple-blinded, split-mouth study was designed. It included patients with a moderate to severe chronic periodontitis with bilateral Grade II furcation involvement of first or second mandibular molars. Each side of mouth was randomly allocated for the treatment with either Bio-Gide American Society of Anesthesiologists GTR or a PRGF or PRGF by itself. Plaque index, gingival index, vertical clinical attachment level, vertical probing depth, recession depth (REC), horizontal probing depth, fornix to alveolar crest (FAC), fornix to base of defect (FBD), furcation vertical component and furcation horizontal component (FHC) were recorded. The current research was conducted to compare the additive effect of combined GTR and PRGF on treatment of furcation bony defects. Altman's nomogram, Kolmogorov-Smirnov test, Friedman test, general linear model, repeated measures, and paired t-test were used as statistical analysis in this research. $P < 0.05$ was considered statistically significant.

Results: Eight patients were finally enrolled for this study. Overall, general and specific clinical and furcation parameters were improved except REC that was deteriorated insignificantly and FAC improved not significantly. Intergroup comparison revealed better improvement of FHC in GTR/PRGF group ($P = 0.02$).

Conclusion: A significant improvement in the Grade II furcation defects treated with either GTR or PRGF/GTR was noticed. Further large-scale trials are needed to reveal differences of mentioned treatment in more details.

Key Words: Bioengineering, guided tissue regeneration, platelet-Rich plasma, tooth, furcation

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INTRODUCTION

Furcation area involvement causes one of the greatest challenges to the success and prognosis of the periodontal treatments. Although not completely hopeless in prognosis, the presence of the furcation involvement significantly increases the chance of tooth loss. This special anatomic location is of paramount importance due to its limited access.^[1] In general, the outcome depends on many confounding factors including affected tooth, cigarette smoking, systemic comorbidities such as diabetes mellitus (DM), and stress.^[2]

Historically, methods such as open and closed root preparation, odontoplasty, open flap debridement, and root resection are suggested; however, none of them gained popularity due to the lack of definite and determinable outcomes.^[1]

First introduced in 1923 by Hegedus, six cases of successfully treated pyorrhea with autograft bone transplantation from tibia to jaw bone were reported.^[3] Since then, the reconstructive methods were vastly developed including auto- and allo-graft, absorbable and nonabsorbable membranes (ieguided tissue regeneration [GTR]), or combination of these methods.^[4] Evolving three decades ago by Karring *et al.* and Nyman *et al.*, GTR method e as more reputable and promising treatment modality.^[5-8] However, a common problem in GTR treatment is about a predictable limited result by several factors such as the furcation morphology, complete access for adequate debridement, and few remaining periodontal cells *in situ* that warrant the succeeds of periodontal treatment.^[7]

Concomitantly, pioneered by Marx in 1998, stimulation of exiting progenitor cells became a hot topic in soft and hard tissue healing. Plateleterich plasma (PRP) is an autologous highly platelet-enriched blood product.^[9] This product is obtained from the centrifuged blood sample which contains a high concentration of a platelet-derived growth factor, insulin-like growth factor, and fibroblast growth factor.^[10] In addition, the lower PH of PRP (6.5–6.7) compared to normal blood (7–7.2) may boost its bacteriostatic and bactericidal properties though the latter is controversial.^[11]

Introduction of platelet-rich growth factor (PRGF) technology dates back to 1999 by Anitua.^[12] This method describes a unique blend of 100% autologous

product along with the absence of bovine thrombin that actually eliminates the risk of infection. It consists of one-step centrifuging of a low-volume harvested patient's blood that will be anticoagulated with the sodium citrate and mixed with the calcium chloride for inducing a jelly product. The final product could be laid on the deficient area. Other beneficial properties of PRGF include: being user-friendly, low cost, considerably lower required blood volume, shorter preparation time (5–10 min), and capability of in-office application.^[13,14]

Periodontal diseases and furcation involvement are increased with age and are aggravated by a systemic disease such as DM.^[15] Population aging and increased prevalence of DM in our country, Iran, are sensed during the recent decade.^[15,16] Exiting literature is scarce for the combination of PRGF (i.e., not PRP) with GTR technique. Hence, the current research was conducted to compare the additive effect of combined GTR and PRGF on the treatment of furcation bony defects.

MATERIALS AND METHODS

Study design and eligibility criteria

The current study was designed as a randomized, triple-blinded (patient, clinician, and biostatistician), split-mouth study. Eight patients who are visited in Periodontology Department of Babol University of Medical Sciences and suffered from a moderate-to-severe chronic periodontitis with bilateral Grade II furcation involvement of the first or second mandibular molars and had at least 3 mm vertical probing depth (VPD) were included in this study.

Exclusion criteria were defined as: (1) a systemic disease and abnormal clotting time or complete blood count report, (2) the confounding medications which interfere with a wound healing (e.g., corticosteroids), (3) a cigarette smoking, (4) the presence of cavity or filling in furcation area, (5) an anatomical complexity such as cemento-enamel projection and bifurcation ridge in selected teeth, (6) Miller's mobility of Grade II and more in selected teeth, (7) the need of the prophylactic antibiotic for the infectious endocarditis, (8) any known allergies to the predetermined materials and any contraindication for periodontal surgery, (9) the presence of the periapical lesions in radiography, and (10) the unwillingness of the patient to accept a periodontal surgery and being a compliant during follow-ups [Figure 1].

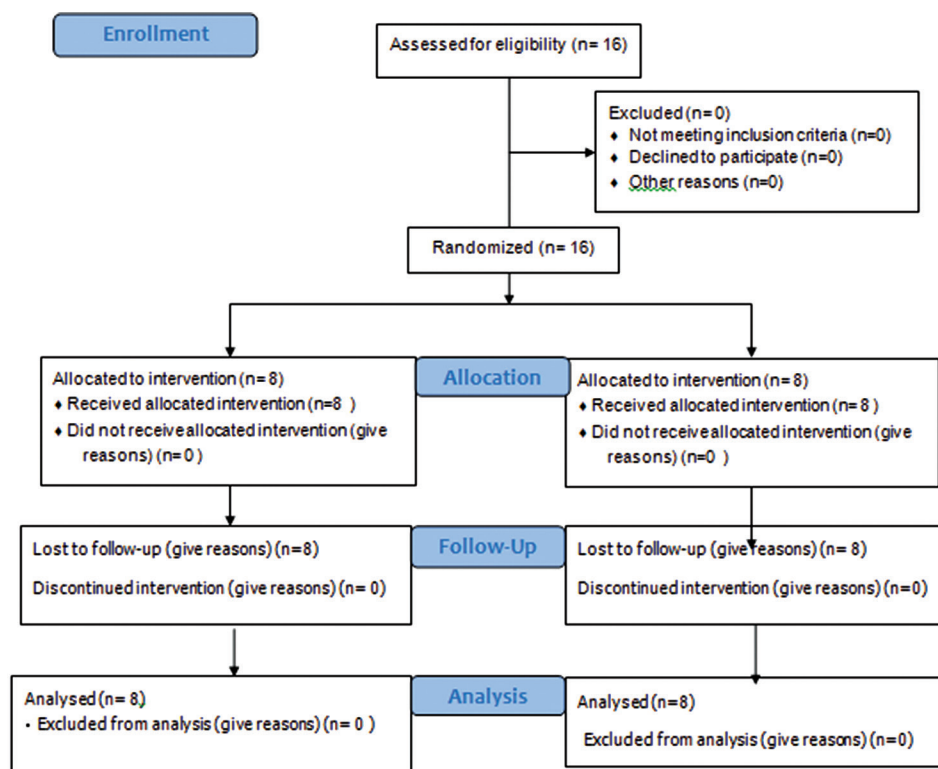


Figure 1: Study design and eligibility criteria flowchart.

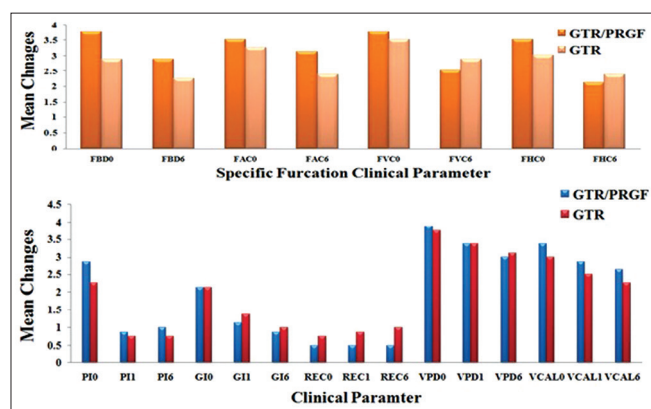


Figure 2: Changing trends of specific furca (upper part) and general clinical parameters (lower part). 0, 1, and 6 denote baseline, at the time of surgery, 1 month, and 6 months after the surgery, respectively.

Randomization and blindness

The patients were assigned for the treat with either a bilayerd collagen barrier (Bio-Gide), (GTR) by itself, or in combination with PRGF (PRGF/GTR). The right side of oral cavity was arbitrarily selected for random allocation. One in-charge clinician performed that the interventions (HE) were unaware of the assignments codes. Further measurements of the periodontal indices were performed by the another clinician (NJ) who blinded to the study arms. A maxillofacial radiologist

blindly reported the osseous changes (SH). This study was approved by the ethical committee of university, and all clinicians undertook Helsinki treaty. It is also registered in the WHO clinical trial registry, branch of the Islamic Republic of Iran (IRCT: 201201231760).

Study protocol

All enrolled patients were approached regarding a written informed consent. Oral hygiene instruction scaling and a root planing (SRP) were provided to all patients for reaching a satisfactory intraoral biofilm level before the surgery. Occlusion was also corrected, if needed. All surgical procedures were performed by the same person (HE) and were as the following: intrasulcular incision with mucoperiosteal flap elevation, debridement of granulation tissue, subgingival SRP, and rinsing with normal saline. Then, the patient received the arms based on the predefined random allocation. Thereafter, the coronally positioned flap was sutured in place with silk 4-0, and the patients were released with antibiotic prescription (penicillin VK, 500 mg, QID), the directions for an oral hygiene (chlorhexidine% 0.12, 5cc, BID) and with the recalls schedule.

Primary and secondary end points

Recording of the baseline measurements was accomplished with a scaled periodontal probe. The

recorded indices were as the followings: the vertical clinical attachment level (VCAL, the distance between the cemento-enamel junction (CEJ) to the depth of pocket), the VPD (the distance from the free gingival margin to the place in which the attached gingiva connect to the tooth; the average of mesiobuccal, midbuccal, and distobuccal measures was taken into account), recession depth (REC, distance from the CEJ to the free gingival margin measured in midbuccal), fornix to the alveolar crest (FAC, distance between FAC at midbuccal or midlingual area), fornix to base of defect (FBD, distance between the fornix and lesion base in midbuccal or midlingual area), furcation vertical component (FVC, the difference of FBD and FAC), furcation horizontal component (FHC, penetration depth to furca from the buccal or a lingual side parallel to the horizon).

The VPD, VCAL, gingival index (GI), plaque index (PI), and REC were recorded at baseline (T_0), at the time of surgery (T_1), and 6 months after the surgery (T_6). The FBD, FAC, FVC, and FHC recorded at first during the surgery (T_0) and 6 months later with the reentry method (T_6).

PRGF preparation

Blood harvesting was performed a few minutes before the surgery (10 ml). Each 4.5 cc of collected blood was mixed in sterilized tubes with 0.5 cc sodium citrate 3.8% as an anticoagulant. The final preparation was centrifuged with a single speed device at 460 g for 8 min (PRGF-Endoret System IV Biotechnology Institute, Vitoria, Spain). Following centrifugation, plasma was divided into parts including the PRGF, the plasma poor in GF, and plasma average in GF.

The PRGF was the buffy coat just above the red blood cells within the tube, and it was meticulously collected with a 100 μ L micropipette and each 1 ml of it mixed with 50 μ L calcium chloride 10%. This resulted in a jelly product saved in a sterilized concealed glass container till the surgery.

Radiologic assessments

The first radiograph was taken with the parallel technique using a PSP Digital Sensor Size 2 (PCT, Soredex; Helsinki, Finland). Bite registration was accomplished using acrylic resin (Duralay, Reliance, worth, IL, USA) that was at first recorded during the radiography to ensure the same occlusion during the recall radiography. Hence, the second radiography that was taken 6 months later was performed with the same kV, mA, exposure time, and same occlusion. Images were recorded as

DICOM series and were processed using Digora for Windows version 2.5 (PCT, Soredex; Helsinki, Finland). The digital subtraction of pre- and post-treatment images and after treatment was done by Photoshop CS6 software (Adobe Systems, California, USA). Serial digital images that are produced in a parallel manner could be superimposed, and a combination of two images can be seen on the screen. When the two images are recorded from the same object and the image intensities of corresponding pixels are subtracted, the difference will produce a uniform image. This technique is known as digital subtraction radiography (DSR).^[17,18] The results interpreted as an osteogenesis if radiopacity was observed and as a resorption if difference of density was seen as radiolucency, otherwise it would be reported as “no change.”

Statistical analysis

We estimated that eight patients should be enrolled to achieve 80% power for a standardized difference of 1.4 between the two study arms which was calculated for the primary end point (vertical clinical attachment loss), using an Altman's nomogram. Continuous data were expressed as mean (\pm standard deviation). A Kolmogorov–Smirnov test was used to assess the normal distribution of the data. A general linear model repeated measures statistics was applied to trace the changing trend of each index within each study arm for normally distributed data; a Friedman test was used. A paired *t*-test was used to assess two-time parameter changes and to compare mean differences between the right and left side of the mouth. Power of the study was calculated with G-power version 3.1.9.2 software. A two-tailed α at $P < 0.05$ was considered statistically significant.

RESULTS

In this clinical trial, a total number of eight patients were finally enrolled, and 16 sites were intervened by either GTR/PRGF or PRGF. Three patients were female and five patients were male their age ranged from 32 to 48 years with a mean age of 40 years.

Radiologic findings

Interpretations of radiographs are displayed in Table 1. Chi-square test did not show any difference between the study arms ($\chi^2 (2) = 2.33, P = 0.31$). A further investigation was executed by quantifying the data with allocation of 0, 1, and 2 points to the respective resorption, no change, and osteogenesis (GTR/PRGF: 2.12 ± 0.83 , GTR: $2 \pm 0.53, t (7) = 0.31$, mean

different = 0.12, confidence interval 95%: 0.81–1.06, $P = 0.76$).

Clinical findings

Data regarding clinical parameters of patients are displayed in Table 2 and Figure 2. Comparison of mean differences between two study arms calculated from T_0 to T_1 for the general clinical parameters and from T_0 to T_6 for specific furca parameters was as the followings: GI: $P = 0.76$, PI: $P = 0.29$, REC: $P = 0.35$, VCAL: $P = 0.1$, FAC: $P = 0.38$, FBD: $P = 0.63$, FVC: $P = 0.05$, and VPD: $P = 0.52$. Improvement of FHC was better in GTR/PRGF group ($P = 0.02$). Overly, the general and specific clinical and furca parameters were improved except for REC parameter that deteriorated insignificantly and for FAC parameter that was improved yet not significantly. Calculated power with effective size = 0.12 and 0.05, first error was 0.05.

DISCUSSION

The current research was conducted on patients with a moderate-to-severe chronic periodontitis with a

Table 1: Osseous changes separated for the study arms

Results	Study arms	
	GTR/PRGF (number of patients)	GTR (number of patients)
Resorption	2	1
No change	3	6
Osteogenesis	3	1

GTR: Guided tissue regeneration; PRGF: Platelet-rich growth factor

Table 2: Comparison of changes in general and specific clinical parameter

Parameters	Study arm, significant	
	GTR/PRGF	GTR
PI*	0.001	0.001
GI*	0.005	0.006
REC**	1‡	0.23‡
VPD**	0.02	0.03
VCAL**	0.05**	0.008
FBD†	0.02	0.05**
FAC†	0.40‡	0.006
FHC†	<0.001	0.01
FVC†	0.005	0.05**

*Friedman test (nonparametric); **GLM-repeated measures; †Paired t-test; ‡Statistically insignificant; **Marginal significance. PI: Plaque index; GI: Gingival index; REC: Recession depth; VPD: Vertical probing depth; VCAL: Vertical clinical attachment level (mm); FBD: Fornix to base of defect (mm); FAC: Fornix to alveolar crest (mm); FHC: Furcation horizontal component (mm); FVC: Furcation vertical component (mm)

bilateral Grade II furcation involvement of the first or second mandibular molars. To avoid the natural differences and the selection bias, a split-mouth design was opted for this study. The study arms consisted of either PRGF with GTR or GTR by itself interventions.

The results supported that Grade II molar furcation lesions by either the same treatment might significantly improve in most clinical parameters measured, such as PI, GI, VPD, VCAL, FBD, FAC, FHC, and FVC when compared to baseline status and the time of surgery of untreated sites. A higher trend was observed in PRGF/GTR group; however, it was not statistically significant.

Baseline gingival recession remained unchanged in GTR and deteriorated in GTR/PRGF group. This condition is somewhat expectable after surgical manipulation which is in commitment with Christgau *et al.*^[18]

The great accumulated concentration of various growth factors in PRGF could stimulate the proliferation and differentiation of periodontal ligament cells and osteoblasts.^[19] In addition, it is assumed that the PRP could stabilize the *in situ* clot due to its thrombin that reacts with fibrinogen, and this in turn facilitates synthesis of collagen in the extracellular matrix scaffold for the migration of cells and thus attachment of these cells.^[20] Remarkable aforementioned advantages of PRGF over other PRP systems persuaded us to apply this method of platelet concentrate preparation.

In general, application of a variety of barrier membranes in regenerative periodontics has led to favorable results. In this study, an absorbable membrane (Bio-Gide) was used as the temporary separator of furcation area from unfavorable cells.^[21,22] Nevertheless, Sammartino *et al.* postulated that the combination of PRP with Bio-Gide membrane is beneficial in healing bone defects of extracted third molar sites compared to the other control groups.^[23]

According to the available literature so far, there is no quite similar previous research to ours. Many combinations of PRP, autogenous platelet concentrate, beta-tricalcium phosphate and GTR are reported with contrasting results.^[18] Similar to Sharma and Pradeep and on the contrary to Anitua and Sammartino *et al.*, a definite role and conclusive results could not asserted based on our findings.^[12,23-25]

These may be due to some reasons:

- Previously, Weibrich and Kleis pointed out that not all available machines provide acceptable

performance.^[26] We used a single-spin device. Dual-spin centrifuges (e.g., Harvest Tec, USA) have been reported to be more effective than single-spin centrifuges. Single-spin centrifuges may produce a mixture of platelet-poor and platelet-rich plasma, hence containing lower concentration of growth factors in a given volume as compared to dual-spin systems.^[10,27] This matter could be one reason for the lack of a significant superiority of PRGF over GTR method

- It is likely that small sample size attenuates the power of the study to differentiate the effects of study arms which is reflected in calculated power (power = 0.05)
- Similar to Christgau *et al.*, we used DSR method to assess osseous changes.^[18] Being qualified intuitively, it may suffer from optimal sensitivity. The same horizontal and vertical geometry of serial images is crucial to ensure the reliability of the method.^[17,18] However, this concern was solved using an impression material and the parallel technique in the current research. Moreover, it is noteworthy to mention that at least 612 months is needed for apparent changes in conventional radiologic images. Our study was limited in just a 6-month follow-up. A quantified method may include animal histological study or measuring bone mass density
- It is controversial that PRGF exerts either osseoinductive or osseoconductive effect. Hence, we entered moderate-to-severe furcation involved sites.^[10] Hence, the treated sites may lack enough viable tissue to be stimulated by PRGF which justifying the remarkable effect of PRP in extracting sites or when it is used in combination with auto- or allo-graft bone that contain necessary substrate cells.

CONCLUSION

The present study evaluated the clinical and the radiographic parameters (using DSR method) after 6 months of treatment and a significant improvement in the Grade II furcation defects treated with either GTR or PRGF/GTR were noticed. A slightly better result of GTR in combination with PRGF was observed, yet this did not reach statistically significant level that needs further large-scale and quantified studies.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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